BOLETIN DE SANIDAD VEGETAL

Fuera de Serie - N.° 17 - 1989

PROCEEDINGS OF A SCIENTIFIC CONGRESS

Barcelona, Spain, 25-28 October 1988

Edited by R. CAVALLORO and V. DELUCCHI

PARASITIS 88

MINISTERIO DE AGRICULTURA PESCA Y ALIMENTACION

DIRECCION GENERAL DE LA PRODUCCION AGRARIA

«PARASITIS 88»

COMMITTEE OF HONOUR

Excelentísimo señor don Carlos Romero Herrera
Ministro Agricultura Pesca y Alimentación-Madrid, Spain
His Excellency Denis Bra Kanon
Ministre de l'Agriculture-Abidjan, Côte d'Ivoire
His Excellency Farama I. Sagna
Ministre du Développement Rural-Dakar, Sénégal
Excelentísimo señor don Joseph Miró I Ardevol
Conseiller de Agricultura Ramaderia i Pesca, Generalitat de Catalunya-Barcelona, Spain

BOARD OF DIRECTORS

President:

Raffaele Cavalloro, CEC, Joint Research Centre-Ispra, Italy

Former President:

Vittorio Delucchi, ETH, Institut für Phytomedizin-Zürich, Switzerland

Vice-Presidents:

Jean-Pierre Bassino, ACTA-Paris, France
Domingo Cadahia, Ministerio Agricultura Pesca y Alimentación-Madrid, Spain

Members:

Angelo Adam, FAO-Roma, Italy
Andrew P. Gutiérrez, University of California-Berkeley, USA
Gabriel Lohoury-Guigui, Sofaco-Abidjan, Côte d'Ivoire
Henri Simonet, Roussel-Uclaf-Paris, France
Antoni Siurana i Zaragoza, Ajuntament-Lleida, Spain
José M. Vives, Departamento de Agricultura Ramaderia i Pesca, Generalitat de Catalunya-Barcelona, Spain

Treasurer:

J. M. Mascherpa, Centre Horticole de Lullier-Genève, Switzerland

Secretary General:

Henry Aeby, Omni-expo-Genève, Switzerland

SECRETARIAT

«Parasitis» International Association
32, rue de Malatrex
CH-1201 **Genève**

STRUCTURE OF THE MEETING

SESSION'S ORGANIZATION

Opening session: Introductory and welcoming addresses

Session 1: Biotechnology

Session 2: Informatic

Session 3: Application, use and transmission of knowledge

Session 4: Public health and hygiene: household pest control

Session 5: The safety of pesticid's use

Session 6: Quality assessment of biological control agents

Session 7: Storage of food

Chairmen: M. H. Aeby, F. Sala, J. Blanco, D. J. Peix, A. P. Gutiérrez, J. Baumgärtner, D. F. González, B. Sánchez Murias, R. Bailly, J. L. Cervigon, J. P. Aeschlimann, D. G. Casanova & E. Celma Calamita.

FOREWORD

R. Cavalloro & V. Delucchi

TABLE OF CONTENTS

PREFACIO

Poco ha cambiado la situación existente desde la celebración de PARASITIS 86 en Ginebra, durante los días 9 a 13 de diciembre de 1986, para tratar sobre los aspectos teóricos de la Protección Integrada (PI) contra las plagas y enfermedades. Los problemas derivados de la utilización de plaguicidas, en la protección de los cultivos, de los animales domésticos, del hombre y su medio ambiente, de los alimentos y productos almacenados, continúan presionando hacia la puesta en práctica y nuevas aportaciones de la PI.

Nuevamente, en Barcelona, la celebración de PARASITIS 88 ha reunido a un grupo de eminentes investigadores y especialistas para ampliar la vista panorámica de la situación actual en los diferentes dominios de la investigación, que es preciso considerar para establecer programas de PI y aportar sugerencias constructivas sobre los futuros desarrollos posibles, a tomar en cuenta, tanto por los investigadores y técnicos responsables de los citados programas, como por los políticos.

En esta ocasión, se han hecho realidades estas aportaciones complementarias, mediante la celebración de PARASITIS 88 con siete sesiones que han sido presididas por: M. H. Aeby, F. Sala, J. Blanco, D. J. Peix, J. Baumgärtner, P. F. González, B. Sánchez Murias, R. Bailly, J. L. Cervigón, J. P. Aeslimann, D. G. Casanova y E. Celma, dentro de las cuales se han presentado 38 contribuciones, a cuyos autores damos las gracias desde estas páginas.

R. Cavalloro
V. Delucchi

OPENING SESSION

INTRODUCTORY AND WELCOMING ADRESSES

Chairman: M. H. Aeby

ADRESSE D'OUVERTURE

R. Cavalloro, Président
Association Internationale «Parasitis»

Autorités, Mesdames, Messieurs, chers Collègues,

En ma qualité de Président de l'Association Internationale "Parasitis" je me réjouis que cette manifestation de Barcelone ait reçu, tant pour le Congrès que pour l'exposition, une attention tout à fait particulière et une réponse aussi large de participants qualifiés.

Cette combinaison, qui nous permet de réunir les forces de la recherche, de l'industrie, de la production et de l'utilisation en matière de protection phytosanitaire, réalise une véritable symbiose entre ces différents domaines, dans le but de trouver et appliquer tous ensemble des pratiques agricoles correctes.

La preuve la plus évidente de la validité, de l'importance et de l'actualité de cette action est précisement votre présence ici et votre disponibilité à nous rencontrer pour débattre les problèmes, confronter les expériences et progresser en étroite collaboration avec un esprit de coopération et de participation active vers le même but: le bien-être de l'homme dans un monde sain et où l'on peut vivre mieux.

L'évolution rapide dans le secteur techno-scientifique est une réalité même en agriculture et vise à l'obtention de récoltes abondantes, saines et de bonne qualité, toujours dans l'optique de faire face aux pressants besoins alimentaires, en améliorant en même temps notre propre existence.

Cette manifestation fait ressortir cet important développement et les dernières réalisations dans le secteur phytosanitaire, en se mettant à l'avant-garde dans l'acceptation prudente des nouvelles technologies, fruit d'un juste progrès, mais toujours dans le respect de l'environnement.

Une stratégie d'intervention en agriculture qui respecte les agro-écosystèmes, ou mieux encore une stratégie de protection intégrée, semble la plus apte, puisq'elle utilise tous les moyens à notre disposition acceptables au point de vue économique, écologique et toxicologique, avec attention particulière au milieu où l'on opère.

C'est dans cette perspective que sont orientés nos travaux de Barcelone pour lesquels je souhaite une complète réussite, féconde de résultats positifs.

Je désire remercier avec gratitude toutes les Autorités qui nous ont honorés avec leur présence, et en particulier les Autorités espagnoles, notamment de la Catalogne et de la ville de Barcelone, qui nous ont accueillis si chaleureusement et qui avec grande sensibilité ont mis tout en oeuvre pour favoriser notre rencontre à fin qu'elle soit profitable et agréable.

A tous les participants mes voeux les plus vifs de bon travail.

Mais je vous invite à vouloir bien réfléchir sur un principe que je crois aujourd'hui important: le meilleur avenir ne sourira pas à celui qui sera technologiquement le plus avancé, mais à celui qui, dans le progrès, saura mieux sauvegarder son milieu, le milieu où il vit.

ALOCUCION DE APERTURA

J. Blanco, director general de la producción agraria
Ministerio de Agricultura, Pesca y Alimentación-Madrid, Spain

Sr. Presidente, Señoras, Señores,

Tengo el honor de poderles dirigir unas palabras de apertura de "Parasitis 88", en nombre del Excmo. Sr. Ministro, que no ha podido hacerlo personalmente en razón de sus compromisos oficiales.

El objeto fundamental de toda política fitosanitaria consiste en proteger a los vegetales y sus productos contra los daños producidos por cualquier agente perjudicial.

Hasta hace relativamente poco, este principio tan general se ha venido practicando en todos los paises sin limitaciones de aplicación de medios, particularmente químicos, como consecuencia de la enorme elasticidad que por razón de sus dimensiones parecía presentar nuestro planeta en relación con el hombre y sus actividades. Pero el ritmo de crecimiento extraordinario y la potencia de las acciones humanas pueden, y de hecho ya se ha producido, romper el equilibrio ecológico de grandes áreas de la tierra.

Todo ello, determinó la necesidad de matizar el postulado básico inicial mediante la incorporación de dos conceptos actuales, adicionales:

- Proteger cualquier tipo de forma viva que destruya o limite la actividad de los agentes perjudiciales
- Proteger a las personas, así como al medio de los peligros derivados del empleo indiscriminado de productos fitosanitarios.

Definidos estos objetivos, evidentemente el medio para conseguirlos reside en lo que se ha venido en llamar "lucha integrada" o "manejo integrado de plagas" (IPM).

Se habla de "protección integrada" en agricultura desde hace más de un cuarto de siglo. Aún cuando durante este periodo de tiempo su denominación ha cambiado varias veces y la forma de entenderlo ha evolucionado, su concepto se mantiene ya practicamente estático desde 1965, en que la FAO celebró un "Symposium sobre la lucha integrada" en Roma.

Es fácil comprender que el concepto tiene una gran complejidad, teniendo en cuenta los numerosísimos factores que intervienen en un ecosistema, por simple que sea, tal como ocurre en los agroecosistemas, la dinámica de poblaciones de un organismo nocivo y la determinación de aquellos umbrales, que son clave en la decisión de la intervención con los medios adecuados de control.

Se encuentran también involucrados en el sistema, la sensibilidad varietal del cultivo, la multiplicidad de organismos nocivos en cuestion, tales como insectos, ácaros, nematodos, hongos, virus o bacterias, entre otros, además de las malas hierbas, y naturalmente, sus antagonistas, es decir, sur enemigos naturales o auxiliares, útiles al hombre. Así mismo, las prácticas culturales ejercen una enorme influencia en el desarrollo de las poblaciones de los organismos nocivos. Finalmente, los innumerables medios de lucha aplicables, bien sean químicos, biológicos o biotécnicos, teniendo en cuenta sus ventajas e inconvenientes y sobre todo los aspectos de la aplicación de los primeros, en relación, como antes anotamos, de una mínima incidencia desfavorable en el medio natural, lo que exige unos conocimientos profundos de todo ello.

Gran parte de estos medios futuros de lucha están basados en la biotecnología, bien sea obteniendo nuevas moléculas plaguicidas mediante las fermentaciones y biotransformaciones, bien sea creando nuevas variedades de plantas y cultivos resistentes a las plagas o a la acción de los herbicidas, aplicando las técnicas de la transferencia de genes.

Por otra parte, en la actualidad parece posible la cuantificación de estos complejos procesos ecológicos y su simulación, gracias al enorme desarrollo de la estadística matemática y de la informática, tanto en la tecnología de los ordenadores como en la de sus aplicaciones.

Todo ello valdría poco si no supiéramos transmitir a los agricultores los conocimientos adquiridos o al menos sus consecuencias. De aquí, la importancia que los problemas de transferencia práctica de estas tecnologías fitosanitarias y su divulgación presentan en el momento actual, dada su complejidad y alta tecnificacion. Felicito a los organizadores de "Parasitis 88" por haber acertado en la programación de estas jornadas, donde se han de tratar tan importantes temas del futuro inmediato, en un momento crucial de nuestras actividades agrarias en que las nuevas tecnologías son imprescindibles para una producción económica y segura para el hombre y su entorno natural.

El Ministerio de Agricultura, Pesca y Alimentación español ha asistido al desarrollo de estas nuevas tecnologías que hacen posible la aplicación del IPM mediante la preparación y concurrencia de sus técnicos en los ámbitos internacionales especializados. Los programas de trabajo se iniciaron con la aparición de las primeras Estaciones de Avisos Agrícolas a partir de 1964 y, concretamente, en 1970 se inició un programa de trabajo formal encaminado a implantar progresivamente las técnicas de lucha integrada en las plantaciones de frutales de la región de Aragón, que dio los primeros frutos y que supuso el fermento de desarrollo de las técnicas en otros cultivos, tales como, algodón, cítricos, olivar y vid. Sin embargo, su aplicación práctica y extensiva a nivel de agricultor es dificil sin la asistencia de técnicos capacitados, por lo que en gran parte la lucha integrada ha permanecido a nivel teórico y solamente aplicado a relativamente pequeñas superficies.

Es fácil comprender la dificultad que presenta para el agricultor medio la realización de una lucha eficaz contra el conjunto de las plagas y enfermedades que afectan a sus cultivos, dentro de este mundo tecnificado que he tratado de esbozar brevemente.

A través de los Boletines e informaciones que periodicamente publican las Estaciones de Avisos, así como de los folletos y otras publicaciones del Ministerio de Agricultura, Pesca y Alimentación y de las Comunidades Autónomas, el agricultor ha dispuesto de una información general sobre prevención y tratamiento de las distintas plagas y enfermedades que pueden presentarse o han hecho su aparición en determinadas áreas geográficas. Estos avisos, muy generales, deben ser aplicados y adaptados por el agricultor a su explotación en particular, no consiguiéndose generalmente los resultados deseados, o bien se utilizan para su control medios y productos en exceso, que conllevan un coste y riesgo ecológico más elevado.

La experiencia acumulada en la aplicación de técnicas de lucha integrada contra plagas de frutales y del algodón, realizada a través de Agrupaciones específicas de agricultores, ha permetido por un lado, racionalizar el empleo de productos fitosanitarios evitando aplicaciones innecesarias, con el consiguiente ahorro y, por otro, incorporar a la lucha contra plagas los nuevos medios de control.

Con estos resultados, se consideró aconsejable intensificar dichas actuaciones y extender los tratamientos integrados a otros cultivos o grupos de cultivos mediante la puesta a punto de las técnicas IPM, la preparación del personal técnico y la mano de obra especializada que ha de dirigir y aplicar tales técnicas, asi como el fomento de la constitución de agrupaciones de agricultores que han de protagonizar la aplicación de la nueva tecnología en las tierras de cultivo agrupadas con esa finalidad.

La diversidad de cultivos y sus rotaciones, que son susceptibles de utilizar este actual sistema de lucha fitosanitaria, aconsejó establecer un plan cuatrienal con carácter experimental y de ámbito nacional, hay renovado. Para ello, el Ministerio de Agricultura, Pesca y Alimentación estableció en la Orden de 26 de Julio de 1983 las actuaciones de la Administración, para el desarrollo de las técnicas de lucha integrada y los incentivos económicos para la promoción de las Agrupaciones para Tratamientos Integrados en Agricultura (ATRIA), contra las plagas de diferentes cultivos o grupos de cultivos, con el fin de alcanzar los siguientes objetivos:

a) Puesta a punto de las técnicas de lucha integrada y utilización racional de los productos y medios fitosanitarios

b) Formación de personal técnico y especializado en la dirección y aplicación de dichas técnicas

c) Fomentar las agrupaciones de agricultores para la realización de tratamientos fitosanitarios integrados (ATRIAS).

En el año 1989 se espera alcanzar en España el número de 250 ATRIAS, que asociarán a más de 50.000 agricultores y una incipiente aplicación de las técnicas de lucha integrada en más de 250.000 Has. Y ésto es posible gracias a la constante formación del personal técnico que ha de dirigir sus actuaciones y el empeño del Ministerio de Agricultura, Pesca y Alimentación en la promoción de la lucha integrada.

No quiero causarles más porque hoy comienzan unas jornadas en que se debatirán estos temas en profundidad, con las aportaciones de los excelentes y experimentados especialistas que participarán en ellas.

Por tanto, sólo me queda dar por abierto el Congreso "Parasitis 88".

SESSION 1

BIOTECHNOLOGY

Chairmen: F. Sala
J. Blanco

THE APPLICATION OF GENETIC ENGINEERING TO PEST CONTROL

C. A. Edwards
Department of Entomology, Ohio State University-Columbus, USA

Summary

There are three main areas where genetic engineering can play an important role in pest control.

1. **Genetic engineering of insect pathogens**

There is considerable potential for pest control in the genetic engineering of insect pathogens, including *Bacillus thuringiensis* and *Beauvaria bassiana*, particularly in the development of toxins such as *B.t.* toxin. Other toxin sources are from root-colonizing of microorganisms such as *Pseudomonas*. Baculoviruses and other viral pathogens show considerable potential in pest control. Microbial metabolites can be engineered to apply to plants and repel insects. Genetic engineering of fungi, protozoa and nematodes all show promise and future potential. Avermectins, produced by soil microorganisms are potent against arthropods.

2. **Genetic engineering of plants for pest control**

Genetic engineering of plants will play a valuable role in pest control in the future. Most gene manipulation in plants is based on *Agrobactericium tumefaciens* or by microinjection. Insect toxins such as those from *B. thuringiensis* can be implanted into plants and provide insect resistance against several orders of insects. Areas with considerable potential include: the genetic development of resistant plant varieties or new varieties with chemical defenses, such as toxins or repellents, including lactones, alkaloids, cyanogenic glucosides and tannins. Additionally, genes from plants which produce natural pesticides such as calamus, big sagebrush, chilcuan, mamey and neem, could be genetically transplanted to crop plants. Many problems in genetic manipulation of crop plants have been resolved.

3. **Genetic engineering of insects**

To date, there has been little successful genetic engineering of insects other than *Drosophila*, but the potential of introducing genes that cause malfunction into pest insects, so they spread through natural populations, and of genetically engineering new strains of parasites and predators of pests is great.

Areas of promise are in the use of DNA restriction enzyme polymorphism to develop insect control agents based on genetically engineering production of peptide neurohormones that influence insect function, and the molecular control of insect development.

4. **The risk of releasing genetically engineered organisms**

Problems in the environmental aspects of the release of genetically engineering organisms are outlined.

1. INTRODUCTION

All of the ways in which genetic engineering can contribute to pest control depend currently upon the ability to transfer genetic material from one cell into another using a common bacteria. The DNA in the cells of higher plants and animals is usually tangled in a large mass inside the chromosomes. In the early 1970s, it was discovered that a bacterium called *E. coli*, has closed circular pieces of DNA or plasmids floating free in its cell liquid. These plasmids are the vehicle that scientists use to transfer genes, between cells. The technique used is to "cut" DNA molecules into gene segments and "paste" the segments into the plasmid. Certain enzymes can break up the DNA of invading viruses to protect the cell and so act as chemical "scissors." These, which are known as restriction enzymes, attack and cut the DNA molecules at specific sites. There are many different kinds of restriction enzymes and a large number of these have been catalogued in terms of the position in the molecule that they will cut. Hence, when a cut is desired at an exact spot along a DNA chain, the appropriate enzyme is selected for the purpose. By using these chemical "scissors", genes which are specific sequences of DNA can be cut out of a complex DNA molecule. Then, the same enzyme is used to cut open the bacterial plasmid. The cut ends of the plasmid and the cut ends of the gene are chemically "sticky," and so they can attach to each other or recombine to form a circular plasmid, containing the new gene. This technique is called "gene splicing" or recombinant DNA technology. The new plasmid carries instructions from the inserted gene to manufacture a new kind of protein. This can thereby produce new strains of organisms which can breed true to the new gene structure.

Theoretically, there is the potential for inserting genes from almost any organism into almost any other organism. However, most progress to date relevant to pest control has been made in the genetic manipulation of microorganisms and plants. Such successes have already led to new microbial and viral preparations for pest control; these techniques are currently being used in plant breeding and enough progress has been made in genetic manipulations on insects to indicate that this also may have great potential for pest control. The aim of this review is not as a technical reference on recombinant DNA techniques, but rather to assess the current potential of various aspects of genetic engineering in pest control.

2. GENETIC ENGINEERING OF INSECT PATHOGENS

Insect pathogens have been known for many years, and extremely large numbers of species of organisms are pathogenic to insects. However, only relatively few of these are effective enough or have been produced successfully in large enough numbers to be used as biological pest control agents. These include: *Bacillus popillae*, a spore-forming, bacterium causing "milky disease" in Coleoptera, *Beauvaria bassiana* a fungus that attacks several groups of insects, *Bacillus thuringiensis* a bacterium that attacks Lepidoptera, and nuclear polyhedrosis viruses (NPV's)that can attack a number of insects in different families. However, technological difficulties in production, stability of preparations and cost have meant that only 1% of the total pest control market is based currently on biological materials (KHACHATOURIANS, 1986).

Insect pathogens are relatively easy to manipulate genetically and not only hold considerable potential in the development of more toxic organisms, with changed host capabilities, that can be produced relatively cheaply and efficiently, but have also reached commercial development. The types of organisms that can be used in this way include:

a) <u>Bacteria</u>

Insect pathogenic bacteria can be divided into two main ecological types: (i) those which reproduce within the susceptible host system; (ii) those which do not reproduce, necessitating repeat applications. There is a third type which can act in either of these ways depending on the circumstances. Over 100 entomopathogenic bacteria have been identified. There are three main types: (i) obligate pathogens e.g. *Bacillus popillae, B. larvae*, (ii) crystalliferous spore-forming bacilli e.g. *B. thuringiensis, B. sphaericus, Pseudomonas aeruginosa, Achromobacter* spp. (iii) facultative pathogens involving a range of species. The most successful bacterial insecticides belong to the genus *Bacillus*. Four species are considered as primary sources of microbial insecticides: *B. thuringiensis, B. popilliae, B. moritai and B. sphaericus*. The two commercially-used species, include *B. thuringiensis* which is cultured *in vitro*, and *B. popilliae*, which is grown *in vivo* on the Japanese beetle (*Popilliae japonica*). *B. thuringiensis* produces entomocidal toxins, α- and ß-exotoxins and δ-endotoxin. The latter, synthesized during sporulation, account for the effectiveness of *B. thuringiensis* as a biological pest control agent. Oral administration of the toxin (insecticidal crystal protein), to Lepidoptera larvae causes cellular damage to the gut epithelium and cessation of feeding. It has been used successfully to control Lepidoptera, with no reports of resistance developing to date, although it is likely that resistance will develop eventually.

This bacterium has been one of the best candidates for genetic manipulation. The active factor which is the insecticidal endotoxin, is a prime target for genetic engineering, because it consists almost exclusively of a single glycoprotein whose ι imary structure is determined by a single gene which can be isolated readily. The nucleotide sequence of its structural and regulatory regions can be established readily and the sequences modified either randomly or in a site-specific fashion, so as to generate groups of genetic variants that can be returned in a biologically active form to a microbial host.

The problems a genetic engineer faces are (i) to increase the potency of the toxin towards major susceptible pests, (ii) to broaden the insecticidal spectrum of activity so that insects showing only marginal susceptibility can be kept in check, and (iii) to reduce manufacturing costs (KIRSCHBAUM, 1985).

Toxin crystals produced by different varieties of *B. thuringiensis* have different types of biological activity. For example, that from var. *kurstaki* is toxic to certain Lepidoptera and has little or no effect on Diptera, whereas *israelensis* toxin is active on Diptera but ineffective on Lepidoptera (BURGES, 1982). The precise mode of action of the toxin is not known but it seems likely that the crystal solubilizes in the insect midgut to a soluble glycoprotein, prototoxin, which then produces a smaller toxin molecule by proteolytic cleavage. This causes midgut cells to rupture and the insect dies. The development of a more potent toxin with a broader host range depends on isolation of the protoxin gene.

Toxicity and host range of the toxic crystals vary. There are two characteristics which can be altered by genetic manipulations. The serological and molecular characteristics of the toxin crystals from various isolates of *B. thuringiensis* have been analyzed and respective DNAs have been sequenced and cloned into *E. coli* and *B. subtilis*. Once a toxin with desired commercial properties has been constructed, the new protein must be manufactured and formulated, and genetic engineering development techniques can be expected to lead to process improvements. Many commercial companies are working on production of *B. thuringiensis* toxins.

A quite different technique, using *Bacillus thuringiensis* subsp. *kurstaki* HD-1 has been developed (GRAHAM, 1988). Two corn-root-colonizing isolates of *Pseudomonas fluorescens* were isolated from agricultural soils. These possess many traits which make them optimal for use as a delivery system in the corn rhizosphere.

Subcloning the *B. thuringiensis* gene into these pseudomonads has been accomplished through introduction on non-self-transmissible plasmids and through chromosomal insertion using Tn5. These genetically-engineered *Pseudomonas* root-colonizers express pesticidal levels of the *B. thuringiensis* toxin protein.

Another method is based on avermectins which are a family of lactone natural products with potent activity against arthropods. They are produced by a soil microorganisms, *Streptomyces avermitilis*. One of these products, abamectin is very toxic to phytophagous mites and insects and is in world-wide development for control of pests of agricultural and horticultural crops. Another, ivermectin (the 22, 23-dihydroderivative of abamectin) is used against ectoparasites of animals. Yet another, closely-related group is the milbemycins. The avermectins probably act on the chloride ion channel in insects and function as antagonists of the inhibiting neurotransmitter α-aminobutyric acid (GABA) to increase chloride ion entry into the post-synaptic region of transmitter cells. Avermectins are potential candidates for genetic manipulation to increase their toxicity range to other insect orders and also to increase their potency.

A technique with considerable potential in crop protection is the use of microorganisms to protect plants (DIMOCK *et al.*, 1988). Microbial metabolites can render host plants resistant to feeding by insects (CLAY, 1988). A product based on this principle has been produced commercially under the trade name InCide™. It uses recombinant DNA techniques to produce strains of microbial endophytes that manufacture microbial insecticides within the vascular systems of crop plants. It is based on manipulation of a bacterium *Clavibacter xyli* subsp. *cynodontis* (CXc) to produce a product which can protect corn (*Zea mays*) from the European corn borer (*Ostridia nubilalis*). Other such products are under investigation and development.

b) <u>**Viruses**</u>

There are about 650 viruses that have been isolated from insects. Of these, about 540 have been obtained from Lepidoptera, 90 from Hymenoptera, and 20 from Orthoptera, Coleoptera and Diptera. During the 1970s, the first commercial viral pesticide was registered. Since then, a tremendous extension in the use of viruses, such as baculoviruses, has been made for control of insect populations on a commercial basis.

Pathogenic viruses are active against a number of pest species of Lepidoptera. The virus becomes active upon ingestion by an insect and reproduces during the development of the disease. Dead insect larvae liberate viruses onto foliar surfaces and into the soil, providing some measure of long-term control. Viruses are rather stable in the soil where they can persist for several years, but unprotected viruses on foliar surfaces have short "half-lives". The viruses can be formulated to optimize storage, wetting, suspension, flow and spraying characteristics.

The nuclear polyhedrosis viruses (NPVs) have received most attention as pest control agents and a number of these have been registered as commercial pesticides which are very safe, since they can infect only invertebrates, and are not phytotoxic.

These insect pathogenic viruses are very suitable for modification and improvement by genetic manipulation because of their small genomes and comparatively simple molecular biology,

Insect viruses are classified into seven families, the most common of which is the baculoviruses. Many DNA recombinant baculoviruses have been constructed and these have expressed different kinds of foreign genes derived from a variety of sources from bacteria to human beings. The Baculoviridae are characterized by a circular double-stranded DNA genome and a rod-shaped enveloped virion. Baculoviruses contain 90-160 kb closed circular double stranded DNA as a genome and probably have more than 100 different genes in the genome. Sequencing and hybridization experiments have shown the genome of unique sequences to be localized tightly and spaced with short sequences of a junction containing a proposed promoter and poly(A) signals. The arrangement of the genes in a baculovirus genome is conserved throughout the various NPVs, even between NPVs that have a relatively low DNA sequence homology.

The circular double-stranded genome, is easily modified by commonly-used techniques such as specific cleavage and ligation. It also has a rod-shaped capsid which allows it to contain extra DNA fragments, a cell line which is susceptible to viral infection; and a polyhedrin gene. This gene is suitable for the insertion of foreign genes, because it is non-essential for viral reproduction, is a large gene with a strong promoter, and is a marker, that is detected easily by light microscopy.

The first steps in the development of a new baculovirus insecticide is to prepare a recombinant baculovirus by introducing foreign genes into the highly expressed polyhedrin gene site. The introduction of peptide toxins that block neuronal function and modify the important peptide hormones are being investigated currently. Infection with such recombinant viruses may cause direct toxicity to insects, alter their behavior, or arrest their development. These effects may occur much faster than with the original parent virus. Stability of baculoviruses in the field depends on polyhedrin occlusion bodies. A recombinant virus still retaining its ability to product polyhedrin is possible, by introducing the foreign gene in the polyhedrin gene site without removing the polyhedrin gene itself. If the promoter of the polyhedrin gene, used for the gene for insect toxin, is replaced with one of the early gene promoters of NPV or with a wild pest insect promoter, which is highly activated in the target organs, such a virus may express the gene for insect toxin at an early stage of infection. If the right gene for insect toxin and the promoter can be isolated and introduced into a baculovirus, the resulting recombinant virus will be likely an efficient viral insecticide. These techniques hold considerable promise for pest control and it seems certain that extensive developments will occur in the near future.

c) **Fungi**

Over four-hundred species of entomopathogenic fungi have been identified and most of these do not have to be ingested to kill insects (KIRSCHBAUM, 1985) There are several entomopathogenic and saprophytic fungi which have been used for practical control of insect populations. The commercial production of these fungi involves ingredients, concentrations, quantities and fermentation methods that are virtually identical to those required for the synthesis of fungal biomass.

The mode of action of these pathogens is that fungal spores adhere to the insect surface, germinate and send out hyphae that penetrate the cuticle, invade the hemocoel and kill the insect. This is through the production of complex toxic metabolites, or through hyphal proliferation and physical disruption of organs. The invading fungus sporulates within the insect and is released when it dies. Some fungi such as *Nomuraea rileyi* infect Lepidoptera, and others such as *Beauveria bassiana* and *Culicinomyces clavosporus* are pathogens of mosquitoes. Spores or conidia can be produced on liquid growth media.

The conidial germlings of entomopathogenic fungi penetrate the insect cuticle by secreting enzymes. Recent research on *Metarhizium anisopliae* has shown that genes control the enzymes concerned and advances have been made on cloning the Pr1 gene and adding genes to the fungal genome. This demonstrates the potential for the genetic engineering of fungal entomopathogens as biocontrol agents (STAPLES *et al.*, 1988).

Many studies are under way in a number of laboratories, to investigate the potential of genetic manipulation of entomopathogenic fungi to increase their lost ranges and toxicity and develop new commercial preparations.

d) <u>Protozoa</u>

Very few protozoa have been mass-produced commercially as pest control agents. The ones currently in experimental production include *Nosema locusta*, to control grasshoppers *Nosema pyraustra* against European corn borer, *Nosema fumiferanae* for control of spruce budworm and *Vairimorpha necatrix* which is targeted against various lepidopterous caterpillars. Commercial production of the protozoa is still an *in vivo* process. Not much work has been done, to date, on genetic manipulation of protozoa but based on their genetic structure they should be good candidates for DNA recombinant techniques.

e) <u>Nematodes</u>

Some entomogenous nematodes appear to produce a protein that counteracts an insect's biochemical response to bacterial infection. When nematode dauer larvae (a nonfeeding juvenile stage) of *Neoaplectana carpocapsae* invade an insect host through its body openings, they molt and burrow through the midgut epithelium into the hemocoel. The nematodes carry gram-negative, faculative anaerobic entomopathogenic bacterium of genus *Xenorhabdus*. This is released into the insects hemolymph where it multiplies rapidly and kills the insect host within 24 hours. The bacteria are not inactivated, even if the insect mounts a defense against the nematode. The *Xenorhabdus* bacteria provide improved growth conditions for the nematode larvae, which mature, invade the fat body and Malpighian tubules, develop into adults, mate, and lay eggs in the corpse.

There are many other entomogenous nematodes but relatively few have been used in experimental or commercial pest control. This is probably because they are produced currently mainly by *in vivo* techniques such as breeding in the wax moth, *Galleria mellonella*. There are promising *in vitro* techniques of production under test. Both the nematodes and bacterium could be potential candidates for genetic manipulation.

3. GENETIC ENGINEERING OF PLANTS

Plant breeding has made major contributions to pest control through the development of strains of a wide range of crops, that are resistant to insect attack. Classical plant breeding depends upon genetic variability. In plant breeding before the development of recombinant DNA techniques, the sources of variability were derived from collecting wild varieties, hybridization and mutagenesis. (Fig. 1) The successful use of plant breeding to produce improved crop varieties has depended upon selecting new useful and viable combinations within that variability. Some of the progress in modern crop improvement can be attributed to past advances in broadening the base of available genetic variability. (GOODMAN, 1988)

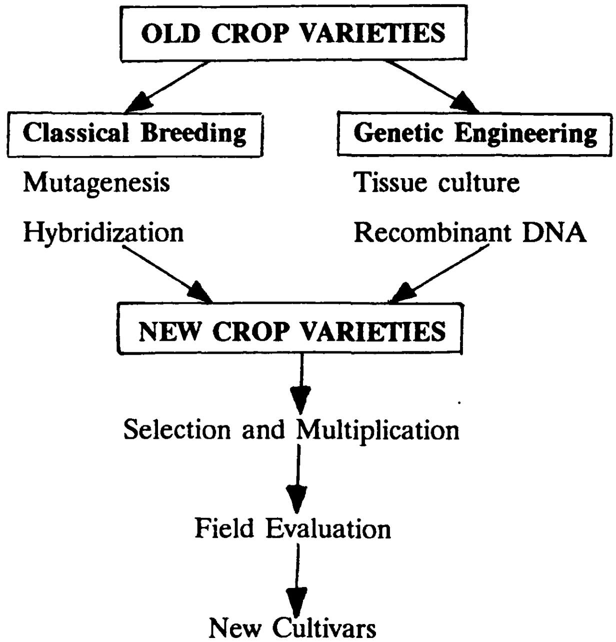


Figure 1. Development of New Crop Varieties

It is now possible to characterize and modify genes at the molecular level, and recombinant DNA technology allows engineering and transfer of genes across biological barriers to create hybridization (GOODMAN *et al.* 1987). Two basic techniques are involved in development of new varieties from (i) tissue culture, (ii) recombinant DNA technology.

The classical methods of gene transfer in higher plants is based on the *Agrobacterium*-mediated gene system of plants of the nightshade family. The *Agrobacterium*-mediated gene transfer systems came from work showing that in crown gall disease, a region of plasmid DNa (the T-DNa) can be transferred to chromosomal DNA in the nucleus, and maintained there in the absence of the bacterium. The earliest success was by taking crown gall tissue into culture and producing whole plants from hormone independent callus cultures. The plants obtained were abnormal because the hormone independence of such material was the result of phytohormone synthesis by enzymes encoded by genes carried by the T-DNA. Tissue culture methods led to use of protoplasts and eventually to direct techniques with explants, such as leaf pieces or stem segments, from which differentiated shoots and subsequently roots could be obtained.

Agrobacterium tumefaciens has been used as the main vector for inserting new genes into plants (BARTON and BRILL, 1984). Virulence is induced in the bacterium by genetic information on the large plasmids, the Ti (tumor-inducing) plasmids. At the time of infection, a segment of the Ti plasmid, called T-DNA, is inserted into the nuclear DNA of the host plant. Genes contained within the T-DNA are functional in transformed cells, and T-DNA gene products are responsible for both hormone independence of crown gall cells in tissue culture and the synthesis of novel metabolites called opines which are simple derivatives of amino acids and keto acids. These are catabolized by *Agrobacterium* as carbon and nitrogen sources.

Tobacco cells containing an intact T-DNA cannot regenerate into normal plants because of hormonal imbalances resulting from the action of T-DNA gene products. However, if the genes responsible for the imbalance are spontaneously deleted from the infected cell, healthy plants containing the remaining T-DNA genes can regenerate. It is also possible to "disarm" T-DNA *in vitro* by deleting experimentally one or more genes of the T-DNA. Tobacco cells that have been transformed with disarmed Ti plasmids are capable of regenerating into healthy plants, and it seems likely that other plants that adapt well to tissue culture can be transformed by similar techniques (BARTON and BRILL, 1984).

Tobacco, petunias and, more recently, tomato, have been the species used most in genetic engineering. With these species, many transgenic individuals can be produced in a single petri dish. The selection systems used yield a high proportion of transformed regenerants, and the plants obtained are normal in growth habit and fertility.

The host range for crown gall among the dicotyledonous species is very wide. However, extensions of the *Agrobacterium* technique to other plant species, even those susceptible to crown gall disease, have been slow. The difficulty in transferring the use of this system to other species has more to do with the difficulties of manipulating the plants than to intrinsic limitations of *Agrobacterium*. Some progress has been made, but the ease and numerical efficiency of *Agrobacterium*-mediated systems for plants in other plant families seems to be limited.

Because of this, genetic engineering some of the important crop species, notably the grass family members such as maize, wheat, and rice, has concentrated on other cellular targets for gene delivery and development of other delivery methods has been investigated in many laboratories. In particular, work on the reproductive biology of plants offers some promise for development of efficient gene transfer systems.

Other methods which do not use *Agrobacterium*, involve delivering recombinant DNA through plant cell walls, across the plasmalemma, and into the nucleus of a cell. Plant viruses and viral nucleic acids can be taken up by protoplasts and direct uptake of DNA by protoplasts also occurs so that the DNA enters the nucleus and is integrated into the chromosomes of the recipient cell (POTRYKUS *et al.*, 1985).

A further method for introducing recombinant genes is by direct microinjection. This has been done with rapeseed (*Brassica napus*) and the target was small somatic embryos from cultures of microspores. For many agricultural species, the only structures from which whole plants can be derived by manipulation in culture are multicellular. The path from such cultures to single cells or protoplasts and back to regenerable cultures and eventually to plants has been extremely difficult. However, some successes are being reported on transmission to crop plants such as maize and soybeans. (KLEIN *et al.*, 1988)

Other techniques used include electroporation and particle acceleration (KIRSCHBAUM, 1987). The universality of the genetic code makes the range of organisms from which genes can be obtained for engineering of plants very wide indeed. To date, genes from bacteria, plant viruses, yeast, mammals, fowl, and an increasingly wide range of cultivated and noncultivated plant species have been expressed in transgenic plants. A relatively simple technique of transferring genes that has been used for cereals, such as wheat, is to make cuts in very dry embryos then place selected DNA in solution on the cut. (STEINBISS, 1988)

An important microbial gene for pest control is that encoding the insecticidal endotoxin protein of *B thurengiensis* (Bt). The expression in transgenic plants of the Bt endotoxin, usually truncated to an active moiety of the native molecule, has given effective insect control in the laboratory and field (FISCHHOFF *et al.* 1987; VAECK *et al.* 1987). As with the microbial form different strains of *B. thuringiensis* confer toxicity to different orders of insects e.g. *B.t. Kurstaki* HD-1 affects Lepidoptera, *B.t. tenebrionis* kills Coleoptera and *B.t. aisawai* is effective against *Heliothis* and *Spodoptera*.

The genetic engineering of *B. thuringiensis* into plants has reached commercial development by two companies and excellent caterpillar control on tobacco has been obtained (MEEUSEN, 1987). Newer work is aimed at implanting genes for resistance to insects into plants. JONES *et al.* (1988) isolated a gene for an enzyme which acylates nornicotine, a major alkaloid of the cultivated species *Nicotinia tabacum*, which is not toxic to *Manduca sexta*. Transfer of the gene for this enzyme from wild, *N. stocktonii* into *N. tabacum* can enable the cultivated plant to synthesize *N-acylnornicotine*, which is highly toxic to *M. sexta*.

Various proteins and enzymes such as chitinases have genes that might be introduced into plants to control insects. Inserting genes that encode protein inhibitors of insect digestive enzymes into plants is another potential control measure (KIRSCHBAUM, 1987).

There appears to be very considerable potential for other approaches to engineering plant resistance to insects. A variety of plant secondary metabolites discourage pests through mechanisms such as mimicking insect hormones, thereby upsetting insect maturation. It is quite feasible to transfer to plants, some of the genetic pathways required to synthesize such metabolites.

Developments are occurring rapidly in genetic engineering of plants and it seems likely that resistant varieties of a number of crops will become available with in the next 4-5 years.

4. GENETIC ENGINEERING OF INSECTS

Very considerable potential for insect control can be seen if genetic engineering of insects becomes feasible. Most of our knowledge of the routine and efficient introduction of exogenous DNA into a host insect's genome is limited to the genus *Drosophila* (HANDLER and O'BROCHTA, 1988). The P-element-transposons from *Drosophila* are the only vectors available for whole insect transformation. Although species-specific gene transformation is not yet possible in non-drosophilid insects, genes can be isolated as cloned recombinant DNA from these species, and introduced into the *D. melanogaster* genome by transformation. For instance, *Drosophilia* has been transformed with the chorion A and B genes from the distantly related species *Bombyx mori*.

Techniques exist for cloning insect cells in bacteria cells and modifying them by site-directed mutation (COCKBURN *et al*, 1984). Genes cloned from *D. melanogaster*, can be inserted into mobile elements and incorporated, without loss of function, back into the *D. melanogaster* genome following microinjection of the mobile element into eggs (RUBIN and SPRADLING 1982). Modified P elements from *D. melanogaster* are regarded currently as the best candidates for transferring genes between species and have already been used to transfer genes between species within the genus *Drosophila* (SCAVARADA and HARTL 1984). It is widely assumed by molecular biologists that general gene transfer systems will be available over the next few years for transferring genes between insect species. It seems likely that the availability of techniques for the genetic engineering of insects will precede ideas on how we can use this technology to our advantage (WHITTEN, 1986).

One example of how genetic engineering might be used for insect pest control is by the production of a single-sex malfunction and release of insects of this sex so as to interfere with natural reproduction in the field.

Another area with potential in insect control concerns insect neurhormones (KIRSCHBAUM, 1985). Insect neuropeptides and peptide hormones, occur in insects in extremely small quantities, which makes their purification difficult, and this limits study of their physical and biological properties. Genetic engineering could play a major role in providing a convenient source of these materials through the expression of cloned genes in microbial hosts. Once purified genes are available there is the possibility of genetically modifying these polypeptides to optimize their insect control properties. Genes encoding polypeptide products could be inserted in microbial pathogens. Use of such organisms to deliver the toxins to insects in the field would overcome the possible instability of free polypeptides in the environment . Such research could lead to the development of chemical antagonists. Knowledge of the amino acid sequence of families of related polypeptides could lead to new insect control agents.

Neurohormones are peptides and proteins that regulate many critical physiological processes of insects including molting, metamorphosis, reproduction and homeostatic processes such as water and salt balance, heartbeat, digestion and the synthesis of proteins, lipids and carbohydrates that circulate in the hemolymph. Disruption of these physiological processes by artificial elevation or suppression of the natural levels of their regulatory neurohormones could seriously impair the functioning and survival of treated insects. Recent advances in genetic and protein bioengineering make it feasible to explore means by which neuroendocrine events might be manipulated as part of a future pest management strategy (KEELEY *et al*, 1988).

Investigations into the molecular basis of insect development could provide new tools for insect control through genetic engineering, as well as providing answers to many fundamental questions about the regulation of development. Since most of our information relates to *Drosophila*, future advances in this area of crop protection demand that we begin to understand the genetics of the molecular control mechanisms in other insects. We need to know if there is stage and tissue-specific gene expression, dictating the stages of metamorphosis and the timing of significant developmental events such as diapause, reproduction, muscle development and degeneration, etc.? There seems considerable potential for insect control by genetic engineering of insects and USDA, some commercial companies, and several universities are already committing considerable funds and effort to research in this direction. Finally, one of the areas of insect genetics that is best understood is that of the development of resistance to pesticides. There seems considerable potential in research into manipulating genes to change susceptibility to insecticides.

5. EVALUATING THE RISK OF RELEASING GENETICALLY ENGINEERED ORGANISMS

There is considerable anxiety among environmentalists and the public as to the safety of releasing genetically engineered organisms into the environment. It has been concluded that decisions to release organisms must be based on the results of limited case-by-case risk assessment studies. Most anxiety relates to the release of genetically engineered microorganisms (G.E.M.'s) which could change their spectrum of activity, have ecological effects or transfer genes to other organisms (FUXA, 1988) There is a perception that genetically released plants involve less environmental risk (Anon, 1987) than the other release of organisms with gene changes (SIMONSEN & LEVIN, 1988; BISHOP, 1988). Many of the regulatory organizations are developing policies and strategies for the release of genetically engineered organisms without environmental risk.

REFERENCES

ANON,- 1987. Regulatory Considerations: Genetically Engineered Plants. Boyce Thompson Institute, Ithaca, NY 106 pp.

BARTON, K.A. and BRILL, W.J. - 1984. Prospects in Plant Genetic Engineering. In Biotechnology and Biological Frontiers. Ed. P.H. Abelson A.A.A.S. 121-131.

BISHOP, D.H.L. - 1988. The release into the environment of genetically engineered viruses, vaccines and viral pesticides TibTech 6 (4) April 1988.

BURGES, H.D. - 1982. Control of insects by bacteria. Parasitology 84:79-117.

CLAY, K. - 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10-16.

COCKBURN, A.F., HOWELLS, A.J., and WHITTEN, M.J. - 1984. Recombinant DNA technology and genetic control of insect pests. In Biotechnology and Genetic Engineering Reviews Vol 2. Ed. G.E. Russell Intercept, Newcastle-upon-Tyne England 69-99.

DIMOCK, M.B., BEACH, R.M., and CARLSON, P.S. - 1988. Endophytic bacteria for the delivery of crop protection agents. Proceedings of Conference on Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Ed. D.W. Roberts and Leslie L. Allee. Boyce Thompson Institute, Ithaca, NY, U.S.A. (in press)

FISCHHOFF, D.A., K.S. BOWDISH, F.J. PERLAK, P.G. MARRONE, S.M. MCCORMICK, J.G. NIEDERMEYER, D.A. DEAN, K. KUSANO-KRETZMER, E.J. MAYER, D.E. ROCHESTER, S.G. ROGERS, & R.T. FRALEY - 1987. Insect tolerant transgenic tomato plants. Biotechnology 5:807-813.

FUXA, J.R. 1988. Environmental Risks of Genetically-Engineered Engineered Entomopathogens. Proceedings of Conference on Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Ed.

D.W. Roberts and Leslie L. Allee. Boyce Thompson Institute, Ithaca, NY, U.S.A. (in press)

GOODMAN, R.M. 1988. Genetic Transfer in Crop Improvement. Proceedings of Conference on Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Ed. D. W. Roberts and Leslie L. Allee. Boyce Thompson Institute, Ithaca, NY, U.S.A. (in press)

GOODMAN, R.M., HAUPTLI, H., CROSSWAY, A., and KNAUF, V.C. - 1987. Gene transfer in crop improvement. Science N.Y. 236:48-54.

HANDLER, A.M. and O'BROCHTA, D.A. - 1988. An Assessment of Gene Transformation in Insects. In Endocrinological Frontiers in Physiological Insect Ecology Ed. F. Sehnal, A. Zabza and D.L. Denlinger. Wroclaw Technical University Press, Wroclaw 1075-1984.

JONES, D., HUESING, J., ZADOR, E. and HEIM, C. - 1987. The tobacco-insect model system for genetically engineering plants for non-protein insect resistance factors. Mob. Ent. 469-478.

KEELEY, L.L., HAYES, T.K., BRADFIELD, J.Y. and SOCK CHUNG, J. - 1988. Neuropeptides in Insect Control. Proceedings of Conference on Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Ed. D.W. Roberts and Leslie L. Allee. Boyce Thompson Institute Ithaca, NY, U.S.A. (In press)

KEELEY, L.L. and HAYES, T.K. - 1987. Speculations on biotechnology applications for insect neuroendocrine research Insect Biochem. 17:639-651.

KHACHATOURIANS, G.G. 1986. Production and use of biological pest control agents TibTech. 6 (5), May, 1986 120-124.

KIRSCHBAUM, J.B. - 1985. Potential implication of genetic engineering and other biotechnologies to insect control Ann. Rev. Entomol. 30: 51-70.

KIRSCHBAUM, J.B. - 1987. Workshop Summary: Potential for Molecular Biological Control of Invertebrates. Mol. Ent. 465-468.

KLEIN, T.M., GRADZIEL, T., FROMM, M.E., and SANDFORD, J.C. - 1988. Factors influencing gene delivery into *Zea mays* cells by high velocity microprojectiles Biotechnology 6:559-563.

MEEUSEN, R.L. - 1987. Insect-resistant Higher Plants: A Case Study of Regulation Under the Coordinated Framework. In Genetically-Engineered Plants Regulatory Considerations. Boyce Thompson Institution. 96-97.

POTRYKUS, I., PASZKOWSKI, J., SAUL, M., PETRUSKA, J., and SHILLITO, R.D. -1985. Molecular and general genetics of a hybrid foreign gene introduced into tobacco by direct gene transfer. Mol. Gen. Genet. 199:169-177.

RUBIN, G.M. and SPRADLING, A.C. - -1982. Genetic transformation of *Drosophila* with transposable element vectors. Science N.Y. 218:348-353.

SCAVARDA, N.J. and HARTL, D.L. Interspecific DNA transformation in *Drosophila*. Proc. Natl. Acad. Sci. USA 81:7515-7519.

SIMONSEN, L. & LEVIN, Bruce R. - 1988. Evaluating the release of genetically engineered organisms. TibTech 6 (4) April 1988.

STAPLES, R.C., ST. LEGER, R.J., BHAIRI, S., and ROBERTS, D.W. - 1988. Proceedings of Conference on Biotechnology. Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Ed. D.W. Roberts and Leslie L. Allee. Boyce Thompson Institute, Ithaca, NY, U.S.A. (in press)

STEINBISS, H.H. 1988. Transfer of genes in crop plants with emphasis on cereals. Parasitis 88, Barcelona. (This volume)

VAECK, M.A. REYNAERTS, H. HOFTE, S. JANSENS, M.DEBEUCKELEER, C. DEAN, M. ZABEAU, M. VAN MONTAGU, & J. LEEMANS. - 1987. Transgeneic plants protected from insect attach. Nature London 32833-37.

WHITTEN, M.J. - 1986. Molecular Biology-Its Relevance to Pure and Applied Entomology Symposium Ann. Ent. Soc. Amer. 79 (5):766-772.

EUROPEAN LABORATORIES WITHOUT WALLS FOR RESEARCH IN AGRICULTURAL BIOTECHNOLOGY

E. Magnien & A. Vassarotti
Division Biotechnology, Comission of the European Communities-Brussels, Belgium

The Biotechnology Action Programme (BAP : 1985-1989) was adopted by the European Community to pool skills and resources from all over the Member States, and to organize transnational research towards specific objectives of Community significance. Particular attention has been given to emerging technologies, at cellular and molecular level, which could possibly increase the capacity of controlling useful living species, in view of their safe and profitable exploitation in agriculture or by industry. Projects selected for funding fell under five main areas, namely :

. information infrastructure,

. enzyme engineering,

. genetic engineering,

. novel methodologies of cell culture,

. risk assessment (containment and release).

The sector of plant biotechnology obviously cuts across the above areas and is deliberately building strength on the combined use of a wide range of advanced technologies. This sector represents the meeting ground for 69 contracting laboratories, which contribute research into 22 transnational projects.

This contractual network has been supplemented with a supporting industrial contact-group, initially constituted of the 43 firms having introduced one or more expressions of interest in BAP projects. In a second phase, the 22 projects have been rearranged by affinity into 11 main research tasks, each requiring a high degree of multidisciplinarity. Such a need is met through the work of the so-called European Laboratories Without Walls (ELWWs), constituted by small groups (from 2-8) of participating laboratories. Each ELWW has embarked on a specific subject related to either genome organization, plant development, symbiotic relationships, defence mechanisms or other traits of agricultural importance.

The partnership in ELWWs is arranged in such a way that molecular biologists are confronted, as much as possible, with geneticists and physiologists who know the functioning of living organisms better, with a view to raising the level of understanding of agricultural species and biological properties thereof. There is, indeed, a distribution of ELWWs which shows core activities with participation of expert groups on vector development, gene expression studies, cell engineering all being surrounded by ELWWs of the "applied" type allowing a regular flow of know-how into specific fields of agricultural importance. In this periphery around the core of developing technologies, some ELWWs can be identified, which deal with host-pathogen relationships in particular.

The essence of the activity supported through BAP in the field of host-pathogen relationships starts with the identification of key molecules at the plant-pathogen interface, and extends to the characterization of their targets, therefore their putative roles, and their genetic determinants which, in turn, give access to regulatory mechanisms. These molecules, whose significance in relation to the establishment of compatible or incompatible interactions remains in certain cases to be elucidated, frequently correspond to hydrolases or to oligosaccharides with eliciting power. Most contracting laboratories have the necessary skills for gene cloning and have managed, in a first step, to identify batteries of genes and to isolate them in different microbes and host-plants.

They operate under four distinct ELWWs, each restricting interest to defined pests or pathogens.

- "Molecular biology of phytopathogenic Erwiniae" describes the objective of a group of five laboratories, which aims at an understanding of the degradation process of plant cellulose and pectins by Erwinia chrysanthemi and E. carotovora, causing soft rot disease. The participants are J.P. Chambost (CNRS, Marseille, F), A. Kotoujansky (INRA, Paris, F), J. Robert-Baudouy (INSA, Villeurbanne, F), A. Toussaint-Pourbaix (ULB, Rhode St. Genèse, B), G.P.C. Salmond (Univ. Warwick, UK). This ELWW is exemplary in combining complementary expertise, which encompasses plant pathology, biochemistry, enzymology and bacterial molecular genetics. These approaches have led to the identification of the many degradative enzymes involved and to the isolation of the corresponding coding sequences as well as other regulatory genes implicated. The understanding of the regulatory mechanisms and of the secretion apparatus required for pectinolysis and cellulolysis is expected in the long run to lead to new control strategies.

- "The molecular pathology of plant-fungi relationships" is a vast area where six contracting laboratories join forces and address, within defined experimental systems, crucial steps such as host-recognition or plant defence reactions. This group is composed of P.J.G.M. de Wit (Univ. Wageningen, NL), S. Fry (Univ. Edinburgh, UK), M. Buiatti (Univ. Florence, I), D. Ingram (Univ. Cambridge, UK), A. Ballio (Univ. Rome, I), R. Wingender-Drissen (M.P.I. Köln, D). It is, in the framework of BAP, a modest approach towards developing molecular methods that may give an insight into some aspects of genetic resistance against diseases. However the ultimate goal, that is to say the understanding and control of the plant defence mechanisms, implies altogether a higher magnitude of effort due to the size and complexity of the task. Nevertheless this ELWW was successful in showing that a research effort in this area can be profitable. For instance, we will mention the isolation of molecular markers distinctive of compatible and incompatible interactions in tomato and also the characterization of the regulatory properties of pathogen-inducible gene promoters in different plants.

- "Ribosome-inactivating proteins as pathogen and pest resistance factors in plants" is a very specific attempt at assessing the potentials of ribosome-inactivating proteins for new crop protection strategies. This small ELWW is composed of two laboratories respectively headed by F. Stirpe (Univ. Bologna, I) and R. Croy (Univ. Durham, UK). The identification, purification and biochemical characterization of ribosome-inactivating proteins is carried out in Bologna while the evaluation of their toxicity to insects and other pests, and the cloning of the corresponding genes is responsability of the British laboratory. At the end, the potential for pathogen control will be conclusively estimated in transgenic crop plants transformed with the genes coding for the supposedly most effective ribosome-inactivating proteins.

- "Biomolecular approaches for the control of beet yellows virus" defines a research project aimed at the development of molecular tools effective for the control of viral infection in sugar beet. The two laboratories participating in this ELWW are respectively headed by R. Hull (John Innes Inst., UK) and J. Brundstedt (DDS, DK). The knowledge acquired on the beet yellows virus genome organization and expression will allow the identification and isolation of highly transcribed viral genes (including the viral coat protein gene). Two alternative strategies will be followed in an attempt to inhibit viral replication and confer resistance to transformed beets : overexpression of either the viral coat protein or of RNAs complementary to beet yellows virus genomic RNA (antisense RNA).

Starting from 1990, the BAP programme will be replaced by BRIDGE to allow an updating and an amplification of activities, those which would more pressingly require the mobilization of research capabilities throughout Europe for the ultimate benefit of agriculture and bio-industries. It is the intention in BRIDGE to organize research support at two discret levels :

- to attack penalising research problems, particularly knowledge gaps, which have been discouraging industrial investments or public adhesion : science-led projects with a moderate funding from the Commission to mobilise European laboratories into ELWWs such as achieved in BAP already ;

- to seize emerging research opportunities where applications of economic and societal interest still have to depend on structural or scale factors :
  major trans-European projects more heavily funded for allowing, within few promising areas, direct transfers of technology.

An arbitrary example of a possible major project could be presented as "the genetic and molecular dissection of resistance to fungal diseases in tomato" ; whereas horizontal studies of various aspects of molecular recognition, host-range and virulence may be more easily tackled in the form of open-ended ELWWs, at a smaller scale and allowing at the same time a reasonable dosage of efforts on different experimental systems and species. The possibilities to materialize such ideas are now left with the experts themselves, who should timely take initiatives to seize the opportunity offered within one year with the launching of BRIDGE.

A programme of this dimension may contribute further to the credibility of biotechnological methods and processes, particularly when applied to agriculture.

Community agriculture is faced with the urgent need to increase its competitiveness while keeping in harmony with the environment. Both objectives, competitiveness and environmental protection, can be either antagonistic as they might have been occasionally in the past, or preferably compatible when based upon judicious technological options. The reconciliation of these equally important issues stays with the right balance of "bio-rational" to chemical practices in agriculture. That is to say with the design of alternative methods in plant nutrition and crop protection that would base productivity on genuine biological processes under genetic control, rather than on external supply of chemical fertilizers or pesticides exclusively. This "bio-rationality" is becoming more conceivable with the advance of modern biotechnology and the demonstration that early applications, such as microbial fertilizers, artificial symbioses or in-built genetic resistances, are already becoming feasible.

RESEARCH ACTIVITIES ON RISK ASSESSMENT WITHIN CEC BIOTECHNOLOGY PROGRAMMES AND REGULATORY ACTIONS

U. Bertazzoni
Instituto di Genetica, CNR, Comission of the European Communities-Pavia, Italy

The research activities carried out under the Risk Assessment sector of the Biotechnology Action Programme (BAP, 1985-1989) cover both the problems of containment and of the release into the environment of genetically modified organisms. This sector, that has emerged as a focal point of interest in Biotechnology, has been expanded considerably in the framework of the revision of BAP (1990-1991) and it is aimed at ensuring the safe use of genetically modified microorganisms, plants and recombinant vaccines.

The new research areas are concerned with the development of specific monitoring techniques, the standardisation of model ecosystems and limited field trials experiments and the study of the stability and the expression of inserted genetic material in released organisms.

The development of specific monitoring techniques includes the setting of methods for the rapid and precise detection of the released organisms in complex biotic systems and for evaluating pathogenic properties and effects possibly brought about by modified organisms. It is also aimed at developing genetic and cellular markers (others than those conferring potentially transmissible antibiotic resistance) which could lead to a prompt identification of released organisms.

The standardisation of model ecosystems concerns the development of micro-ecosystems, to be used at the laboratory and glasshouse level, for understanding the behaviour of modified organisms. In this context it is also important to standardise the parameters to be evaluated within the microcosm experiments. In the development of small scale field trial tests, it is necessary to consider the different soil and environmental conditions.

It is also important to measure the survival rate of genetically modified organisms under actual field conditions and to determine the competitive differences with non-engineered organisms and the extent of possible impact on other species.

Concerning the problem of stability of genetic material inserted in released organisms, of capital importance is the evaluation of the possible horizontal transfer of genes from modified organisms to other organisms. To this end, the expression of the inserted gene in microcosm and field trial experiments should be evaluated.

In order to achieve the conditions for a biological containment, the organisms to be released should be constructed with limited potential of survival in the environment. Finally the stability of vaccines obtained by recombinant DNA techniques and inserted into viable vectors should be carefully evaluated, as well as their possible spread to other related species.

In the future Biotechnology programme, called BRIDGE (1990-1994) the assessment of risks possibly associated to the release of modified organisms will be covered under the sector foreseen for normative research. The research activities will complement the efforts initiated in BAP and will focus mainly on microorganisms, recombinant vaccines and transgenic animals.

MOLECULAR AND PHYSIOLOGICAL PARAMETERS AS AIDS IN SELECTION FOR TEMPERATURE TOLERANCE

N. Marmiroli
Institute of Genetics, University-Lecce, Italy

G. Di Cola
Institute of Genetics, University-Parma, Italy

M. Komjanc, V. Terzi, A. M. Stanca, P. Martiniello
Experimental Institute for Cereal Research-Fiorenzuola d'Arda, Italy

C. Lorenzoni
Institute of Plant Genetics, Catholic University-Piacenza, Italy

Application of genetic engeneering and gene cloning techniques to monocotiledons, in particular to cereals suffers of two major drowbacks: 1) the useless of the plant plasmid vector Ti and 2) the impossibility of rigenerating plants from cell or tissue cultures. These facts are actually limiting the generation of transgenic plants and in particular of plants with exogenous genes able to increase or potentiate the resistance to environmental stress.

Under these circumstances the genetic approach acquires a particular importance since the identification and selection of genes involved in stress tolerance and their transfer to the progeny through the sexual reproduction remains the only possibility for genetic improvement.

The study of gene-regulation during stress response, with particular emphasis at the high temperature stress has led to the identification of several gene-products induced during the stress which are called heat stock proteins (hsp).

Here is reported the genetic variability in the hsp patterns found by studing five barley cultivars with different climatic habit. The differences could be indicative of: a) a genetic polymorfism in some of the hsp genes or b) the existance of a different regulation in the various cultivars for the hsp synthesis.

Synthesis of hsp in the five cultivars is also dependent upon the particular stress regimen; we report here a comparison of the following temperature treatments:1) exposure at 35 °C for three hours, 2) exposure at 40 °C for three hours and 3) exposure at 40 °C after a pretreatment at 35 °C.

The possibility of a genotipic effect in the heat stress response have been extended at the physiological level by analyzing such parameters as the growth rate and the electolytes leakage which are indicative of the general condition of the plants. These parameters were measured after the same temperature treatments as for hsp analysis to see whether there is a correlation between the hsp pattern and the plant physiological condition.

The results obtained with the electrolyte leakage test showed that induction of hsp at a sub letal temperature protected the plant membranes from loosing electrolytes in condition of a severe temperature stress.

No particular differences were found among the five genotypes analyzed. The measure of the crop growth rate by typical regrowth test performed on young and adult plants showed that a significant level of thermoprotection was afforded by a mild temperature pretreatment (35 °C). The death of the plants was almost totally prevented by hsp induction and accumulation. The thermoprotection afforded by the 35 °C pretreatment was ifluenced by the genotypes: different levels of thermoprotection were found among the five cultivars.

In conclusion this study shows how the molecural and physiological analysis can differentiate among barley cultivars those genotypes which have a potential for sentitivity or resistance to heat stress. Moreover the study give a correlation between the thermotolerance afforded by, and the presence/absence of a particular hsp pattern.

These two facts can help in predicting possible hybrid lines in which the segregation of a given hsp pattern could be correlated with specific levels of thermotolerance by means of physiological tests like those utilized here.

BIBLIOGRAPHY

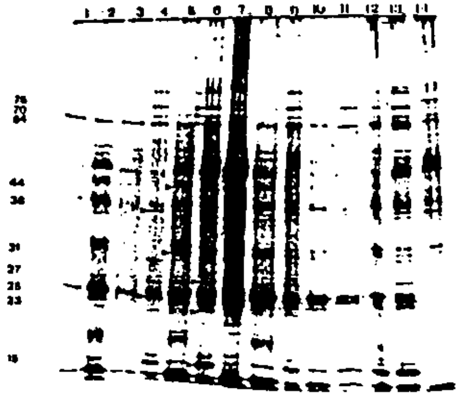
1. Li, G.C. and Werb, Z. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts
   Proc. Natl. Acad. Sci. USA 79: 3218-3222

2. Lin, C.Y., Roberts, J.K. and Key, J.L. 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization
   Plant Physiol 74; 152-160

3. Marmiroli, N., Restivo, F.M., Odoardi Stanca, M., Terzi, V., Giovanelli, B., Tassi, F., and Lorenzoni, C. 1986. Induction of heat shock proteins and acquisition of thermotolerance in barley seedlings (Hordeum vulgare)
Genet. Agr. 40: 9-25

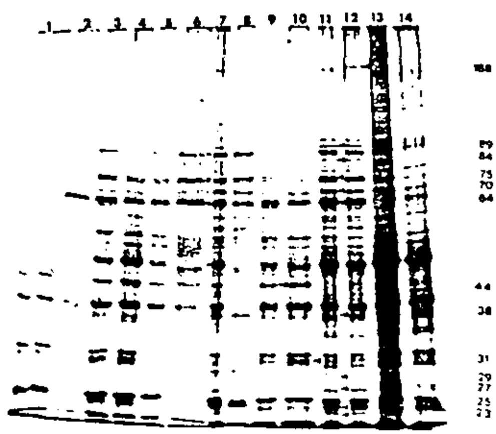
4. Wu, M.J. and Wallner, S.J. 1984. Heat stress response in cultured plant cells. Heat tolerance induced by heat shock versus elevated growing temperatures
Plant Physiol 75: 778-780

5. Xiao, C.M. and Yura, T. 1985. High temperature-induced thermotolerance in pollen tubes of Tradescantia and heat shock proteins
Plant Physiol 78: 887-890



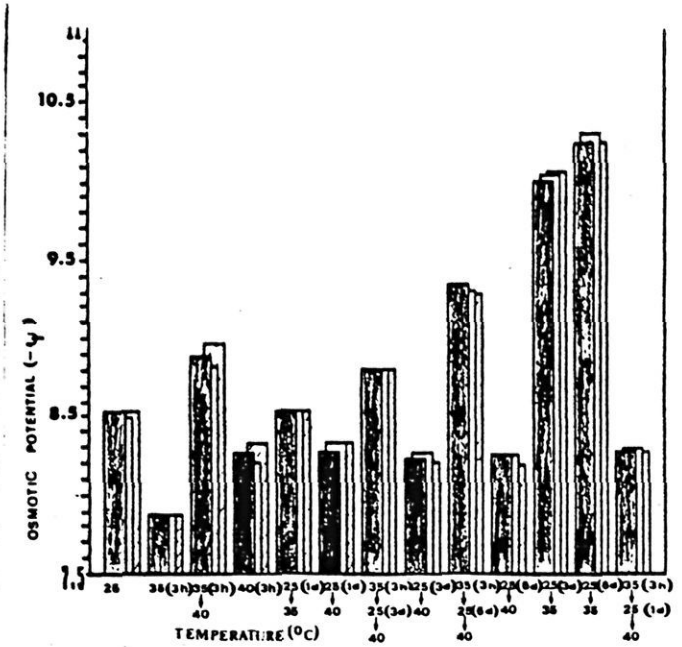
Electrophoretic separation of proteins labeled under various temperat
regimens after 15 days of growth at 25 °C; three cultivars are compared

1 = 35 °C (3 hrs)	25 °C (6 days)	40 °C (3 hrs)	cv IGRI
2 = "	25 °C (3 days)	"	"
3 = 35 °C (3 hrs)	25 °C (1 day)	40 °C (3 hrs)	"
4 = "	40 °C (3 hrs)		"
5 = 40 °C (3 hrs)			"
6 = 35 °C (3 hrs)			"
7 = 25 °C (3 hrs)			"
8 = 35 °C (3 hrs)	25 °C (6 days)	40 °C (3 hrs)	cv HURON
9 = 35 °C (3 hrs)	25 °C (3 days)	"	"
10 = "	25 °C (1 day)	"	"
11 = 35 °C (3 hrs)	40 °C (3 hrs)		"
12 = 40 °C (3 hrs)			"
13 = 35 °C (3 hrs)			"
14 = 25 °C (3 hrs)			"



Electrophoretic separation of proteins labeled under various temperatur
regimens after 15 days of growth at 25 °C; three cultivars are compared.

1 = 35 °C (3 hrs)	25 °C (6 days)	40 °C (3 hrs)	cv ONICE
2 = "	25 °C (6 days)	"	IGRI
3 = 35 °C (3 hrs)	25 °C (3 days)	40 °C (3 hrs)	ONICE
4 = "	25 °C (3 days)	"	IGRI
5 = 35 °C (3 days)	25 °C (1 day)	"	ONICE
6 = 35 °C (3 hrs)	"	"	IGRI
7 = 35 °C (3 hrs)	40 °C (3 hrs)		ONICE
8 = 35 °C (3 hrs)	"		IGRI
9 = 40 °C (3 hrs)			ONICE
10 = "			IGRI
11 = 35 °C (3 hrs)			ONICE
12 = 35 °C (3 hrs)			IGRI
13 = 25 °C (3 hrs)			ONICE
14 = 25 °C (3 hrs)			IGRI

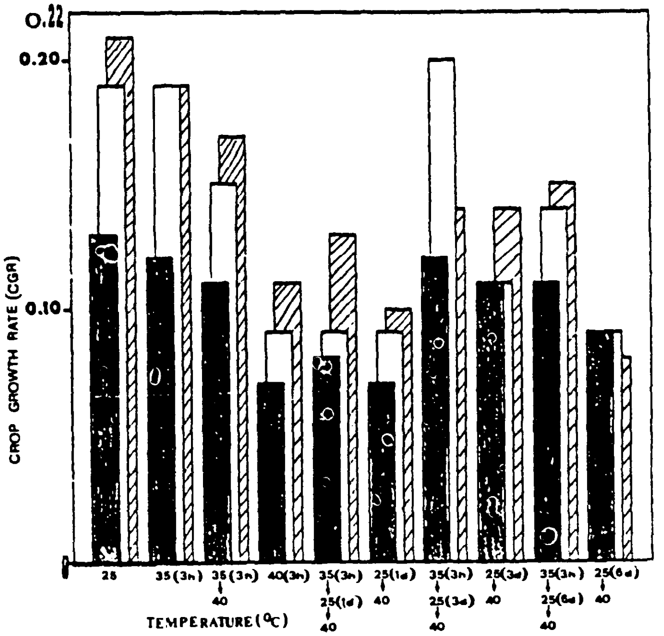


<u>Effect of various temperature treatments upon the Osmotic potential in barley cultivars</u>

In each histogram the osmotic potentials, after different temperature regimen, are indicated. The plant were growth at 25 °C for 15 days before temperature treatments. E) Osmotic potentials for the cultivars: Igri (black), Onice (white) and Huron (///); the osmotic potential is expressed in bars.

Within the three cultivars the response to most of the different temperature treatments is similar except for the following temperature regimens: 35 °C (3h)-> 40 °C and 35 °C (3d and 6d) in which the three cultivars gave quite different values of osmotic potential. These two temperatures conditions correspond to those effective also in inducing a significant and lasting thermoprotection. Therefore a correlation exists between induction of hsp, increase of osmotic potential and aquisition of thermotollerance.

Osmotic potential is calculated according to the following equation: $\Psi = (RT/M)\ln a_w$; a_w = water activity in the sealed chamber measured with a thermocouple psychrometer as described by Richard and Ogata, 1958.



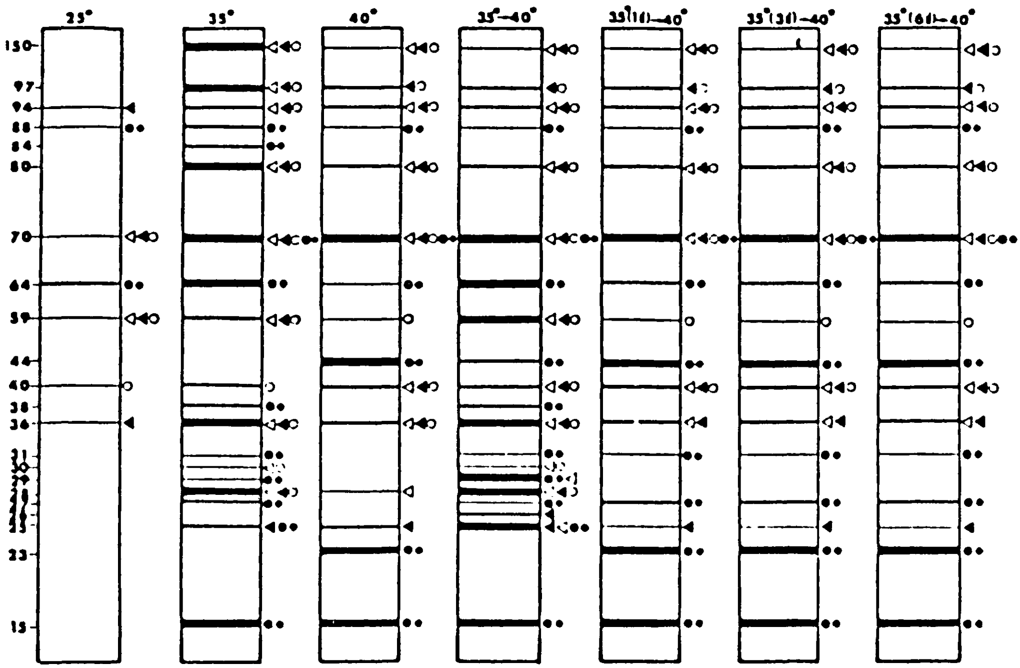
Effect of various temperature treatments upon the Crop Growth Rate (CGR) 15 days after returning the plants in the greenhouse at 25 °C

CGR = $(ss_2 - ss_1) / (t_2 - t_1)$; $g/m^2/d$, da Kvet et al., 1971

Each histograms represents a temperature regimens as indicated. The CGR is calculated for the cultivars: IGRI (black), ONICE (white) and HURON (///).

The temperature stress 25°--> 40 °C for three hours reduced CGR significantly in all the three cultivars; the other two treatments 25°--> 35° and in particular 35°--> 40° had minor effects.

Other evidences on the lasting of the thermoprotection afforded by temperatures pretreatment are shown by the CGR values in plants pretreated at 35 °C (three hours) after one, three and six days.



Drawing of the major variations observed during the various temperature treatments in the five cultivars (Huron, Tipper, Igri, Georgie, Onice).

The symbols ◁ (Tipper), ◀ (Huron), O (Georgie), ● (Onice), ✱ (Igri) are pointed to protein bands which correspond to stress induced or stress potentiated proteins.

Report on the Session 1: BIOTECHNOLOGY

F. Sala
Istituto di Botanica, Università-Parma, Italy

Scientists, agriculturalists, industries and consumers have meet at the Biotechnology Section of the "Parasitis 88" meeting to discuss the production of the most advanced biotechnological tool for the exploitation of plants in agriculture.

There are many fields in which biotechnology has produced, and is presently producing at high rate, exploitable knowledge with plants and with the organisms that interfere with plants: insects, fungi, bacteria and viruses.

The first presentation was by Dr. C. Edwards of the Department of Entomology of the Ohio State University, USA, who emphasized that there are several biological ways through which insect control can be acheved.

One is that of exploiting insect pathogens, such as Bacillus thuringiensis and Beauvaria. Different varieties have different insect specificity. Genes for the toxins have already been cloned and are being modified by genetic engineering. Insect viruses are also of high potential and some of them are already in commercial or experimental production. The same is true for fungi that parasitise insects. They can be commercialized and spread as spores and are omenable for modifications with the genetic engineering techniques.

An other area of promise for the near future is in the introduction of genes that cause malfunction into pest insects. These may include genes for neurohormones, such as neuropeptides, or genes that control insect development.

Dr. H. Van Mallaert of the Plant Genetic System, Gent, Belgium, describe the tremendous work that has been done in this leading laboratory with the genes for the Bacillus thuringiensis toxins.

This laboratory has cloned several delta-endotoxin genes, each encodring a protein with a specific insecticidal spectrum. The cloned genes have been inserted into plant expression vectors and transferred to plants, using the Agrobacterium mediated T-DNA transfer system.

With this system they expect to prepare transformed plants that express the gene only following insect attack. In fact, the TR promoter used in one of the constructions is stimulated by insect wounding.

Dr. H.H. Steinbiss, from an other leading laboratory, the Mox-Planck Institut für Zuchtungsforschung of Köln, F.R. Germany, emphasized the fact that newer and simpler methods are presently being described for the transfer of gene into higher plants.

These avoid the use of Agrobacterium tumefaciens and of the "in vitro" culture of protoplasts or plant tissues and may lead, in the near future to the development of methodologies which will be of universal application.

The next challange is that of directing genes into specific sites on the chromosomes. This might be possible through events of homologous recombination with appropriate gene constructions.

Dr. H.H. Steinbiss also proposed that we may get rid from the necessity to integrate gene in the celluler genome by considering the use of plant viruses as carrier of the desired gene.

Appropiately engineered plant viruses may be inoffensive for the plant but may amplify the gene copies active in the recipient cell.

One problem may be determined by the fact that plant viruses may not go through the sexual event. However this could be overcome by treating seed preparations with the virus.

Finally, Dr. E. Magnien and Dr. U. Bertazzoni presented the initiatives that the Commission of the European Communities are carrying on in the field of promotion of research activities and on risk assessment in the field of pest control and biotechnology in agriculture.

The BEP programme, followed by the BAP and, hopefully, by the BRIDGE programme are good examples of the decision of the European Communities to encourage transnational cooperation and collaboration with the industrial research, up to the pre-competitive level of the exploitation of the invention.

Dr. U. Bertazzoni described the research activities of the Communities on risk assessment covering the problems of continement and of release into the environment of genetically engineered organisms.

The Communities is also working on draft directives airming at regulating the release of genetically modified organisms. The proposals are based on a case-by-case decision after revision of potential hazard and benefits to the society.

SESSION 2

INFORMATIC

Chairman: D. J. Peix

HARDWARE AND SOFTWARE DEVELOPMENTS ENABLING COMPUTER-INTEGRATED AGRICULTURE

D. Holt
Agricultural Experiment Station, University-Urbana, USA

Developments in computer and telecommunications technology will make it possible to link the diverse farm, industry, business, financial, government, and consumer components of agriculture much more closely. Computer-integrated manufacturing can serve as a model for computer-integrated agriculture. Agriculturalists will plan, implement, and manage agricultural operations with the help of powerful computer workstations linked in a vast network of shared computer capabilities. Several evolutionary trends in computer technology are setting the stage for broader, more effective use of computers in agriculture. The most important task for agriculturalists is to develop software that will allow each participant convenient, cost-effective access to and effective use of the agricultural information of the world.

1 INTRODUCTION

People tend to think about the agriculture of a nation as a large number of farms of different types. Actually, however, the agriculture of a developed or a developing nation is an enormously complex, geographically dispersed, loosely organized conglomerate of producers, processors, marketers, and consumers of agricultural products, linked with input industries, financial institutions, educational institutions, farm and commodity organizations, professional societies, and public and private research and development groups.

Now these great agricultural enterprises, functioning in the global economy, find themselves in competition with other similarly organized entities around the world. At stake are the greatest commercial prizes in history, the international markets for food, feed, fiber, and renewable sources of fuel and chemical feedstocks. To the extent that nations compete effectively by improving their overall agricultural efficiency and productivity, they will not only capture shares of agricultural markets but will also help drive the scourge of hunger from our planet and make the world's people much less dependent on non-renewable resources.

While international competition is an inescapable reality of modern agriculture, international cooperation is equally important. More rapid transfer and more effective use of good information will help all agriculture become more efficient and productive and will improve quality of agricultural products. It will help each nation serve domestic agricultural needs and find an appropriate niche in the global agricultural economy.

In an article in Science (Holt, 1985), I described an imaginary farm of the future, in which the farm family uses computer technology to monitor and manage production and marketing enterprises, as well as many everyday activities in the home. In that scenario, computers helped the farm family integrate and coordinate many different activities. The farm of the future will be integrated at several levels. It will epitomize integrated pest management, integrated enterprise management, integrated farm management, and integrated family management.

Now we can take the concept of computer-integrated management up in the hierarchy of systems to the level of world agriculture. In the future, the diverse components of world agriculture will be integrated in an efficient, unified system by a computer-aided, agricultural decision support network. The concept of computer-integrated manufacturing provides a model upon which we can begin to define the concept of computer-integrated agriculture.

2 THE INDUSTRIAL MODEL

In modern manufacturing firms, computer-aided design capabilities, sensors, programmable robots with machine vision, hearing, and touch, other electronically controlled machines, expert systems, and office automation software and hardware are networked to form computer-integrated manufacturing (CIM) systems. These systems enable manufacturing firms to detect and respond rapidly and effectively to changes and emerging opportunities in markets and business conditions.

Computer-integrated manufacturing systems are characterized by much improved communication and coordination among research and development, procurement, production, distribution, marketing, and financial management components of firms. Benefits include greater efficiency and productivity; reduced inventories of inputs, parts, and products; shorter product development time; maximum product and service differentiation; greater market penetration; and, most important, more effective use of each employee's creative talents.

Many of the principles of computer-integrated manufacturing are applicable to agriculture, which is, after all, the largest, most complex, most hi-tech, and most important manufacturing industry of all. Because the components of agriculture are so diverse and so widely dispersed across the landscape, computer integration of agricultural activities promises to be even more beneficial than the integration of typical manufacturing industries.

3 COMPUTER-INTEGRATED AGRICULTURE

On the farms and in the other components of the agricultural infrastructure of the future, the physical, chemical, and biological status of soils, crops, animals, and people; environmental conditions in fields, processing facilities, storage facilities, livestock buildings, factories, homes, and offices; and operational status of mechanical and electrical systems will be monitored by sensors mounted in buildings, facilities, and organisms; mounted on machines and equipment; and connected to microprocessors and computers.

This information plus detailed information on past and potential financial transactions and financial status will be stored in electronic format in computers. Other relevant information will be collected from outside each operation, where it will reside in local, state, regional, national, and international databases, readily accessible through computer networks and various information retrieval systems.

Powerful portable and desktop computers will be the workstations from which human managers and other decision makers manage agricultural and agriculture-related enterprises and activities of the future. These workstations will be equipped with fully integrated packages of expert systems and other items of computer software, accessible locally and through telecommunications networks. Managers will communicate with the computer system in natural language, at times merely by spoken word. Just as machines are extensions of limbs and muscles, the computerized management information system will be a powerful extension of the agricultural decision-maker's mind.

Transactions involved in purchasing and selling inputs and products of the farms and agribusinesses of the future will take place on the computer network, aided by expert systems. Network software will match quantity, quality, and price specifications of sellers and purchasers, debit and credit accounts, and compute the least-cost means and routes of transfer. Using sophisticated agricultural software, producers will "lock in" profits at the beginning of production cycles by forward pricing of purchases and sales and will monitor the system continuously to identify ways to increase profit by changing production and marketing strategies.

Once decisions are made concerning the operation of the farm, agribusiness, or other agricultural operation of the future, the manager will implement them or they will be implemented automatically using machines and devices that are largely controlled by computers. Some of these will be conventional farm and industrial machines and devices, but they will be equipped with elaborate electronic controls and microprocessors to guide, control, and adjust them. Others will be exotic machines and devices, such as robots, able to perform intricate tasks very precisely, with little or no help or direction from humans. They will operate 24 hours per day when needed, in cold and heat, dark and light, and under wet and dry conditions.

The microprocessors of the future will have the enormous speed and memory of today's mainframe computers. They will be found in personal computers and in agricultural machines, robots, and devices. Minicomputers will be communication nodes that broker information on huge, highly integrated communication networks. At the top of this hierarchy of devices will be the mighty supercomputers of the future, programmed to permit levels of analysis and prediction undreamed of today.

Just as rivers, railroads, and highways provide conduits through which people and goods move throughout a nation, the telecommunications networks' linking computers will be conduits for communication and transfer of agricultural information. The networks of the future will provide easy, almost instantaneous, and often automatic connections among agricultural network participants. Information to be shared will be transferred so rapidly and conveniently that a participant will be able to use databases and decision-aid software residing in the memory of someone else's computer as readily as that in his/her own computer.

The integration provided by the network will enable the computer components of computer-integrated agriculture to function as a giant, shared computer. At the same time that it fosters and improves decentralized decision-making, this computer-aided agricultural decision support system will integrate individual agricultural operations into coordinated and efficient national and international enterprises, generating unprecedented levels of agricultural flexibility, responsiveness, efficiency, quality, and productivity.

As the computerized telecommunications networks develop, a vast market for agricultural information will develop in parallel. Agriculturalists will be enabled to access a enormous number of information sources and information processing tools and select the alternatives that meet their specific needs and their quality and price specifications. Competition in this agricultural information market will lead to continuous improvement in the quantity, quality, and usefulness of information and information tools.

The computer network and the information and information tools accessible through it will be an absolutely essential part of each farm business, each agribusiness, every agricultural institution and agency, and every policy-making group, even more so than the telephone and mail networks of today.

4 THE IMPORTANCE OF COMPATIBILITY

To achieve a beneficial level of integration, many different types and brands of computer hardware and software will have to function and communicate compatibly. Recognizing this, several private sector and government groups are working to develop standards and protocols that will enable universal computer connectivity and compatibility. These standards and protocols are facilitating computer integration of manufacturing and service industries and will be essential to efficient, effective integration of agriculture.

Telecommunications networks are moving toward the universal acceptance of computer communications standards. Examples of protocols under consideration are TCP/IP (Transmission Control Protocol/Internet Protocol) and the OSI (Open Systems Interconnection) protocol. Some firms installing CIM (Computer Integrated Manufacturing) systems are converging on MAP (General Motors' Manufacturing Automation Protocol) and some office systems are accepting TOP (Technical and Office Protocol). Protocols and standards should be flexible and should change to accommodate improvements in technology, but universal acceptance of some basic standards and protocols will greatly facilitate computer-integrated agriculture.

5 THE IMPORTANCE OF RAPID TRANSMISSION

Just as the computer-aided decision support system for agricultural management will require fast computers, it will require fast communication of information between computers. Current computer networks, such as BITNET, transfer data at 1.2 to 56 kilobits (thousands of bits) per second. It takes about eight bits to encode a number or a letter. At existing transmission rates, rapid communication is limited to relatively short, typed messages and documents.

Since much of the information generated by computers will be in graphic form, transmission of pictures will be very important. To communicate some information, including the results of some simulations, will require transmitting moving pictures. To transmit moving black and white pictures digitally will require transmission rates of 10 to 60 Mbits (million bits) per second. For color pictures, the required rate is 320 to 1920 Mbits per second.

NSFNet, which currently links the five National Centers for Supercomputing Applications in the USA, permits transmission at about four Mbits per second. Researchers have achieved microwave transmission rates of gigabits (billions of bits) per second experimentally. An extensive fiber optic, microwave station, and satellite communication network, plus ongoing improvement in communications hardware and software will enable the high rates of transmission required to serve users of the computer-aided agricultural decision support system.

6 EVOLVING TECHNOLOGY OF COMPUTERS

Computer technology and the organization of computer use has evolved in important ways. From carrying cards to mainframe systems, we changed to working at "dumb" terminals attached to mainframes or minicomputers. The dumb terminals evolved into desktop computers, which now are becoming powerful workstations with even greater power and flexibility than the mainframes of yesteryear. These workstations will continue to become more powerful, flexible, portable, and easy to use.

The mainframes have not disappeared, but are evolving into minicomputers and supercomputers, available for computational jobs that cannot be addressed by even the most powerful of workstations. They now function as extremely powerful peripherals to workstations, available when the need arises. Their computational speed has increased from megaflops (millions of floating point operations per second) to gigaflops (billions) per second, and computers with teraflop (trillions) speed are contemplated.

supercomputers. Among a number of specialized roles, supercomputers and mass storage devices can function as the file-servers and interfaces for vast information repositories, enabling computer users to access these repositories; search, find, sort, retrieve, and process information very rapidly; and effectively integrate information into their own decision-making environment.

Since early computers were large and expensive, information was processed centrally, with many users sharing a single computer. As computers became smaller, more powerful, and less expensive, it became economic and convenient to go to distributed processing, with most of the information being processed at users' personal computers or workstations. At best, however, computer users cannot have all the databases and other applications they need stored in their workstation computers.

In addition to sharing databases and other software, computer users often need to communicate directly to others working at other computers. To meet these needs, computers are being linked in an extensive, interconnected hierarchy of local-area, broad-area, and "back-bone" networks. When fully networked and integrated, the entire system of interconnected computers will function as if it was one vast and powerful computer.

Until recently only one application could be loaded into the random-access memory (RAM) of a desktop or portable computer at one time. Thus, for example, if one was using a word-processing program and wished to switch to a database application, it was necessary to store the document being processed, log out of the word-processing program, load the database software from a storage device, and proceed through menus to the desired database activity. Standard operating systems such as MS/DOS were only designed for performing one task at a time. This limitation is being addressed in two ways.

With the advent of multi-tasking operating systems, it is now possible to load several applications into RAM and switch back and forth among them at will. When one switches out of and then back to an application, he/she resumes activity at the point at which work was interrupted. As this trend continues and is enhanced by the development of networks, it will be possible to switch among applications residing not only in the RAM of one's own computer, but in that of other computers as well. This will greatly facilitate analytic, design, and planning activity requiring frequent and simultaneous access to several databases and other applications.

The need to be able to carry on several computer activities concurrently is also addressed by parallel processing, in which several processors are controlled by one operating system. The operating system analyzes computational tasks and assigns them to processors to achieve as much simultaneous computation as possible, thus increasing speed and productivity. While parallel processing is currently employed in supercomputers and prototypes, the principles will eventually be applicable in networks of microcomputers and perhaps even in workstation computers.

Parallel processing, other capacity improvements, and networking will hasten an evolutionary change from single operating systems to more user-friendly, host operating systems to multiple-guest operating environments. The large nodes on the computer-aided, agricultural decision support network, including Universities, government agencies, and large private firms, will need to provide very sophisticated, convenient multiple guest operating environments. This will allow them to make their greatest potential contribution to computer-integrated agriculture. The combination of multi-tasking operating systems on workstations and multiple quest operating systems on minicomputers and mainframes will enable the rapid switching among applications that was described earlier in preceding paragraphs.

Another important evolutionary change is in the way information is displayed on computer screens. The change is progressing from text displayed and manipulated as separate lines (line mode) to text/screen mode to text <u>or</u> graphic mode to text <u>and</u> graphic mode (combined). Further refinement of the combined text/graphic mode leads to text/graphic windows. Improvement in graphics capabilities and digital data transmission rates will enhance and diversify the manner in which information can be represented, transferred, and displayed.

The manner in which humans communicate data, instructions, and questions to computers has evolved from cards and tapes to keyboards. The mouse adds another dimension of power and flexibility to this communication process, and, combined with graphic interfaces, enables those who cannot type to use computers effectively and efficiently. Successful prototype systems for voice communication with computers exist and will continue to be refined. Voice communication will render computer use much easier and more efficient and will enable many more people to use computers. It will be an important technological component of computer integrated agriculture.

The next evolutionary step in communicating with computers will be to connect man-made electronic computers directly to bio-electronic computers (brains), so that the man-made computer becomes a direct extension of human thought processes. Such a monumental scientific and technical achievement is within the realm of possibility.

6 RESEARCH AND DEVELOPMENT NEEDS

Most of the research on hardware components of the system, except agricultural sensors, has been and will be conducted by non-agricultural private firms and other groups outside of agriculture. Much general purpose software that will be useful in agriculture, such as spreadsheet and database management, wordprocessing, and telecommunications software, has originated and will continue to originate outside agriculture. Some agricultural researchers and educators should interface with these efforts to stay abreast of technological changes and to assure that the general purpose hardware and software will meet the unique needs of agriculture.

The telecommunications technology that will enable computer-integrated agriculture is being developed and implemented rapidly by telecommunications firms and other groups. National and international computer communications protocols and standards are being negotiated and adopted. By the time a significant amount of agricultural software is available for use on networks, the networks will be in place. These networks, of course, will serve many other purposes and will continue to be extended, improved, and refined indefinitely.

Agricultural research, development, and education people should focus on the following:

6.1 Designing the system.

Through the systems engineering approach, the international computer-aided agricultural decision support system should be designed to provide agricultural information in the most usable form at the lowest cost. Ultimately the system should be self-supporting through subscription fees, connect-time charges, software purchases, etc. An important part of this effort will be determining at what levels the system should be organized, how it can best utilize existing and future networks, identifying the unique roles that can and should be played by various participants, and developing and adapting protocols and standards that enable easy communication among those who are suppliers to and users of the system.

6.2 Developing sensors.

Sensors must be developed to measure and monitor variables that characterize the status of agricultural systems. These will range from sensors that monitor the environment within agricultural buildings and facilities to sensors that measure important plant and soil variables as a machine operation is underway. New and improved sensors will be needed for agriculture-related industrial operations as well.

6.3 Developing agricultural software.

The following categories of software must be developed to enable participants to use the computer-aided agricultural decision support system effectively.

6.3.1 Intelligent software interfaces that enable participants to access, evaluate, sort, and integrate information provided by other participants.

One of the biggest needs is for publicly supported agricultural researchers and educators to develop software interfaces that provide users ready access to university agricultural libraries and other public agricultural information repositories. Among other challenges, as much of the available text and graphic information as possible must be put in electronic format, thereby enabling rapid and efficient searching, sorting, evaluation, analysis, and integration of information be participants.

6.3.2 Prototype knowledge-based systems, including expert systems, to help participants make decisions and manage operations.

These computer programs will serve to summarize and make useful the huge agricultural knowledge base, including the part of it that now exists only in the minds of experts and experienced practitioners of agriculture. Through this effort, the great progress being made in basic research on artificial intelligence can be translated into powerful agricultural management tools. Conceptually sound prototypes developed in publicly supported research efforts can be further developed and commercialized by the private sector or, when appropriate, can be employed directly for public institutional purposes, including extension and teaching.

6.3.3 Computer-aided instructional materials ranging from mini-course modules to fully integrated curricula.

Such computer-aided instructional software should be highly interactive and take advantage of the latest computer graphics technology to provide text; black and white, still and motion pictures and other graphics; and sound. Computer-aided instructional materials will be much enhanced by an infusion of artificial intelligence technology. Provision of high-quality instructional software will revolutionize agricultural instruction at all levels.

CONCLUSION

By application of rapidly developing computer and telecommunications technology, the diverse components of world agriculture can be integrated into a well-coordinated system. Benefits of computer-integrated agriculture include greater efficiency and productivity, abundant supplies of safe, high-quality, relatively inexpensive agricultural products, better protection of the environment, greater conservation of natural resources, and improved quality of life.

BIBLIOGRAPHY

Holt, D. A. - 1985. Computers in production agriculture. - Science 228:422-427.

EXPERT SYSTEMS AND IPM: AN OVERVIEW

N. D. Stone
Virginia Polytechnic Institute & State University-Blacksburg, USA

H. Saarenmaa
The Finnish Forest Research Institute-Helsinki, Finland

Summary

In recent years, expert systems have proliferated in business and industry, often improving productivity and increasing profits. Concomitantly, expert systems for agriculture and natural resource management have emerged and generated considerable excitement from their promise of improving the decision making process by delivering more and better expertise. However, building expert systems to help control pests in managed ecosystems has been difficult due to the inherent uncertainty in these systems. Expert system developers have, therefore, faced new challenges and discovered novel uses for artificial intelligence (AI) technology in these domains. This paper addresses the use of AI technology in integrated pest management (IPM). Several application areas are discussed: (i) rule-based expert systems, primarily for tactical management, (ii) model-based reasoning systems which add robustness and flexibility to rule-based systems, (iii) AI-based methods of simulation; (iv) the role of databases and geographic information systems in intelligent decision support systems; and (v) the potential application of intelligent safety systems to implement IPM programs in the future. It is concluded that artificial intelligence systems have the potential to fulfill the early promises of IPM, helping farmers and foresters make better pest management decisions by providing a true system-level understanding and integration of knowledge.

1 INTRODUCTION

More than ten years ago, a computer program called MYCIN was written that could diagnose bacterial infections in humans with accuracy and consistency as good as the best human doctors (YU *et al.*, 1985). It was a program that used artificial intelligence (AI) to enable the computer to reason to a solution and defend its conclusions. The system was essentially a model of the problem solving heuristics of human experts and represented the first major success of a field of applied AI research called expert systems. Since that time, expert systems have been developed to solve complex problems in many disciplines, particularly in business and the military. Today, expert systems are changing the ways in which businesses make decisions, train employees, monitor and operate equipment, and plan for the future.

A similar trend has occurred in agriculture and natural resource management. Expert systems have been proposed or built that help farmers decide what to plant (PLANT *et al.*, 1987), whether to apply pesticides (STONE *et al.*, 1986b), when to irrigate and harvest (LEMMON, 1985), how to market their crops (UHRIG *et al.*, 1987), and whether to purchase crop insurance (HELMS *et al.*, 1987). In the agricultural sciences, expert systems are being used to help design and interpret simulation models (LOGAN, 1988), to monitor experimental aparatus (JONES *et al.*, 1986), and to teach. In a broader context, expert systems in combination with more traditional computer problem solving applications can provide an essential integration of information needed to design and implement true integrated pest management programs. The potential of these systems and other related AI techniques is just beginning to be realized.

1.1 DEFINITION

Conceptually, expert systems are fairly simple. They are artificial intelligence computer programs that solve complicated problems by mimicking the problem solving techniques of human experts. They work by storing expert *knowledge* about a well defined problem type, not merely data about specific problems, and then applying reasoning methods to that knowledge to come up with solutions to particular cases. For example, in the MYCIN system, the knowledge base consisted of premise-action rules like (after VAN MELLE, 1985):

Rule 035	**premise:**	the gram stain of the test organism is gramneg
		and the morphology of the test organism is rod
		and the aerobicity of the test organism is anaerobic
	action:	there is suggestive evidence that the identity of the test organism is bacteriodes.

When the system is run, data about specific tests are entered and information is generated—the gram stain is gram-negative. This information is evaluated based on the knowledge (in the rule) that if the gram stain is gram negative and the organism is an anaerobic rod-type bacterium, then the organism is probably *Bacteriodes.*

While conventional programs can be written to emulate expert reasoning, for two simple reasons, expert systems represent something quite different. First, the type of information stored in the computer is symbolic, rather than algorithmic; and second, the use of that knowledge base for problem solution is automated through the inferencing procedure or *inference engine*, rather than specifically described in procedural code. Symbolic representation allows the computer to manipulate objects, like the "test organism" above, that may have properties like "morphology" or "aerobicity". In short, it provides a better syntax for dealing with ideas and knowledge than traditional typed variables in conventional programming languages. Automated inference, meanwhile, frees the knowledge engineer to concentrate on accurately representing the expert's knowledge, without worrying precisely how those pieces of knowledge fit together.

1.2 EXPERT SYSTEMS AND PEST MANAGEMENT

Expert systems have tremendous potential in pest management in at least two ways, one quite apparent, the second much more profound. First, they provide a way to package and deliver to decision makers the best information and expertise available about solving difficult problems. Many pest management decisions are made without the benefit of the most up-to-date information and knowledge simply because because that knowledge is scarce, limited to a single person with years of experience, or known only to researchers. Problems like pest identification, pesticide selection, and timing of field operations can be improved in many cases by providing more and better information to producers.

The second way expert systems and its parent field, artificial intelligence (AI), are influencing agriculture is by providing computer systems capable of fulfilling the ultimate goals of integrated pest management (IPM)—that is, capable of providing the system-level understanding and synthesis required to make intelligent decisions about cropping systems, from economically sound pest management to implementation of alternative crops and agronomic strategies.

In this paper we present an overview of the methodology for building expert systems. In particular, we explore the knowledge processing techniques that can be used to implement an IPM system, and we discuss recent and current developments in expert systems applied to pest management.

2 RULE-BASED EXPERT SYSTEMS

Rule-based expert systems are the most common type of knowledge-based system (Fig 1). They represent knowledge about a problem domain in rules, independent *if...then* statements that define relationships between observations and conclusions. The rules are processed by an inference engine which selects them in a logical sequence, building a chain of if...then statements that eventually lead to a final conclusion or solution to the problem. The knowledge-base is the core of the system; it contains the domain-specific expertise in the form of rules. The inference engine compares the rules with facts supplied by the user, storing the facts in the working memory. Then it chains the rules with each other to make conclusions, thereby adding more facts to the working memory.

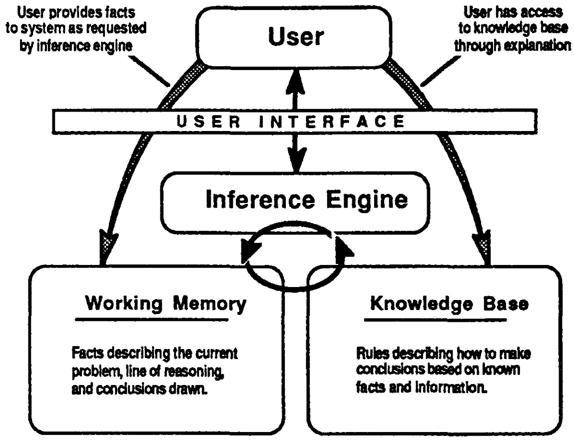


Figure 1. Basic design of an expert system. The inference engine compares what its knowledge base with the specific information about the problem at hand, asking questions of the user as it goes. As new information is gained the cycle repeats until the system gives its advice.

By keeping a record of the chain of rules processed, a rule-based expert system can explain its conclusions. The explanation usually consists of a translation of the rules fired and the order in which they were chained together. For example, a rule from an expert system recommending control actions for cotton insect pests might be,

Rule-x: **If** the BWV_infestation of ?field is above_threshold
and the CFH_infestation of ?field is near_threshold
then recommendation is tank_mix for BWV and CFH.

This, could be translated into an explanation for why a tankmix was recommended for a particular field:

> Since the boll weevil infestation is above the economic threshold and the cotton fleahopper infestation is near the economic threshold, spraying for both pests using a tankmix is recommended.

Other common explanation capabilities enable expert systems to explain why some conclusion was eliminated, or why some piece of information is being requested during a consultation.

Note that the explanation facilities, although they operate on rules, are separate from the rule-base. This separation of the knowledge-base from the inferencing and explanation facilities is a key feature of knowledge-based systems in general and expert systems in particular, and it requires that the inferencing scheme used be generic. That is, the expert system knows how to solve problems generally, given knowledge in the form of rules. Without the rules, what is left an empty shell, the skeleton upon which the expert system is built. Generally, all that is required to build an expert system is to select or design an expert system shell (there are several currently available commercial shells for a wide variety of computers), and to enter the knowledge-base in the form of rules in the proper syntax for the shell selected. The shell provides the knowledge representation methods, inference capability, explanation, and user interface to the developer.

Problems in pest management that have been addressed by rule-based expert systems include: identification of key beneficial and noxious organisms (MICHALSKI *et al.*, 1983; BLANCARD *et al.*, 1985; WOOLLEY & STONE, 1987; LERENARD, 1988; HOSHI & KOZAI, 1988, CERVO *et al.*, 1988), pesticide selection (JONES *et al.*, 1986; STONE *et al.*, 1986b; JENKINS & LYONS, 1988), braod tactical recommendations in grape systems (SAUNDERS *et al.*, 1987), and tactical advice in well-defined areas like weed control (BLONNIGEN, 1988; MARAN *et al.*, 1988).

2.1 LIMITATIONS

Despite the elegance of rule-based expert systems, they have proven inadequate for many large applications, for example those that require adaptive capabilities, simulation, or large amounts of static knowledge (HAYES-ROTH, 1985; FIKES & KEHLER, 1985). When the number of rules increases into the hundreds, the rule-base becomes unwieldy, difficult to modify or extend without unanticipated side-effects.

Furthermore, the rule-based knowledge representation itself includes inherent limitations. Rules directly relate the current status of a problem to conclusions that can be drawn; however, they do not describe the mechanism(s) involved. Sets of rules in a rule-base, therefore, represent correlative or compiled knowledge (KOTON, 1985), rather than causative knowledge. Rule-based systems are often termed *shallow* systems as a result; they reason without knowledge of the underlying mechanics of the system. The deeper understanding of the problem is only implicit. It follows that rule-based systems must anticipate all possible descriptions of a problem to be robust. If unanticipated situations arise, rule-based systems will fail. They are not adaptive, nor do they know their limits.

3 MODEL-BASED KNOWLEDGE SYSTEMS

To deal with the inherent limitations of rule-based systems, researchers in AI and expert systems developed another kind of knowledge-based system that includes a deep model of the problem domain as well as a shallow advisory system that uses the deep model as its basis. In agriculture and natural resource management, such systems that use simulation models and rule-based reasoning have been termed integrated expert systems (COULSON *et al.*, 1987), although this term more commonly refers to expert systems linked with deep models and databases (STONE *et al.*, 1986b; STONE, 1987) and a few been developed to varying degrees (MCKINNION, 1988, STONE & TOMAN, 1988). In industry, these systems have been called model-based reasoning systems (INTELLICORP, 1986). The model-based reasoning (MBR) system gets its information from two sources, from the user's description of the problem and from the deep model's knowledge about how such a problem might come about. The deep model is used to evaluate the probable cause of the user's problem. Rules are then used, as in a classical rule-based system, to determine a solution from the causes identified. Theoretically, the number of rules can be reduced and the rules be made more general by adding a deep model of the domain separate from the expert problem solving strategies.

MBR systems are more robust than purely rule-based systems because they give advice based on the probable cause of a problem, they do not go directly from symptoms to advice (Fig 2). By use of the deep model, the MBR can determine what fundamental problems are most likely to create the observed symptoms. Classical expert systems require this step to have been worked out in the creation of the rules. Reasoning from first principles allows MBR systems potentially to hypothesize several causes for observed phenomena (INTELLICORP, 1986), or even to handle multiple simultaneous faults (DEKLEER & WILLIAMS, 1987).

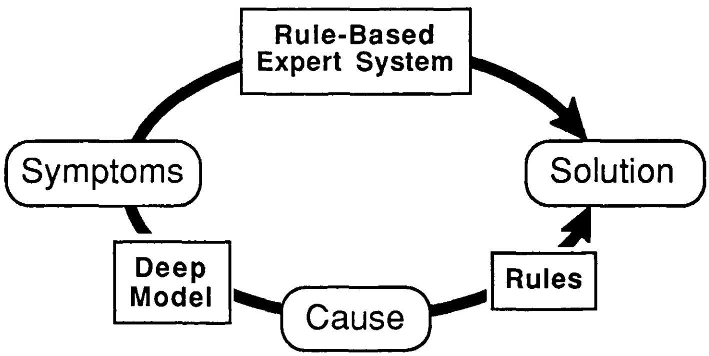


Figure 2. Comparison of rule-based versus model-based reasoning systems. Rule-based systems relate symptoms directly to solutions; model-based systems rely on a deeper understanding of the system tc determine one or several hypothetical causes for the symptoms before trying to solve the problem.

3.1 DEEP MODELS

The difficulties in designing an MBR are constructing the model and making it accessible to the reasoning process. Models can take several forms, from classical simulation models in languages like FORTRAN to rule-based, frame-based, or object-oriented models. Each of these can be used to describe the structure and function of a system such as an automobile, factory, organization, plant, animal, or ecosystem, and each can be connected to a reasoning system to create a model-based knowledge system.

Many problems in pest management would seem to be well suited to an MBR approach, either because models of the natural system already exist, or because the complexity of managed ecosystems makes it virtually impossible to enumerate in rules all the possible problems that can occur. However, the most commonly found models of pest management systems, simulation models written in FORTRAN, are also the most difficult to link to a reasoning system in a dynamic way. Whereas the models that integrate most easily with reasoning systems, models written using AI languages or structures, are only just now being built for agricultural systems. Depending on the level of linkage between the reasoning component and the model, such systems can be classified as hybrid AI/traditional systems (low-level linkage), integrated systems, or model-based reasoning systems (complete linkage).

3.1.1 ALGORITHMIC SIMULATION

Since the early 1960's, simulation models have been a classical method to incorporate knowledge of dynamic behavior in a system. In the 1970's and early 1980's, simulation models were at the heart of many IPM programs funded through the National IPM and CIPM programs (HUFFAKER, 1980; FRISBIE & ADKISSON, 1986). Without much modification, a simulation model can be connected to a reasoning system that runs it and interpretes its results. However, the reasoning system treats such a model—that is normally written FORTRAN or another procedural language—as a black box and is not capable of explaining or reasoning about the processes within the model.

An example of a hybrid system is the GOSSYM/COMAX system for cotton management (LEMMON, 1985; MCKINNION, 1988). A rule-based expert system is used to set up simulation runs of the GOSSYM model (BAKER *et al.*, 1983) of cotton growth as a function of weather, irrigation, and fertilizer applications. The simulation output is then examined by the expert system in order either to determine input variables for another simulation run, or to recommend an irrigation/fertilizer application schedule. Obviously, since the inner workings of the model are not accessible by the expert system, the model must be assumed to work correctly. There is no adaptation or feedback capability between the reasoning component and the deep model in such hybrid systems.

3.1.2 FRAME-BASED MODELLING

Frames are another common knowledge representation form in expert systems. Frames have names and *slots* to hold related pieces of knowledge. In many ways, frames are like records in a database, while slots are like fields. However, the slots of frames can hold much more than data. For example, consider the frame called **Pesticide** in Fig 3. It has slots that describe the compound: a COMMERCIAL_NAME slot, a LBS_ACTIVE_ACRE slot holding a number, and a FORMULATION slot holding a value selected from a list. A LABEL slot holds the filename of a text file containing the label information, and a related slot, called PRINT_LABEL, contains a program to print the text file named in the frame's label slot. Obviously, slots can hold many different types of information, even code. Slot values are often restricted to some type of information or value class. These restrictions are shown in parentheses in Fig 3. For each pecticide to be included in the knowledge base, a new copy of the empty frame—a new *instance*—is created and values placed in its slots. Thus, several different instances of each frame can be created in a knowledge base by entering different values into the slots. Notice that some slot values, like the program in the PRINT_LABEL slot, will work for all instances of the frame and so do not need to be changed when creating new instances; they are defined at the frame level.

Frames can also have slots that refer to other frames or instances. The **Insecticide** frame (Fig 3), for example, has a slot called A_KIND_OF. The value of the A_KIND_OF slot is the **Pesticide** frame. By defining this relationship, instances of the **Insecticide** frame inherit all the **Pesticide** slots in addition to locally defined slots like INSECTS, a list of insects with their associated crops for which the insecticide has been registered. Value classes can also be frames. The PESTICIDE_TYPE slot of **Pesticide** has the value class, "instance of **P_type**". The value placed in this slot must be one of the instances of the **P_type** frame, e.g., **Pyrethroid** or **Organophosphate**. One can access the slots of this secondary frame. For example, the **P_type** frame has a

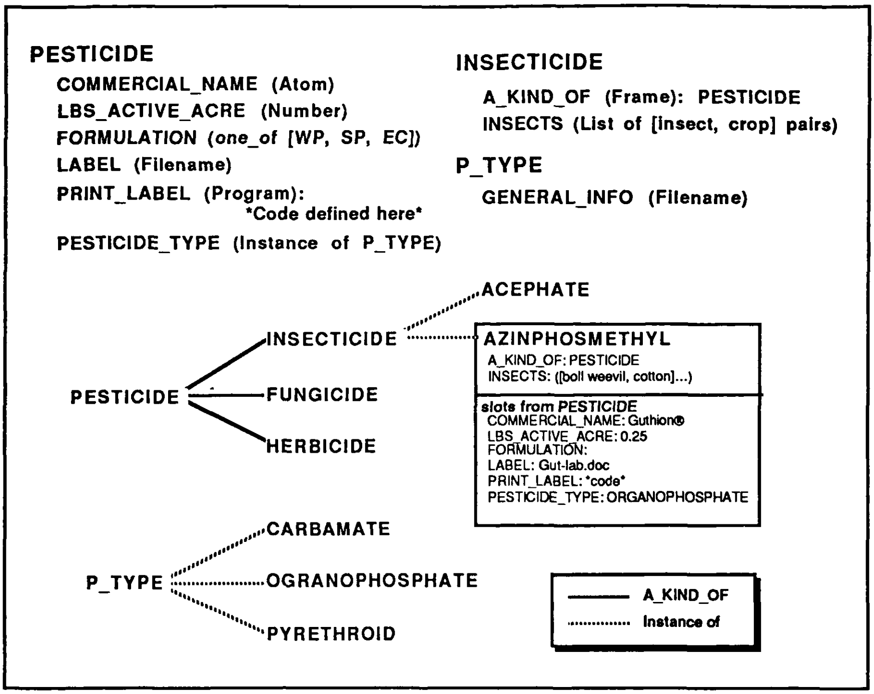


Figure 3. Example of frame-based representation using pesticides. Three frames are defined at the top. Each has slots that hold values of different types (shown in parentheses). Frames can refer to one another, creating relationships that can be expressed in a hierarchy, as shown in the lower portion. Instances are frames filled in with specific values. See text for further explanation.

slot called GENERAL_INFO; so, if one wants to know about the general class of insecticides that includes Guthion®, one can refer to the GENERAL_INFO of the PESTICIDE_TYPE of **?Insecticide** where COMMERCIAL_NAME of **?Insecticide** is "Guthion". This would search all instances of the **Insecticide** frame for one with the commercial name, Guthion; it would then read the frame value from the PESTICIDE_TYPE slot and access the GENERAL_INFO slot of that frame. All this allows very flexible access to information, even if some information is missing, like the generic name of Guthion.

Frame-based models are generally descriptive, using frames to define the components of a system, with slots to hold the relationships among components. SAARENMAA (1988), for example, used a frame-based model to describe the taxonomic and behavioral relationships among the pests complex attacking trees in Finnish forests. Each frame described a natural group such as a pest species. This model used the notion of inheritance to represent taxonomic relationships. The slots of frames higher in the taxonomic hierarchy were inherited by all frames declared as members of the parent frame (lower in the hierarchy) unless specifically redefined. For example, the family of bark beetles had the slot TREE_UNIT_INJURED with the value "Phloem". This was inherited down to all the bark beetle species. However, in ambrosia beetles this slot value was overwritten by the value "Sapwood", because these beetles are an exception in the group and do not eat phloem. In this way, a whole textbook of entomology could be coded efficiently, and the relationships described could be used by a reasoning system.

3.1.3 OBJECT ORIENTED SIMULATION

Object-oriented programming (STEFIK & BOBROW, 1986; PASCOE, 1986) takes the frame-based model one step further. Procedural code is added but the entire program is still contained in the slots of frames. Instances of frames are called *objects*, and program code or procedures are called *methods*. Simulation occurs when a method fired by one object instructs another object to fire its own methods. The program procedes through a series of such *messages* between objects. Methods can also be self-activating, for example, performing some function like modifying a display when the value of a particular slot changes. Reasoning systems can be added to an object-oriented system by creating methods that perform inference about the system when they receive a message.

The great advantages of object-oriented programming result from the correspondence between the natural system and the modelled system. Because objects in the simulation contain their own code, they are autonomous. And because all methods are triggered by messages, objects actually react and respond to the other objects in the model—much the same way that organisms act and react to stimuli in their environment. Behavioral modelling and building models that depend on the spatial relationships of objects are particularly well suited to this style of programming (LOEHLE, 1987; SAARENMAA *et al.*, 1988). In pest management, the area of biological control is most likely to benefit from this new style since the outcome of biological control programs depends so heavily on parasite behavioral traits and the spatial heterogeneity of the environment (MAKELA *et al.*, 1988).

Building an object-oriented model of a natural system involves much the same tasks as building a frame-based representation of the system. However, the objects must be selected based more on functional and behavioral distinctions than on hierarchical relationships. For example, MAKELA *et al.* (1988) found it more convenient to classify the insects in their object-oriented host/parasite model into male and female classes before discriminating species or even families because female insects shared many behaviors. It was simpler to define behaviors like oviposition, calling, and search at this higher level, than to break each species down into male and female classes after detailing the taxonomic hierarchy.

3.2 APPLICATIONS

An overview of MBR in natural-resource management is provided by SAARENMAA (1988) and the underlying concepts have been reviewed by STONE (1987). There are few examples of model-based knowledge systems so far. They have been employed in fault diagnosis in electrical engineering (DEKLEER & WILLIAMS, 1987) and in the military (WHITEHEAD & ROACH, 1987). Object-oriented simulation models in ecology have been created only recently (GRAHAM, 1986; MAKELA *et al.*, 1988; SAARENMAA *et al.*, 1988; FOLSE *et al.*, 1988).

4 EXPERT SYSTEMS AND DATABASES

Knowledge acts on information, and information is derived from data. Ultimately, an expert system's capabilities depend on access to data. Pest identification, for example, requires only a description of the pest organism—data easily at hand—while farm planning requires reams of descriptive data as well as economic trends and predictions. Pest management problems can be characterized by the degree to which they depend on information spread out in time and space (Fig 4). Tactical decisions (areas 1, 3), like whether or not to spray an insecticide to control thrips in cotton, are frequently made based on current sampling information. However, longer term strategic decisions (2, 4), such as whether to use biological control agents in a control program, are made based much more on historical trends, forecasts, and expectations of long-term economic gain.

Currently, farmers are advised to make most pest management decisions based at the field level. Sampling provides information and decisions are made for each field individually. However, more and more pest problems have been seen to have a strong area-wide component (areas 3 & 4). This trend can be seen in the development of farmer cooperatives for pest control (e.g., PHILLIPS *et al.*, 1980), the emphasis on migration and movement in pest control research, and the continuing emphasis on quarantine, control, and eradication programs. Making good decisions about area-wide pest management depends both on a good understanding of the spatial dynamics of the pest populations in relation to control measures, and on being able to coordinate a control effort in space as well as in time. Many pest eradication efforts, for example, have been severely handicapped because of poor coordination of area-wide efforts (DAHLSTON, 1988).

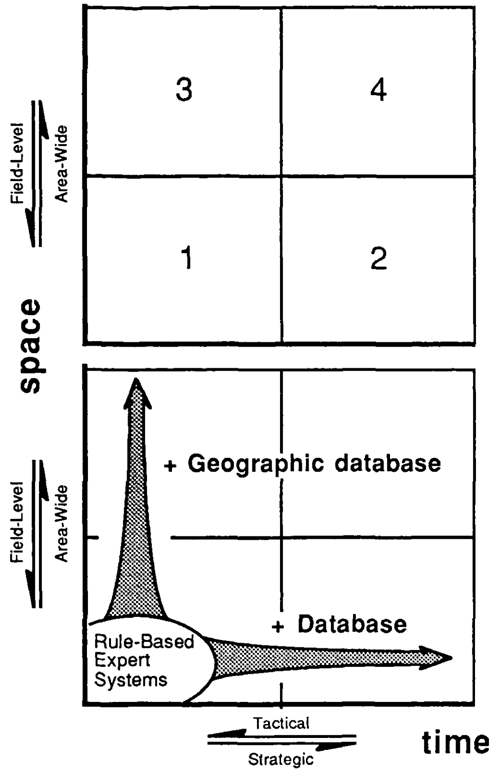


Figure 4. Classification of pest management problems by their dependence on information spread out in time and space. Classical expert systems work best on narrowly defined problems, with the problems fully describable from information in the present. Systems that wish to address more strategic or areawide problems require access to a database or geographic information system.

Rule-based expert systems work best on problems for which the solution can be based on a description of the immediate circumstances (Fig 4). Extending expert systems to deal with area-wide or strategic planning questions requires access to databases. Furthermore, model-based systems, though they introduce adaptiviness and extend the scope of expert systems into simulation, prediction, and planning, running models demands more data of another sort. In short, for most real-life applications large amounts of plain data are needed (FOX & MCDERMOTT, 1986).

4.1 TIME-BASE DATA

Historical data can comprise records of past pest levels and treatments as well as meteorological records. These serve as input to simulation or reasoning. Most of today's knowledge systems must take standard database records and map them into the slots of frames before they can reason about them. Commericial object-oriented data base management systems (DMBS) allow direct access to the data and are just making their entry into the marketplace. An agricultural example is the COTFLEX system developed by Stone and colleagues for cotton production advice in Texas (STONE *et al.*, 1986b; STONE & TOMAN, 1988).

COTFLEX contains three advisors that deal with different aspects of cotton farm management. One, the pest management advisor, is a modular expert system that gives advice on how to control three key insect pests of cotton on a field by field basis. COTFLEX was developed to prototype stage on a Pyramid 90x computer running UNIX and is now being modified for delivery and field tested on an IBM PC/AT-compatible microcomputer under MS-DOS. A Prolog-based expert system shell, Arity Prolog, was used in combination with C and Prolog code to achieve a dynamic link between a database manager and the reasoning component.

The system's evaluation of the severity of a pest infestation depends to a large degree on what has happened in the past. For example, if a spray has recently been made to control *Heliothis* populations in a field, the system interprets new information about that field in relation to evidence of insecticide resistance. Or, when assessing a borderline infestation of an insect pest, the system determines whether the pest population has been increasing or decreasing, information that requires a record of past field information. The system's database is used both as a record-keeper and as a time-dependent database for access by the reasoning system. COTFLEX stores previous user-supplied information and system recommendations, so it can track the progress of each field over the course of the full production season.

The database in COTFLEX was written using C-language structures and linked dynamically to the frame-based Prolog environment. Information is stored in C structures, very similar in form to the records found in a relational database system. When the user selects a field during a consultation with COTFLEX, the database is checked, updated if necessary, and the relevant information in the database for that field is transferred into Prolog functors, similar to frames, which are then used by the inference engine.

4.2 SPATIALLY-REFERENCED DATA

Pest monitoring data have a spatial component, and in many cases, this spatial information is extremely important to making proper decisions. Database systems that store spatially-referenced information along with visual representations of the relevant region are called geographic information systems (GIS). Maps are used as the fundamental organizing theme in the database. Areas or polygons with like attributes are identified on the maps and information about those areas is stored. Traditional database management fuctions like sorting, finding intersections and unions, and providing reports are standard features of these systems, except that they are applied to identifiable regions on maps. GIS systems have been available for many years on mainframe computers and have been used mainly as computerized mapping and analysis tools. More recently, GIS systems have been linked with AI tools and expert systems to add a reasoning component to their map-based analysis capabilities (ROBINSON *et al.*, 1987; COULSON *et al.*, 1987).

There are many ways to connect a GIS with an expert system. The most elegant way is to store all the data in object-oriented form, including the methods needed for display and analysis. This approach has been used in a static way by SAARENMAA *et al.* (1988) in simulating animal behavior with rule-based reasoning. However, to date, no program with the analytical and mapping power of classical GIS systems has been developed with an embedded reasoning component. Some researchers are attempting the linkage (TOMAN, 1988); however, the data representation schemes used by AI programmers and by the traditional GIS programs are not currently compatible.

5 INTEGRATION AND DELIVERY

Providing decision support for pest management programs can be accomplished by building expert systems addressing discrete problems within the overall management domain. However, to provide good advice on the integrated management of pests, one cannot take an isolated approach. Too often, the advances in one aspect of crop production has been the cause of serious problems in other areas. While expert systems show great potential in addressing specific, well defined problems, the test of their success in pest management will be how well they can be used to deal with complex and poorly bounded problems.

Currently, two types of systems have been built or proposed to deliver broad based decision support in agriculture. One, the FLEX approach mentioned above (STONE *et al.*, 1986b; STONE & TOMAN, 1988), is based on the integration of databases, simulation models, and modular expert systems in a software environment or shell for farm level decision support. The second is based on dedicated computer hardware systems or *expert workstations.*

5.1 SOFTWARE INTEGRATION

The farm-level expert system (FLEX) approach was developed out of a project to build an expert system to help cotton farmers in Texas manage their crop and their farms. Initially, the system was designed in a modular way to access databases and simulation models of the cotton crop and its major pests in the region, thereby providing sound objective information, expert advice about pest management, and the most accurate predictions available on how pest populations were changing and affecting the crop (STONE *et al.*, 1986a). Integration of these elements occurred through the development of a functional model of the overall decision making system involved in cotton farming (FRISBIE *et al.*, 1987; STONE *et al.*, 1987). Fig 5 shows a part of the function model in COTFLEX. The diagram is essentially a blueprint for how different types of knowledge and information are used in conjuction with modular expert systems to come up with advice about a variety of problems. The delivery of such a complex system does depend on fairly sophisticated hardware; however, COTFLEX has been implemented on an MS-DOS microcomputer with one megabyte of memory and a 40 MB hard disk.

5.2 EXPERT WORKSTATIONS

Expert workstations support the decision making of experts by providing them with access to a diversity of systems under a single uniform interface (FAUGHT, 1986). Expert workstations are different from plain expert systems in that they assume that the user is an expert of the field like in the case of decision support systems. The central methodology behind expert workstations is MBR. A typical expert workstation provides access to AI based models and databases that the user can run and combine to support his/her decision making. Expert workstations already exist in biotechnology (INTELLIGENETICS, 1986), geology (SHELL-HOLLAND, 1987), aerospace engineering (FIKES & KEHLER, 1985). Work is underway in forestry (LOH, 1988; SAARENMAA, 1988).

6 EXPERT SYSTEMS AND IPM

In addition to the capability of AI systems to deliver powerful decision support tools to the pest management community as described above, there is yet the promise of more powerful systems to come—systems that monitor and reason about IPM systems in real time and that fulfill the vision of the first proponents of integrated pest management. But to look ahead, one must first look back to the fundamental goals of IPM in its formative years.

SMITH & REYNOLDS (1966) [from WEARING (1988)] defined IPM as "a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury". The key is the maintenance of pest populations at levels below those at which economic injury occurs. Two tasks are involved, both of which require a thorough understanding of the pest/plant interaction.

First, managers must be able to identify when injurious pest levels are likely to occur (*Problem Recognition*); and second, managers must know how to readjust the system to prevent economic damage (*Problem Solution*). Both recognition and solution require considerable expertise. Both require an understanding of the system itself. In addition, the former requires the ability to reason about the future course of the system and the risks involved. The latter requires an inventory of available tactics and knowledge of their effects under different circumstances as well as any constraints on their use. Problem solution also is dependent on a clearly stated objective such as "maximize long-term profit", or "minimize the probability of farm failure".

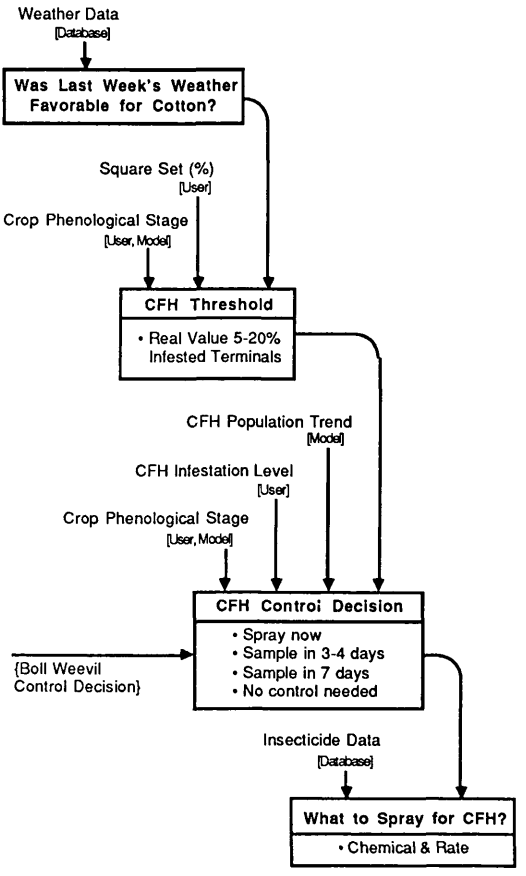


Figure 5. A functional model representation of the decision process involved in deciding how to respond to cotton fleahopper infestations in cotton. Each box represents a decision; lines represent information flow and text outside of boxes represent the sources of information required. (Modified from Stone *et al.*, 1987).

This level of complexity and interdependence of biological and economic systems has led repeatedly to the conclusion that IPM decisionmakers need systems-level tools to help them make good decisions. Since the 1960's, there have been two types of tools emphasized: tactical decision rules based on sampling, and computer simulation models. The former removes the complexity of the system by providing clear implementable rules to follow in the field. The economic threshold is the best example. The latter, simulation models, give the user some control over the complexity, and provide some level of predictability.

However, economic thresholds have been virtually impossible to estimate in many cases, especially where there are multiple pests or a long period of time in which pest populations cause economic damage. There is simply too much complexity to summarize in a simplistic decision rule. And simulation models have not yet been built with enough reliability to be truly useful at the tactical level. As GETZ & GUTIERREZ stated (1982):

> Perhaps the most important challenge that needs to be met in computer-based agroecosystem management in general, and integrated pest management in particular, is how to best utilize data and information of questionable reliability in conjunction with output from models that have not been extensively validated, in order to assist the agriculturalist in making crucial management decisions.

They saw the need for more flexible decision aids that could deal with uncertain information and with different types of knowledge. The challenge still faces us, but today the tools we need are at hand and they come from the types of systems we have been discussing.

Getz and Gutierrez also recognized the centrality of the decision maker. IPM is implemented throught decision rules. Without computers, those rules tend to be simplistic, like the economic threshold strategy. With computers and expert systems, those rules can become dynamic systems describing much more comprehensive and adaptive knowledge.

The key point is that management decisions are the heart of IPM. Making proper management decisions implicitly means considering all possible tactics in compatible ways and controlling pests only when the economic return outweighs all the associated costs. In effect, ***IPM has redefined what a proper pest management decision Is***. If we can design a computer decision aid that makes fundamentally correct decisions, it will be doing IPM.

Ultimately, making proper decisions about pest management means doing the two things required by Smith and Reynolds: monitoring a complex ecosystem to identify problems before they become economically damaging, and adjusting the system using management strategies that are compatible with overall system performance.

The integrated management of multiple agricultural pests using multiple tactics may seem to have no analog in industrial systems. However, the term "safety system" (CORCORAN *et al.*, 1981) that is in use in nuclear facility management provides such a model. A safety system is a type of fault diagnosis system that monitors changes in the power plant and intervenes when abnormal changes take place.

Fault diagnosis has been the classical application area of MBR. Recently, the merger of model-based knowledge systems and safety systems has created the term "intelligent safety system" (KLEIN & FININ, 1987). It provides a perfect formula for an IPM knowledge system. Intelligent safety systems (Fig 6) are MBR systems that include a monitoring component and which anticipate faults and recommend corrective action before the fault causes unacceptable damage. The analogy to IPM is clear. If IPM is dedicated to maintaining pest populations at levels below those causing economic damage, then an IPM practitioner must perform the same tasks as an industrial safety system: monitoring, comparing observed system behavior to what is expected, and making whatever corrections are required to prevent problems from causing unacceptable levels of damage.

Implementing IPM as an intelligent safety system is not a small task. First of all it requires very good deep models for plant growth and development, and plant/herbivore interactions. When good models are available, reasoning on them is straightforward. There are numerous techniques available by which generating diagnostic nets can be automated and variables to be monitored can be pinpointed. Validation of our present models and development of new, AI-based models for this purpose is underway. Until such models can be constructed to explicitly deal with uncertainty, however, their use will be limited. AI-based models are well suited to this goal.

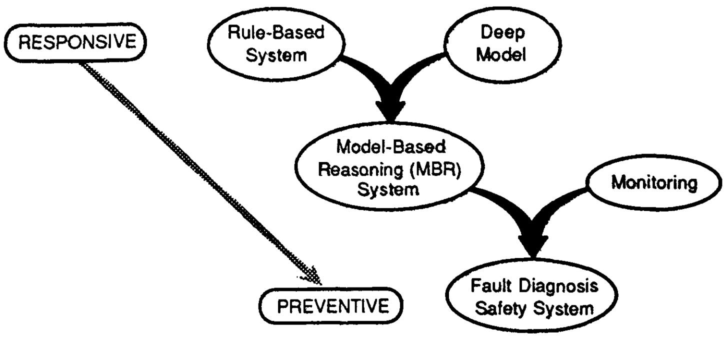


Figure 6. Relationships among and progression from rule-based expert systems to intelligent safety systems. Because rule-based systems depend on descriptions of symptoms, they are inherently responsive systems. Safety systems are preventive, monitoring their environment and seeking to correct problems before they become economically damaging.

In conclusion, a knowledge system implementation of IPM is a merger of different types of information systems. Formerly, separate models and systems have been built to solve problems in different phases of IPM decision making. We would like to reverse this trend and push toward the development of intelligent safety systems for pest management. The fundamental challenge is to build deep models that describe what is known about the dynamics of complex managed ecosystems. There is a rich basis for such an effort in the classical simulation models developed over the last fifteen to twenty years in crop and pest management. Once such models have been constructed in forms dynamically accessible to inferencing systems, it will be possible to reason about the fundamental causes of observed events in the natural system, and to suggest management tactics and strategies to guide decisionmakers, thereby bringing about the true implementation of IPM.

7 REFERENCES

BAKER, D. N., LAMBERT, J. R. & MCKINION, J. M. - 1983. GOSSYM: A simulator of cotton crop growth and yield. South Carolina Exp. Sta. Tech. Bull. No. 1089.

BLANCARD, D., BONNET, A. & COLÉNO A. - 1986. TOM: Un système expert en maladies des tomates. P. H. M. Revue Horticole **261**: 7-13.

BLONNINGEN, P. - 1988. Weed control in sugar weed cropping. pp. 481-491 in Knowledge-based systems in agriculture. Prospects for application. International DLG-Congress for Computer Technology. Frankfurt am Main—Bad Soden a.Ts., June 19-22.

CERVO, R., MARACCHI, G. & MIGLIETTA, F. - 1988. Diagnosis of pests and diseases in olives. pp. 473-480 in Knowledge-based systems in agriculture. Prospects for application. International DLG-Congress for Computer Technology. Frankfurt am Main—Bad Soden a.Ts., June 19-22.

CORCORAN, W., FINNICUM, D., HUBBARD, R. III, MUSICK, C. & WALTZER, P. - 1981. Nuclear power plant safery functions. Nuclear Safety **22**: 20-35.

COULSON, R. N., FOLSE, L. J. & LOH, D. K. - 1987. Artificial intelligence and natural resource management. Science **237**: 262-267.

DAHLSTEN, D. L. (ed.) - 1988. Eradication of Exotic Pests: An Evaluation with Selected Case Histories. Yale University Press, (in press).

DEKLEER, J. & WILLIAMS, B. C. - 1987. Diagnosing multiple faults. Artificial Intelligence **32**: 97-130.

FAUGHT, W.S. - 1986. Applications of AI in engineering. Computer **19**(7): 17-27.

FIKES, R. & KEHLER, T. - 1985. The role of frame-based representation in reasoning. Comm. ACM **28**(9): 904-920.

FOLSE, L. J., PACKARD, J. M. & GRANT, W. E. - 1988. AI modelling of animal movements in a heterogeneous habitat. Ecol. Modelling (in press).

FOX, M. S. & MCDERMOTT, J. - 1986. The role of databases in knowledge-based systems. Carnegie-Mellon University, Robotics Institute, Tech. Rep. 3, 23 pp.

FRISBIE, R. E. & ADKISSON, P. L. (eds.) - 1985 Integrated Pest Management on Major Agricultural Systems. Texas Agricultural Experiment Station MP-1616. 743 pp.

FRISBIE, R. E., STONE, N. D., RICHARDSON, J. W. & SANSONE, C. - 1987. COTFLEX: Organizing, developing, and implementing a cotton expert system for total crop management. Pages 193-194 in Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis TN.

GETZ, W. M. & GUTIERREZ, A. P. - 1982. A perspective on systems analysis in crop protection and insect pest management. Ann. Rev. Entomol. **27**: 447-466.

GRAHAM, L. A. - 1986. HAREMS: A generalized data base manager and simulator for barrier island feral horse populations. Coop. Park Studies Unit Tech. Rep. No. 32, 49 pp.

HAYES-ROTH, F. - 1985. Rule-based systems. Comm. ACM **28**(9): 921-932.

HELMS, G., RICHARDSON, J. W., KNIGHT, T. O., RISTER, M. E. & STONE, N. D. - 1987. COTFLEX: An expert system to provide cropmix and farm policy advice to producers. pp. 197-201 in Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis TN.

HOSHI, T. & KOZAI, T. - 1988. Disease and pest diagnosis for tomatoes. pp. 457-472 in Knowledge-based systems in agriculture. Prospects for application. International DLG-Congress for Computer Technology. Frankfurt am Main—Bad Soden a.Ts., June 19-22.

HUFFAKER, C. B. (ed.) - 1980. New Technology of Pest Control. John Wiley and Sons, New York. 500 pp.

INTELLICORP - 1986. Model-based reasoning in the KEE and SimKit systems. IntelliNews **2**(2), 11 pp.

INTELLIGENETICS - 1986. An overview of StrateGene. Workstation for Genetic Engineering Applications. 4 pp.

JENKINS, J. J. & LYONS, J. P. - 1988. Use of an expert system to derive pesticide groundwater contamination recommendations. Proc. AAAI/ASAE Workshop: Integration of Expert Systems with Conventional Problem Solving Techniques in Agriculture, August 10-12, San Antonio, Texas.

JONES, P. & HALDEMAN, J. - 1986. Management of a crop research facility with a microcomputer-based expert system. Trans. Am. Soc. Agric. Eng. **29**(1): 235-242.

JONES, P. JONES, J. W., EVERETT, P. A., JOHNSON, F. A. & SPRENKEL, R. K. - 1986. Development of an expert system for making insect control decisions in soybeans. pp. 77-83 in Proc. Internat. Conf. Computers in Agric. Ext. Programs, Florida Coop. Ext. Serv. & Inst. Food and Agric. Sci., Univ. Florida.

KLEIN, D. & FININ, T. - 1987. What's in a deep model? A characterization of knowledge depth in intelligent safety systems. pp. 559-562 in Proc. 10th IJCAI-87.

KOTON, P. A. - 1985. Empirical and model-based reasoning in expert systems. pp. 297-299 in Proc. 9th IJCAI, Los Angeles.

LE RENARD, J. - 1988. SEVP—Diagnosis in plant protection for 17 types of crop. pp. 422-456 in Knowledge-based systems in agriculture. Prospects for application. International DLG-Congress for Computer Technology. Frankfurt am Main—Bad Soden a.Ts., June 19-22.

LEMMON, H. E. - 1985. COMAX: an expert system for cotton crop management. Science **233**: 29-33.

LOEHLE, C. - 1987. Applying artificial intelligence techniques to ecological modelling. Ecol. Modelling **38**: 191-212.

LOGAN, J. A. - 1988. Toward an expert system for development of pest simulation models. Environ. Entomol. **17**(2): 359-376.

LOH, D.K. - 1988. Integrated natural resource automation—the knowledge shop. Resource Technology 88. International Symposium on Advanced Technology in Natural Resource Management. Fort Collins, Colorado, June 19-23, 1988. (in press).

MAKELA, M. E., STONE, N. D. & VINSON, S. B. - 1988. Host-parasitoid population dynamics in a heterogeneous environment. pp. 228-233 in Proc. SCS Multiconference on AI and Simulation: The diversity of applications. Soc. Comp. Simul. Internat., San Diego, Calif.

MARAN, L. & PIKE, D. R. - 1988. WEAS—Plant protection advisory system. pp. 82-97 in Knowledge-based systems in agriculture. Prospects for application. International DLG-Congress for Computer Technology. Frankfurt am Main—Bad Soden a.Ts., June 19-22.

MCKINNION, J. M. - 1988. Farm application of GOSSYM/COMAX. Proc. AAAI/ASAE Workshop: Integration of Expert Systems with Conventional Problem Solving Techniques in Agriculture, August 10-12, San Antonio, Texas.

MICHALSKI, R. S., DAVIS, J. H., BISHT, V. S., SINCLAIR, J. B. - 1983. A computer-based advisory system for diagnosing sybean diseases in Illinois. Plant Disease **4**: 459-463.

PASCOE, G. A. - 1986. Elements of object-oriented programming. Byte **11**(8): 139-144.

PHILLIPS, J. R., GUTIERREZ, A. P. & ADKISSON, P. L. - 1980. General accomplishments toward better insect control in cotton. pp. 124-153 in HUFFAKER, C. B. (ed.), New Technology of Pest Control. John Wiley and Sons, New York.

PLANT, R. E., WILSON, L. T., ZELINSKI, L., GOODELL, P. B. & KERBY, T. A. - 1987. CALEX/Cotton: An expert system management aid for California cotton growers. pp. 203-206 in Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis TN.

ROBINSON, V. B., FRANK, A. U. & H. A. KARIMI. - 1987. Expert systems for geographic information systems in resource managment. AI Applic. in Nat. Res. Mgmt. **1**(1): 47-57.

SAARENMAA, H. - 1988. Model-based reasoning in ecology and natural resource management. Resource Technology '88. International Symposium on Advanced Technology in Natural Resource Management. Fort Collins, Colorado, June 19-23, 1988. 11 pp. (in press).

SAARENMAA, H., STONE, N.D., FOLSE, L.J., PACKARD, J.M., GRANT, W.E., MAKELA, M.E. & COULSON, R.N. - 1988. An artificial intelligence approach to simulating animal/habitat interactions. Ecological Modelling (in press).

SAUNDERS, M. C., HAESELER, C. W., TRAVIS, J. W., MILLER, B. J., COULSON, R. N., LOH, K. D. & STONE, N. D. - 1987. GRAPES: an expert system for viticulture in Pennsylvania. AI Applic. in Nat. Res. Mgmt. **1**(2): 13-20.

SHELL-HOLLAND. - 1987. Geological modelling workbench. Carnegie Group, 1 p.

SMITH, R. F. & REYNOLDS, H. T. - 1966. Principles, definitions and scope of integrated control measures in Baden-Württemberg, West Germany. Z. Angew. Entomol. **77**: 398-401.

STEFIK, M. & BOBROW, D. G. - 1986. Object-oriented programming: themes and variations. AI Magazine **6**: 40-62.

STONE, N. D. - 1987. Artificial intelligence approaches to systems modelling. Am. Soc. Agric. Engin. Paper No. 87-1083, 21 pp.

STONE, N. D. & TOMAN, T. W. - 1988. COTFLEX: An integrated expert and database system for decision support in Texas cotton production. Proc. AAAI/ASAE Workshop: Integration of Expert Systems with Conventional Problem Solving Techniques in Agriculture, August 10-12, San Antonio, Texas.

STONE, N. D., COULSON, R. N., FRISBIE, R. E. & LOH, D. K. - 1986a. Expert systems in entomology: three approaches to problem solving. Bull. Entomol. Soc. Am. **32**(3): 161-166.

STONE, N. D., FRISBIE, R. E., RICHARDSON, J. W. & COULSON, R. N. - 1986b. Integrated expert system applications for agriculture. pp. 836-841 in Proc. Internat. Conf. Computers in Agric. Ext. Programs. Florida Coop. Ext. Serv. & Inst. Food and Agric. Sci., Univ. Florida.

STONE, N. D., FRISBIE, R. E., RICHARDSON, J. W. & SANSONE, C. - 1987. COTFLEX: A modular expert system that synthesizes biological and economic analyses: the pest management advisor as an example. Pages 194-197 in Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis TN.

TOMAN, T. W. - 1988. An intelligent geographic information system for the landscape-level simulation of natural systems. Senior Thesis, Honors Program in Computer Science, Texas A&M University, 47 pp.

UHRIG, J. W., THIEME, R. H. & PEART, R. M. - 1987. Grain marketing alternative selection: an expert system approach. pp. 830-835 in Proc. Internat. Conf. Computers in Agric. Ext. Programs. Florida Coop. Ext. Serv. & Inst. Food and Agric. Sci., Univ. Florida.

VAN MELLE, W. - 1985. The structure of the MYCIN system. pp. 67-77 in B. G. Buchanan and E. H. Shortliffe (eds.) Rule-Based Expert Systems: The MYCIN experiments of the Stanford Heuristic Programming Project, Addison-Wesley Publishing Company, Menlo Park.

WEARING, C. H. - 1988. Evaluating the IPM implementation process. Ann. Rev. Entomol. **33**:17-38.

WHITEHEAD, J. D. & ROACH, J. W. - 1987. HOIST: A second generation expert system based on qualitative physics. Grand Prize Winner, Texas Instruments Paper Contest: AI Industrial Automation. Awarded at AAAI-87, Seattle, Washington.

WOOLLEY, J. B. & STONE, N. D. - 1987. Application of artificial intelligence to systematics: SYSTEX—A prototype expert system for species identification. Syst. Zool. **36**(3): 248-267.

YU, V. L., FAGAN, L. M., BENNETT, S. W., CLANCEY, W. J., SCOTT, A. C., HANNIGAN, J. F., BLUM, R. L., BUCHANAN, B. G. & COHEN, S. N. - 1985. An evaluation of MYCIN's advice. pp. 589-596 in B. G. Buchanan and E. H. Shortliffe (eds.) Rule-Based Expert Systems: The MYCIN experiments of the Stanford Heuristic Programming Project, Addison-Wesley Publishing Company, Menlo Park.

APPROACHES TO MODELLING THE SPATIAL DYNAMICS OF PESTS AND DISEASES

W. Van Der Werf, W. A. H. Rossing & R. Rabbinge
Department of Theoretical Production Ecology, Agricultural University-Wageningen, Netherlands

M. D. De Jong
Centre for Agrobiological Research-Wageningen, Netherlands

P. J. M. Mols
Department of Entomology, Agricultural University-Wageningen, Netherlands

SUMMARY

Spatial distribution patterns and dispersal process play an important role in the population dynamics of pests and the epidemiology of diseases. To account for this, different approaches can be taken. Three possible approaches are illustrated in this paper by means of case-studies. The first example concerns the flight of fungal spores in a forest. The problem is treated with diffusion and flux equations which describe the behaviour of spore masses but not that of individual spores. In the second example, predation and egg production by carabid beetles is simulated on the basis of a motivational and behavioural model which explains walking paths and attack rates in terms of the beetle's gut satiation. In the third example, the processes underlying the spread of viruses in sugarbeet are studied, using a simulation model which describes the walking, feeding and virus transmission activities of individual aphids as well as the progression of disease in individual plants. The degree of complexity simulated and the concomitant computing time increases from the first example to the third. Each of the approaches presented here has its merits and shortcomings. The choice of the approach depends upon the aim of the study and the available experimental data for model construction and validation.

1. INTRODUCTION

Biological populations vary in density over their habitat. This spatial heterogeneity results first of all from spatially variable abiotic conditions such as soil type, microclimate and availability of nutrients. Furthermore, individuals aggregate to find mates or protection. Limited dispersal of offspring results in aggregation as well. Finally, interactions with spatially distributed competitors, predators or food adds to spatial variability. Spatial distributions and spatial dynamics need to be studied and simulated to obtain a good insight into population density and its fluctuations in time and space.

The most appropriate approach to accounting for spatial heterogeneity depends on the purpose of the study. Statistical methods are adequate when determining minimum sample sizes for the estimation of disease severity or pest attack (DAAMEN, 1986a,b; WARD *et al.*,1985a,b, 1986). This approach deals with **dispersion**, i.e. the pattern of distribution of organisms over patches (SOUTHWOOD, 1978), but not with the process that leads to it, **dispersal**. Methods to describe dispersion have been adequately treated by SOUTHWOOD (1978) and by UPTON and FINGLETON (1985) while RABBINGE *et al.* (1989) describe methods to account for the effects of spatial variability in simulation models.

This paper will concentrate on the dynamic simulation of dispersal. In studies of temporal dynamics of pests and diseases insight into spatial dynamics may be crucial to explain processes on the population level and to allow the construction of realistic models (KAREIVA and ODELL, 1987). Furthermore, in a variety of systems, spatial dynamics *per se* is the topic of the study, e.g. in the case of the spread of diseases in crops.

Three examples will be given of explanatory dynamic simulation models which incorporate a detailed treatment of spatial dynamics to explain system behaviour in time and space. The first example - distribution and flight of fungal spores inside a forest - applies diffusion and flux equations without making reference to the behaviour of individual spores. In the second example - predation by carabid beetles - detailed observations on behaviour of individuals are integrated in a motivational and behavioral model as part of a population model to calculate predation and beetle population dynamics. In the last example, we describe a first attempt to model the spread of viruses in a way that accounts for the walking and feeding behaviour of the vector.

2. MODELLING AERIAL SPORE TRANSPORT OF SILVERLEAF FUNGUS, *Chondrostereum purpureum*

2.1 Introduction

'American' black cherry, *Prunus serotina*, was introduced into the Netherlands between 1920 and 1950 to improve the understory of coniferous forests on poor sandy soils. However the species became a serious competitor for native tree species and hampered forest regeneration. Cutting blackcherry trees and inoculating the stubs with silverleaf fungus, *Chondrostereum purpureum*, was considered as a possible means of biocontrol. Before this could be undertaken, the possible

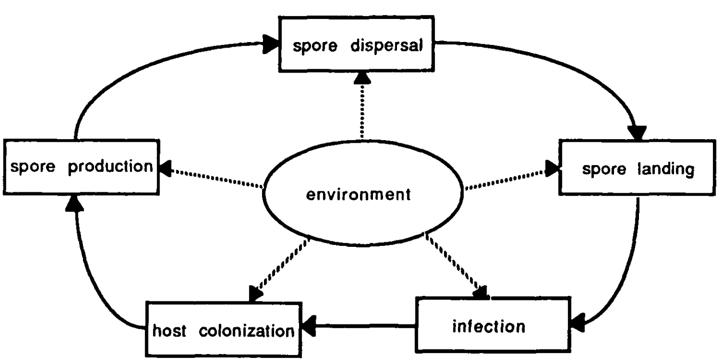


Fig. 1: Schematic presentation of life cycle of *Chondrostereum purpureum*.

escape of spores of the fungus to susceptible *Prunus* species outside forests, e.g. ornamental trees and fruit trees, had to be quantified (WAGENMAKERS, 1984; DE JONG and SCHEEPENS, 1985; DE JONG, 1988; DE JONG *et al.*,1988). To accomplish this, the life cycle of the fungus was divided into five main phases (Fig. 1). The processes in each phase were quantified. Here the approach adopted to quantify spore dispersal is outlined.

DE JONG (1988) described the dispersal of spores outside the forest with the Gaussian plume model while he calculated the place and extent of spore emission from the forest by a quantitative evaluation of the relevant spore exchange mechanisms, based on crop micrometeorological studies of GOUDRIAAN (1977, 1979). Spores can be emitted from the forest vertically by turbulent diffusion and horizontally with wind currents (mass flow) downwind of the forest and diffusion. The importance of horizontal emission decreases with forest size. Vertical and horizontal spore emission are functions of forest size and weather, notably wind speed and temperature profile. DE JONG used a multi-layer model of the forest for his calculations. Here, the essential elements of the model are illustrated by means of a one-layer model.

2.2 One-layer model.

As a first approximation the forest is treated as a single vegetation layer. It is assumed that the spore concentration is the same over the whole forest area. The spore concentration in the forest is the result of seven exchange processes with the environment (Fig. 2). Along the vertical axis the density of spores (spores m^{-2} forest area) changes due to (1) turbulent exchange with the atmosphere, (2) sedimentation from the atmosphere, (3) sporulation by *C. purpureum* basidiocarps, (4) sedimentation on the ground and (5) deposition on branches and needles. In the horizontal plane spores are transported due to (6) air mass flow (wind) and (7) turbulent diffusion.

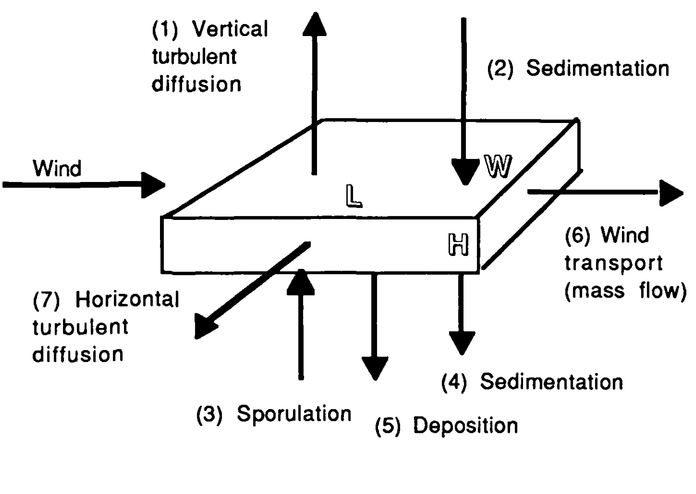


Fig. 2: Box representation of one-layer approach to modelling spore emission from a forest. H: height; L: length and W: width of forest.

Accounting for these seven processes the net rate of change of spore density (spores m^{-2} s^{-1}) in the forest can be formulated as:

$$H * C_i' = - R_{turb} + R_{sedin} - R_{sedout} + R_{spor} - R_{dep} - R_{wind} - R_{dif} \qquad (1)$$

where H is the height of the trees, C_i the spore concentration in the forest and C_i' the net rate of change of C_i.

R_{turb} is the rate of vertical turbulent spore exchange between the forest and the atmosphere,
R_{sedin} is the rate of spore sedimentation into the forest from the atmosphere,
R_{sedout} is the rate of spore loss from the forest by sedimentation on the ground,
R_{spor} is the rate of spore production by *C. purpureum* basidiocarps
R_{dep} is the rate of spore loss from the forest by deposition on needles, branches and soil,
R_{wind} is the rate of sidewards spore emission due to mass transport by the wind, and
R_{dif} is the rate of sidewards spore emission due to turbulent diffusion.

The rate of vertical turbulent spore exchange between the forest and the atmosphere depends on the difference in spore concentration as driving force and the exchange resistance r (with dimension s m^{-1}):

$$R_{turb} = (C_a - C_i)/r \qquad (2)$$

where C_a is the spore concentration in the atmosphere.
The rate of spore sedimentation is proportional to spore concentration and the sedimentation velocity (m s^{-1}).

$$R_{sedin} = V_{sed} * C_a \qquad (3)$$
$$R_{sedout} = V_{sed} * C_i \qquad (4)$$

R_{spor} is a function of temperature and basidiocarp area, derived from data of GROSCLAUDE (1969).

The rate of spore deposition in the forest depends on wind speed, u (m s^{-1}), the deposition efficiency of needles, branches, etc., e_{dep} (-), the area of branches, needles, etc. with respect to ground area, A (m^2 m^{-2}) and the spore concentration, C_i:

$$R_{dep} = u * e_{dep} * A * C_i \qquad (5)$$

The rate of horizontal mass transport of spores is

$$R_{wind} = H * C_i * u/L \tag{6}$$

in which L is the length of the forest. The rate of horizontal diffusion equals

$$R_{dif} = C_i/r_h \tag{7}$$

where r_h is the resistance to horizontal diffusion, which increases linearly with the size of the forest.

Typical values for the parameters in these equation are: R_{spor} = 20 spores m^{-2} s^{-1}, C_a = 100 spores m^{-3}, r = 20 s m^{-1}, V_{sed} = 1 mm s^{-1}, u = 2 m s^{-1}, e_{dep} = 0.01, A = 2 m^2 m^{-2} and r_h = 1000 s m^{-1}.

Now equation 1 can be written explicitly

$$H * C_i' = R_{spor} + Ca/r + v_{sed} * C_a - (1/r + V_{sed} + u * e_{dep} * A + u * H/L + 1/r_h) * C_i \tag{8}$$

or, more briefly

$$H * C_i' = b - a * C_i \tag{9}$$

Here b denotes rates independent of C_i while a denotes rates that vary with C_i. At equilibrium , when C_i'=0, C_i equals b/a:

$$C_i = (Rprod + Ca/r + V_{sed} * C_a)/(1/r + V_{sed} + u * e_{dep} * L + u * H/L + 1/r_h) \tag{10}$$

$$C_i = (20 + 100/20 + .001 * 100)/(1/20 + 0.001 + 2 * 0.01 * 2 + 2 * 17/250 + 1/1000)$$

$$C_i = 25.1/0.228 = 110 \text{ spores } m^{-3}$$

For this steady state the contribution of the seven transport processes to spore emission from the forest can be calculated with Eqs. 2 - 7. Some examples of results from the one-layer model are given in Fig. 3.

This simple model gives a reasonable first impression of the importance of the different processes in spore dispersal. Apparently, under the conditions chosen, sporulation, turbulent vertical transport, horizontal mass flow and deposition are the only processes of practical importance. At very low wind speeds sedimentation may be also important.

The assumption of a homogeneous spore density over the whole forest area is not justified. When air without spores is blown into the forest, C_i will be very low at the upwind side while the calculated value $C_{i,eq}$ is reached at some distance in the forest. How large is this distance? This question could be answered by horizontal compartmentalization of the forest.

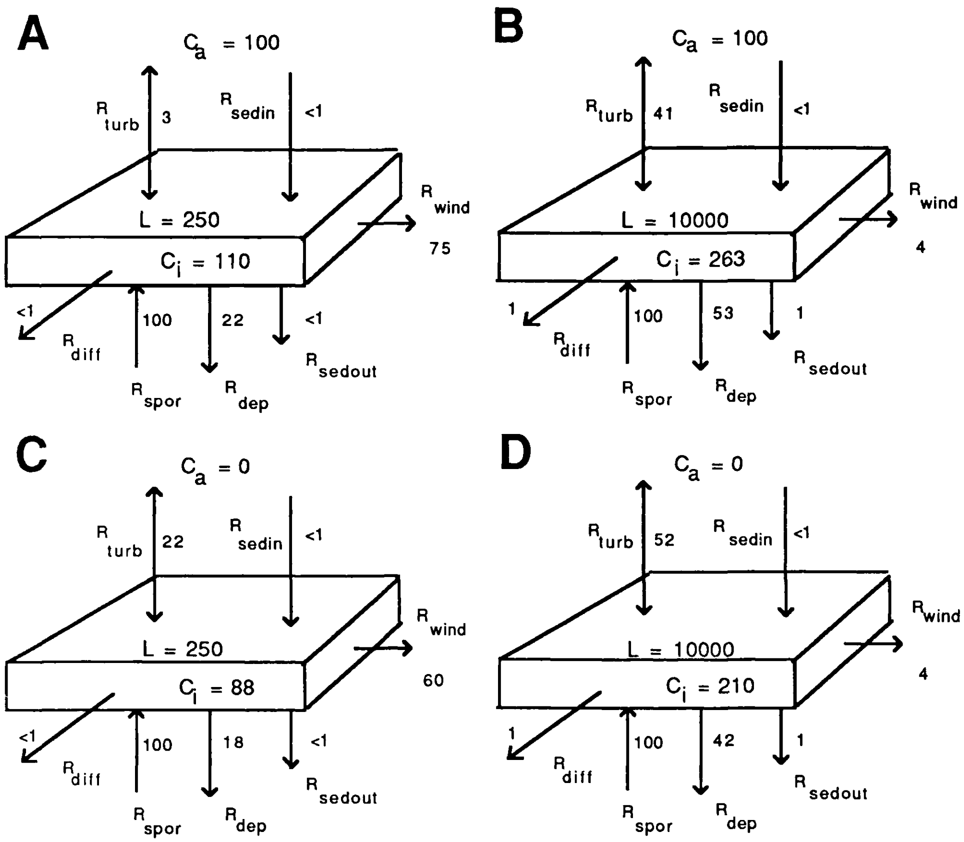


Fig. 3: Spore emission from a forest calculated with the one-layer model for two forest sizes and two atmospheric spore concentrations. A: L = 250 m and C_a = 100 spores m^{-3}; B: L = 10000 m and C_a = 100 spores m^{-3}; C: L = 250 m and C_a = 0; D: L = 10000 m and C_a = 0. In- and effluxes are expressed in %.

However, a rough estimate may be obtained in a simpler way. According to Eq. 1, the partial derivative of C_i with respect to time is:

$$\delta C_i/\delta t = \{ R_{spor} + Ca/r + V_{sed} * C_a - (1/r + V_{sed} + u * e_{dep} * A + u * H/L + 1/r_h) * C_i\}/H \qquad (11)$$

The air traverses the forest at a rate dx/dt = u where x denotes distance along the wind axis with x = 0 at the upwind side of the forest. Therefore δt equals $\delta x/u$, so that

$$\delta C_i/\delta x = \{ R_{spor} + C_a/r + V_{sed} * C_a - (1/r + V_{sed} + u * e_{dep} * A + u * H/L + 1/r_h) * C_i\}/(H * u) \quad (12)$$

or shorter:

$$\delta C_i/\delta x = (b - aC_i)/(H * u) \quad (13)$$

Equation 13 describes an exponential convergence of C_i to $C_{i,eq}$ along the wind axis. The equilibrium concentration is closely approximated when x equals 3 times H * u/a, i.e. in the example given in Fig. 3A (L = 250 m and C_a = 100 spores m^{-3}) at: x = 3 * 17 * 2/0.228 = 447 m

Apparently the equilibrium concentration is not reached in this small forest. The error which results from this mistake is small because C_a and $C_{i,eq}$ differ little. For the large forest of Fig. 3B the assumption that air leaving the forest at the lee edge contains spores at the equilibrium concentration is fulfilled (x << L). However, in this situation the assumption implicitly made in Eq. 5 that spore emission through mass flow affects the spore concentration in the whole forest is not fulfilled. Again this problem could be solved by horizontal compartmentalization. Nevertheless, the one-layer approach outlined here allows a useful first approximation of spore emission under different circumstances. Spore concentrations and fluxes can be easily obtained with a pocket calculator. However, for a deeper insight into the spore dispersal processes in a forest a multi-layer approach is necessary.

2.3 Four-layer model

A more realistic representation of the system is obtained by distinguishing three vertical forest layers: (1) a spore production layer near the ground, (2) a stem layer and (3) a crown layer in which spores may be deposited on needles and branches (Fig. 4). Above the forest, distinction is made between a thick upper atmosphere layer without spores and a thinner boundary layer, just above the forest, in which air movement is slowed down by drag exerted by the canopy. The free atmosphere does not take part in the spore exchange processes and is excluded from the model equations. The resulting four-layer model is essentially the same as the one-layer model described above. The only difference is that rates are now calculated for four layers in stead of one, resulting in a matrix formulation of the problem (WAGENMAKERS, 1984; DE JONG, 1988).

With the matrix model the distribution of spores at equilibrium can be calculated while the equilibrium concentrations can be used to calculate the horizontal and vertical emission rates. However, the calculation of an equilibrium state of the system is only valid when the circumstances remain constant long enough to establish equilibrium. This will rarely be the case because wind speed and direction, the main driving forces in this system, fluctuate in time and space. Therefore, a dynamic simulation approach would be appropriate. In a dynamic simulation model horizontal dimensions could be added.

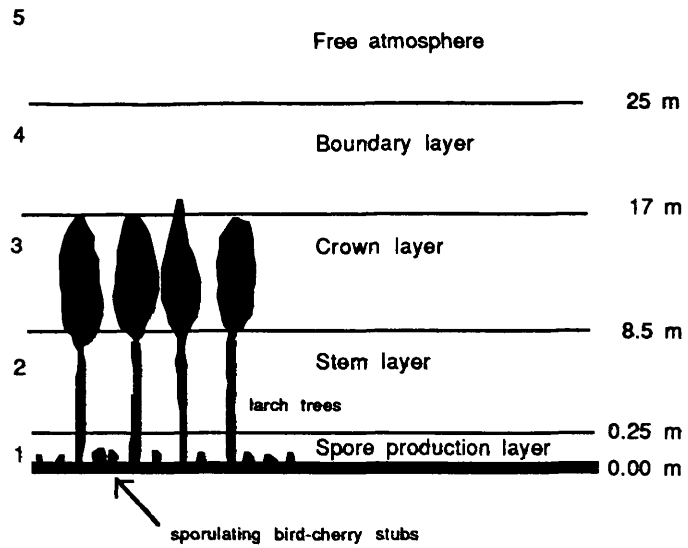


Fig. 4: Schematic representation of aerial layers in a forest in which *Prunus serotina* is controlled with *Chondrostereum purpureum*

The four-layer model, as described, enables an analysis of risks associated with biocontrol of black cherry. The transport of airborne material within forest or crop canopies plays a vital role in disease epidemiology and constitutes a factor of importance in many other fields, e.g. spraying techniques for pesticides and transport of pollen or air pollutants. Moreover, calculation of the spread in the environment of genetically engineered organisms, e.g. viruses (CORY and ENTWISTLE, 1988; HUBER, 1988), has become an important issue recently. Simulation and systems analysis may help to quantify risks and gain insight into the processes involved.

3. MODELLING DISPERSAL OF THE CARABID BEETLE *Pterostichus coerulescens* AND ITS CONSEQUENCES FOR PREDATION

3.1 Introduction

In many cases food of predators, as of other animals, is not distributed randomly throughout the habitat but shows a more or less aggregated pattern (SOUTHWOOD, 1978; UPTON and FINGLETON, 1985). As many predators actively search for food, the pattern of movement in relation to the density and distribution of the prey greatly affects the rate of feeding and as a consequence the rate of reproduction and the spatial and temporal dynamics of the population in the field.

The predation strategy of the carabid beetle *Pterostichus coerulescens* L. (=*Poecilus versicolor* Sturm) has been studied in relation to prey availability, to analyse its effect on egg production and survival of the species (MOLS, 1979, 1983, 1986, 1987 and in prep.). The beetle lives in heathland

and poor grassland where it hunts for aphids, caterpillars and maggots. The internal factors governing the beetle's behaviour are analyzed and related to components of behaviour. The results are integrated in a simulation model to predict predation, consumption and egg production under different sets of external conditions such as prey density, prey distribution and temperature.

3.2 Modelling motivation and predatory behaviour of *Pterostichus coerulescens*

In many species behaviour is governed by some internal 'motivational drive' which is related to gut content (HOLLING, 1966; FRANSZ, 1974; RABBINGE, 1976; SABELIS, 1981; KAREIVA and ODELL, 1987). For *P. coerulescens* the satiation level of the gut, defined as the actual gut content divided by the potential gut content or gut capacity, is a useful measure for the 'motivation' of the beetle (MOLS, 1987). The potential gut content can not exceed a physical maximum but its actual size depends on the volume of other organs and tissues. The actual gut content changes by ingestion, excretion and resorption. The rates of change are predominantly affected by ambient temperature and day length, the latter determining the pre-oviposition period. Thus the physiological drive for behaviour, i.e. the satiation level or its complement, hunger, results from various internal states which in their turn are affected by the amount of prey ingested and climatic conditions. A simulation model is developed in which the various relations are quantitatively introduced.

3.2.1 Methods

The motivational model

In the relational diagram of Fig. 5 the state variables describing feeding and egg production of the carabid beetle are represented. The amount of food ingested depends on the predator's hunger. After digestion in the gut, part of the food is excreted as faeces, the remainder being taken up in the haemolymph. The assimilated food is used for maintenance processes, converted into egg material in the ovaries or stored as fat. From the ovaries full-grown eggs move into the oviduct from where oviposition takes place

The five state variables defining the internal state of the predator (gut content, weight of haemolymph, ovaries, eggs in the oviduct and reserves) change with rates depending on internal and external conditions. The ovaries start to grow as soon as a critical day length of 14 h and an average daily temperature of 10 ^{o}C are exceeded. After a pre-oviposition period eggs are laid during a period of one to two months, depending on temperature. After this period the ovaries regress while most assimilated food is stored in reserves to be used during diapause. During the breeding season the ovaries monopolize the assimilated food. The concomitant increase in volume may limit full expansion of the gut, thus causing a reduction of the potential gut content during the oviposition period. Temperature is the most important external condition affecting rates of change of the internal state.

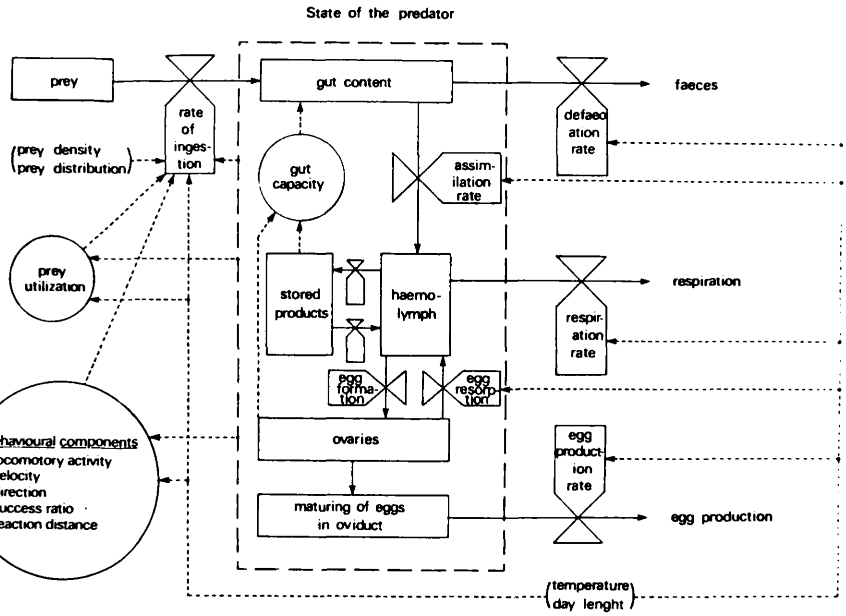


Fig. 5: Relational diagram of the motivational model of *P. coerulescens* and the behavioural components. Rectangles represent states, valves rates and circles auxiliary variables following the conventions of FORRESTER (1961). Solid lines indicate flow of matter, dotted lines flow of information.

The motivational model is verified by comparing the simulated egg production with actual data of experiments in which beetles are kept in small containers with abundant food supply to exclude aspects of searching behaviour. Model results agree with the observed egg production (not shown here; MOLS, in prep.).

Modelling predatory behaviour of *P. coerulescens*

MOLS (1986) analyzed walking behaviour of *P. coerulescens* with video equipment. Three types of walking behaviour can be distinguished based on average linear displacement and walking velocity (Fig. 6):

a. Straight walk. This type of walk is associated with a relatively high walking velocity (3 to 5 cm s^{-1}). It occurs if hunger exceeds 95%.
b. Random walk. The walking velocity is between 2 and 3 cm s^{-1}. This type of walk occurs when the satiation level is below 95% and results in a winding walking path.
c. Tortuous walk. The walking velocity is low (1 cm s^{-1}) and the walking path is very winding. This type of walk is found just after consumption of a prey. The duration of tortuous walk is a function of the satiation level (Fig. 7). At satiation levels of less than 10% this behaviour lasts about 11 minutes, while at satiation levels between 70 and 80% the duration is only 2 minutes.

A more detailed description of movement of the beetle, necessary for simulation of prey searching behaviour, is obtained by measuring the velocity and direction of walking during successive short periods of time, 'time steps'. The velocity of walking as a function of satiation level is shown in Fig. 8,

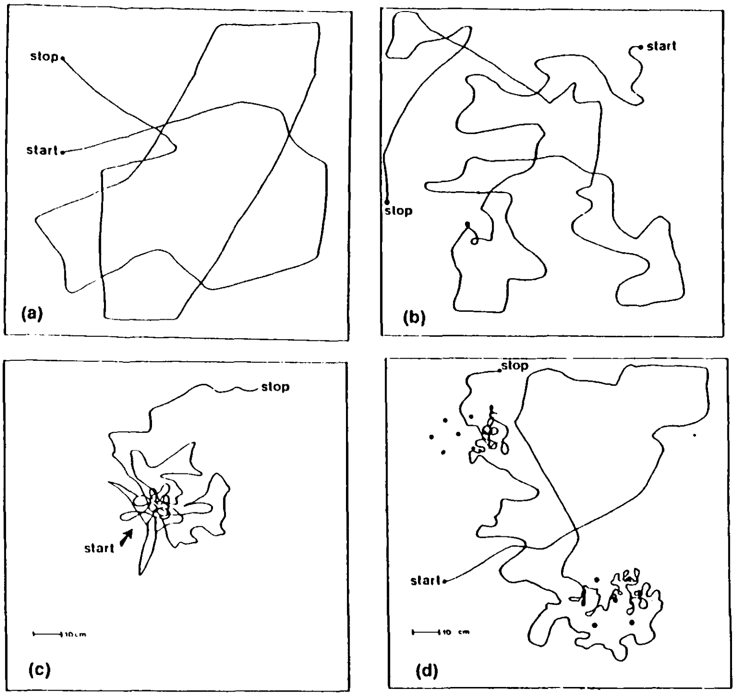


Fig. 6: Walking patterns found in *P. coerulescens:* (a) straight walk, (b) random walk, (c) tortuous walk and (d) combination of straight, random and tortuous walk in an area with prey (indicated by dots).

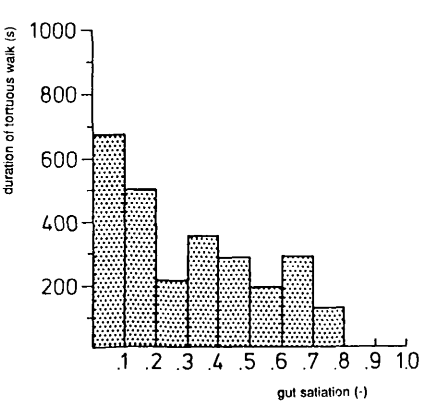


Fig. 7. Duration of tortuous walk behaviour of *P. coerulescens* following consumption of prey, as a function of the satiation level.

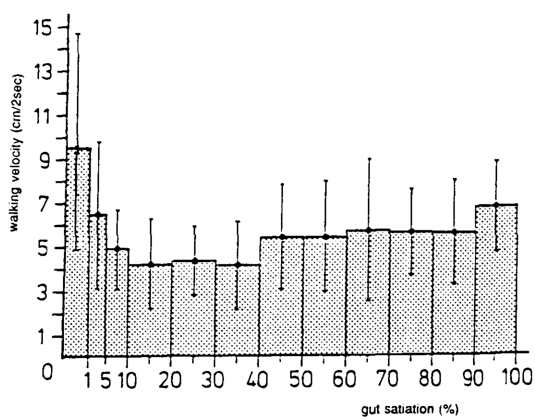


Fig. 8. Relation between the gut satiation and walking velocity of *P. coerulescens* for 'straight walk' and 'random walk'. Bars represent standard deviations.

not considering tortuous walk. Below a satiation level of 5%, walking velocity increases rapidly. The relative direction of walking is measured as the angular deviation from the direction in the previous time step. If a small time step is chosen, autocorrelation exists between subsequent angular deviations, which complicates the analysis unnecessarily. A large time step yields insufficient resolution. By iterative calculations it was found that a time step of 2 seconds is most suitable, yielding satisfactory walking paths without autocorrelation between successive angular deviations.

Using this time step, frequency distributions of angular deviation are constructed from the video recordings for beetles with various gut satiation levels. The frequency distributions are used to fit the parameters of the Tukey distribution, a symmetric statistical distribution characterized by three parameters (Fig. 9).

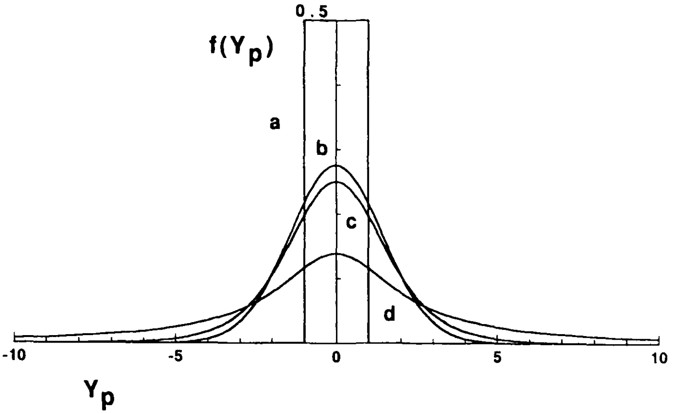


Fig. 9: Tukey probability distribution for four values of the kurtosis-parameter λ. a: $\lambda = 1$ (uniform); b: $\lambda = 0.14$ (normal); c: $\lambda = 0$ (logistic) and d: $\lambda = -0.85$ (Student's t_1 or Cauchy). The Tukey distribution is defined by:

$A = \mu + \sigma . Y_p$ where

$$Y_p = \begin{cases} (p^\lambda - (1-p)^\lambda)/\lambda & \text{when } \lambda \neq 0 \\ \ln\left(\frac{p}{1-p}\right) & \text{when } \lambda = 0 \end{cases}$$

A is the angular deviation (in radians), μ is the mean change in direction, σ is a scale parameter which determines the variance of A together with λ, while the kurtosis is determined by λ. The variable p is drawn from a standard uniform distribution.

Further analysis (MOLS, 1987) shows that the parameters determining the shape of the distribution can be predicted from the walking velocity of the beetle, low velocities coinciding with a wider range of angular deviations:

$\sigma = EXP(-0.173 * V + 0.208)$ $r^2=0.69$

$\lambda = -0.0661 * V + 0.2$ $r^2=0.49$

where V is the linear displacement (cm) per time step of 2 s (see Fig. 9 for further explanation).

Thus, in the model, the motivational state of the predator determines the velocity of walking which in turn determines the concomitant probability distribution of angular deviations. By drawing from the distribution, the potential dispersal during one time step can be calculated. The actual dispersal depends on the activity of the beetle, i.e. the fraction of time the predator is walking, is negatively correlated with the satiation level.

Given the distribution of prey, predation occurs if the distance between the predator and the nearest prey is less than the reaction distance, the largest distance at which a prey is recognized. Experiments by MOLS (in prep.) show that the percentage beetles reacting to prey decreases with increasing distance to the prey and that the reaction is hardly affected by the level of gut satiation.

Not all encounters with prey result in prey consumption. The success ratio, defined as the proportion of encounters resulting in prey consumption, depends on the satiation level of the predator because a hungry predator is more eager to attack a prey and will continue the attack longer.

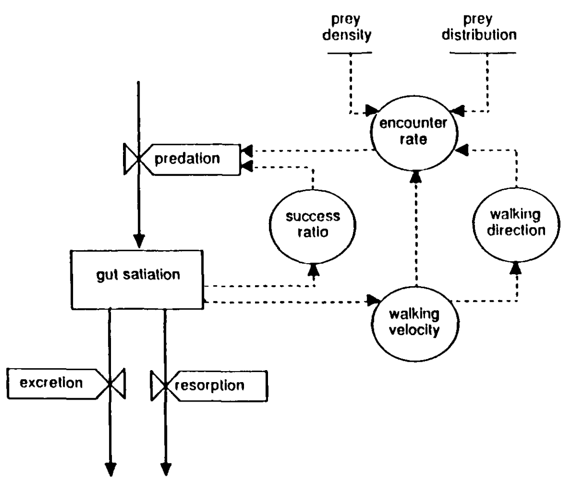


Fig. 10: Diagram illustrating relations between the motivational model (represented by the state variable gut satiation) and the behavioural components. For explanation see text.

These components of behaviour are integrated in a computer model which is linked to the model describing the motivation of the predator (Fig. 10). Prey is offered in various distributions. For *P.*

coerulescens the most important prey items are small caterpillars, aphids and maggots (HENGEVELD, 1980), which exhibit a very low walking velocity compared to the beetle's. Therefore dispersal of the prey can be neglected in the model.

The simulation model describing the internal state of the carabid and its interaction with predatory behaviour is run for aggregated and random prey distribution. Aggregated prey occur in circular clusters, each cluster containing 20 prey of 2 mg each. The distribution of prey within the clusters is random and the clusters are distributed at random over a large area. Prey density in the clusters is not affected by possible cluster overlap. Temperature is 20 °C. Overall prey densities and cluster diameters are varied to study the effect on the beetle's egg production during a season of 51 days. Each run is repeated at least ten times with different 'seeds' for the random number generator in the behavioural model. The average model outputs represent estimates of population averages because interaction between beetles is absent due to their low density.

3.2.2 Results

Model results (Fig. 11) show that the effect of prey density on average egg production is represented by saturation type curves which resemble the Holling type 2 functional response. Aggregated prey distributions result in higher average predation and egg production than random distributions. The greatest egg production is obtained with a cluster diameter of 40 cm. The representation of these results in Fig. 12 shows that cluster diameters from 40 to 160 cm result in similar egg productions. Cluster diameters below 40 cm result in a lower average egg production due to a low rate of prey encounter. At cluster diameters above 160 cm, egg production is low as the dispersion pattern of prey approaches the random distribution. Tortuous walk after finding a prey, which leads to more frequent visitation of previously searched area, constitutes a disadvantage to the beetle when prey is distributed at random.

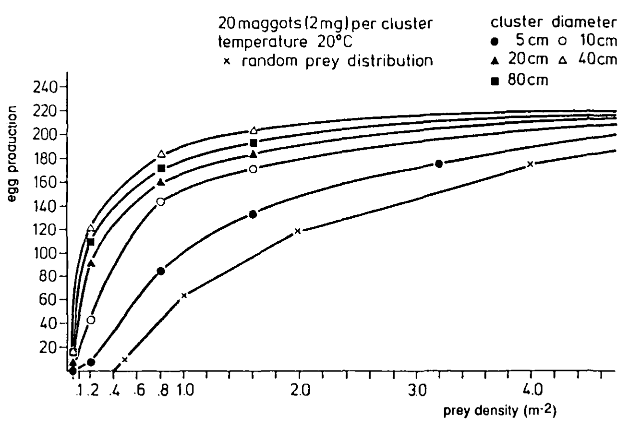


Fig. 11. Average simulated egg production of *P. coerulescens* during a 51 day period as a function of prey density for random and aggregated prey distribution. Temperature is 20 °C. Curves are eye-fitted to the simulated results; see also Fig. 12.

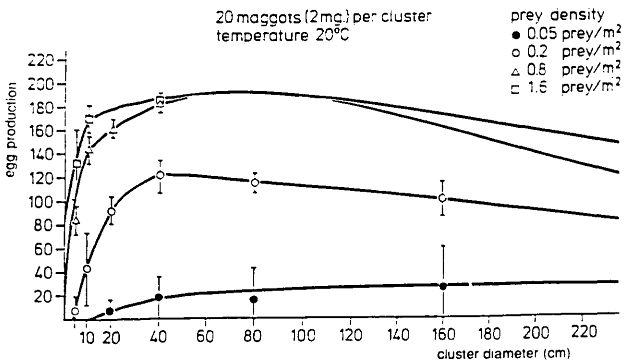


Fig. 12. Average simulated egg production of *P. coerulescens* during a 51 day period as a function of cluster diameter at various overall prey densities. Each cluster contains 20 prey of 2 mg each. Temperature is 20 °C. Each value represents the average of 10 runs with the stochastic model of walking behaviour. Bars represent standard deviations. Curves are eye-fitted to the simulated results Data pertain to the same simulation results as used in Fig. 11.

3.3 Simplification of stochastic motivation and behavioural model by means of compound simulation

The models described above can be used to analyze the effect of *P. coerulescens* on the dynamics of a pest population. Due to the stochastic nature of the model for walking behaviour and the small time-step (2 s.) this requires many simulation runs and a large amount of computer time. To avoid this, average values for predation by individual beetles, calculated with the stochastic model, could be used as input for the predator-pest model. Such an approach assumes that the model outcome resulting from average input values is identical to the average outcome with variable inputs. This is generally only true when the variation in input parameters is small or when the model is linear in its variables. In this case, however, there is a large variation in behaviour between individual beetles, especially at low prey densities (Fig. 12) while several relations (a.o. the functional response) in the model are non-linear. Thus averaging before simulation is impossible. This problem is addressed by compound simulation (FRANSZ, 1974; RABBINGE et al., 1989)

3.3.1 Methods

The population of beetles is divided into three classes with walking velocity as classifying criterion. Within a class the beetles are assumed to behave identically. To account for the aggregated distribution of prey, each class is subdivided into two, corresponding to beetles inside and outside a prey cluster. Thus individual beetles belong to one of six classes (Fig. 13).

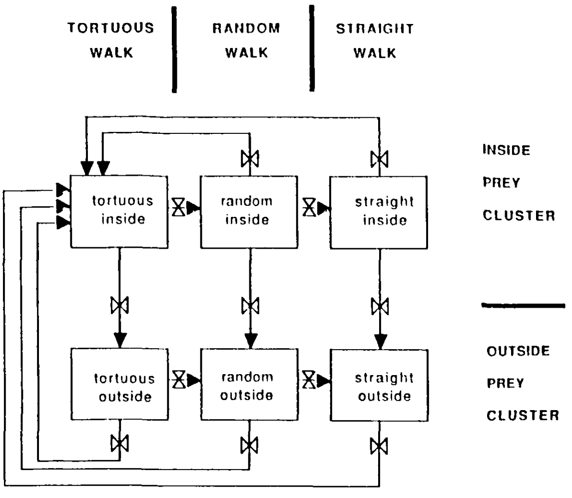


Fig. 13. Schematic representation of behavioural classes of the predator distinguished in the compound simulation model.

The complex stochastic model describing the walking behaviour of individual beetles is replaced by relations, calculated from a large number of runs with this model, which describe residence time within a prey cluster as a function of beetle walking velocity and cluster diameter (Table 1). The rate of encounters with prey clusters and prey within a cluster is approximated with analytical equations for the rate of predation on sessile prey, developed by SKELLAM (1958) and SABELIS (1981).

Table 1: Average number of steps before leaving a cluster, as a function of cluster radius and predator walking velocity, computed with the stochastic model for walking behaviour of *Pterostichus coerulescens.*

cluster	----------------Predator velocity (cm s^{-1})---------------					
radius (m)	0.25	0.50	1	2	4	8
0.1	245	59	16	5	2	1
0.2	919	209	53	12	4	2
0.4	3335	886	189	40	9	3
0.8		3176	718	141	26	7
1.6			2635	524	88	19
3.2				2021	307	52
6.4					1144	163

3.3.2 Results

To check the validity of the simplifications, the compound simulation model is run at prey densities varying between one and 100 individuals per m^2. Prey, weighing 0.7 to 2 mg, is distributed at Each time step, the simulation model is run for each class of beetles separately and beetle numbers in each class are adjusted according to the outcome. Values for output variables of interest are obtained by averaging the outputs of the classes, weighed by the respective number of beetles. In this way the stochastic model of the individuals' walking behaviour is replaced by a deterministic model in which the motivational state of all beetles in a class changes by the same average rate.
random. Temperature is 20 ^{o}C yielding an egg production period of 51 days. Competition between carabids is assumed absent. Egg production per carabid is found to be in close agreement with the results of the stochastic model for predation.

Next, the effect of dividing the beetle population into 6 classes by two criteria is evaluated for an overall prey density of 5 m^{-2}, each prey weighing 2 mg. Once per day, the initial prey density is restored. In Fig. 14 the fraction of total simulation time spent to 'random walk' or 'tortuous walk' is shown. 'Straight walk' is not represented in the figure as this type of behaviour does not occur under the simulation conditions. Although tortuous walk occurs after each prey consumption, it is maintained during 11 minutes at most. Overall, tortuous walk is found during less than 5% of the time and mainly within prey clusters. The beetles predominantly exhibit random walk. As expected, the time spent in clusters increases with cluster size and density.

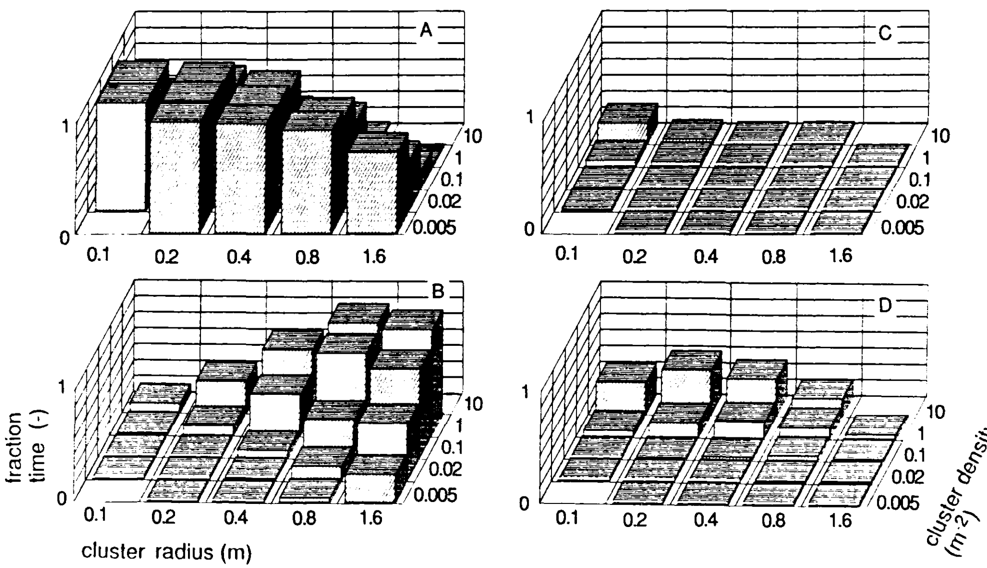


Fig. 14: Fraction of time an individual carabid spends in each of the classes of the compound simulation model as a function of cluster radius (m) and cluster density (m^{-2}) at an initial prey density of 5 m^{-2}, each prey weighing 2 mg. The initial prey density is restored at the onset of each simulated day. The graphs shown pertain to (A) random walk outside a cluster; (B) random walk inside a cluster; (C) tortuous walk outside a cluster and (D) tortuous walk inside a cluster. As 'straight walk' behaviour never occurred, the respective graphs are omitted. Not all combinations of the independent variables are represented.

Preliminary runs show that at low prey densities most time is spent to straight walk behaviour as gut satiation is low. The results suggest that, when prey density is constant, the behavioural classes can be removed from the model as beetles predominantly exhibit one type of behaviour. Then, only the location of the predator determines predation, consumption and egg production.

BAARS (1979) followed radioactively labelled *P.coerulescens* in heathland and found alternatingly straight and tortuous walk. From the insight in the dispersal of the beetle gained by the approach presented here, this can be explained by the strongly aggregated distribution of prey in such habitats. Experiments of the type BAARS (1979) carried out are extremely time consuming and do not yield information on the causes of the phenomena observed. Detailed experiments in combination with systems analysis and simulation leads to an understanding of the underlying processes and conclusions with greater general applicability.

4. MODELLING DISPERSAL OF APTEROUS GREEN PEACH APHID, *Myzus persicae*, AND VIRUS SPREAD IN SUGARBEET

4.1 Introduction

Virus yellows is an economically important disease of sugarbeet, *Beta vulgaris*, throughout the world, causing yield losses up to 60% (DUFFUS, 1973). The disease can be caused by three different viruses: beet yellows virus (BYV), beet mild yellowing virus (BMYV) and beet western yellows virus (BWYV). These viruses can occur singly or in mixed infections. BYV is a closterovirus (BAR-JOSEPH *et al.*,1979) while BMYV and BWYV are luteoviruses (DUFFUS, 1973; DUFFUS & RUSSELL, 1975). The only important vector of these viruses is the green peach aphid, *Myzus persicae*.

The disease typically occurs in patches which vary in size from 1 to 30 m diameter, depending on the date of infection (VAN DER WERF, 1988). Each patch is presumably initiated by one infectious aphid which infects one or few beet plants and starts a vector colony in the early stages of crop growth, during May or June. Subsequently, a reservoir of infectious plants is formed around the primarily-infected plant(s) while the aphid population builds up. Aphid population growth is promoted by the improved nutritional quality of virus-infected plants. Massive aphid dispersal and virus spread occurs after mid-June when adjacent plants have made leaf-contact. The aphid populations collapse in July due to predation, diseases, parasitism and decreasing suitability of the beet plant, resulting in an arrest of virus spread.

Spread of viruses by vectors constitutes a complicated three-species interaction between a plant-host, a virus-pathogen and an insect-vector. Often natural enemies are also involved in this interaction as they can reduce the number of vectors and alter their behaviour (ROITBERG *et al.*, 1979). Because of this complexity, analysis of processes in the field is difficult and observations can often be explained in more than one way. Modelling the dispersal and feeding behaviour of *M. persicae* in relation to the development of plant infectivity, provides a means of studying the importance of the various processes which are involved in virus spread. Such an approach complements field studies by integrating results of experiments on the process-level. The latter experiments concern e.g. walking and feeding behaviour of the vector, virus transmission characteristics, latency period and incubation period.

Here a brief account is given of such a simulation study of virus spread. A more detailed description of the model and a program listing is given by RIESEBOS (1988).

4.2 Structure of the model

The model describes a 12 x 12 m^2 area with 1152 sugarbeet plants (7.8 plants m^{-2}) and a time-varying number of aphids. All plants have the same number of leaves and are equidistantly spaced. The status of each plant and each aphid is characterized by a memory structure encompassing a small number of variables. For a plant, these variables describe (1) the infection date, (2) the number of healthy leaves (i.e. those emerged before infection), (3) the proportion of leaves infected, (4) the presence of symptoms and (5) the number of aphids on the plant. Variables describing an aphid are (1) the time of birth, (2) the time of virus acquisition, (3) the time of latest displacement, (4) the position in the field, and (5) morph, apterous (wingless) or alate (winged).

Model results are compared to data from an experiment in which the effect of sowing date and number of introduced *M. persicae* on spread of BYV was studied (VAN DER WERF, 1988). In this experiment three plants located near the centre of each plot were infected with BYV on 23 June. On these plants 2, 9 or 65 *M. persicae* were released on 25 June. The plants had been sown on either 18 April (regular) or 20 May (late). Each of the six treatments was replicated in four plots.

Momentaneous temperatures are calculated by fitting sinusoids between measured minimum and maximum temperatures (ANONYMUS, 1986). Plant leaf number, which provides an indication of physiological age, is a function of accumulated temperature (MILFORD *et al.,* 1985a,b; VAN DER WERF, 1988).

BYV is a semi-persistent virus. In accordance with this, aphids in the model loose the ability to transmit virus at moulting. An aphid moults every 36 hours. Simplifying from SYLVESTER (1961), the retention period (RP) is taken as exactly 12 h. following acquisition. Based on data of SYLVESTER (1956a,b) and HEATHCOTE and COCKBAIN (1964), the acquisition feeding period (AFP), i.e. the feeding time needed for an aphid to acquire virus from an infected plant, is set at 2 h. while the transmission feeding period (TFP) is estimated as 1 h. Whenever the feeding time (which in the model equals the time not spent to walking) on a leaf equals or exceeds the TFP or AFP, virus acquisition or transmission occurs, provided that a non-viruliferous aphid feeds on an infectious leaf or an infectious aphid on a healthy plant. *M. persicae* shows preference for young beet leaves (JEPSON, 1983). Various degrees of aphid preference for heart leaves (those smaller than 10% of their final area) can be simulated (see below).

Following infection, plants become infectious after a latency period (LP). The LP increases linearly with time, from 4 days in the seedling phase to 12 days in old plants (VAN DER WERF, 1988). Counting from infection, symptoms become apparent after the incubation period (IP), which is influenced by plant physiological age and temperature. The IP increases from *ca.* 3 weeks in June to 2 months in August (VAN DER WERF, 1988). Aphids can acquire virus from the infectious leaves on infected plants, irrespective of the presence of symptoms. No virus can be acquired from a healthy or a latent infected plant or from those leaves on an infectious plant that appeared before infection.

Aphid population dynamics is not simulated in this model because insufficient data are available to do this with sufficient precision, due to the difficulty of quantifying predation.and immigration. Therefore, if a population dynamics module were included it would be difficult to determine whether possibly aberrant model outcomes with respect to virus spread resulted from errors in the virus dissemination part of the model or from incorrectly simulated population dynamics of the vector. To avoid these problems, the model mimics the population development observed in the field. Every simulation-day, simulated aphid numbers are compared to field-counts made in the centre of the plot or interpolations from these numbers. When the difference exceeds 10%, new aphids are added or existing ones removed to compensate the difference. Newly added aphids are 0 h. old and non-viruliferous, mimicking births. They are borne on plants which are already infested by aphids, in proportion to the number of aphids already present on the plant. Aphids are removed at random, assuming that predators do not discriminate between aphid instars and cause a fixed relative mortality rate, irrespective of aphid density. (This means that predators aggregate in areas with high aphid density, approximately in proportion to the number of aphids present). Winged aphids leave the field. No immigration occurs.

Predation by ladybirds reduces the number of viruliferous aphids in the field. It is difficult, however, to estimate the impact of predators on the basis of predator density (FRAZER, 1988). Therefore, the predation rate in the model, 1% predation per hour, was determined in another way, by iteratively maximizing the agreement between the simulated and the observed age distribution of the aphid population, throughout the season. At lower relative predation rates than .01 $h.^{-1}$ the proportion of older nymphal stages was overestimated while at higher relative predation rates the proportion of older stages was too low.

The dispersal and feeding behaviour of *Myzus persicae* plays a central role in the model. Aphid dispersal activity is expressed by the variable P, the proportion of aphids walking within an hour. When the number of leaves is smaller than 12, P has a small value, 0.025. This gives expression to the almost complete absence of dispersal in young crops, observed in the field (VAN DER WERF, 1988), which is probably due to the high nutritional quality of young plants as well as to lack of leaf contact between them. When the number of leaves is greater than 12, P is calculated as

$$P = .01 \times (65 - \frac{N}{2.5}) \qquad (14)$$

where N is the number of leaves on the plant. As 2 to 3 leaves emerge each week, aphid activity decreases slowly during the season. Equation 14 is based on calculations of the proportions of newly-emerged, expanding and fullgrown leaves on the beet plant throughout the season (MILFORD *et al.*, 1985b) and estimates of the dispersiveness or aphids on these three types of

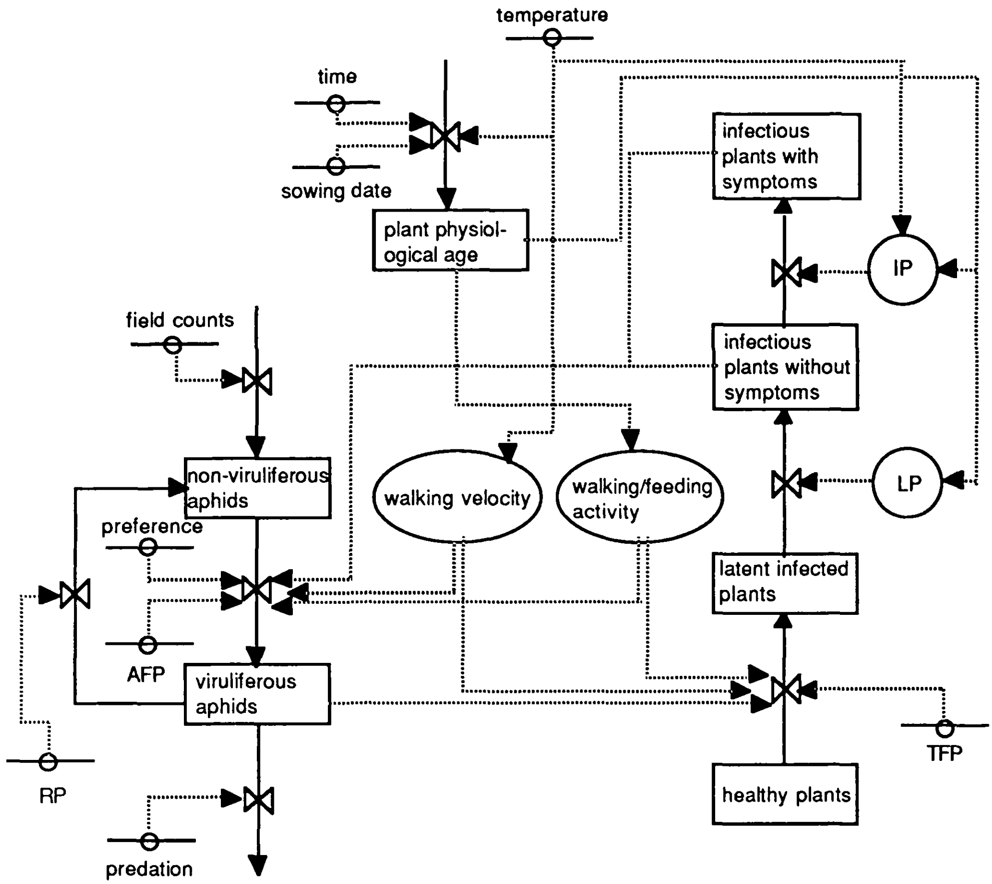


Fig. 15: Relation diagram of model of virus spread by Myzus persicae in sugarbeet, drawn according to conventions of FORRESTER (FORRESTER, 1961; DE WIT and GOUDRIAAN, 1978). Boxes ( ) represent states, solid arrows ( ) represent fluxes between states, valves ( ) in the solid arrows denote rates while hatched arrows ( ) pointing to valves denote control of rates by state variables. Circles or ovals denote auxiliary variables. External variables, not influenced by the model, are denoted by .

leaves (JEPSON, 1983), assuming a homogeneous distribution of aphids over the plant (RIESEBOS, 1988).

The distance covered each hour (D; linear displacement), is an optimum type function of aphid age, adult aphids walking more rapidly than young or aged ones, and temperature:

$$D = D_{max} \times \left(1 - \left(\frac{A - 25}{25}\right)^2\right) \times \left(1 - \frac{9}{4} \times \left(\frac{T - 30}{30}\right)^2\right) \qquad (15)$$

where A is denotes the age of the aphid in days and T the temperature in °C. The value of D_{max} is 125 cm, in accordance with data of FERRAR (1968). The walking direction is drawn from a uniform distribution over $(-\pi, \pi)$

The main components and relations of the model are summarized in the relation diagram of Fig. 15. Runs with the model were made with two purposes: (1) to compare simulation results with field data and thus test our conception of the system, and (2) to determine which parameters have the largest effect on model behaviour and should therefore be studied in more detail experimentally.

The model is written in the programming language C (KERNIGHAN & RITCHIE, 1978). The time step is one hour.

4.3 Results

Simulation results and field data are compared in Figs. 16A and B which show the time-course of the number of yellowed plants per plot for each of the six treatments. Fair overall agreement exists between the two figures. Agreement is very good in 4 treatments but the model underestimates virus spread resulting from release of two *M. persicae* in late-sown sugarbeet while it overestimates the spread resulting from release of 65 *M. persicae* in early-sown sugarbeet. This suggests that parameters related to plant age are more important in reality than they are in the model in its present form.

The overall similarity of simulated and observed spread is a promising result as model structure and parameter estimates on the process level are in agreement with the literature. Nevertheless, further model validation is needed to obtain a better insight in possible shortcomings of the model.

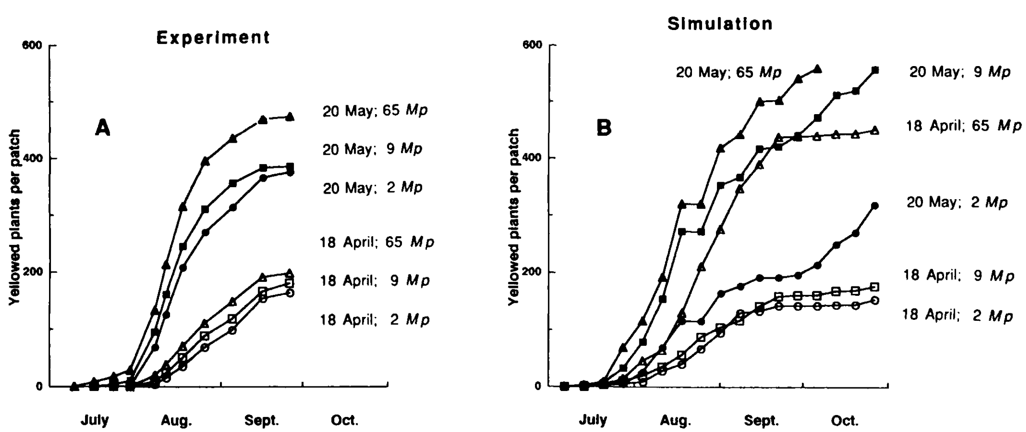


Fig. 16: Comparison of observed (A) and simulated (B) increase of number of BYV-infected plants with symptoms, in relation to crop sowing date and numbers of *Myzus persicae* introduced on 25 June

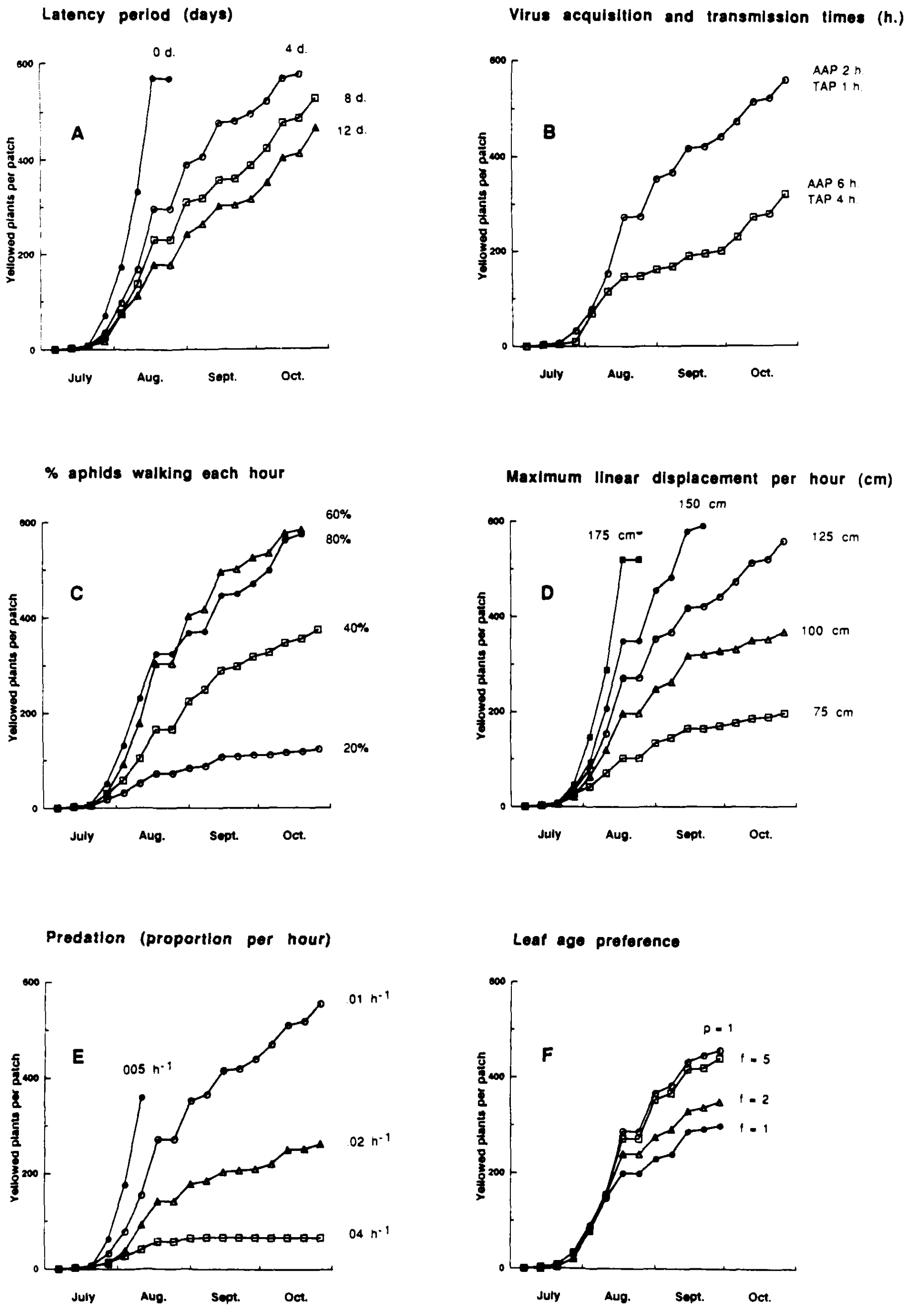
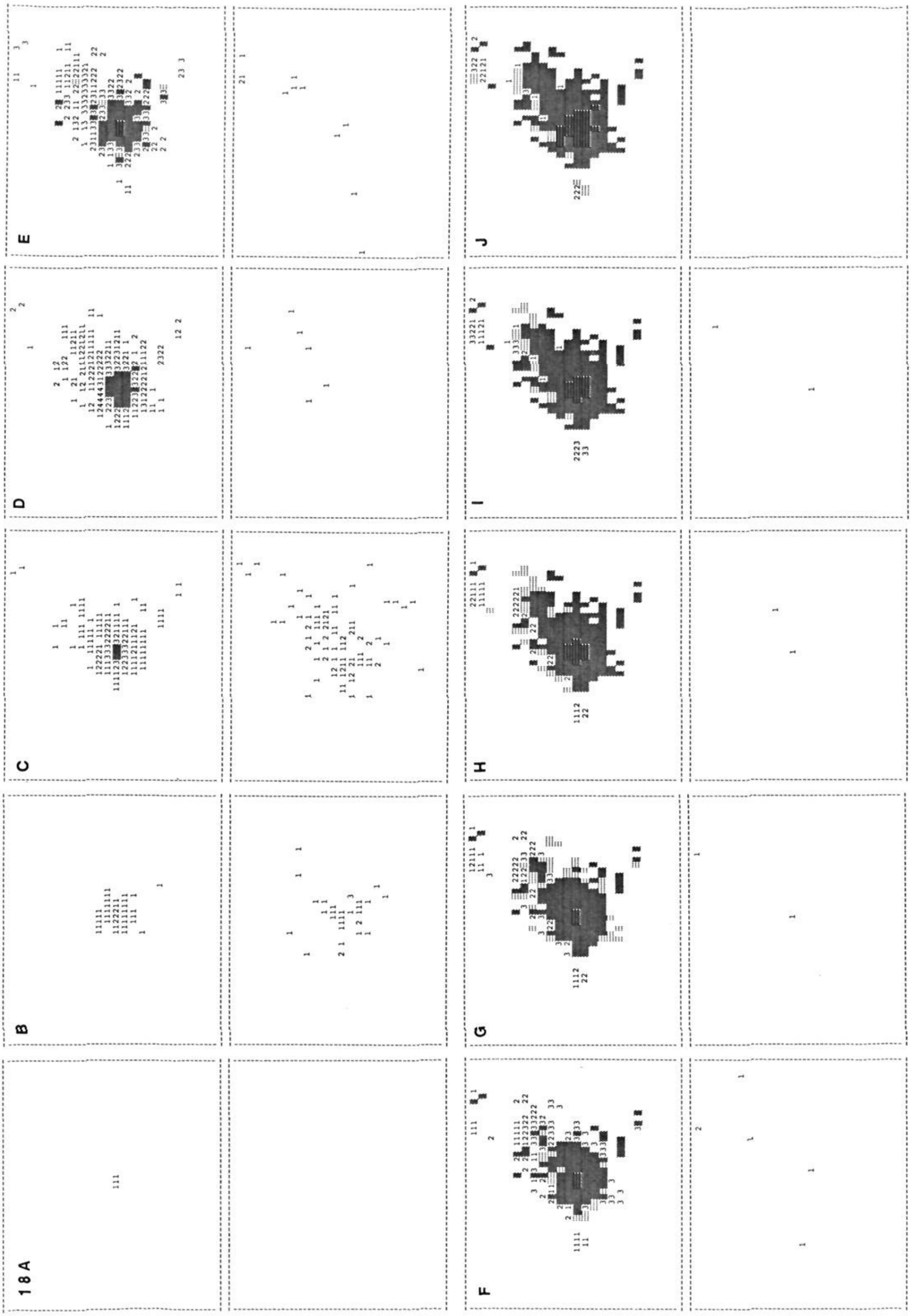


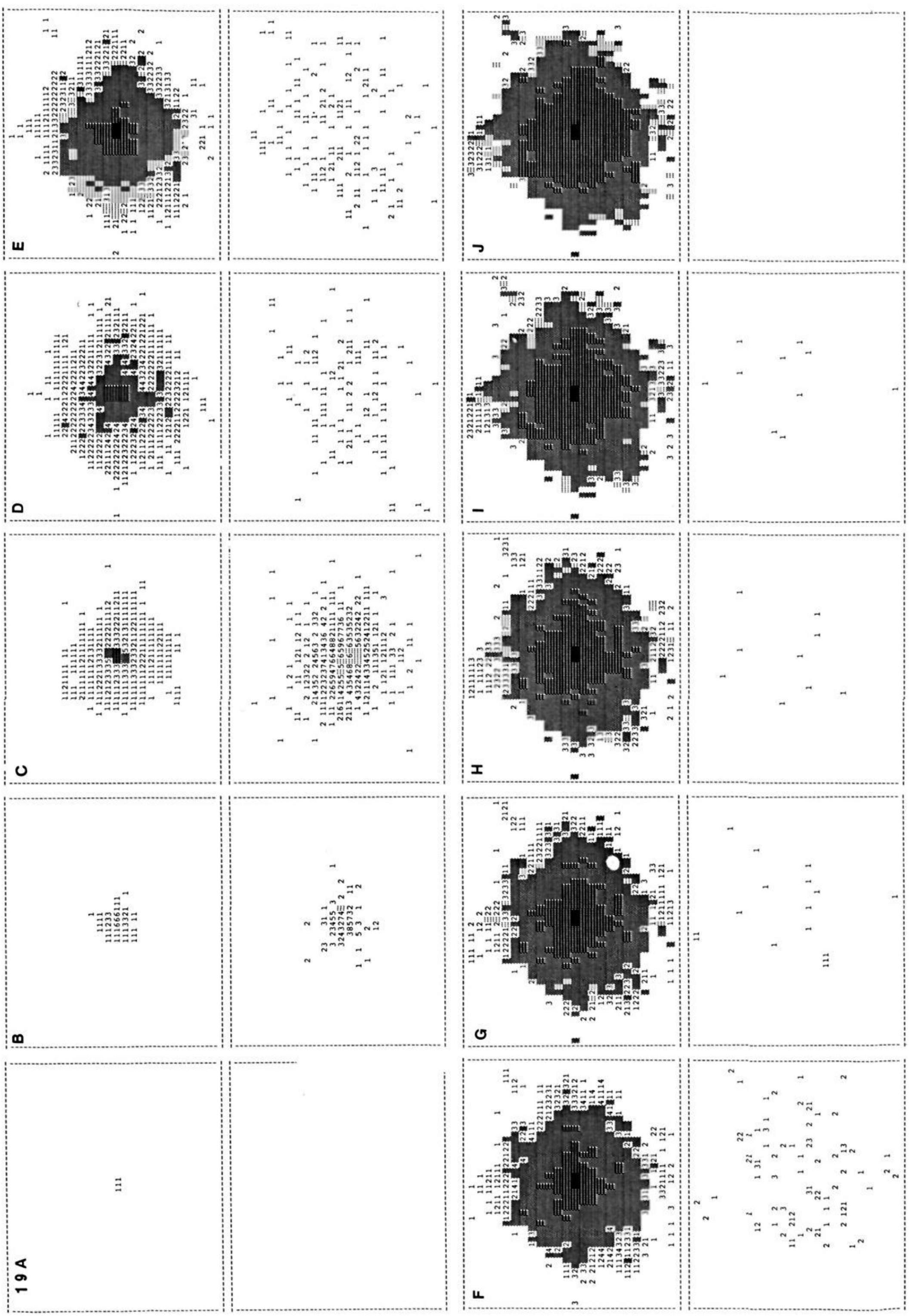
Fig. 17: Sensitivity analysis of model of virus spread by *Myzus persicae* in sugarbeet. (A) latency period, (B) virus transmission characteristics, (C) walking activity, (D) walking distance per hour, (E) relative predation mortality and (F) leaf age preference as indicated by the factor f_{pref} in the formula $p = f_{pref} \times Y$, where p is the proportion of aphids feeding on young leaves and Y is the proportion of young leaf area on the plant.

Sensitivity analysis provides a means to determine the influence of model structural components and parameter estimates on the results. Here a 'fine' sensitivity analysis (RABBINGE *et al.*, 1989) for six parameters is presented (Fig. 17A-F). The analysis applies to sugarbeet sown on 20 May and infested with 9 *M. persicae*. For the LP reasonably precise values were available so that Fig.17A illustrates its theoretical importance. The LP has a large impact in our model which confirms results obtained with more basic epidemiological models (ZADOKS and SCHEIN, 1979). For the other five parameters, only rough estimates could be derived from the literature. The figures 17B-F give therefore an indication of the uncertainty in predicted epidemics resulting from the imprecision of the parameter estimates in the model. For instance, fig. 17B shows that virus acquisition and transmission times have a considerable effect on spread. While several authors determined feeding times needed for the acquisition and transmission of beet viruses under greenhouse conditions with several species of test plants, possible influences of sugarbeet host plant quality (age) in the field have been neglected. Therefore, those studies may have limited relevance for the prediction of processes occurring in the field. Aphid behaviour, here expressed by two parameters, activity and distance covered, has been very little studied, resulting in a considerable uncertainty about model predictions (Fig. 17C,D). Predation appears to be a particularly important factor in the model (Fig. 17E), indicating that it deserves more attention in experimental research, the more so because in the model predators influence only the proportion of aphids which are viruliferous and not the total number. Thus, in reality, the impact of natural enemies on virus spread may be even greater than indicated by the simulation results. The effect of leaf age preference is relatively small (Fig. 17F).

Figs. 18 and 19 show the spatial distribution of the aphids and the virus-infected plants at two-week intervals in sugarbeet sown on 18 April and 20 May, respectively. Aphids become more numerous and infect a greater number of plants in the late-sown crop. These plots clearly show the temporal separation of the presence of the aphids and the resulting increase in the number of plants with symptoms. Virus spread occurs when the aphids are numerous and disperse over the plot, predominantly between mid-June and mid-July in this case. Symptoms only become obvious at the end of this period. Spraying against aphids at this point is useless as further spread will be negligible, while plants infected at this advanced development stage suffer little damage.The appearance of a 'secondary' focus in top right-hand corner of Fig. 18 is worth noting. In field studies this would generally be regarded as the result of virus spread by alatae, 'hopping' in the field. This simulation shows that such a secondary patch may be also initiated by apterous aphids.

Figs. 18 and 19 (next pages): Simulated spatial distribution of aphids and virus-infected plants at two-week intervals in sugarbeet sown on 18 April or 20 May. In the model the plots are inoculated with BYV on 23 June and infested with 9 *Myzus persicae* 2 days later. Dates: (a) 23 June; (b) 7 July; (c) 21 July; (d) 4 August; (e) 18 August; (f) 1 September; (g) 15 September; (h) 29 September; (i) 13 October and (j) 27 October. The upper plot of each date denotes the spatial arrangement of BYV-infected plants in a 12 x 12 m^2 field plot. Numbers indicate the percentage of infected leaf area on plants without symptoms: (1) 0-10%, (2) 10-20%, etc. Plants with symptoms are indicated by shaded blocks, the four degrees of shading indicating percentages infected leaf area of (⁙) 0-30%, (▒) 30-60%, (▓) 60-90% and (█) 90-100%. The lower plot denotes the number of aphids per plant. Numbers of aphids per plant exceeding 9 are indicated by a shaded area (⁙).


18 A
B
C
D
E
F
G
H
I
J


19 A
B
C
D
E
F
G
H
I
J

5. CONCLUDING REMARKS

The three spatial models of population dynamics, described in his paper, simulate the processes underlying system behaviour at different integration levels. The first model - spore dispersal in a forest - describes the system with diffusion and flux equations which apply to spore 'masses', while neglecting the behaviour of individual spores. In the second example - predation by carabids - a detailed analysis of the individuals' motivation and resulting predatory behaviour is made. Subsequently, general rules derived from this analysis are used in a compound simulation population model which distinguishes six classes of beetles but no individuals. In the third example - spread of beet viruses - the behaviour of the individual virus vectors is simulated. Computation time increases with the amount of detail included, from example 1 to 3.

The level of complexity that must be simulated in a given case, depends upon the aim of the study and on the available experimental data and computing resources. In the risk analysis pertaining to biocontrol with silverleaf fungus, simulation of individual spore trajectories would have been cumbersome while enormous computing times would have been needed to account for the large number of individual spores involved. The simpler approach adopted by DE JONG (1988) suffices to obtain the desired estimates of spore escape. In the case of the carabids, detailed information on the beetle's motivation and predatory behaviour was obtained in studies aiming at a better understanding of the survival strategy of the species in a temporally and spatially variable habitat. To evaluate the potential for natural aphid control by populations of a closely related *Pterostichus* species, living in arable lands and exhibiting similar behaviour as the one studied in heathlands, the complicated models of the motivational and behavioural processes had to be discarded because of their short time step (2 s.). The most important trait, the beetle's residence time in a prey colony, is captured in a two way table with the beetle's walking velocity and the prey colony's diameter as entries. This enables compound simulation, distinguishing only six classes of beetles. Finally, in the beet virus model, the state and behaviour of the individual vectors is accounted for. This is necessary because in this case every single vector has a potentially large impact, each infective puncture leading to the infection of a whole plant. The relatively large time step in this model (1 h.), which is made possible by the large size of the objects the vector is encountering, beet plants, allows a detailed representation of the system.

Simulation models offer the opportunity to integrate large amounts of information, gathered on the process level, in a simulation model with behaviour comparable to the real world. In this way the correctness of the conception of the system can be tested. It is often found that the behaviour of systems which are commonly regarded as 'well understood' cannot be easily predicted. Thus, gaps in knowledge are located. If, on the other hand, model predictions are satisfactory, the model can be simplified to a tool for decision making. Another merit of the modelling approach is that imaginary experiments can be performed. Such imaginary experiments can be used for orienting experimental research (beet virus model) or risk analysis (silverleaf fungus model). Experiments with the carabid model suggest that *Pterostichus* species have potential to suppress aphid outbreaks in arable crops.

Models including spatial dynamics and variability have been developed more recently than 'homogeneous' models, due, in part, to the large amounts of computing time needed. If a homogeneous model for the development of a disease is converted into a 'heterogeneous' model, accounting for 1000 interacting patches in a field, the number of state variables and rate equations in the model increases also a 1000-fold. An even greater number than that is probably needed in heterogeneous models to account for migration. As computers have become faster (some researchers even use 'supercomputers'; ONSTAD, 1988) the possibility for simulating pests and diseases in a spatially realistic way becomes much greater. This results in the ability to account for density-dependent processes in a more realistic way such that better population models may result.

ACKNOWLEDGEMENTS

Mrs. Drs. P.S. Wagenmakers, Ir. P. Link and Ir. W. Riesebos made important and highly esteemed contributions to the models described in the sections 2, 3 and 4, respectively. We are also grateful for their assistance in the preparation of this paper. Dr. D.I. Rouse gave valuable criticism on the final draft.

REFERENCES

ANONYMOUS, 1986. Maandoverzichten van het weer, meteostation Swifterbant, issued by the Department of Physics and Meteorology, Agricultural University, Wageningen, the Netherlands.

BAARS, M.A., 1979. Patterns of movement of radio-active carabid beetles. Oecologia 44, 125-140.

BAR-JOSEPH, M., S.M. GARNSEY AND D. GONSALVES, 1979. The closteroviruses: a distinct group of elongated plant viruses. Adv. Virus Res. 25, 93-168.

CORY, J.S. and P.F. ENTWISTLE, 1988. Ecological considerations and research ramifications of releasing genetically manipulated viruses. Proc. 18th int. Congr. Entomol., 252.

DAAMEN, R.A., 1986a. Measures of disease intensity in powdery mildew (*Erysiphe graminis*) of winter wheat. 1. Errors in estimating pustule number. Neth. J. PL. Path. 92, 197-206.

DAAMEN, R.A., 1986b. Measures of disease intensity in powdery mildew (*Erysiphe graminis*) of winter wheat. 2. Relationships and errors of estimation of pustule number, incidence and severity. Neth. J. PL. Path. 92, 207-222.

DE JONG, M.D., 1988. Risk to fruit trees and native trees due to control of black cherry (*Prunus serotina*) by silverleaf fungus (*Chondrostereum purpureum*). PhD. thesis, Agricultural Univ. Wageningen, the Netherlands, 138 pp. (in Dutch; summary and description of computer programs in English).

DE JONG, M.D. and P.C. SCHEEPENS, 1985. Analyse van risico bij bestrijding van Amerikaanse vogelkers (*Prunus serotina*) met (*Chondrostereum purpureum* voor planten waartegen de bestrijding niet is gericht. Internal Rep. nr. 58 of Centre for Agrobiological Res., Wageningen, the Netherlands, 35 pp.

DE JONG, M.D., P.C. SCHEEPENS and J.C. ZADOKS, 1988. Risk analysis for biological control. A Dutch case study in biocontrol of *Prunus serotina* by means of the fungus *Chondrostereum purpureum*. Submitted.

DUFFUS, J.E., 1973. The yellowing virus diseases of sugar beet. Adv. Virus Res. 18, 347-386.

DUFFUS, J.E. and G.E. RUSSELL, 1975. Serological relationship between beet western yellows and beet mild yellowing viruses. Phytopath. 65, 811-815.

FERRAR, P., 1968. Interplant movements of apterous aphids, with special reference to *M. persicae* (Sulz.) (Hom. Aphididae). Bull. entomol. Res. 58, 653-660.

FORRESTER, J.W., 1961. Industrial dynamics. MIT-press, Boston.

FRANSZ, H.G., 1974. The functional response to prey density in an acarine system. Simulation Monographs, PUDOC, Wageningen, 149 pp.

FRAZER, B.D., 1988. Coccinellidae. In: A.K. MINKS and P. HARREWIJN (Eds.): Aphids, their biology, natural enemies and control. Elsevier, Amsterdam, p. 231-247.

GOUDRIAAN, J. , 1977. Crop micrometeorology: a simulation study. Pudoc, Wageningen, the Netherlands, 249 pp.

GOUDRIAAN, J., 1979. MICROWEATHER simulation model, applied to a forest. In: S. HALLDIN (Ed.): Comparison of forest water and energy exchange models. Int. Soc. Ecol. Modelling (ISEM), Copenhagen, 47-57.

GROSCLAUDE, C., 1969. Le plomb des arbres fruitiers. VII. Observations sur les carpophores et les spores du *Stereum purpureum*. Ann. Phytopath. 1, 75-85.

HEATHCOTE, G.D.and A.J.COCKBAIN, 1964. Transmission of beet yellows virus by alate and apterous aphids. Ann. appl. Biol. 53, 259-266.

HENGEVELD, R., 1980. Poliphagy, oligophagy and food specialisation in ground beetles (Coleoptera, Carabidae). Neth. J. Zool. 30, 564-584.

HOLLING, C.S., 1966. The functional response of invertebrate predators to prey density. Mem. entomol. Soc. Canada 48, 1-86.

HUBER, J., 1988. Assessing the risks of releasing genetically modified insect pathogens. Proc. 18th int. Congr. Entomol., 250.

JEPSON, P.C., 1983. A controlled environment study of the effect of leaf physiological age on the movements of apterous *Myzus persicae* on sugar-beet plants. Ann. appl. Biol. 103, 173-183.

KAREIVA, P. and G. ODELL, 1987. Swarms of predators exhibit 'preytaxis' if individual predators use area-restricted search. Amer. Nat. 130, 233-270.

KERNIGHAN, B.W. and D.M. RITCHIE, 1978. The C programming language. Englewood Cliffs: Prentice-Hall, 228 pp.

MILFORD, G.F.J., T.O.POCOCK and J. RILEY, 1985a. An analysis of leaf growth in sugarbeet. II. Leaf appearance in field crops. Ann. appl. Biol. 106, 173-158.

MILFORD, G.F.J., T.O.POCOCK, J. RILEY and A.B. MESSEM, 1985b. An analysis of leaf growth in sugarbeet. III. Leaf expansion in field crops. Ann. appl. Biol. 106, 187-203.

MOLS, P.J.M., 1979. Motivation and walking behaviour of the carabid beetle *Pterostichus coerulescens* L. at different densities and distributions of the prey. Miscell. Papers Agric. Univ. Wageningen 18, 185-198.

MOLS, P.J.M., 1983. Simulation of the motivation and egg production of the carabid beetle *Pterostichus coerulescens* L. Rep. 4th Symp. Carabids 1981, 35-43.

MOLS, P.J.M., 1986. Predation in the carabid beetle *Pterostichus coerulescens* L. In: P.J.DEN BOER, L. GRUM & J. ZYSZKO (Eds.): Feeding behaviour and accessibility of food for carabid beetles. Rep. 5th ~Meeting Europ. Carabidologists. Warsaw Agric. Univ.-SGGW-AR, 49-58.

MOLS, P.J.M., 1987. Hunger in relation to searching behaviour, predation and egg production of the carabid beetle *Pterostichus coerulescens* L.: Results of simulation. Acta Phytopathologica et Entomologica Hungarica 22, 187-296.

ONSTAD, D.W., 1988. Simulation model of the population dynamics of *Ostrinia nubialis* (Lepidoptera: Pyralidae) in maize. Environm. Entomol. In press.

RABBINGE, R., 1976. Biological control of the fruit tree-spider mite. PUDOC, Wageningen, 228 pp.

RABBINGE, R., S.A. WARD & H.H. VAN LAAR (Eds.), 1989. Simulation and systems management in crop protection. PUDOC, Wageningen. (in press).

RIESEBOS, W., 1988. Een model voor de verspreiding van beet yellows virus door *Myzus persicae*. M.Sc. thesis, Department of Theoretical Production Ecology, Agricultural University, Wageningen, the Netherlands, 140 pp. + appendices (in Dutch).

ROITBERG, B.D., J.H. MYERS and B.D. FRAZER, 1979. The influence of predators on the movements of apterous pea aphids between plants. J. Anim. Ecol. 48, 111-122.

SABELIS, M.W., 1981. Biological control of two-spotted spider mites using phytoseiid predators. Part 1. Agricultural Research Report, 910, PUDOC, Wageningen, 242 pp.

SKELLAM, J.G., 1958. The mathematical foundations underlying line transects in animal ecology. Biometrics 14, 385-400.

SOUTHWOOD, T.R.E., 1978. Ecological methods. 2nd ed., Chapman and Hall Publ., London, 524 pp.

SYLVESTER, E.S., 1956a. Beet yellows virus transmission by the green peach aphid. J. econ. Entomol. 49, 789-800.

SYLVESTER, E.S., 1956b. Beet mosaic and beet yellows virus transmission by the green peach aphid. J. Amer. Soc. Sugar Beet Technol. 9, 58-61.

SYLVESTER, E.S., 1961. Re-evaluation of retention of beet yellows virus by green peach aphids under fasting and feeding conditions. Virology 14, 467-479.

UPTON, G.J.G. and B. FINGLETON, 1985. Spatial data analysis by example. Wiley, New York, 410 pp.

VAN DER WERF, 1988. Yellowing viruses in sugarbeet; epidemiology and damage. Ph. D. thesis Agric. Univ. Wageningen, the Netherlands, 152 pp.

WAGENMAKERS, P.S., 1984. Simulatie van de sporeverspreiding van *Chondrostereum purpureum* in een bos. M.Sc. Thesis, Agicultural University, Department of Theoretical Production Ecology, Wageningen, the Netherlands. 58 pp.

WARD, S.A., R. RABBINGE and W.P. MANTEL, 1985a. The use of incidence counts for estimation of aphid populations. 1. Minimum sample size for required accuracy. Neth. J. Pl. Path. 91, 93-99.

WARD, S.A., R. RABBINGE and W.P. MANTEL, 1985b. The use of incidence counts for estimation of aphid populations. 2. Confidence intervals from fixed sample sizes. Neth. J. Pl. Path. 91, 100-104.

WARD, S.A., K.D. SUNDERLAND, R.J. CHAMBERS AND A.F.G. DIXON, 1986. The use of incidence counts for estimation of aphid populations. 3. Population development and the incidence-density relation. Neth. J. Pl. Path. 92, 175-183.

ZADOKS, J.C. and R.D. SCHEIN, 1979. Epidemiology and plant disease management. Oxford Univ. Pr., New York, 427 pp.

AN OVERVIEW OF ECONOMIC ANALYSIS IN AGRICULTURAL SYSTEMS

U. Regev
Department of Economics & the Monaster Economic Research Center, University-Beersheva, Israel

P. Rieder
Department of Agricultural Economics, Federal Institute of Technology, ETH-Zürich, Switzerland

Abstract

Economic systems in different parts of the world are divided into: (1) market economies; (2) developed economies controlled by centralized bureaucracies; and (3) economies of developing countries. The paper discusses the principles of agricultural policy in these three types of economies with special reference to the paradox of famine in the face of food surpluses, farmer income support mechanisms, and agriculture-related ecological problems from an economic viewpoint.

The traditional goal of economic analysis in crop production is to find the profit maximizing production decisions and to analyze the impact of these decisions on the social and ecological environments. In pest management, economic analysis followed a similar direction, resulting in economic action thresholds and other pest management decision rules. The conflicts between the farmers profit maximization objective and the long term aim of the society to obtain inexpensive food products and keep the environment clean is especially obvious in pest management. Economic analysis predicts that these conflicts invariably lead to overuse of chemical pesticides.

Economic analysis of pest management decisions under uncertainty has pointed that pesticide application is more frequent and with larger doses when the pest status is more uncertain. In choosing pest management strategy the farmer selects the one perceived by him as more risk reducing. However, a risk reducing strategy in the short run may prove to be risk increasing in the long run.

*Thanks are due to A. P. Gutierrez for his help in developing and crystallizing some of the ideas in this paper, and to U. Egger for his help in many economic aspects of the paper.

1 The role of Economic Analysis in Agriculture

Economics is sometimes defined as the science of satisfying human material wants. As such it is concerned with the allocation of scarce resources and is conventionally categorized into normative and positive economics. The first is aimed at finding optimal decision rules according to predefined goals, subject to technological, resources, social, or other constraints, and prescribing them to private firms or public agencies. Positive economics on the other hand deals with analyzing economic behavior with the dual purpose of scientific understanding of the policy implications of such an analysis. Another conventional categorization is into macro and micro economics. The first category – macro-economics – deals with global issues of the economy such as the national product, the rate of growth, unemployment, the quantity of money and the rate of inflation, as well as international trade and the exchange rate. As far as the agricultural sector in concerned, three broad categories of macro economics can be distinguished (McCalla and Josling 1985):

- Development of the agricultural sector with regards to its efficiency and income level in relation with the rest of the economy (Mundlak, 1988; Rieder, 1987).
- Issues of food supply and consumption and their link to available incomes.
- The role of international trade and resource transfer in the search for increased productivity and income in the agricultural sector (Gardner, 1988).

The second category – micro economics – analyses the behavior of consumers, producers, a single household, a firm, or a market for a single good. Here one can find analyses of the consumer's behavior and its implication for the demand of a given product, as well as analyses of the production process, which transforms goods and services into forms desired by the consumers. The production function, which relates the output (a desired product) to a set of inputs (resources), is the corner stone of such analysis. Prices, determined in a competitive economy, serve as signals for profit maximizing producers and utility maximizing consumers, as well as for government's policy making directed at enhancing efficiency or affecting income distribution. Agriculture is characterized by its intensive use of land and water resources, its dependence on the natural environment and reciprocal negative effects on the environment. This makes the agricultural production process more susceptible to environmental risk and uncertainty factors on the one hand, while causing ecological disruptions and other external damages to the society on the other.

A major contribution of economics to the analysis of the environmental issues is based on the paradigm of external diseconomies and/or common property resource. Briefly illustrated, we will find that in a commonly owned fishing lake, the fish population will degrade due to over-utilization (high rate of fishing) and lack of concern for future harvests. Unless some collective organization is established and actions taken to maintain the collective interests, the common property will be over-exploited over time. This is known in the economic literature as the 'free rider' problem where individuals, persons or firms, try to take advantage of collective actions by pursuing their own interests. (Baumol and Oates, 1975). The ecological problem in agriculture is of similar nature, but in this case the society as a whole is hurt by the externalities of agricultural production, and thus should be involved in the collective action. These issues are further complicated when the ecological damages spread beyond a single country (pollution of rivers, air and soil). In all the above situations, competitive market forces fail to provide the proper incentives to sellers and buyers, which result in misallocation of resources. This can be partly corrected by public and government intervention.

The above discussion explains partially why the agricultural sector is so often subject to a high degree of government intervention. This makes the economic analysis most important in pointing out the optimal (some will say least damaging) policy for government intervention, in order to achieve environmental and economic objectives. Real agricultural policies of governments vary greatly in different economic systems which can be classified as (Egger, 1988):

1. Market Economies
2. Centrally Planned Economies
3. Economies of Developing Countries

The functioning of these systems is summarized in Table 1. For our purpose it is of interest to see how decisions are made to develop and formulate agricultural policy. Because the process of decision making differs between economic systems, they are analyzed separately in an outline form.

1. **Market Economies**

- Pressure groups of producers and traders maintain a very strong support for agriculture. They argue that this support is justified by the social goods produced by agriculture and its natural disadvantage when compared to the rest of the economy. This support provides incentives for over production which results with increasing food surpluses problems.
- Political interests within the agricultural sector are very heterogeneous. Privileged members profit more from the groups' political influence.
- Consumers and taxpayers are badly organized. The distribution of support costs and benefits between consumers and producers differs from country to country, depending on the specific agricultural policy.
- The behavior of international agricultural markets is dominated by the following protectionist rules: exports are motivated by surplus production which causes low prices, and imports are taxed at the borders to maintain the internal support prices.

2. **Centrally Planned Economies**

- Agricultural production is determined by central Bureaucracy planning, and the cooperative farmers' formal self-determination is ignored.

- Low labor performance in collective farms arises from insufficient consideration of producers interests and incentives.
- Inefficient production and resource allocation are commonplace (Hayami, 1988)
- Food deficits in years of bad harvests are covered partly by privately produced commodities, and mostly by buying considerable amounts of (mainly) grains on international markets. World markets are greatly influenced by such erratic and unpredictable large demands.

3. **Economies of Developing Countries**

- Farmers' interests are hardly organized. Land oligarchies maintain most uneven distribution of land and wealth.
- A centralized class of bureaucrats controls agricultural policy. They use agricultural exports for self-enrichment.
- There is no national capitalist class to limit government spending. Potential opposition groups are given certain privileges as a clientele and are integrated into the system in this way.
- Well organized urban interests prevail, as evidenced by low food prices. The large administrative bureaucratic apparatus is sustained by the agricultural sector.
- The farmers' income cannot improve because of the substantial discrimination of the agricultural sector. Production remains on a low level insufficient to fulfill the basic food requirement of the population.
- As a consequence, food imports increase whereas exports, which consist largely of raw materials, are marketed in saturated and fluctuating low price markets.

To understand and explain farm structure and agrarian strategy in a country, political constraints and interest groups have to be taken into consideration. The crucial role which the government plays in each system is evident from the above and private interests can prevail only by influencing governmental institutions and policies. Thus, economic analysis should focus first on governmental institutions and activities affecting the agricultural sector.

On the micro level, economists apply econometric methods to estimate production functions in the agricultural sector using various optimization methods *e.g.* linear and non-linear programming, dynamic programming and optimal control techniques, to find solutions to farmers' economic decision problems Such a solution comprises the optimal crop mix, the minimum cost inputs mix and output size (Rieder, 1985; Anderson, 1986). An especially important issue is that of the the effects of various government support programs on the farmers behavior and welfare, and their negative impact on resource allocation in agriculture, resulting in serious inefficiency because of agricultural price distortions (Schultz, 1978; Jörin and Rieder, 1984; Lichtenberg and Zilberman, 1986a; World Bank, 1986; Kettunen, 1987; OECD, 1987).

Risk has been long recognized as having major effects on economic decision making. Von Neuman and Morgenstern (1953) formulated the axiomatic basis of economic behavior under conditions of uncertainty. Their paradigm of expected utility, though under attack from within and outside the economic field, is still the basis for many important postulates and theorems of economic behavior and is applied in many fields such as welfare analysis, investment theory, financial portfolio selection and the theory of the firm. In the latter, Sandmo (1971) and Batra (1974) showed that risk averse firms[1] produce less under price uncertainty, than they do under deterministic conditions. Furthermore, under the commonly accepted hypothesis that risk aversion decreases with wealth, increasing price uncertainty leads to a reduction of output, and an increase in fixed costs will also reduce the output of the firm. Feder (1977) generalized these results to incorporate any uncertainty parameter in the production function and applied them to analyze the impact of risk on pest management decisions.

2 Economic contributions to Pest Management

Since the 40's when chemical pesticides for agricultural use started to spread, we observe an ever increasing trend of global pesticide use as well as the magnitude of pest damage. Do such trends contradict the economic basis of pest management decisions? What is the economic explanation for this apparently suicidal path and how does the economic science react to the recent growing public concern about the environmental and health consequences of pesticide use? Some answers and explanations are given below.

Pest management problems have been historically formulated and analyzed in a classical static profit maximization framework, and pesticides were included as inputs in the production function of a single pest control agent against a single pest (Hall and Norgaard, 1973; Talpaz and Borosh 1974). The concepts of economic and action thresholds (Headley, 1972; Gutierrez *et.al.*, 1979; Lazarus, 1983; Moffitt *et.al*, 1984; Plant, 1986) have transformed the solution to the problem into simplified decision rules, to be used by a single farmer with or without the aid of sophisticated research station support. Two major difficulties stem from this approach. The first is that, for the sake of simplicity, important aspects of the pest management problem are often ignored, so that the resulting decision rule is not applicable to real world situations. Secondly, pest management decisions produce harmful side–effects in the form of environmental pollution and health risks, which leads to conflicting interests of farmers and the society at large. These aspects are further discussed below.

Another approach specifically recognized the dynamic elements of the pest control problem, and used Bellman's *principle of optimality* to solve a discrete multiperiod decision problem by dynamic programming (Shoemaker 1973, 1981). This approach is especially suitable for selecting among discrete pest control strategies (*e.g.* comparing IPM with conventional strategies). Non–linear programming is ap-

[1] A risk averse firm will prefer a lower risk project over a higher risk one, if they yield the same expected profits. An econometric method for estimating producers' risk attitudes is suggested by Antle (1987)

plied to pest management problems incorporating the dynamic nature of the problem in a discrete time frame (Regev *et. al.*, 1976). The dynamic nature of the problem is also tackled in a continuous time framework by using 'optimal control' techniques (Pontryagin, 1964). These techniques were adopted to explore the optimal exploitation of renewable resource problems (Cropper *et.al.*, 1979).

The perceived benefits of pesticide use by the farmers is, that it maintains stabilized high yield. However, it also involves social costs resulting from the detrimental effects of pesticides on human health, pollution of the environment (air soil and water), as well as the disruption of ecological equilibria of pests and their natural enemies in the agro-system (van den Bosch, 1978). Neo-classical economic analysis has demonstrated that these side effects of pest management decisions belong to a large group of economic problems (production of public goods), where competitive market forces are insufficient to correct or optimally solve the problem (Baumol and Oates, 1975). Researchers are then called to evaluate the effects of pesticides on human health and the environment, and government intervention is needed to regulate their use. General criteria for determining the degree and form of public regulation are required to obtain a balance between social benefits and costs (Regev *et.al.*, 1976; Miranowski, 1980; Heimlich, 1982). This poses a challenging task for the economists – that of evaluating and measuring the benefits and costs of pesticides to the farmers and the society at large (Lichtenberg and Zilberman, 1986b; Antle, 1988).

Pest management is multiseasonal problem in its nature, thus it requires to consider short run *vs.* long-run objectives. Current pest control decisions have long-run impact on long term survival of the pests and their ability to develop resistance to pesticides. This must be taken into account for long-run profit maximization (present value of profits over time), since current pesticide use will reduce the effectiveness of pesticides and profits in the future (Gutierrez *et.al.*, 1979a). These long term effects of current pesticide use are generally **not** taken into consideration by the profit maximizing farmer in a competitive economic environment, since they are beyond his control. He optimizes his own short–run profits, which leads to overuse of pesticides seen from the standpoint of the society as a whole (Hueth and Regev, 1974; Taylor and Headley, 1975; Sarhan *et.al.*, 1979; Lazarus and Dixon, 1984). These points are demonstrated mathematically in Appendix 1, and illustrated in Figure 1.

Analysis of multiple pests, and the interactions of pests with their natural enemies is far more complicated, both on the theoretical and the applied levels. It is well known that pest resurgence commonly occurs when a new or formerly unknown pest appears following chemical control (van den Bosch, 1978; Huffaker, 1980). Feder and Regev (1975) dealt with a single predator–prey situation on a theoretical level, where they showed conditions for long run damages by the attempted control of a pest.

Integrated pest management (IPM) has been developed as a viable alternative to the failing and environmentally damaging conventional pest control methods (Huffaker and Smith 1980). Comparing IPM with conventional pest control methods requires going beyond the marginal analysis, often used for the optimization of conventional pest control systems. Shoemaker (1982) used dynamic programming to analyze alternative control methods. Lichtenberg (1987) found in the case of rice field mosquito control that biological control was clearly superior economically over chemical control. However, the true comparison lies in the hands of the farmers who have to make such decisions. For them, uncertainty and risk aversion play a major role in the choice between alternative control methods.

2.1 The Impact of Uncertainty on Pest Management Decisions

For quite a long time, it was intuitively argued in the literature that uncertainty increases pesticide use (Carlson, 1970; Norgaard, 1976). This argument is based on the scenario that when a risk averse farmer observes the slightest sign of pest, he does not know whether it will simply die out, or will grow and destroy his crop. Consequently, farmers rather apply a relatively inexpensive pesticide, rather than take the risk. In this sense standard pest management procedures are much like insurance policies (Mumford and Norton, 1986). Several empirical works support the hypothesis of increased pesticide use resulting from uncertainty (*e.g.* Carlson, 1979; Cochran, 1985), but others showed no consistent increases or reductions of pesticide use resulting from risk consideration (Greene *et.al.*, 1985; Plant, 1986; Moffitt, 1986). Contrary to the effect of price uncertainty, that reduces firm's output and inputs (Sandmo, 1971), Feder (1979) proved theoretically that uncertain pest density, or uncertain damage per pest, induces higher pesticide use. However, if the efficiency of the pesticide is uncertain, pesticide use may either increase or decrease. Regev (1988) shows that uncertainty in the efficiency of pesticides is likely to increase pesticide use, however, uncertainty in the damage–pest relationship is likely to reduce it.

Uncertainty has an undisputable effect on the choice of pest control strategies. Clearly when risk is increasing, pest control strategies which are risk reducing are more likely to be selected, even if they yield slightly lower expected profits than others which bring higher but more uncertain profits. The loss in expected profits by the selected strategy could be regarded as insurance premium. However, many strategies which quickly reduce pests, such as chemical pesticides, are accepted by farmers as risk reducing, but may in fact increase risk in the long run as a result of increased resistance and disruption of the natural ecosystem. Here again, the long term risk increasing effects of pesticide use result from the combined action of all pesticide users and not individual action, which therefore cause the farmer to ignore them. This impact, is of particular importance, since it calls for a specific set of remedies by the government.

Crop insurance against pest damage would appear to lower risk and help farmers to select environmentally safer pest control strategies, and would seem to be a solution. However, crop insurance may cause a risk averse farmer to adopt a riskier cropping program, thus exacerbate not reduce the problem (Lazarus and Swanson 1983). This is known in the economic literature as the moral hazard effect by which acquisition of insurance changes the behavior of the decision makers so that unfavorable events are more likely to occur.

3 Farmers response to risk in different economic systems

Risk in agriculture arises either from fluctuations in natural conditions or in markets. In order to reduce the risks arising from natural conditions, farmers increase the use of pesticides and other chemicals, as explained above. The different agricultural policies in each of the three economic systems determine the extent of market instability and their effects on the farmers in each country. The economic situation of the farmers in different systems concerning the use of chemicals is now discussed assuming no biological-technological progress. Concerning production and market sources of risk in agriculture, the farmers behavior differs in the three economic systems:

1. In the Market Economies farmers are protected against the world markets fluctuations by guaranteed fixed prices on a relatively high level. They determine production quantities accordingly at high levels, and minimize production risks by overuse of low cost chemicals.

2. In the Centrally Planned Economies agriculture is also decoupled from world markets and prices are stabilized, but on a low level. Because of the socialist ideology, the producer incomes depend only partly on the agricultural output, which together with the often insufficient supply of chemicals, explains the lack of farmers interest to use such inputs in order to lower risk.

3. In the Developing Countries farmers face both the risk of being exposed to unstable world markets and that of discrimination by national price policy against agriculture. Most farmers are too poor to buy the necessary chemicals, whereas large farmers cultivate the overwhelming part of total arable land in a very extensive way, using cheap labor and land. Therefore the use of chemicals is limited to large plantations producing for export to the world markets (e.g. tropical fruits and beverages).

The above scenarios are likely to change in the near future following the currently going negotiations in GATT (the Uruguay Round). These are expected to result in a certain reduction, or at least a freeze, in the protection of agriculture. Consequently, guaranteed real prices will be lowered in the Market Economies, resulting in diminishing food surpluses in the industrial countries. Direct payments linked with ecologically motivated restrictions in production may be accepted by governments in the future as the policy measure for maintaining a minimum income for farmers. Secondly, the introduction of market elements in Centrally Planned Economies (especially USSR and China) will increase producer prices and raise agricultural production.

3.1 Agricultural options for the use of chemicals

The reduction of surpluses in the Industrial Countries and the less erratic demand of Centrally Planned Economies should increase the prices in agricultural world markets, and the farmers will face the following new situations:

1. Market Economies: The larger share of directly transferred income to the farmer disconnects his income from the risk of fluctuating yields. Considering social pressure and restrictions of certain chemicals, this will lead to a reduction in the use of chemicals.

2. Centrally Planned Economies: Price incentives will both improve the supply of inputs and the marketing channels necessary to increase production. If governments provide such services the use of chemicals will rise.

3. Developing Countries: Increased prices on the world market provide incentives to increase production. Nonetheless, poor farmers cannot increase their production without better access to resources - first of all land, then water and capital. However, an increase in chemicals usage can be expected if agrarian reforms take place.

Generally it can be concluded that stable incomes decoupled from fluctuating yields and governmental restrictions on the use of chemicals will diminish the use of these inputs, conceived by the farmers to be risk reducing inputs in agricultural production. There is some evidence of such future tendencies in the Market Economies. In the other parts of the world a likely increase of the use of chemicals depends on various preconditions of agricultural policy, among which the political will of the bureaucratic state class to implement agricultural reforms is preponderant.

Finally, it should be observed that natural ecosystems are adapted to long term sustainability, while agricultural ecosystems, in contrast, are managed for short-run profit maximization. To develop sustainable agriculture, the agroecosystem must be maintained with a long term perspective. This can be accomplished only through a forward looking national agricultural policy. Anything less will results in depletion of the nonrenewable agricultural resources (Gutierrez and Regev, 1983).

Table 1: Characteristics of Economic Systems

Economic systems / Criteria	Market Economies	Centrally Planned Economies	Bureaucratic Developing Societies
Decision Making	Decentralized; individual profit and utility maximization; private ownership of production resources	Centralized; collective ownership of production resources; partly private use of land	Both, centralized and decentralized; private and public property of production resources plus traditional collective forms of property
Motivation system	private profits	solidarity insufficient; therefore replaced by an individual incentive system	various value-systems: traditional and imported; distribution on the basis of personal relations
Co-ordination	market; competition; governmental intervention in markets.	planning; closed economy; different price systems; some market elements	central planning and price-systems; barter and monetary economy
Control systems	market mechanism; increasing governmental intervention	within bureaucracy	lacking control of bureaucracy by markets or political system

Appendix

The Pollution Problem

In this Appendix it is shown how pollution and resistance lead the farmer to increase pesticide use beyond the socially optimal levels.

The individual farmer's problem is:

$$\max_{\vec{x}} \pi(\vec{x}) = \max_{\vec{x}} P \cdot Y(\vec{x}) - \sum_{j=1}^{J} w_j x_j \qquad (1)$$

where:
P is the product price
$\pi(\vec{x_i})$ is the profit function
$\vec{x} = x_1, \ldots, x_J$ are the production inputs
$Y(\vec{x})$ is the production function
w_j is the cost per unit of the j-th input, where $j = r$ denotes pesticide input

Necessary conditions for internal solution:

$$P \cdot \partial Y / \partial x_j - w_j = 0, \text{ for all } j = 1, \ldots, J \qquad (2)$$

When the production function $Y(\vec{x})$ is assumed to be concave, the conditions in (2) are also sufficient, which implies that $P \cdot \partial Y / \partial x_j$ is a decreasing function.

The society includes pollution as part of the cost of producing food, thus its problem is:

$$\max_{\vec{x_i}} \sum_{i=1}^{I} \pi_i(\vec{x_i}) - L(\sum_i x_{ir}) \qquad (3)$$

where:
$\vec{x_i} = x_{i1}, \ldots, x_{iJ}$
x_{ij} is the j-th input applied by the i-th farmer, $i = 1, \ldots, I$
x_{ir} is a chemical pesticide input applied by the i-th farmer
$L(\cdot)$ is the costs of pollution to the society, and $\partial L / \partial x_{ir} > 0$
$\pi_i(\cdot)$ is the profit of the i-th farmer.

Necessary conditions for internal solution are

$$\partial \sum_i \pi_i(\vec{x}_i) / \partial x_{ij} = P \cdot \partial Y_i / \partial x_{ij} = \begin{cases} w_j & \text{for all } i,\ j \neq r \\ w_r + \partial L / \partial x_{ir} & \text{for all } i,\ j = r \end{cases} \qquad (4)$$

Since $P \cdot \partial Y / \partial x_j$ is a decreasing function, comparing (2) for j=r with the second line of (4), the individual solution for chemical pesticide (x_r) obtained from equation (1), is greater than the social solution obtained from equation (3).

The Resistance Problem

Since for many pest problems pest resistance to pesticides develops in relatively long time period, we deal with a multi-seasonal pest management problem. The individual farmer's problem:

$$\max_{\vec{x}_t} \sum_t \beta_t [P \cdot Y(\vec{x}_t, R_t) - \sum_j w_j x_{tj}] \tag{5}$$

where:
$\vec{x_t} = x_{1t}, \ldots, x_{jt}, \ldots, x_{Jt}$
x_{jt} is the j-th input applied at the t-th season

The objective of farmers' cooperative, on the other hand, takes into consideration the collective effect on resistance:

$$\max_{\vec{x}_{1t},\ldots,\vec{x}_{It}} \sum_t \beta_t \sum_{i=1}^{I} [P \cdot Y_i(\vec{x}_{it}, R_t) - \sum_j w_j x_{ji}] \tag{6}$$

Subject to:

$$R_{t+1} = g(\sum_{i=1}^{I} x_{rit}, R_t) \tag{7}$$

where:
$\vec{x_{it}} = x_{it1}, \ldots, x_{itj}, \ldots, x_{itJ}$
R_t is the 'stock' of pest resistance at the beginning of season t
$g(\sum_{i=1}^{I} x_{rit}, R_t)$ is resistance development, and
$\partial g / \partial x_{rit}) > 0$

The individual farmer does not consider $g(\cdot)$ in his pest management decisions, since it is a function of the collective action of his fellow farmers, and his decision has only negligible effect. This is especially true when there are many farmers and the pest in question is mobile between seasons.

Necessary conditions for the solution of (5):

$$P \cdot \partial Y / \partial x_{jt} = w_j \quad \text{for all } j = 1, \ldots, J; \text{ and for all } t. \tag{8}$$

Necessary conditions for the solution of (6):

$$P \cdot \partial Y / \partial x_{jit} = \begin{cases} w_j, & \text{for all } i, t; \text{ and for all } j \neq r \\ w_r \quad + \lambda_t \cdot (\partial g / \partial x_{rit}) & \text{for all } i, t; \text{and } j = r \end{cases} \tag{9}$$

Where λ_t is the shadow price of resistance 'stock', and is thus non-negative. Comparing (8) with (9), it is clear that here again, the collective solution yield a pesticide use level which is lower than the private solution. The analysis of this resistance problem is similar to that of the former pollution problem, and Figure 1 illustrates this problem. However, in this case, the farmers themselves are damaged (in the long run), and their lack of concern to resistance development may rise from short sight approach, but also from lack of institutional arrangements for collective action.

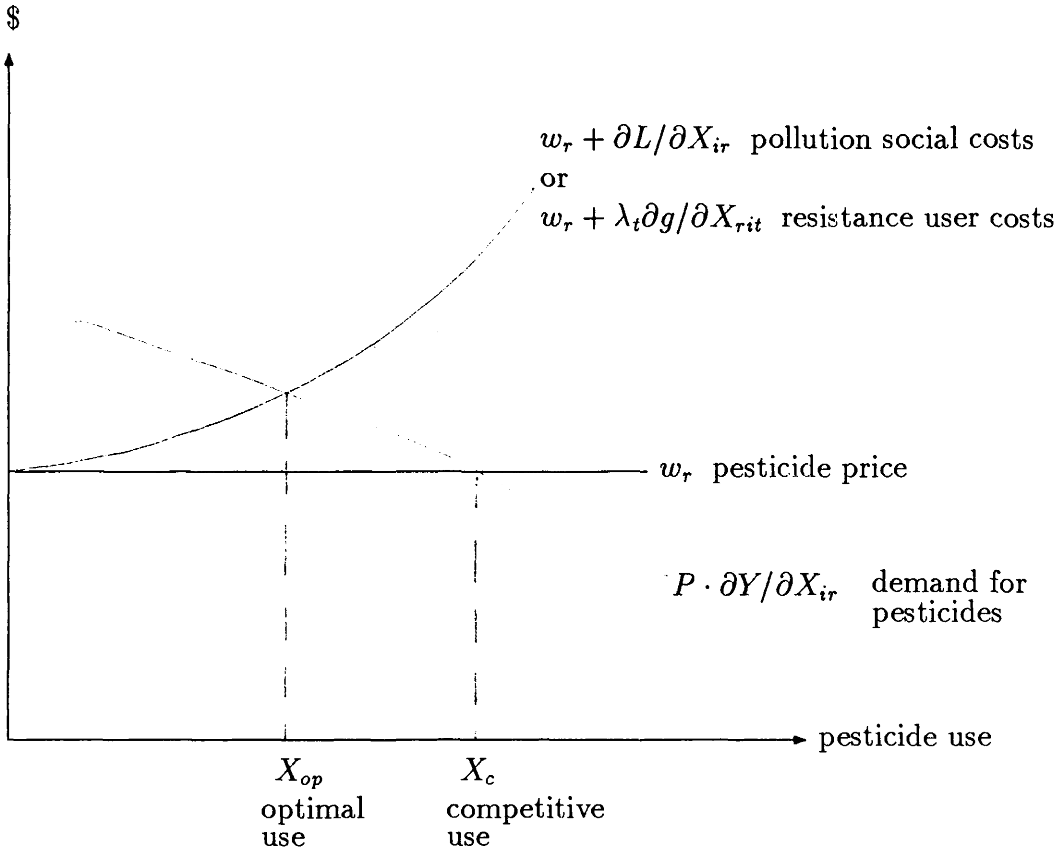


Figure 1: The effect of externalities on overuse of pesticides

References

[1] Anderson, K. & Ayami, Y. - 1986. *The Political Economy of Agricultural Protection: East Asia in International Perspective.* Allen & Unwin, Sidney.

[2] Antle, J.M. - 1987. Econometric estimation of risk p¨oducers' risk attitudes. *American Journal of Agricultural Economics*, 69:509–522.

[3] Antle, M.J. - 1988. *Pesticide Policy, Production Risk, and Producer Welfare.* Resources for the Future, Washington, D.C.

[4] Batra, N.R. & Ullah, A. - 1974. Competitive firm and the theory of input demand under price uncertainty. *Journal of Political Economy*, 82:537–548.

[5] Baumol, W. & Oates, W. - 1975. *The Theory of Environmental Policy:Externalities, Public Outlays, and the Quality of Life.* Prentice Hall, Englewood Cliffs, New Jersey.

[6] Carlson, G.A. - 1970. A decision–theoretic approach to crop disease prediction and control. *American Journal of Agricultural Economics*, 52:216–222.

[7] Carlson, G.A. - 1979. Pest control risks in agriculture. In J.A. Roumasset, J. Boussard, and I. Singh, editors, *Risk, Uncertainty and Agricultural Development.*, pages 199–209, College, Laguna, Philippines; Southeast Asian Regional Center for Graduate Study and Research in Agriculture. New York: Agricultural Development Council.

[8] Cochran, M.J., Robison, L.J. & Lodwick, W. - 1985. Improving the efficiency of stochastic dominance techniques using convex set stochastic dominance. *American Journal of Agricultural Economics*, 67:289–95.

[9] Cropper, M., Lee, D., & Pannu, S. - 1979. The optimal extinction of a renewable natural resource. *Journal of Environmental Economics and Management*, 6:341–349.

[10] Egger, U. - 1988. *Agrarstrategien und ihre Erfolgsbeurteilung mit Hilfe eines Indikatorensystems.* PhD thesis, ETH, ETH–Diss. No. 8613, Zürich.

[11] Feder, G. -1977. The impact of uncertainty in a class of objective functions. *Journal of Economic Theory*, 11:504–512.

[12] Feder, G. - 1979. Pesticides, information, and pest management under uncertainty. *American Journal of Agricultural Economics*, 61:97–103.

[13] Feder, G. & Regev, U. - 1975. Biological interactions and environmental effects in the economics of pest control. *Journal of Environmental Economics and Management*, 2:75–91.

[14] Gardner, B. - 1988. Recent studies of agricultural trade liberalization. In *XX International Conference of Agricultural Economists*, International Association of Agricultural Economists, Buenos Aires, Argentina.

[15] Greene, C.R., Kramer, R.A., Norton, G.W., Rajotte, E.G., & McPherson, R.M. - 1985. An economic analysis of soybean integrated pest management. *American Journal of Agricultural Economics*, 67:567–72.

[16] Gutierrez, A.P., Regev, U. & Shalit, H. - 1979a. An Economic Optimization Model of Pesticide Resistance: Alfalfa Weevil – An Example. *Environmental Entomology*, 8:101–107.

[17] Gutierrez, A.P., Wang, Y. & Regev, U. - 1979b. An optimization model for lygus hesperus (*Heteroptera: Miriadae*)damage in cotton:the economic threshold revisited. *The Canadian Entomologist*, 111:41–54.

[18] Gutierrez, A.P. & Regev, U. - 1983. The Economic of Fitness and Adaptedness: The Interaction of Sylvan Cotton (*Gossypium hirasutum L.*) and the boll weevil (*Anthonomus grandis Boh.*). An Example. *Acta Œcologica/Œcologia Generalis*, 4:271–87.

[19] Hall, D.C., & Norgaard, R.B. - 1973. On the timing and application of pesticides. *American Journal of Agricultural Economics*, 55:198–201.

[20] Hayami, Y. - 1988. *Japanese Agriculture under Siege: The Political Economy of Agricultural Economy.* MacMillan, London.

[21] Headley, J.C. - 1972. Defining the Economic Threshold. In R.L. Metcalf, editor, *Pest Control Strategies for the Future*, National Academy of Science, Washington, D.C., 100–108.

[22] Heimlich, R.E., & Ogg, C.W. - 1982. Evaluation of soil-erosion and pesticide-exposure control strategies. *Journal of Environmental Economics and Management*, 9:279–88.

[23] Hueth, H. & Regev, U. - 1974. Optimal agricultural pest management with increasing pest resistance. *American Journal of Agricultural Economics*, 56:543–52.

[24] Huffaker, C.B., (ed.) - 1980. *New Technology of Pest Control*, Wiley & Sons, New-York and Toronto.

[25] Huffaker, C.B. & Smith, R.F. - 1980. Rationale, Organization, and Development of a National Integrated Pest Management Project. in C. B. Huffaker, editor, *New Technology of Pest Control*, Wiley & Sons, New-York and Toronto.

[26] Jörin, R. & Rieder, P. - 1984. Economic effects of political decisions in agricultural markets on producers, consumers, and taxpayers. In *Proceedings of IV-th European Congress of Agricultural Economics*, Kiel.

[27] Kettunen, L. (ed.) - 1987. *Supply Management by Government in Agriculture*, Wissenschaftsverlag Vauk Kiel.

[28] Lazarus, W.F. & Dixon, B.L. - 1984. Agricultural pests as common property: control of the corn rootworm. *American Journal of Agricultural Economics*, 66:456–65.

[29] Lazarus, W.F. & Swanson, E.R. - 1983. Insecticide use and crop rotation under risk: rootworm control in corn. *American Journal of Agricultural Economics*, 65:738–47.

[30] Lichtenberg, E. - 1987. Integrated versus chemical pest management: the case of rice field mosquito control. *Journal of Environmental Economics and Management*, 14:304–312.

[31] Lichtenberg, E. & Zilberman, D. - 1986a. The welfare economics of regulation in revenue–supported industries. *American Economic Review*, 76:1135–1142.

[32] Lichtenberg, E. & Zilberman, D. - 1986b. The Econometric of Damage Control: Why Specification Matters. *American Journal of Agricultural Economics*, 62:261-273.

[33] McCalla, A.F. & Josling, T.E. - 1985. *Agricultural Policies and World Markets.* Macmillan, New York and London.

[34] Miranowski, J.A. - 1980. Estimating the relationship between pest management and energy prices, and the implications for environmental damage. *American Journal of Agricultural Economics*, 62:995–1000.

[35] Moffitt, I., Hall, D. & Osteen, C. - 1984. Economic thresholds under uncertainty with application to corn nematode management. *Southern journal of agricultural economics*, 16:151–157.

[36] Moffitt, L.J. - 1986. Risk–efficient thresholds for pest control decisions. *Journal of Agricultural Economics*, 37:69–75.

[37] Mumford, J. & Norton, G. - 1986. Economics aspects of integrated pest management. In V. Delucchi, editor, *Integrated Pest Management Protection Integree QUO VADIS?*, Parasitis, Geneva, Switzerland.

[38] Mundlak, Y. - 1988. Agricultural growth and world development. In *XX International Conference of Agricultural Economists*, International Association of Agricultural Economists, Buenos Aires, Argentina.

[39] Norgaard, R.B. - 1976. Integrated economics and pest management. In J. C. Apple and R. F. Smith, editors, *Integrated Pest Management*, Plenum, New York.

[40] OECD - 1987. National policies and agricultural trade. Paris.

[41] Pontryagin, L.S., Boltyanskii, R.V., Gambreldzi, R.V. & Miskenker, E.F. - 1964. *The Mathematical Theory of Optimal Processes*, Pergamon Press, Oxford.

[42] Plant, R.E. - 1986. Uncertainty and the economic threshold. *Economic Entomology*, 79:1–6.

[43] Regev, U. - 1976. Economic conflicts in plant protection: individual *vs.* social decisions. *EPPO Bulletin*, 6:265–273.

[44] Regev, U. - 1988. The impact of risk on pest management strategies. Monaster Center discussion paper.

[45] Regev, U., Gutierrez, A.P. & Feder,G. - 1976. Pests as a common property resource: a case study of alfalfa weevil control. *American Journal of Agricultural Economics*, 58:186–97.

[46] Rieder, P. - 1985. Experience with milk quota systems in Switzerland. *European Review of Agricultural Economics*, 12:473–479.

[47] Rieder, P. & Egger, U. - 1987. International agricultural markets and malnutrition in the third world. *Food Review International*, 3:33–69.

[48] Sandmo, A. - 1971. On the theory of competitive firm under price uncertainty. *American Economic Review*, 61:65–73.

[49] Sarhan, M.E., Howitt, R.E. & Moore, C.V. - 1979. Pesticide resistance externalities and optimal mosquito management. *Journal of Environmental Economics and Management*, 6:69–84.

[50] Schultz, T. - 1978. On economics and politics of agriculture. In T. W. Schultz, editor, *Distortions of Agricultural Incentives*, Bloomington: Indiana University Press.

[51] Shoemaker, C.A. - 1973. Optimization of agricultural pest management i, ii, and iii. *Mathematical Bioscience*, 16:143–75, 17:357–65, 18:1–22.

[52] Shoemaker, C.A. - 1981. Applications of dynamic programming and other optimization methods in pest management. *IEEE Transactions on Automatic Control*, ac-26:1125–1132.

[53] Shoemaker, C.A. - 1982. Optimal integrated control of univoltine pest populations with age structure. *Operations Research*, 30:40–61.

[54] Talpaz, H. & Borosh, I. - 1974. Strategy for pesticide use: frequency and applications. *American Journal of Agricultural Economics*, 56:769–75.

[55] Taylor, C.R. & Headley, J.C. - 1975. Insecticide resistance and the evaluation of control strategies for an insect population. *Canadian Entomology*, 107:237–42.

[56] Van den Bosch, R. - 1978. *The Pesticide Conspiracy.* Doubleday & Company Inc., Garden City, New-York.

[57] Von Neuman, J. & Morgenstern, R. - 1953. *Theory of Games and Economic Behavior.* Princeton University Press, Princeton, New-Jersey.

[58] World-Bank - 1986. World development report. Washington D.C.

Report on the Session 2: INFORMATIC

D. J. Peix
Producción e Industrias Agroalimentarias, Generalidad de Cataluña-Barcelona, Spain

A. P. Gutierrez
Division of Biological Control, University of California-Berkeley, USA

The four papers in this session dealt with the role of mathematics and computers in the future of agricultural pest management, with an emphasis on improving decision making by farmers and farm policy makers. The session began with a view of a worldwide agriculture of the future, in which computer-based information played a key role in coordinating and enhancing the profitability of international agriculture. We then heard about some of the computer software that will be needed to make this vision of the future a reality: expert systems, artificial intelligence applications and computer simulation models of crop-pest agroecosystems. Last, we discussed the economics of agricultural pest management, focusing on the need for better incentives to bring pest management practices more in line with the long-term goals and needs of society.

In this vision of the future presented by Dr. Holt, farms of the future will have access to computer and communication technology that will unite the components of what is today the most distributed of all major industries, agriculture. Coordinating such a vast and dispersed industry as agriculture will certainly create the potential for better planning, better dissemination of information and pest management advice, and a truly global agricultural system. However, this vision is dependent on the development of computer software systems to deliver better expertise and ecosystem-level understanding of pest management systems.

A system-level understanding of agricultural pest management can come only from basic research and the development of simulation models of crop--pest systems. In Dr. Stone's presentation, a review of past and current research in the development of expert systems for agriculture, he stressed the need for building such models and linking these models with reasoning programs or experts systems to help farmers make more informed decisions about managing their crops. By developing expert systems that incorporate models of the natural system, and that thereby can determine the fundamental causes of pest problems, we can design integrated pest management programs that act to prevent problems from reaching the point of economic loss, rather than developing programs that tend to respond only after a problem has appeared.

The third presentation focused on the state-of-the-art in developing computer simulation models for pest systems. Ultimately, our ability to develop expert decision aids for farmers today and in the future depends on the creation of valid computer models of agroecosystems. Dr. Van der Werf discussed the development of pest models in crop systems in which spatial heterogeneity is present. This research represents a dramatic new emphasis in simulation, linking concepts of behavioral modelling at the individual level with classic population-level modelling, and providing a framework for the analysis and interpretation of the spatial patterns of pest damage and spread in agricultural areas.

Finally, we discussed the constraints inherent in pest management due to economic forces. Dr. Regev clearly demonstrated that due to the uncertainty of agricultural production and the externalities in our market systems, the economic goals of individual farmers are fundamentally at odds with the long term goals of society. Pest management policies adopted by farmers tend to be more polluting than people as a whole would like, yet legislative regulations to correct this imbalance have proven virtually impossible to implement. Unless this basic conflict between society and its agricultural producers can be resolved, pest management programs of the future will continue to pollute at unacceptable levels.

In conclusion, pest management programs of the future will be increasingly supported by computer simulation models, expert advisory programs, and an international information network. Farmers, however, even provided with the best and most current information on their industry will still tend to be at odds with society regarding their apparent overuse of pesticides. Information systems and computer networks of the future must therefore be supported by innovative and enlightened agricultural policies to create an agricultural system that is in harmony with long-term ecological and economic stability.

SESSION 3

APPLICATION, USE AND TRANSMISSION OF KNOWLEDGE

Chairmen: J. Baumgärtner
D. F. González

THE 10 YEAR RESEARCH AND DEVELOPMENT PLAN FOR THE APPLICATION OF REMOTE SENSING IN AGRICULTURE STATISTICS

M. J. Meyer-Roux
Joint Research Centre, Commission of the European Communities-Ispra, Italy

MOTIVATIONS AND OBJECTIVES

Important decisions concerning agriculture are made by the Commission -on the European level, but the quantity and the quality of agricultural information, especially on crop production, is not always adequate to its uses.

This is mainly due to the fact that despite the progress which has already taken place in the integration of Community statistics, national systems of agricultural statistics still differ in their approach to conventional surveys and the resources they have available.

Remote Sensing seems to be the most promising technique for upgrading the agriculture statistical system. In order to achieve rapid improvements, avoid duplications of work as well as methods which differ too greatly, the Commission is setting up a R & D project aimed at the introduction of remote sensing in the E.C. statistics system for agriculture. The Joint Research Centre is responsable for this plan, the guidelines being defined according to the priorities of DG VI and Eurostat.

The primary necessity is to distinguish, identify and measure the area of crops of significant importance. The second is to estimate production in time to make decisions. When such basic statistics are available, then forecasting can be considered.

The results of this plan should fit into a framework which we can call an Advanced Agriculture Information System (see Figure 1). This system will be based on new information such as remote sensing data from high resolution and low resolution satellites and on more elaborate methods of interpretation such as agrometeorological models.
In the research plan, emphasis will be placed upon remote sensing, but in operational use this technique is seen as complementary to more conventional data. The semi-operational projects within the plan will test this kind of complementary.

The R, D & D plan

While the R, D & D effort is thought to extend over 10 years, this proposal considers only the 5 first years in some detail. During this period, the plan should already lead to an integration of new methods in the E.C. agriculture information system. Priority is therefore given to conditions where remote sensing by existing sensors can lead to operational use. Cereals and oil seeds which generally grow on flat terrain and large fields will be considered first. When feasible, vineyards and olive trees will also be dealt with because of their economic importance.

Taking into account priorities, methods to be developed and requirements of satellite data, the plan has been organized in 7 actions (Table I). Actions 1-5 each correspond to a given operational objective. Action 6 is a support (ground surveys) necessary to all the actions. Action 7 consists of several long-term research items, not connected with one particular operational objective.

For each action, a specific method will be developed and research will be undertaken in support of the semi-operational objective (actions 1-2-4-5) or as the very core of the action (actions 3-7): see Table 2.

In respect to the shift towards operational applications, the actions concern three different time schedules:

- Short term (3-5 years)
  precise inventories of acreage at the regional level (action No. 1), monitoring and alarm for specific crops (action No. 2) and rapid estimates on the European scale (action No. 4).
- Medium term (5-10 years)
  improvement or development of yield prediction models (action No. 3), integration of several methods and data handling chains (action No. 5).
- Long term (more than 10 years)
  use of all weather sensors, GIS and expert systems (action No. 7).

The shift towards the operational applications is visualized as essentially progressive, through a geographical extension of the test zones considered in the semi-operational phase.

After about 5 years:

- some techniques will be sufficiently developed that they can be implemented at OSCE or in the national statistical offices.
- some long-term research will have to be continued in the JRC.
- most of the methods will be nearly operational but in comparison with conventional surveys will require higher technical skills. These methods will be implemented in the structure which will seem the most suitable. It could be either the JRC, OSCE or a private contractor of DG VI.

The financial contribution to this plan should be sufficient to cover the work of approximately 12 scientists within the remote sensing programme of the JRC plus contracts for research and semi-operational projects. The part covered by contracts will differ according to specific actions described in the following sections and will also change in time. As an average, approximately two third of the total contribution should be carried out through contracts or cost shared actions.

1. ACTION No. 1 : REGIONAL INVENTORIES

1.1 Aim

The purpose of this action is to obtain precise annual inventories for the main crops of interest at the regional level.

FIGURE 1 - Diagram of an Advanced Agriculture Information System.

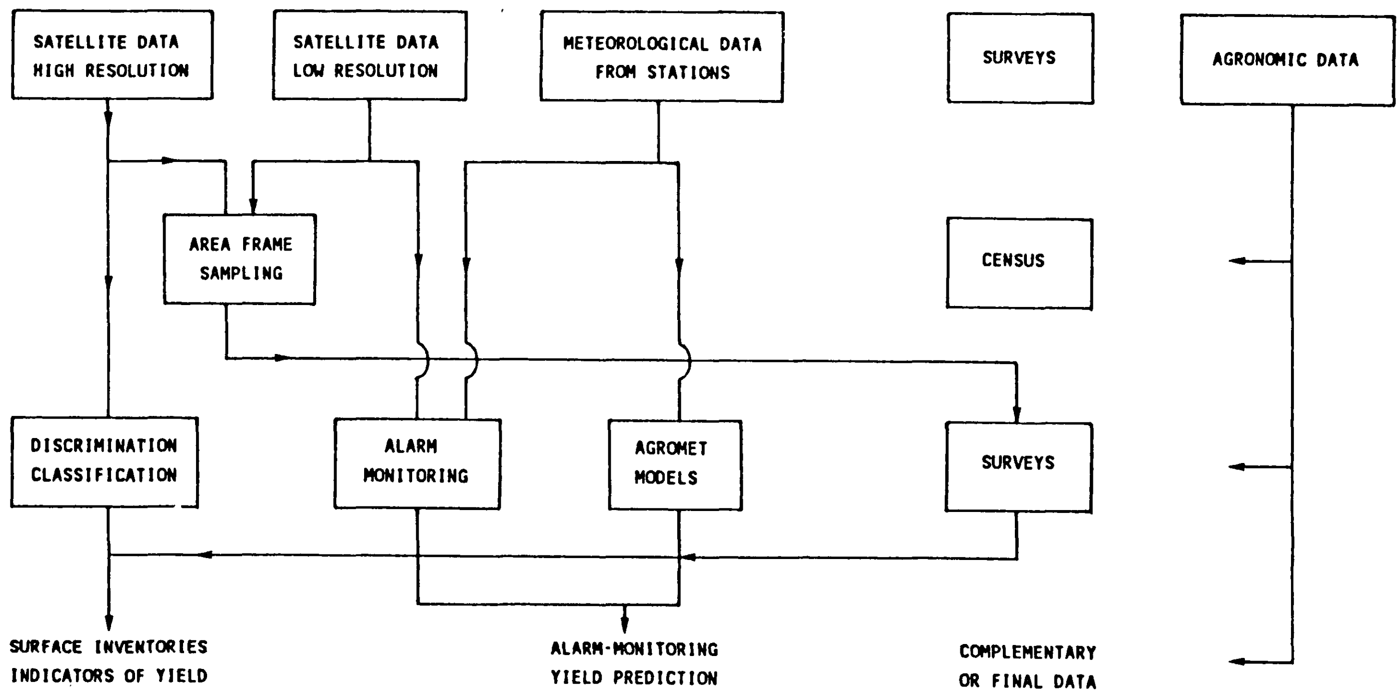


TABLE I - Outline of the agriculture project.

ACTION	MAIN ORIENTATION	GEOGRAPHIC LOCALIZATION	INPUT	OUTPUT
1. Regional inventories (acreage).	Semi-operational.	3-4 selected administrative regions.	High resolution satellite data.	Precise regional inventories (plus localized statistics).
2. Vegetation conditions and yield indicators.	Semi-operational plus research.	Selected regions and sampling of sites, then all of Europe.	Low resolution satellite data (mainly AVHRR).	Vegetation monitoring, alarm for main crops.
3. Models of yield prediction.	Research.		Low resolution satellite data. High resolution satellite data. Meteorological data.	Yield prediction.
4. European rapid estimates of acreages and potential yield.	Semi-operational.	Sampling of some 40 or 50 sites through Europe.	High resolution satellite data.	Estimates of surface and prediction of yield at European level for main crops.
5. Advanced agriculture information system.	Semi-operational and research.	Sampling of some 40 or 50 sites through Europe.	All data available.	Integration of preceding methods with conventional ones.
6. Area frame sampling associated surveys.	Support for other actions.	Whenever it is needed, mainly on 3-4 regions and 40-50 sites.	Satellite imagery to built area frame.	Support documents or data for other action.
7. Long-term research.	Research.		New satellite data (microwaves..). New analysis methods (expert systems, GIS..).	Improved inventories and yield prediction.

TABLE II - Methods and specific research.

ACTION	METHOD	ACCURACY STATEMENTS	SPECIFIC RESEARCH
1. Regional inventories.	Regression estimate.		Improved classification and sampling strategies.
2. Vegetation conditions and yield indicators.	Spatial or temporal comparison of V.I. and T_s (integrated indices..).	Comparison with conventional methods.	- Radiometric + geometric correction - Resolution needed - Modelling of curves - Evapotranspiration.
3. Models of yield prediction.	- Improvement of actual agromet models . better repres. of stations. . improving the data. - Integration of sat. data in agromet models. - Deriving agronomic parameters (ETR). - Direct relationship.	Comparison with conventional methods.	Special emphasis on vine and olive production for conventional models.
4. Rapid European estimates of acreages and potential yield.	Computer - assisted photo-interpretation.	Assessment by specific sampling surveys.	
5. Advanced agriculture information system.	Integration of all available results.	Economic comparisons between methods.	
6. Area frame sampling associated surveys.	Stratification - segmentation - sample-surveys on site or surveys with farmers.	Accuracy statements of inventories.	- Automatized procedures. - Sampling scheme. - Type of surveys.
7. Long term research.	- Using new sensors with the preceding methods. - GIS - expert systems.	Comparison of results or timeliness.	

TABLE III - Cost of the project: Evolution of the specific credits of the project, taking 1992 as a reference.

ACTIONS	1987		1988		1989		1990		1991		1992	
	STAFF JRC	SPECIFIC CREDITS (K Ecus)	STAFF JRC	SPECIFIC CREDITS (K Ecus)	STAFF JRC	SPECIFIC CREDITS (K Ecus)	STAFF JRC	SPECIFIC CREDITS (K Ecus)	.STAFF JRC	SPECIFIC CREDITS (K Ecus)	STAFF JRC	SPECIFIC CREDITS (K Ecus)
No. 1	0.5	60	3	32	3	20	3	18	3	17	3	16
No. 2	0.5	30	2	17	2	25	2	22	2	21	2	20
No. 3	-	-	1	7	1	12	1	16	1	16	1	15
No. 4	-	-	1	19	1	20	1	20	1	24	1	26
No. 5	-	-	2	10	2	10	2	12	2	12	2	13
No. 6	-	-	1	-	1	-	1	-	1	-	1	-
No. 7	-	10	-	15	-	13	-	12	-	10	-	10
Coordination + support	1	-	2	-	2	-	2	-	2	-	2	-
Specific credits: % of the 1992 level		100 46		100 67		100 80		100 90		100 95		100 100
Number of staff	2		12		12		12		12		12	

- Specific credits would not include cost of the staff at the JRC
- The cost would increase progressively from 1987 to 1992

When possible localized statistics and crop maps will be obtained as a byproduct.

1.2 Method

The only known method which can give an estimate with an accuracy statement is the "regression estimate" method. It combines ground observations on segments of 50 to 100 ha. with a complete classification of high resolution satellite data.

Such a regression estimate not only gives an assessment of accuracy with the final estimate but takes care of slight differences between what is observed by satellite and the abstract definition of the category or class associated with the statistic.

1.3 Input data

This methods requires a random sample of segments for the given region or strata of the region. The ground observations have to be recorded geometrically.

Complete coverage of the region with high resolution satellite data (TM, Spot) is needed, incompleteness giving generally poor results.

1.4 Accuracy statements

The methodology used generates an accuracy statement. To derive the economic usefulness of remote sensing analysis, prices of images and survey costs must be integrated. Taking the French study as a guideline, to be cost effective the efficiency of the technique must be at least 2. This comparison is of course irrelevant when a very cheap accurate method can be used, such as a mail census, to obtain regional inventories.

If timeliness is not the main objective, results should be obtained before the 1st November so that they can be integrated in the final estimates which are generally made at the end of the crop year.

Localized statistics and crop maps will be considered as an added advantage of the method if they can be obtained.

1.5 Specific research

This action can be considered as semi-operational since it is in use in Canada and the U.S.A. The efficiency of the method must however be studied according to:

- type of crops
- size and shape of field
- relief
- phenological calendar
- cloud cover
- number of coverage with satellite images
- type of sampling strategies
- improved classifications (textural - per field ...).

1.6 Where and how to test this method

This action No. 1 will be carried out in 3 to 4 administrative regions of the European Community (Nuts 2) of approximately 20.000 km^2 with estimates determined also for districts (Nuts 3) included in this region.

It should be subcontracted, the role of JRC being to coordinate the action. JRC will also develop new means of classifications and in general the research needed as well as portable programs which could be implemented in statistical offices.

After the first three years, action No. 1 will continue on a shared costs basis so that with the same amount of money 1 region more should be included each year (6 in 92).

2. ACTION No. 2 : VEGETATION CONDITIONS AND YIELD INDICATORS

2.1 Aim

The purpose of this action is to demonstrate the usefulness of low resolution satellite data, to monitor the vegetation, to set an alarm for crops and to obtain yield indicators for some crops.

2.2 Method

The two essential indicators which can be derived from meteorological satellites such as the NOAA' series with AVHRR sensors are the vegetative index and the surface temperature.

The vegetative index, particularly the normalized difference, can be used:
- for a broad stratification according to land use (1)
- to determine phenological stages for groups of crops (2)
- to detect an abnormal development or state of the vegetation such as a drought (3).

The surface temperature can be used:
- to give an improved spatial representation of air temperature given by meteorological stations, or even to replace air temperature for some applications (4)
- to determine critical situations such as frost (5)
- to determine or derive real evapotranspiration through a better knowledge of the water budget (6).

Most of these applications use a spatial comparison of data, particularly (1) and (4). Most of them need a temporal comparison, especially (2) and (3). Alarm monitoring can sometimes be carried out using only AVHRR data with this kind of spatial or multitemporal comparison. Others will need to incorporate external data such as air temperature, especially for stress related to evapotranspiration such as the integrated stress-degree-day method.

2.3 Input data

These methods require frequent data of the vegetative index at 14h30 and the surface temperature at 2.30 and 14.30. Raw data from AVHRR cannot be used directly; they must be radiometrically and geometrically corrected.

For most applications, weekly or ten day data should be sufficient (except for frost, ...) but to obtain such a product daily data are necessary because of cloud coverage.

The requirements for the resolution is 1 km for specific products. For general purpose ones a 5 or 10 km resolution is more suitable. This will have to be defined according to the application.

For meteorological applications there were no needs for archives and most of the receiving stations did not have such a service. On the other hand, the defined method for agricultural applications requires historical data.

2.4 Accuracy statements

For each kind of application some comparison will have to be made with more conventional methods. In general accuracy statements are difficult because of the low resolution and the difficulty of having representative ground observations for 1 km^2 or more.

2.5 Specific research

One field of research will be to improve the quality of the vegetative index and the surface temperature (radiometric and geometric corrections).
- Another important one will be to assess the best resolution needed for different kinds of applications.
- Research in modelling normal curves of vegetative indexes (V.I.) should be a way of detecting changes or abnormal conditions. This should be related with a better comprehension of the significance of such data (Ts. and V.I.).
- Deriving real evapotranspiration should be an important goal of the research linked at action No. 2.

2.6 Where and how to test the methods

Action No. 2 should be carried out on the 3 to 4 administrative regions already chosen for action No. 1. It should also be studied on the 40 or 50 sample sites throughout Europe which will be defined in action No. 4.

Because of specific research being carried out, research teams should also have a certain freedom in the choice of their test sites because of available ground observations or specific conditions.

Research work should be carried out mainly by national research teams by means of contracts. The role of the JRC should be to ensure that historical data are kept or stored and to organize a data bank of refined products either in house or as an adviser to other organisations. This implies a coordination of methods used to obtain refined products which is also a main role of the JRC in this action.

3. ACTION No. 3 : MODELS OF YIELD PREDICTION

3.1 Aim

Agrometeorological models of yield used operationally have not been very precise until now. The aim of action No. 3 will be to give a better forecast of yield either by improving agromet models or by directly relating remote sensing data to yield.

3.2 Method

This research will use different methods:

- for all crops an improvement can be obtained with remote sensing data to expand more precisely meteorological data coming from stations, using normal agromet models (1)
- remote sensing data for a given region can be integrated as meteorological variables in a statistical model such as the agromet model used by OSCE (2)
- remote sensing data can give more agronomically oriented parameters which can be integrated with meteorological data. Special attention will be given to real evapotranspiration
- direct relationship between parameters such as vegetative indexes and yield can be found.

When there are no agromet models for a specific crop their creation will also be included in action No. 3.

3.2.1 Improvement of actual agromet models

Data coming almost instantaneously from meteorological stations are not always quite suitable. Meteorological stations were not chosen for agricultural applications and the quality of instantaneous data can also be a problem. Low resolution satellite data can be used to improve the representativeness of the stations.

They can also be used when there is a lack of data; rain for example can be derived from meteosat or AVHRR imagery (type of observed clouds).

3.2.2 Integration of satellite data in a statistical agromet model

Monthly or 10 day rainfall and temperature are the main input in the OSCE agromet model. An integrated vegetative indice or surface temperature from AVHRR could be introduced in such a model. This requires historical data which could be available in the future.

3.2.3 Introduction of more agronomic oriented parameters

Remote sensing data could be used to derive agronomic parameters such as Stress-degree-day or real evapotranspiration. This requires a combination of remote sensing data at low resolution with external data such as air temperature.

This will also require development of large-scale models taking these new parameters into account. Special attention will be paid to evapotranspiration.

3.2.4 Direct relationship between yield and remote sensing data

For certain phenological stages, high resolution satellite data and specially vegetative indices can be well correlated to the final yield. For low yields one image at the heading stage can be sufficient, more often a series of images will be necessary for comparison with normal curves or the normal senescence line.

3.3 Input data

Meteorological data and observation of phenological stages will be necessary. For method *3.2.1*, images of low resolution satellites are generally sufficient. For *3.2.2* and *3.2.3* data on a tape are necessary but they will be interpretated on a 10 day-basis or monthly basis. For method *3.2.4* high resolution satellite data and the corresponding type of crop are necessary.

3.4 Accuracy statement

Most agromet models can be tested with historical data using results from the last 15 or 20 years. This is not possible with remote sensing since data from only a few years are available. The accuracy statement will be based on several locations instead of years.

3.5 Specific research

Most of the methods presented in 3.2 are at a research stage and have not been tested experimentally on the large scale. Most of the research will deal with expanding local experiments to large scale procedures which often means simplifying to deal with available data.

Special attention will have to be paid to permanent crops where even simple agromet models do not exist. This is particularly important since for such crops acreage does not change quickly and annual production depends mainly on yield changes.

3.6 Where and how to test the method

Most of the methods will be created by agronomic and statistical studies. They will have to be tested on administrative regions or test sites where ground observations are sufficiently precise.

The cost of this action should increase in 1989 -1990 when the first models can be implemented and tested.

4. ACTION No. 4 : RAPID EUROPEAN ESTIMATES OF ACREAGES AND POTENTIAL YIELD

4.1 Aim

With a rapid analysis of images of a sample of some 50 sites throughout Europe, the goal is to obtain rapid estimates of the acreage of main crops and their conditions and potential yield.

The aim is also to test an unsupervised method whcih could be applied to the assessment of foreign crops of countries outside the European Community where conventional surveys cannot be made.

4.2 Method

Using a sample of about 50 sites in the European Community it becomes realistic to obtain and purchase 5-6 coverages of high resolution satellite data during the annual growing season.

Analysis will be carried out by unsupervised methods where computer-assisted photointerpreting will play an important role.

Basic knowledge of the sites will be useful but results should not depend upon recent ground observations.

Estimates or yield forecast will be based on external knowledge of the last year's inventories and rapid analysis of changes. The sample of sites should be used to monitor the changes and not the level of production.

4.3 Input data

Repetitive coverage with high resolution satellite data is necessary. Sites will be chosen for their representativeness but also in order to maximize the coverages.

4.4 Accuracy statements

During the present plan, sampling surveys will be set to check the results since the test sites are related to image frames and not to administrative regions where statistical data exist.

Some accuracy statements will then be made for each test site but also at the European level. The error analysis should define what is related to the sampling scheme and what is related to the unsupervised method.

4.5 Specific research

This project is semi-operational and should benefit from research carried out in actions No. 1 and No. 3. Improvements of procedures over time will be important in terms of both accuracy and timeliness.

4.6 Where and how to test the methods

This action will be tested with some 40 or 50 sample sites throughout Europe. It should be subcontracted to national teams for ground observations but should be limited to a single subcontractor for image analysis.

The cost should increase regularly since approximately 10 test sites could be added each year from 1988 to 1992.

The Joint Research Centre should follow up some sites to test the procedures and conduct the error analysis in order to present an operational scheme, number and localization of sites, to give European or national estimates.

5. ACTION No. 5 : ADVANCED AGRICULTURAL INFORMATION SYSTEM

5.1 Aim

The aim is to test the integration and interactivity of different methods: conventional ones and new ones using remote sensing techniques.

Actions No. 1 to 4 are studied independently to test their accuracy. In action No. 5 all methods will be used on the same sites to test an advanced agricultural information system which would fulfil different requirements, give inventories but also forecast, and will assess the quality but also give the cost effectiveness of the different methods.

This action will prepare a transition to an operational system.

5.2 Method

All methods where the output of one action will be an input to another will be developed. For example, knowledge of inventories obtained with high resolution satellite data will help in the use of repetitive coverage of low resolution images. Agromet models can have an output such as the phenological stages by sums of temperature. Such an output can help to decide the meaning of a low or high vegetative indice. Some ground observation of a specific phenological stage can be used as an input for an agromet model.

All these methods cannot be defined precisely since they depend on the timeliness and the form of documents or data obtained by the other actions.

5.3 Input data

All available data will be used as presented in the scheme of the advanced agricultural information system, i.e.:
- high resolution satellite data
- low resolution satellite data
- meteorological data from the ground stations
- surveys
- agronomic data such as soil types or characteristics of crops.

5.4 Accuracy statement

The precision of the combined procedures in giving inventories or forecasts will be given.

However the role of the analyst is much more important in this case and there is often a trade off between the higher accuracy and the timeliness of the method, specially for an action which is operationally oriented.

5.5 Specific research

In this action there should not be basic research, but communication of data. Use of data bases and geographic information systems will be specially important even for semi-operational procedures.

5.6 Where and how to test the methods

Since procedures will have to be tested on a large range of conditions, the 40 or 50 sites defined for action No. 4 will also be used in this case. Selected administrative regions will also be tested but in shared cost actions.

As for action No. 4 the cost should increase regularly since approximately 10 test sites could be added each year from 1988 to 1992.

6.1 Aim

All actions require a survey either because it is part of the method (actions No. 1 and 5) or because the results must be checked. Most of the surveys will be integrated in action No. 6 to avoid duplication and to give common guidelines.

Because of the diversity of listframes used in the different countries to perform sampling surveys and because the plan must test remote sensing possibilities, ground observations and surveys in general will be sampled out of an areaframe based on satellite data.

The objective of action No. 6 will be first to support other actions but also to precise the method of areaframe sampling and associated surveys.

6.2 Method

In area sampling surveys the statistical unit is a portion of territory called a segment. The region is considered as a list of n segments out of which is pulled a sample n. Acreage of crops or non agricultural cover types can be associated to segments as can the number of farms, the cattle, economic items, ...

For land cover, direct ground observation can be performed by enumerators; for others, farmers must be involved. Different methods are then used; closed, open or weighted segments.

If it is possible to define a complete statistical system with this technique, this method is efficient if basic and up-to-date knowledge of the land cover allows a good stratification of the region in order to produce an efficient sampling design.

Remote sensing images are definitely an adequate tool for this work.

6.3 Input

Different documents can be used to build an area frame, but the main documents will be satellite images. There are no precise requirements for producing such images. For stratification objectives they must show the types of landscape and must also show roads and specific features so that someone can go to the defined segment and enumerate the right fields.

6.4 Accuracy statement

It is possible to obtain some knowledge of the quality of the stratification from the accuracy of the overall results. A better way is to make a complete classification of the administrative region.

By a variance analysis of the results of the surveys it is possible to check the best allocation of samples between the strata.

6.5 Specific research

Building an area frame involves tedious photointerpretation. We will try to automate this work.

To obtain the best results in terms of economy, the size and shape of the segments will be studied.

Each specific survey will also have to be studied since in Europe when they exist they are generally sampled out of a census which is statistically quite different.

6.6 Where and how to test the method

The method will be tested on the three or four administrative regions and on the 40 - 50 sites.

7. ACTION No. 7 : LONG TERM RESEARCH

7.1 Aim

This action will consider problems the solution of which may improve the efficiency of the European Agriculture Information System significantly but which require long term effort. A typical example of such problems is the cloud coverage which may limit the potential of remote sensing in Northern countries drastically, and which calls for the development of new sensors having an all-weather capability.

The main objective of this action will therefore be to support the resarch on microwave sensors, and especially the SAR (Synthetic Aperture Radar), which may bring three types of benefits in the system:
- all weather capability
- improvement in crop discrimination
- unique potential for some definite parameters, e.g. soil moisture.

Moreover, the importance of an optimum integration of remote sensing data with data from other sources (exogeneous data) will call for the implementation, during the semi-operational project, and a fortiori in the operational phase, of so-called Geographic Information Systems. This will require specific effort to evaluate existing GIS concepts and adapt them to our needs.

Finally, decision making processes within the European Agriculture Information System will be based on the handling of a great amount of data and the performance of some advanced models, for which the introduction of expert systems could be useful. This action will deal with the development and implementation of these systems as and when necessary.

7.2 Implementation

As far as the studies of the SAR are concerned, the Agriculture project will speficy its needs which will be integrated and managed within the Microwave Remote Sensing project of the JRC.

This effort will include:
- basic research (e.g. microwave signature of crops)
- radar data acquisition campaign by airborne sensors
- exploitation of the SAR data from ERS-I (from 1990).

The same scheme will be used for the research on GIS and expert systems where the requirements of the Agriculture project will be integrated to more general efforts of the JRC in these fields.

DATA REDUCTION AND ANALYSIS

I. P. Woiwod & J. N. Perry
Rothamsted Experimental Station-Harpenden, Great Britain

Summary

Rational and environmentally sensitive pest control (IPM) usually requires the accumulation of quantitative information either from synoptic pest monitoring, remote sensing, field sampling or a combination of all three. The amount of information acquired can be very extensive and will usually need some form of analysis and summary before use by agricultural advisers or farmers. Some practical and statistical considerations of pest monitoring are reviewed. Amongst topics covered are computer mapping for summary of synoptic monitoring data, statistical aspects of remote sensing, field sampling, transformation of counts, experimental design of field experiments, fixed precision and sequential sampling, and binomial sampling.

1. Introduction

Integrated Pest Management (IPM) is now widely used to describe systems which employ rational, and environmentally-sensitive, pest-control strategies. As concerns about the impact of agriculture on the environment increase it is desirable that such strategies should integrate all available techniques of pest control, to maximise the profitability to the farmer and simultaneously to reduce the chemical input into the environment to a minimum (Burn *et al.*, 1988). An important requirement in many such schemes is the accumulation of quantitative information, by monitoring pest populations and the damage they cause, so that forecasting systems can be developed. The necessity and timing of control can then be assessed accurately and the effect of any control measures monitored, to aid future improvement of the system.

The data collected in any monitoring or forecasting scheme can be considerable. They will always require some analysis and summary to be of practical use to farmers, agricultural advisors or other members of the agricultural industry. In this condensed form data can help rational decisions to be made on the need for, and timing of control measures. The type of information required and the method by which it is obtained will naturally vary, according to the ecology of the pest in relation to the crop and economic considerations. However, there are various statistical and practical principles which apply widely to monitoring systems and their analysis, some of the more important of which will be outlined here.

2. Scale of Monitoring

The first consideration in any monitoring scheme is to chose an appropriate scale of sampling in order to acquire the desired information. Two rather different scales of sampling are commonly employed in IPM and related systems. These can be briefly categorised either as 'synoptic scale' or 'field scale'. By 'synoptic' we mean that the information is collected in a similar way over a wide area to provide a general overview, whereas 'field scale' is collected in a small area, usually an individual farm field, and is more concerned with providing detailed information at a local scale. In the past there has been some controversy over the relative usefulness of the two approaches, but this is mainly due to a misunderstanding of the different roles of the two types of sampling scale, since the information provided is nearly always complimentary; both approaches being appropriate in certain circumstances.

2.1 Synoptic Monitoring

Large-scale, synoptic monitoring is particularly useful for wind-borne or highly migratory pest species, where information on population increases or pest outbreaks in one area may be relevant to the likelihood of subsequent problems elsewhere. The data from such large scale monitoring can also provide the necessary information on pest phenology for the development of forecasting systems, for example to predict flight times from accumulated or threshold temperatures (Thomas *et al.*, 1983, Worner, 1988).

Synoptic monitoring systems are not trivial to set up, partly because of the labour and hence cost involved, but also because long distance coordination is required which in many cases involves cooperation across political boundaries. However, several successful schemes have operated throughout the world. Two good examples are the well-known Desert Locust (*Schistocerca gregaria* (Forsk)) and African Armyworm (*Spodoptera exempta* (Wlk)) forecasting schemes which have been operating in Africa for many years (e.g. Betts, 1976); the former based on field reports of pest development and outbreaks, the latter on a more formal network of light traps.

In Europe the best example of synoptic pest monitoring is to be found in the networks of 12.2 metre high suction traps now operating in many countries, e.g. France, Great Britain, Holland, Belgium, Spain, Switzerland and Italy, and are informally coordinated under the name 'Euraphid' (Taylor, 1981). The original network was set up in Great Britain to supply samples for the Rothamsted Insect Survey (RIS) (Taylor, 1977, Woiwod *et al.*, 1984, Taylor, 1986). The RIS and subsequent suction trap networks were developed to monitor the aerial populations of the economically important and highly migratory group of insects, the aphids. Currently the RIS coordinates a network of 24 traps throughout Great Britain (Woiwod *et al.*, 1988), many of which have been in operation for 20 years. As up to 300 species are identified on a daily basis the resulting database is considerable and growing.

2.1.1 Dynamic Computer Mapping

As soon as the RIS network was in place the problem arose, as with other synoptic systems, of understanding and presenting large data sets obtained from an irregular set of sample points. Although there are many formal statistical approaches to the analysis of such data we have found that one of the most useful in the first instance is to display the situation in the form of a computer density distribution map (Woiwod & Tatchell, 1984).

There are several powerful mapping packages available for such work of which we have used three. SYMAP from the Laboratory of Computer Graphics Harvard (Woiwod, 1979), SURFACE II developed by the Kansas

Geological Survey (Sampson, 1975) and UNIMAP (UNIRAS A/S, Søborg, Denmark). These packages show a development of power and complexity which mirrors advances in computer graphics, with SYMAP output being by overprinting on line printer, SURFACE II designed for output to high quality graph plotter, whereas UNIMAP takes full advantage of colour graphics. Despite these differences most powerful mapping packages work on a similar principle where the irregular sample data is interpolated onto a regular grid matrix which is then displayed graphically, usually as a contour map. There are many methods for carrying out the interpolation but a common approach is to use some form of distance-weighted mean. If an empirical model of the variance in spatial trends in the data can be fitted then a more analytical method known as Kriging (Clark, 1979) can be employed and errors of estimation can be obtained. However, for insects where populations are highly mobile the extra computations required for such analyses is unlikely to be worthwhile (Woiwod, 1979).

2.1.2 Remote sensing

Remote sensing is becoming an increasingly important area of interest in agricultural research as satellite information becomes more readily and cheaply available. The scale at which remote sensing is carried out depends on the requirement but it can be used in a synoptic way even at field level. Already, valuable information on local vegetation quality patterns is being provided as an aid to locust forecasting (Hielkema *et al.*, 1986) and, although it is doubtful if it will ever be possible to assess pest populations directly, it may well be possible to estimate crop damage for some pests and diseases. Although these prospects are exciting, a major drawback is the massive amount of data being recorded routinely by satellites which can be reduced to a manageable state only by mapping, data-selection and careful statistical analysis (Fuller, 1983).

Data for remote sensing is usually recorded as a spectral intensity in each of several bands. Each scene consists of millions of spatially defined pixels, in each of which intensity is measured. The pixels represent small areas of the earth's surface, usually resolved to squares with sides measuring tens of metres. *Ad hoc* techniques of analysing images include: contrast stretching, in which intensities are replaced by functions of intensities; de-striping, to remove artefactual banding effects; smoothing, using moving averages of neighbouring pixel intensities; edge enhancement by manipulating residuals from smoothed data; and the use of well-known multivariate techniques, including principal component analysis, to reduce the dimensionality of the data. Discrimination between land uses involves marking sets of pixels to form training sets using some independent non-satellite-based source of information. Kittler (1983) reviews some discrimination methods. Alternatively, representations of fields, or other natural entities, may be built up using segmentation procedures, by spatially combining neighbouring pixels until lack of homogeneity is demonstrated (see Rosenfeld, 1984). Additionally, texture measures are useful, especially to detect boundaries (see review by Haralick, 1979). Since, statistically, neighbouring pixels can be expected to be positively correlated; Besag (1986) suggested an iterative classification method based on a model built to incorporate these spatial dependencies. However, examples of sensible uses of statistical techniques to improve the analysis of real satellite data in applied situations are, as yet, rare. Examples of advances and worthwhile analyses are in papers by Campbell *et al.* (1983), Kershaw (1987) and Battese *et al.* (1988).

3. Field Monitoring

Although synoptic monitoring can often provide useful information on pest phenology and likely infestation rates there is usually considerable field-to-field variation and at some stage in any IPM scheme pest populations will need to be assessed in individual fields. The details of how this is done will vary for each species in each crop. However, the statistical methodology for such sampling, which allows for local spatial variability, the best way to transform the data, techniques for sampling to a defined level of precision, and methods for the assessment of populations with the minimum of labour, are all well-known in the field of biometry. Below we outline some of these considerations which might be less well-known to some entomologists in the field.

3.1. Sampling

Ever since Beall, in 1935, found non-random spatial distributions of insects in fields, entomologists have had to consider the most efficient way to sample a crop. Crucial questions involve the size and number of units comprising the sample, size and shape of the plots within which samples are taken, and the size of the experiment when treatments are involved. Often sub-division of a field will be hierarchical. There may be blocks of several plots, within which conditions are relatively less variable than between different blocks, and each plot may be divided into several sub-plots. Ideas of components of variance, which are still extensively used in plant and animal breeding trials, are rarely used nowadays to aid the choice of sampling procedures in Entomology. This might be thought surprising. An introduction to the techniques of analysing components of variance was given, as long ago as 1935, in Yates and Zacopanay's wide-ranging general study. In this, the yield over several plots, within each of which several sampling units were taken, was assumed to have a variance which was made up of two additive components. One component was assumed to relate to the variation from sample unit to sample unit; the other to affect all units within the plot equally, but to vary from plot to plot. This model may be extended to allow variance components to be estimated for more complex cases, such as those involving a hierarchy of sources-of-variation of increasing area. A knowledge of these components based on information from preliminary samples taken prior to an experiment or survey, enables comparison of different proposed sampling schemes. Early examples were due to Marshall (1936), Bartlett (1936) and Ladell (1938). Other papers, such as Fleming and Baker (1936), Jones (1937) and Meyers and Patch (1937) were limited in their approach, being based on an earlier methodology which originated in the pioneering paper of Mercer and Hall (1911). Likewise, Beall (1939) used different methodology of less convenient application. The power of the method of components of variance was best demonstrated in the classic paper by the American entomologist Chester I. Bliss in 1941. Bliss' proposal to sub-divide large areas into successive agglomerations of sample units, in a hierarchical scheme, gave rise to techniques useful both to assess the spatial pattern of pests on crops, and to provide information especially useful in the planning of experiments. An identical scheme was reinvented by Greig-Smith in 1952 and soon became the foremost method used by plant ecologists to measure the spatial pattern at several scales. Perhaps Bliss' method deserves to be used more than is the case at present. Beall (1939), Bliss (1941) and Anscombe (1950) deal with errors in sampling which may be due to the workers who take the sample, a problem which deserves more attention when developing sampling strategies for field surveys (Harrington, 1987).

3.2. Transformations, generalized linear models and Taylor's power-law

The first transformation of entomological data was used in 1936 by Marshall and, in two separate papers, by Bartlett. Both authors used the square root transformation to stabilize the variance, because, if counts of insects had a Poisson distribution, their variance would be proportional to their mean. Under these circumstances the analysis of variance without transformation would not be valid. Bartlett mentioned that if, instead, variance was proportional to mean-squared (much more likely in practice) then a logarithmic transformation was appropriate to stabilize it. In another paper during that year he warned that variance might be a quadratic function of the mean, but gave no evidence in support. However, stabilization of variance is only one reason for transforming entomological data; another is to achieve additivity of effects, which is another requirement of the assumptions underlying the analysis of variance. C.B. Williams in 1937 showed that the logarithmic transformation not only stabilized variance efficiently for the highly variable, long-tailed distributions which often occur in ecological and pest management data, but also reasoned that count data was multiplicative in nature and that, a *priori*, one might expect that similar differences in environment would produce similar percentage increases in a catch, rather than similar numerical increases. Of course, for multiplicative data, a logarithmic transformation is suitable because it ensures additive effects on the transformed scale. Williams recommended the use of log(n+1) instead of log(n) to avoid problems with logarithms of zero. He noted that this modified transformation is proportional to the square-root transformation for low counts and approximately equal to the standard logarithmic transformation for large counts. Since Poisson data is only encountered at low densities, the use of Williams' log transformation effectively makes the square-root transformation redundant. More sophisticated transformations, originally proposed by Beall and based on the negative binomial distribution, have rarely been used, because in practice the parameter **k** of the negative binomial distribution cannot be assumed constant (Taylor *et al.*, 1979), and extra replication is required to estimate **k** from preliminary samples. Hence fortuitously, for entomological count data, the same transformation ensures additivity as usually provides an adequate stabilization of variance. More sophisticated analyses have recently become possible using the ideas of Nelder and Wedderburn (1972) and Nelder and Pregibon (1987); these are usually referred to in the statistical literature as generalized linear models. The benefit of using generalized linear models is that a separate function can be specified to achieve an additive model from that which is used to achieve homogeneity of variance.

In his 1941 paper Bliss pre-dated one major discovery by a decade; he also in that paper pre-dated another major discovery, that of the power-law relationship between variance and mean for animal count data, by two decades. The power-law relationship which states that log (sample variance) is a linear function of log (sample mean) was fitted by Bliss for statistical convenience, but is usually attributed to Taylor (1961), because he realised its ecological implications and importance and demonstrated its wide application to a range of species from different taxa. While the power-law relationship implies that the most efficient transformation to ensure variance stabilization is also a power-law, in general with non-integer exponent, it is seldom necessary to use this degree of sophistication, and a logarithmic transformation will usually suffice. However, the power-law has been used increasingly to develop efficient sampling schemes for agriculture and fisheries. Taylor *et al.* (1978, 1979, 1980) and Taylor *et al.* (1983) demonstrated its near-universal application. Taylor (1984) and Taylor *et al.* (1988) give examples of this use. Perry (1981, 1987) investigated statistical aspects.

A frequently recurring problem in analyses of Taylor's power-law has not yet been satisfactorily resolved. Consider the power-law plot of a species with $b > 1$, which the great majority of species display. The fitted power-law regression line will cross the Poisson line, where sample variance and sample mean are equal, at some density, usually a relatively low population density for the species concerned. Taylor *et al.* (1978) and Taylor (1986) have argued that at this low density the data are usually indistinguishable from a spatially random distribution which would generate a Poisson series of counts, even though the condition, that variance and mean are equal, is only necessary, but not strictly sufficient. They also argue on biological grounds that, at still lower densities, there will be even less interaction between individuals of the species concerned, and that there is therefore no biological reason why the spatial distribution of the individuals should be anything but random at these lower densities. The consequence of this is that as mean density decreases from the point representing the intersection between the power-law regression line and the Poisson line, the apparent plotted relationship will change, and the data will follow the Poisson line, giving rise, overall, to a broken stick, or split-line type of relationship. Compounding this biological reason for the change of relationship is a statistical reason. At the sort of densities we are considering the data consists of low integer counts, predominately zeroes, and usually with the rest of the sample consisting exclusively of ones, but with possibly a few higher values. As density decreases, the proportion of the sample which are zeroes or ones increases, and, again because of the discrete low integer nature of these counts, it becomes impossible for the sample to express 'regularity', the condition for which sample variance is less than sample mean. Compounding these problems is a further one concerning the quality of the data. At low densities the variance of log (sample variance), the dependent variate, is relatively high; also, because of the discrete nature of the counts for usual sample sizes, the number of possible distinct values of sample mean and variance that it is possible for the sample to express are relatively few. The scatter of the data for these low densities therefore appears both relatively large and coarse. Thus, it can be relatively difficult to detect the problem of the change in the relationship and of data following the Poisson line at lower densities. Our advice to overcome this problem is to plot the data and visually inspect it, so that it can be seen where the power-law regression line intersects with the Poisson line, and then, prior to fitting any regression relationship, to discard all points with lower densities than the density at which the intersection is thought to occur. This is, admittedly, a subjective process, and should be tackled with some caution. It is, however, vastly preferable to simply fitting a regression line as if there was no change in relationship; such a practice would underestimate the true slope of the regression and lead to erroneous conclusions, such as those probably drawn for this reason by Downing (1986).

3.3. Design of experiments

Too much has been written about the design of experiments for us to attempt a review here. The text book by Cochran and Cox (1957) is still a classic. In Entomology, little use is made of designs more sophisticated than the simple randomized block; except for the use of split-plots, when subsidiary treatments can be applied conveniently to small areas which are parts of main plots, and latin squares, when it is suspected that spatial trends may exist across fields in unknown directions. However, a fresh look needs to be taken at experimental designs in Entomology, due to recent interest in assessing environmental effects of pesticides and in investigating the role of spatial heterogeneity in the stabilization of field populations. Both of these studies involve experiments with relatively large plot size. The consequent difficulty of obtaining enough land and man-power to ensure adequate replication is a problem which occurs increasingly frequently. Another area where more research is needed concerns the use of preliminary samples taken from each plot prior to treatment application, and the use of this information to analyse final insect numbers, usually in pesticide trials. Work is needed to assess whether it is sufficient to assume that the mortality acts proportionately, in a density-independent fashion (when an analysis of the difference between the logarithmically transformed initial and final counts would be all that is required) or whether, instead, the mortality operates in a density-dependent fashion, in which case a full analysis of covariance should be performed.

3.4. Fixed precision sampling, fixed precision sequential sampling, and sequential sampling

Sampling schemes where the precision is defined in terms of the achieved standard error of an estimate as some given percentage of the estimate have been used for a long time. Excellent discussions of these fixed precision sampling schemes are provided by Ruesink (1980), Taylor (1984) and Finch *et al.* (1975, 1978). Taylor's power-law is recommended as the most efficient way to derive such a scheme; the value of the exponent of the relationship must be known and is usually calculated from preliminary samples. Green (1970) proposed a sequential sampling design in which sampling is terminated when a defined level of precision is achieved; again the power-law was recommended. All such methods require knowledge of the underlying relationship between the variance and mean of a set of samples of animal counts, and as it has been shown that the power-law provides a simple species-specific relationship at a given sampling scale (Taylor, 1984, Taylor *et al.*, 1988) then it is usually the best choice.

Sequential sampling methods test a specific hypothesis to determine whether or not a pest population exceeds some threshold density, above which action would be recommended. Sequential sampling was originally due to Wald (1945); the first use for pests was by Oakland (1950) in fisheries; the first plan for insect populations was given by Morris (1954). Pieters (1978) and Shepard (1980) give reviews. To derive a plan, the form of the distribution counts must be known. For this reason, Perry and Taylor (1988) have warned that most existing plans, based on negative binomial distributions with common parameter **k** for each density, may not be appropriate, because **k** is density dependent. Perry and Taylor use the new Adès family of distributions to derive a plan which appears to work much better in practice.

3.5. Binomial sampling

Binomial sampling, sometimes giving what are termed incidence counts, are used when the cost of counting samples exactly is prohibitive which is often the case in routine IPM monitoring. The relationship between the mean density and the proportion of sample units uninfested is determined by preliminary sampling. Future density estimates may be derived using this relationship, by noting merely what proportion of sample units are occupied, usually a much quicker process than direct counting. The technique appears to have been developed independently, over many years, in several disciplines, as noted by Perry (1987). Several relationships have been tried (see Kuno, 1986) but the best one is due to Kono and Sugino (1958) although it was later independently discovered by Gerrard and Chang (1970). A variant of this method, which accounts for density dependence of the negative binomial parameter **k**, was given by Wilson and Room (1983). Some further statistical work on incidence counts needs to be done to cope with the difficulties of estimation from relationships in which both variables are subject to error. Binns and Bostanian (1988) provide an interesting modification.

References

Anscombe, F.J. (1950). Soil Sampling for potato root eelworm cysts. *Ann. appl. Biol.*, **37**, 286-295.

Bartlett, M.S. (1936a). The square root transformation in analysis of variance. *Journal of the Royal Statistical Society Suppl.*, **3**, 68-78.

Bartlett, M.S. (1936b). Some notes on insecticide tests in the laboratory and in the field. *Journal of the Royal Statistical Society Suppl.*, **3**, 185-194.

Battese, G.E., Harter, R.M. and Fuller, W.A. (1988). An error-components model for prediction of county crop areas using survey and satellite data. *Journal of American Statistical Association*, **83**, 28-36.

Beall, G. (1935). Study of arthropod populations by the method of sweeping. *Ecology*, **16**, 216-225.

Beall, G. (1939). Methods of estimating the population of insects in a field. *Biometrika*, **30**, 422-439.

Besag, J. (1986). On the statistical analysis of dirty pictures. *Journal of the Royal Statistical Society, Series B*, **149**, 259-302.

Betts, E. (1976). Forecasting infestations of tropical migrant pests: the Desert Locust and the African Armyworm. In: Rainey, R.C. (Ed.) *Insect Flight, Symposium of the Royal Entomological Society of London*, **7**, 113-134. Blackwell Scientific Publications, Oxford.

Binns, M.R. and Bostanian, N.J. (1988). Binomial and censored sampling in estimation and decision making for the negative binomial distribution. *Biometrics*, **44**, 473-483.

Bliss, C.I. (1941). Statistical problems in estimating populations of Japanese beetle larvae. *J. Econ. Ent.*, **34**, 221-232.

Burn, A.J., Coaker, T.H. and Jepson, P.C. (Ed.) (1987). *Integrated Pest Management.* Academic Press, London.

Campbell, N.A., Hick, F.R. and Carlton, P.T. (1983). Evaluation and development of the techniques for crop inventory in the wheatbelt of western Australia using satellite data. 16th International Symposium on Remote Sensing of the Environment: Buenos Aires.

Clark, I. (1979). *Practical Geostatistics.* Applied Science Publishers, London.

Cochran, W.G. and Cox, G.M. (1957). *Experimental designs* (2nd edition) Wiley, New York.

Downing, J.A. (1986). Spatial heterogeneity: evolved behaviour or mathematical artefact. *Nature, Lond.*, **323**, 255-257.

Finch, S., Skinner, G. and Freeman, G.H. (1975). The distribution and analysis of cabbage root fly egg populations. *Ann. appl. Biol.*, 79, 1-18.

Finch, S., Skinner, G. and Freeman, G.H. (1978). Distribution and analysis of cabbage root fly pupal populations. *Ann. appl. Biol.*, 88, 351-356.

Fleming, W.E. and Baker, F.E. (1936). A method for estimating populations of larvae of the Japanese beetle in the field. *J. Agr. Res.*, 53, 319-331.

Fuller, R.M. (Ed.) (1983). Ecological mapping from ground, air and space. *ITE Symposium No.10.* Natural Environment Research Council, Swindon.

Gerrard, D.J. and Chiang, H.C. (1970). Density estimation of corn root worm egg population based upon frequency of occurrence. *Ecology*, 51, 237-245.

Green, R.H. (1970). On fixed precision level sequential sampling. *Res. Popul. Ecol.*, 12, 249-251.

Grieg-Smith, P. (1952). The use of random and contiguous quadrats in the study of the structure of plant communities. *Ann. Bot.*, 16, 293-316.

Haralick, R.M. (1979). Image texture survey. *Proceedings of the IEEE*, 67, 786-804.

Harrington, R. (1987). Varying efficiency in a group of people sampling cabbage plants for aphids (*Hemiptera: Aphididae*). *Bull. ent. Res.*, 77, 497-501.

Heilkema, J.U., Roffey, J. and Tucker, C.J. (1986). Assessment of ecological conditions associated with the 1980/81 desert locust plague upsurge in West Africa using environmental satellite data. *Int. J. Remote Sensing*, 7, 1609-1622.

Jones, E.W. (1937). Practical field methods of sampling soil for wireworms. *J. Agr. Res.*, 54, 123-134.

Kershaw, C.D. (1987). Discrimination problems for satellite images. *Int. J. Remote Sensing*, 8, 1377-1383.

Kittler, J. (1983). Image processing for remote sensing. *Philosophical Transactions of the Royal Society of London, A*, 309, 323-335.

Kono, T. and Sugino, T. (1958). On the estimation of the density of rice stems infested by the rice stem borer. *Japanese Journal of Applied Entomology and Zoology*, 2, 184-188.

Kuno, E. (1986). Evaluation of statistical precision and design of efficient sampling for the population estimation based on frequency of occurrence. *Res. Popul. Ecol.*, 28, 305-319.

Ladell, W.R.S. (1938). Field experiments on the control of wireworms. With Appendix. The information supplied by the sampling results, by W.G. Cochran. *Ann.appl.Biol.*, 25, 341-389.

Marshall, J. (1936). The distribution and sampling of insect populations in the field with special reference to the American bollworm *Heliothis Obsoleta Fabr.* *Ann. appl. Biol.*, 23, 133-152.

Mercer, W.B. and Hall, A.D. (1911). The experimental error of field trials. *J. Agr. Sci.*, 4, 107-132.

Meyers, M.T. and Patch, L.H. (1937). A statistical study of sampling in field surveys of the fall population of the European corn borer. *J. Agr. Res.*, 55, 849-871.

Morris, R.F. (1954). A sequential sampling technique for spruce budworm egg surveys. *Can. J. Zool.*, 32, 302-313.

Nelder, J.A. and Wedderburn, R.W.M. (1972). Generalized linear models. *Journal of the Royal Statistical Society, Series A*, 135, 370-384.

Nelder, J.A. and Pregibon, D. (1987). An extended quasi-likelihood function. *Biometrika*, 74, 221-232.

Oakland, G.B. (1950). An application of sequential analysis to whitefish sampling. *Biometrics*, 6, 59-67.

Perry, J.N. (1981). Taylor's power-law for dependence of variance on mean in animal populations. *Appl. Statist.*, 30, 254-263.

Perry, J.N. (1987). Iterative improvement of a power transformation to stabilise variance. *Appl. Statist.*, 36, 15-21.

Perry, J.N., and Taylor, L.R. (1988). Families of distributions for repeated samples of animal counts. *Biometrics*, 44, Sept.88 (in press).

Pieters, E.P. (1978). Bibliography of sequential sampling plans for insects. *Bull. Entomol. Soc. Amer.*, 24, 372-374.

Rosenfeld, A. (1984). Image analysis problems, progress and prespects. *Pattern recognition*, 17, 3-12.

Ruesink, W.G. (1980). Introduction to sampling theory. In *Sampling methods in soybean entomology.* (M. Kogan and D.C. Herzog, eds), pp. 61-78. Springer-Verlag, New York.

Sampson, R.J. (1975). SURFACE II graphics system. *Kansas Geological Survey Series in Spatial Analysis,* No.1, Univ. Kansas, Lawrence.

Shepard, M. (1980). Sequential sampling plans for soybean arthropods. In *Sampling methods in soybean entomology.* (M. Kogan and D.C. Herzog, eds), pp. 79-93. Springer-Verlag, New York.

Taylor, L.R. (1961). Aggregation, variance and the mean. *Nature, Lond.*, **189**, 732-735.

Taylor, L.R. (1977). Aphid forecasting and the Rothamsted Insect Survey. *Journal of the Royal Agricultural Society of England,* **138**, 75-97.

Taylor, L.R. (Ed.) (1981). EURAPHID Rothamsted 1980. Rothamsted Experimental Station, Harpenden, U.K.

Taylor, L.R. (1984). Assessing and interpreting the spatial distributions of insect populations. *Ann. Rev. Entomol.*, **29**, 321-357.

Taylor, L.R. (1986). Synoptic dynamics, migration and the Rothamsted Insect Survey, Presidential Address to the British Ecological Society, December 1984. *J. Anim. Ecol.*, **55**, 1-38.

Taylor, L.R., Perry, J.N., Woiwod, I.P. and Taylor, R.A.J. (1988). Specificity of the spatial power-law exponent in ecology and agriculture. *Nature,* 332, 721-722.

Taylor, L.R., Taylor, R.A.J., Woiwod, I.P. and Perry, J.N. (1983). Behavourial dynamics. *Nature, London.*, **303**, 801-804.

Taylor, L.R., Woiwod, I.P. and Perry, J.N. (1978). The density-dependence of spatial behaviour and the rarity of randomness. *J. Anim. Ecol.*, **47**, 383-406.

Taylor, L.R., Woiwod, I.P. and Perry, J.N. (1979). The negative binomial as a dynamic ecological model for aggregation and the density-dependence of k. *J. Anim. Ecol.*, **48**, 289-304.

Taylor, L.R., Woiwod, I.P. and Perry, J.N. (1980). Variance and the large scale spatial stability of aphids, moths and birds. *J. Anim. Ecol.*, **49**, 831-854.

Thomas, G.G., Goldwin, G.K. and Tatchell, G.M. (1983). Associations between weather factors and the spring migration of the damson-hop aphid, *Phorodon humuli*. *Ann. appl. Biol.*, 102, 7-17.

Wald, A. (1945). Sequential tests of the statistical hypotheses. *Annals of Mathematical Statistics*, 16, 117-186.

Williams, C.B. (1937). The use of logarithms in the interpretation of certain entomological problems. *Ann. appl. Biol.*, 24, 404-414.

Wilson, L.T. and Room, P.M. (1983). Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. *Environ. Entomol.*, 12, 50-54.

Woiwod, I.P. (1979). The role of spatial analysis in the Rothamsted Insect Survey. In: N. Wrigley (Ed.) Statistical applications in the spatial sciences, 268-285, Pion, London.

Woiwod, I.P., Tatchell, G.M. and Barrett, A.M. (1984). A system for the rapid collection, analysis and dissemination of aphid-monitoring data from suction traps. *Crop Prot.*, 3, 273-288.

Woiwod, I.P., Tatchell, G.M. Dupuch, M.J., Macaulay, E.D.M., Parker, S.J., Riley, A.M. and Taylor, M.S. (1988). Rothamsted Insect Survey - nineteenth annual summary. *Rothamsted Report for 1987, Part 2*, 195-229.

Woiwod, I.P. and Tatchell, G.M. (1984). Computer mapping of aphid abundance. *Proceedings of the 1984 British Crop Protection Conference: Pests and Diseases*, 2, 675-683.

Worner, S.P. (1988). Evaluation of diurnal temperature models and thermal summation in New Zealand. *J. Econ. Ent.*, 81, 9-13.

Yates, F. and Zacopanay, I. (1935). The estimation of the efficiency of sampling, with special reference to sampling for yields in cereal experiments. *J. Agric. Sci.*, 25, 545-577.

SIMULATION TECHNIQUES APPLIED TO CROPS AND PEST MODELS

J. Baumgärtner
Division of Phytomedicine, Institute of Plant Sciences, ETH-Zürich, Switzerland

A. P. Gutiérrez
Division of Biological Control, University of California-Berkeley, USA

Abstract

The complexities of crop production and integrated pest management require a systems approach for research and implementation. In this review analytical and operations research approaches are separated from the simulation approach, which is further subdivided into an engineering and a demographic approach. The review focuses exclusively on simulation models and considers primarily the demographic approach. The discussion of the latter is preceeded by a brief review of statistical methods which are fundamental in research and implementation of integrated pest management programs. The demographic approach is built on ecological principles, such as the concept of physiological time, single species population dynamics, population interactions, and a comprehensive concept of requisite acquisition and allocation. The level of complexity introduced into the different models depends on the need of the pest management system. Phenological models are useful for determining timing of events, but are considered less useful than population models which include abiotic and density-dependent factors. These are the building blocks for models on population interactions which can eventually be embedded in a comprehensive agroecosystem model. This model includes multi-species and multi-trophic interactions, permits the evaluation of vital requisites for population development and yield formation, and enables the researcher to take into account the socio-economic environment. Such models can be simplified to fit into an economic decision making environment or they can be summarized for tactical decision making.

1 INTRODUCTION

Modern pest control, as we know it, began with the synthesis of organic insecticides. Since that time three major phases in pest control philosophy can be distinguished. In the first phase, pest control specialists moved away from older ecologically oriented methods, and began to focus entirely on individual pests and looked at chemical control to solve pest problems. This approach proved unsatisfactory, however, because it soon became obvious that it was incompatible with other means of pest control and contributed to environmental degradation (VAN DEN BOSCH, 1978; BROWN, 1978). The unilateral reliance on chemical pest control measures was responsible for pest resurgence and outbreaks of secondary pests (FLINT & VAN DEN BOSCH, 1981). In addition, pesticides applied frequently, caused the development of resistance in pest populations making chemical pest control less efficient and further aggravating the negative aspects of the pest problems. In the second phase, pest management specialists recognized this and attempted first to substitute pesticides with other control measures or to use less disruptive chemicals that could be integrated and applied in a comprehensive concept of pest management (STERN *et al.*, 1959; WOODS, 1974; METCALF & LUCKMANN, 1975). An important advancement during this period was the adoption of the notion that measures should be undertaken only if pest densities reached or passed a predefined threshold (i.e., an economic threshold). In economic terms, the threshold was where the marginal gains equal the marginal control costs (HEADLEY, 1972). Both the integration of pest control measures and the concept of the economic threshold required that the production system rather than an individual pest population be taken into account (HUFFAKER, 1980; DELUCCHI, 1987; BAUMGÄRTNER & DELUCCHI, 1988).

The third phase of pest control was stimulated by recognition of the needs for a holistic or systems oriented approach for research and implementation of pest management concepts (HUFFAKER & CROFT, 1976). Further, it has been recognized that crop production is a biological process with economic objectives (WINTER, 1978) which would suggest that to develop sound pest management systems a profound knowledge of the relevant processes and their evaluation in an economic context is a prerequisite. This is especially true if our goal is to develop optimal management policies for agroecosystems. In general, the traditional methods of agricultural experimentation were found insufficient for obtaining the basic knowledge, and for integrating the results into a coherent management system. Hence the principles of systems analysis, commonly used in other areas of science and engineering, appeared to provide a more efficient scheme for guiding the research and the implementation of the results (RUESINK, 1975; HUFFAKER & CROFT, 1976; HUFFAKER, 1980; GETZ & GUTIERREZ, 1982).

In an agroecosystem context, systems analysis has been defined as the application of those techniques (both quantitative and qualitative) that enhance our understanding of the interactions between components of the crop-pest system and their relationship to the environment (the 'outside' world) and management practices. GETZ & GUTIERREZ (1982) identify three different approaches: the analytical approach (fundamental subsystem structures are emphasized and models are used to develop qualitative principles in ecology and resource management theories), the simulation approach (stressing biological realisms and model completeness), and the operations research approach (models are designed to address specific management problems and the analysis is geared towards obtaining quantitative solutions) (Fig.1). In this paper, we stress the simulation approach.

Readers interested in the application of operations research techniques to the solution of pest management problems will find references in the review of GUTIERREZ & GETZ (1982). In the analytical approach, the mathematical models usually have closed form solutions. These models have had little impact in pest management because too many details of the biology must be left out to maintain mathematical tractability, hence we discuss such models only in the context of their mathematical relationship to simulation models. Our review will emphasize specific problems in agroecosystem design and management rather than addressing general principles of system structure and function.

In the simulation approach, the problem is often formulated as sets of complicated differential equations that cannot be solved analytically, but which can be discretized (difference equations) and programmed on a computer to evaluate the results numerically. Many of these models have had considerable impact on the development of pest management systems. The simulation approach (Fig. 1) is based primarily on mechanistic principles that historically have been introduced in two different ways. The first is an engineering approach where the scientists build models with relational diagrams and often use high level simulation languages to study the temporal dynamics (DE WIT & GOUDRIAAN, 1978). In the second or demographic approach, the structure and the dynamics of the system are based on population theory. Populations of plants, plant parts, arthropods, weeds and diseases are the components of the agroecosystems, and their relationships to each other define the structure of the system, and hence their spatial and temporal dynamics (GILBERT *et al.*, 1976; GUTIERREZ & WANG, 1977; GUTIERREZ *et al.*, 1984). An overview on the state of the art has been written by HUFFAKER (1980) while much of the relevant mathematics are reviewed in CURRY & FELDMAN (1987).

Of the two simulation approaches, we focus on the underlying principles and applications of the demographic approach. Emphasis is give here to principles of temporal dynamics, while the spatial dynamics is discussed by VAN DER WERF *et al.* (in this symposium). Our review will be preceeded by a brief discussion of statistical methods that have proven important in agroecosystems analysis and in the development of integrated pest management programs. Fig. 2 shows how this review proceeds.

2 STATISTICAL MODELS

In traditional agricultural research, crop physiologists and agronomists have assigned different levels of one or more treatments to plots arranged according to elaborated rules of experimental designs, and subjected the data to analysis of variance tests for significant differences. Workers in pest management on the other hand, were often more interested in determining relationships between pest density and final yields, and used typically bivariate regression models for this purpose. In general practical considerations seriously limit the number of treatments and levels in the ANOVA approach, while a bivariate regression model was often found to be too restrictive for expressing pest impact on yield (SCHAUB *et al.*, 1988; BAUMGÄRTNER *et al.*, 1988c). Both methods have serious limitations imposed by the complexity of the system and the spatio-temporal processes underlying the biological interactions. Furthermore, any changes in system design and pest management strategies are likely to have profound effects on the structure and dynamics of whole agroecosystems.

To assess such effects, workers developing statistical models first sought to gauge the state of a system, and then attempted to make predictions about its future dynamics. Typically, they estimated densities of system components, such as number of plants, plant organs, arthropods, and weeds, or the incidence of diseases and other factors. What resulted was a two-dimensional matrix of samples and components (PIELOU, 1980). To analyze the data, multivariate analyses rather then bivariate methods were used as a first step. This procedure permits limited examination of the system's structure. Adding a temporal dimension, however, makes the analysis considerably more complex and often intractable. DIAMOND & CASE (1986) have recently edited a book on the state of the art of analyzing communities using these methods. Failure to account for the temporal behavior of a system is a serious limitation because yield formation is greatly influenced by the dynamics of both biotic (arthropod and weed dynamics as well as pathogen epidemics) and abiotic factors (soil nutrient and water and weather) that change over time. Researchers using multivariate statistics often overlook the limitations inherent in their approach when they try to infer causal relationships from data obtained in field sampling. The history of agroecosystem design and management suggests, however, that their collegues who construct dynamic population models on general ecological principles apply a better method. In the next chapter some of these principles, fundamental in crop physiology or animal demography, will be discussed briefly. Attempts to overcome some of the difficulties of the statistical approaches are described here, using specific applications as illustrations.

An attempt to explain the importance of single environmental factors and management inputs with a statistical analysis of controlled field experiments is that of BOWER & KALDOR (1980). Their work is a good example of the potential and limitations in a randomized block design which was used to evaluate chemical control of codling moth, *Cydia pomonella* (L.), and the the side effects of pesticides on spider mite and predator population densities (Tab. 1). The authors analyzed the three variables separately and found statistically significant differences between treatments. Thus their work provides important information on the selectivity of pesticides in a multi-species environment, but does not explain the dynamics of predator-prey interactions (which was not the purpose of their investigation).

SCHAUB *et al.* (1988) assessed the heteropteran fauna in Swiss apple orchards subjected to different intensities of insecticide treatments and other management procedures using ordination and classification methods (Fig. 3). Orchards are separated according to species composition and intensity of management measures. Orchards with frequent or occasional pesticide input (orchards under supervised or integrated pest control) appear in the center of the figure, while neglected orchards are located peripherally. The analysis indicates that locally dominant species are responsible for separating them spatially. The method was useful for summarizing a big data set and to put forward research hypotheses, but was of limited value for addressing specific management problems.

The limitations of static approaches, however, can be overcome at least to some extent with regression models that include dynamical properties of system components. BIERI *et al.* (1987) for example, related the yield of canning peas (*Pisum sativum*) not to a pea aphid (*Acyrthosiphon pisum* Harris) density, but to the calculated number of aphid-daydegrees experienced by the crop. SCHULTHESS *et al.* (1988) went further and expressed cassava (*Manihot esculenta*) yield losses (as a percentage) at the end of the dry season as a function of the cumulative number of mealybug (*Phaenacoccus manihoti* Mat.-Fer.)-days, divided by the initial plant weight to account for the influence of plant size at the beginning of plant-herbivore interaction (Fig. 4). ENTWISTLE & DIXON (1987) on the other hand, estimated the daily losses in wheat yield (d(t), as a percentage of yield loss) due to the presence of the grain aphid *Sitobion avenae* Fabricius. The final yield (as a percentage of the potential yield) was then expressed as the product of the daily calculated (1-d(t)/100), and put into a multiple regression model to evaluate the effects of insecticide applications. This model improved profitability decision making compared to prophylactic spraying.

The model of BIERI *et al.* (1987), SCHULTHESS *et al.* (1988) and of ENTWISTLE & DIXON (1987) incorporated temporal elements in the regression function, They are less successful, however, in applying principles of crop physiology and animal demography to complex agroecosystems operating under highly variable conditions, PEDIGO *et al.* (1978) and BARFIELD *et al.* (1987) for example, stress the importance of multiple stress factors in yield formation. These limitations were overcome to some extent by BAUMGÄRTNER *et al.* (1988c) who used a more comprehensive regression model to analyze rice production in Madagascar and included principles from crop physiology and arthropod demography. Although the authors found it difficult to incorporate the temporal behavior with sufficient detail, the model provided some insight into the processes affecting yield formation under various agrotechnical practices. Thus the model proved useful for planning pest management strategies in fields with highly variable yield potentials attacked by a multi-species pest complex.

Among other statistical methods, path analysis and spectral analysis also have been used to incorporate temporal processes and structural complexity in agroecosystem research, but their applicability seems to be limited. Path analysis, used widely by population genetists, has been applied only in crop physiology, e.g. in research on growth of apple trees (DÖRFEL & NEUMANN, 1973). PLATT & DENMAN (1975) point to the potential of spectral analysis in ecological research and discuss some of the reasons that prevented ecologists from making use of this method. In addition they criticized ecologists for investing to much effort in reductionism, for having an aversion to taking a holistic view, and for having a tendency to suppress variabilities inherent in ecological systems. Two examples of applications of spectral analysis in research on the biology of pest organisms are given here. LAUGA & ALAUZET (1983) used the method to investigate the age specific fecundity of the weevil *Pissodes notatus* Fabricius, and TAYLOR (1986) identified periodic variation in moth catches and found lunar, annual and four-year cycles.

3 DYNAMIC COMPONENT MODELS

The concept of physiological time

Plants, arthropods and diseases are poikilothermic organisms. Therefore, their rates of development are related to temperature (eqn.1) and temperature needs to be taken into account when investigating their temporal dynamics. STINNER *et al.* (1974), GILBERT *et al.* (1976) and CURRY & FELDMAN (1987) review the concept and use of physiological time in population models. The units of physiological time in eqn. (1) are based on proportional development

$$\int_{t_0}^{t_1} r\{T(t)\}\, dt = 1 \qquad \text{rate integration} \qquad [1]$$

According to eqn. [1], the time required to complete a particular life stage has accrued when the integral of the developmental rates r over the time period (t_0 to t_1) is equal to 1. Any value less than 1 is interpreted as the proportion of development that has been completed during a particular period.

Eqn. [1] indicates that the rate r is not constant but depend on the temperature T which may vary through time (t). A review of models including a discussion of temperature T(t) profile is far beyond the scope of this review, and readers are referred to the work of GILBERT *et al.* (1976), ALLEN (1978), and CURRY & FELDMAN (1987) for an historical and quantitative review of the concept. Note also that there are difficulties in representing accurately the temperature profile temporally and spatially (BAUMGÄRTNER & SEVERINI, 1987). These difficulties together with a general need to base models of complicated biology on difference equations (GILBERT *et al.*, 1976; GUTIERREZ & WANG, 1977; CURRY & FELDMAN, 1987) force modelers to reformulate eqn. [1] in discrete form as follows

$$\sum_{1}^{nt} r\{T(t)\}/n \approx 1 \qquad \text{rate summation} \qquad [2]$$

If chronological time is expressed in units of days, and n is the number of time increments per day, nt is the number of increments in t days. The developmental phase is completed once the sum reaches unity.

Several functions have been proposed to describe the r(T) relationship. Eqn. (3) is based on the work of LOGAN *et al.* (1976) and is presented here as an example of a nonlinear and asymmetrical r(T) function that is valid for the whole temperature range permitting poikilothermic development

$$r(T)=c_1 \cdot \{\exp[c_2 \cdot (T-T_0)]-\exp[c_2 \cdot (T_m-T_0)-c_3 \cdot [(T_m-T_0)-(T-T_0)]]\} \qquad [3]$$

where c_1, c_2, c_3 are constants, T_0 is the lower and T_m the upper temperature threshold for development.

Over a restricted range of temperature, the developmental rate r is usually proportional to the temperature above the lower developmental threshold T_0 and the following linear model with the constant c_4 can be used

$$r(T) = c_4 \cdot (T - T_0). \qquad [4]$$

Once the temperature T_i for for each time increment is known the instantaneous rates of either eqn. [3] or eqn. [4] can be built into eqn. [2]. If eqn. [4] is selected the subsequent function predicts that a life phase is completed after D days if the sum of effective temperatures (T_i-T_0) reaches the value of the thermal constant C. In this model physiological time is defined as degrees ^{0}C per day above a threshold T_0 or day-degrees and not on the basis of proportional development. Below this threshold no development occurs.

$$\sum_{i=1}^{D} (T_i - T_0) = 1/c_4 = C. \quad \text{for} \quad (T_i > T_0) \qquad [5]$$

Eqn. [5] is a powerful tool for simulating poikilothermic development and has found wide applications in research and implementation work. GILBERT *et al.* (1976) discuss the model in a demographic context, while CAMPBELL *et al.* (1974) compute the reliability of estimations for c_4 and C when estimated from controlled experiments. More recently, GUTIERREZ *et al.* (1984) used the ratio of food supply-demand to modify this model to include nutritional effects on developmental time.

Given the above relationships to temperature, individuals born at the same time exhibit different developmental rates. The most widely used methods to handle variation in development times are time distributed processes (MANETSCH, 1976; DE WIT & GOUDRIAAN, 1978). WAGNER *et al.* (1984) and CURRY & FELDMAN (1987) summarize a third approach based on a biophysical model for the r(T) function.

Population dynamics

In many theoretical studies or in models for populations achieving a stable age distribution (e.g., pathogens), the net per capita growth rate (z=births-deaths) can be built into a reasonably simple function describing the population dynamics of species N

$$dN/dt = z \cdot N. \qquad [6]$$

The number N of individuals at any point in time (N_t) may be obtained by integration, given the initial density N_0

$$N_t = N_0 \cdot \exp(z \cdot t) \qquad [7]$$

Methods for estimating the intrinsic rate of increase (z) for pathogens are described by ZADOKS & SCHEIN (1979), while KREBS (1972) discusses the models that are required for arthropods. The intrinsic rate of increase for arthropod populations is generally estimated from age specific life tables obtained under controlled conditions, and is assumed to be the maximum rate under a non-limiting environment. Life tables of field populations are widely used by entomologists and plant population ecologists (KREBS, 1972; SILVERTOWN, 1982). HARCOURT (1970) for example, constructed an age specific life table for a planting of early market cabbage. Because the factors responsible for losses are known, HARCOURT (1970) could assess their importance in monetary units, in the field under study. For arthropods, age specific life tables are constructed to estimate the intrinsic rate of increase [6] under given host plant characteristics and environmental conditions. The studies can be used to define geographical zones favoring arthropod outbreaks (KREBS, 1972), or to test plant cultivars for resistance (SCHULTHESS *et al.*, 1987). A series of such life tables permits a key factor analysis (VARLEY *et al.*, 1973), which has provided insight as to factors controlling the dynamics of plant and arthropod populations. The method has limitations, however, that restrict its use in agroecosystem design and management, so we do not discuss it further here.

In most cases the age structure of a field population is not constant through time and another type of model is required to represent the demographic processes (HUGHES, 1963; GILBERT *et al.*, 1976). A convenient and concise mathematical description is the VON FOERSTER (1959) equation (WANG *et al.* 1977, GUTIERREZ & WANG, 1977; CURRY & FELDMAN, 1987)

$$dN/dt + dN/da = -\mu(a) \cdot N(t,a) \qquad \text{for } t, a>0 \qquad [8a]$$

and

$$N(t,0) = \int_0^\infty \Omega(a) \cdot N(t,a)\, da \qquad \text{for } t>0 \qquad [8b]$$

with an initial age distribution of N(0,a) for a>0. $\mu(a)$ is the age dependent mortality function and $\Omega(a)$ is the age dependent birth rate. Eqn. [8a] represents the aging and death of cohort, i.e. of those individuals born at the same time, while eqn. 8b describes the birth rof new individuals.

In most situations, however, μ and Ω are not only functions of age a, but vary also with changing environmental conditions. Constant within life stage mortalities generally permit analytical solutions (WANG *et al.*, 1977; NISBET & GURNEY, 1980). However, if complex functions are required to represent $\mu(.)$ and $\Omega(.)$, eqn. [8] cannot be solved analytically and the model must be discretized and evaluated numerically (i.e., via simulation models, WANG *et al.*, 1977).

Population interactions, functional and numerical response models

Population interactions have a great influence on population development of arthropods (BEDDINGTON *et al.*, 1976; GUTIERREZ *et al.*, 1981a,1984) and on functions $\mu(.)$ and $\Omega(.)$ in eqn. [8], We ignore age structures and use the simple form of TOFT's (1986) equations to express population interactions in functional and numerical response models

$$dN/dt = R(N) - F(N,P)\cdot P$$
$$dP/dt = G\{F(N,P)\} - v\cdot P \qquad [9]$$

F(N,P) is the functional response describing the relationship between the number of prey (hosts) captured per predator (parasitoid), while R(N) is the rate of increase of the prey (host) in absence of predators (parasitoids). G{F(N,P)} is the numerical response of the predator (parasitoid), while v is the death rate of predators (parasitoids) unrelated to prey (host) presence. Many functional response models have been proposed, and a thorough discussion of them goes far beyond the scope of this work. As an example we present the global form of the model by FRAZER & GILBERT (1976) for parasitoids. During the time interval Δt, the number of hosts attacked (N_a) by the number P of parasitoids with a per capita demand rate b and a search rate a is

$$N_a(t) = N_t\cdot\{1\text{-}\exp[[-P\cdot b\cdot\Delta t/N_t]\cdot[1\text{-}\exp(a\cdot N_t/(-P\cdot b\cdot\Delta t))]]\} \qquad [10]$$

where N_t is the host density at time t. If host depletion does occur a different model is used or eqn. [10] has to be modified accordingly (KLAY, 1987; GUTIERREZ *et al.*, 1988c) transforming the host-parasitoid model into a predator-prey model. Recently much emphasis has been given to incorporation of behavioral elements and spatial dynamics in functional response models (BAUMGÄRTNER *et al.*, 1988b).

The functional response estimates the mortality rate of the prey(host), and permits reproduction of predators(parasitoids). During the transfer of biomass or energy from one trophic level to the next higher one some material is inevitably lost because the organism has to cover the costs of converting material and of basic maintenance processes. For non overlapping generations, BEDDINGTON *et al.* (1976) presented a nonlinear and a linear model to represent predator reproduction as a function of prey numbers attacked

$$G[F(N,P)] = \lambda/e \cdot [N \cdot (1-F(N,P) \cdot \kappa - \nu] \qquad [11]$$

where λ, e, and ν = constants (e = the biomass per egg, ν = constant depending on maintenance energy requirements and the proportion of food that is assimilated), and κ = proportion of prey eaten that may depend on prey density. The model is greatly simplified for parasitoids because an attacked host is converted typically into a parasitoid. In classical demography eqn. [9] and some form of eqn. [10] have been formulated for numbers of individuals interacting with each other, usually at the second trophic level. In formulating multitrophic population models, however, this procedure is difficult to use, particularly for formulating plant-herbivore interactions and for including vital requisites other than mass or energy.

A comprehensive multi-species model for requisite acquisition and allocation

GUTIERREZ *et al.* (1981a, 1984, 1987b) used prey mass rather than numbers and formulated a metabolic pool model that placed functional and numerical responses into a comprehensive framework of population interactions. The comprehensiveness stems from two attributes: First, the metabolic pool model provides a coherent framework for linking functional and numerical responses over several trophic levels. It permits the use of a common acquisition/allocation function for all components of agroecosystems including the plant population, its subunits and the socio-economic environment (Fig. 5). From a theoretical point of view, a link between crop physiology and classical population theory is established, while the unified structure of the models facilitates model development. Second, the model is not restricted to mass or energy flow between populations, but it also incorporates into population interactions the effects of acquisition and allocation of other vital requisites, such as nitrogen and water. According to this model the assimilation rate (dA/dt), also comprising available reserves, becomes

$$dA/dt = M_{i-1} \cdot f\{M_{i-1}, M_i, T, b(.), a(.)\} \cdot \{1-\beta\} - \gamma(T) \cdot M_i + \zeta \cdot Q. \qquad [12]$$

M_i is the mass of the population on the trophic level i, β is the proportion of acquired food that is either excreted or lost in conversion, γ represents temperature (T) dependent maintenance costs per unit mass, and ζ denotes the proportion of reserves Q available. In the model of food allocation by a coccinellid predator, BAUMGÄRTNER *et al.* (1987) separated conversion costs from requirements of maintenance. f(.) is the functional response model that includes both the per capita demand rate b(.) which depends on food quality and the search rate a(.) which is built on behavioral elements. Note that the model includes a genetically defined per capita demand rate b as the major driving variable in f(.). If model [12] is constructed for modelling the energy flow, b can vary according to the need of the organism to acquire additional vital requisites, such as nitrogen and water. GUTIERREZ *et al.* (1987b) applied this concept to the cassava ecosystems model, while theoretical justification for incorporating genetically controlled demand rates as major driving forces in population interactions is given by GUTIERREZ & REGEV (1983).

4 SIMULATION MODELS IN AGROECOSYSTEM MANAGEMENT

Populations are the important components of agroecosystems and they should therefore be put into the center of agroecosystem research work. Once the theoretical basis for population development and interactions has been established, management models can be constructed by incorporating intrinsic parameters, density dependent factors and abiotic factors (Fig. 6). This makes the models more realistic but increases their complexity at the same time. At the extreme, such models are research tools only, claiming to integrate all relevant aspects of the system under study and to focus attention on the main gaps in present operational knowledge (DE WIT & PENNING DE VRIES, 1985). As a consequence, they are difficult to use for solving specific pest management problems and some workers have therefore simplified them to create a more tractable framework for economic optimization work (e.g. SHOEMAKER, 1980; REGEV *et al.*, 1988), including the evaluation of regional production potentials and constraints (DE WIT & PENNING DE VRIES, 1985).

In most cases, however, practical considerations dictated another and more pragmatic procedure for developing and implementing simulation models. Different management problems require different levels of understanding, hence different types of the simulation model (Fig. 2). For example, workers and systems managers interested only in seasonal occurrences of a pest may base their model exclusively on physiological time. They need population models to examine population interactions or to address specific fundamental problems of agroecosystem design and management. For this reason in the modern literature there has appeared quite a number of simulation models with specific applications.

Descriptive phenological models

Phenological models make use of physiological time to introduce realistically a temporal dimension into methods for decison making in crop and pest mangement. For example, CHARMILLOT (1980) wanted to know the beginning and the end of the first flight of the codling moth *C. pomonella* in Swiss apple orchards. He assumed that moth development is proportional to temperatures above a threshold of 10^{0}C and calculated the sum of effective temperatures, beginning on January 1. Above this physiological time horizon he plotted empirically obtained flight curves (Fig. 7). He compared three pest management strategies. Given the presence of the codling moth, a farmer can apply control measures against the first individuals, which are likely to give birth to the economically important next generation, or alternatively, he can wait until the number of eggs reaches or passes a predetermined action threshold of 1% of infested fruits before chemical control measures are undertaken. Both strategies require fewer treatments than the traditional (classical) method based on calendar time.

Phenology is the study of the timing of recurrent biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species (LIETH, 1974) and has guided many agricultural activities since ancient times (WAGGONER, 1974). To explain the relevant events, however, the definition of an appropriate time horizon is often not sufficient and more ecological principles need to be considered in model building.

Analytical phenological models

WELCH *et al.* (1978) tried to explain flight phenologies with simplified population models, i.e. respecting time varying age structures and variabilities in individual developmental times, but ignoring migration and death rates. Normalized birth rates control the sequence of multivoltine population development. These attributes were used to parametrize a time invariant distributed delay model (MANETSCH, 1976), operating on physiological time expressed in degree days. An example of this approach is the flight phenology of the summerfruit tortrix, *Adoxophyes orana* F. v. R., simulated with a time-varying distributed delay model (BAUMGÄRTNER *et al.*, 1988a, Fig. 8). The delay model became the core of the Predictive Extension Timing Estimator (PETE) permitting phenology predictions of various pest and disease developmental stages. This model stimulated interest in rapid processing and delivery of biological and environmental information to farmers. In 1974, the PMEX computer system for real-time management of agricultural pests became available to Michigan apple growers, and was soon followed by similar systems elsewhere (WHALON & CROFT, 1984). The organization of such a system (Fig. 9) was conceptualized by RUESINK (1975) and modified by FLINT & VAN DEN BOSCH (1981).

The delay model also became a useful tool for fundamental research work on pest insects, such as the study of the influence of microclimate and spatial distributions on the flight phenology of the leaf miner *Phyllonorycter blancardella* F. BAUMGÄRTNER & SEVERINI (1987) linked time-varying distributed delays with temperature-dependent developmental rates predicted by the function of LOGAN *et al.* (1976, eqn. 3). The results are given in Fig.10 and show how microclimatic differences and spatial distributions help explain the complicated flight patterns often observed under field conditions.

Analytical phenological models can explain the timing of events, but fail to represent important population attributes. Among these are population density or mass that represents the temporal dynamics and the yield formation of the system under study or management. VANSICKLE (1977) introduced attrition (losses) into MANETSCH's (1976) delay model, and extended thereby the practical application of the model beyond phenology to the modelling of interacting field populations (GUTIERREZ *et al.*, 1984).

Single species population models

Single species population models without age structures are often constructed by phytopathologists. Usually eqn. [6] is modified to account for proportional plant tissue infected as a function of initial inoculum density, lesion growth rates, and maximum infestation levels (ZADOKS & SCHEIN, 1979). GESSLER & BLAISE (1988) added recently an empirical host growth function and latent periods in disease development to eqn. [6]. The model predicted satisfactorily the epidemic of various diseases and was concluded to be a valuable tool for epidemiological research.

REDDY *et al.* (1987) used the dynamic simulator of cotton growth and development built by BAKER *et al.* (cit. in REDDY *et al.*, 1987) to investigate the effect of herbicides on cotton growth, development and yield. Herbicides can inhibit root growth, and when this effect was incorporated into the model reduced cotton performance was predicted. The authors concluded that improper application of herbicides was one of the contributing factors for the cotton yield decline observed in the United States since 1965. Recently, a simulation model for alfalfa was used to investigate injury potential by a leaf mining insect (SAWYER & FICK, 1987).

CARTER *et al.* (1982) constructed a comprehensive population model for cereal aphids, including time varying age structure [eqn. 7]. However, a simpler model based on eqn. [6] predicted population build-up until the late milky stage of the crop (RABBINGE & MANTEL, 1981). This is an important capability because the final yield loss can be expressed as a function of pest density at this stage (RABBINGE & RIJSDIJK, 1983). The intrinsic growth rate in eqn. [6] can be modified according to differences in crop productivity and field characteristics. The model is compact and can be incorporated into the EPIPRE disease predictor algorithms used for decision making in cereal pest management.

Single species population models represent satisfactorily the dynamics of a particular population (inluding plants and their subunits). They permit decision making with respect to a particular component of the system and provide solid ground for further work on population interactions. We consider therefore the development and evaluation of realistic single species population models (Fig. 6) the most promising building block for models in agroecosystem research.

Predator-prey(host-parasitoid) interactions

Population models developed for overlapping generations have provided insight into biological control operations, both in the field and in greenhouses. Under the latter conditions several models on acarine predator-prey systems, and on population interactions between aleyrodid hosts and parasitoids were developed. These studies have shown the dynamics of the relevant population interactions and have rationalized the use of predatory mites and parasitoids as biological control agents in greenhouses (BAUMGÄRTNER & YANO, 1988; BAUMGÄRTNER *et al.*, 1988b).

For field conditions, DOVER *et al.* (1979) constructed population models for interactions between European spider mites (*Panonychus ulmi* KOCH) and predatory phytoseiid mites (*Amblyseius fallacis* GARMAN) in Michigan's apple orchards. It was concluded that the model represented satisfactorily the dynamics of the acarine predator-prey system, and a summary of the results complements empirical field studies on mite-predator-interactions. The results were summarized in a diagram for use by apple growers (Fig. 11, WHALON & CROFT, 1984). Fig. 11 shows both the results of simulation studies as well as the empirically derived decision rules. Different contour lines are shown that indicate whether biological control is sufficient to control mite populations.

WAAGE *et al.* (1985) built insecticide mortality into a widely used host-parasitoid population model representing discrete generations. The results are interesting from a practical standpoint; an insecticide affecting both populations for example, may or may not create pest resurgence, depending on the timing of the application and the pest stage measured.

Models on interactions between arthropods are of great interest in research work and in implementation of biological control programs. The use of these models, however, is limited because plant growth and development is not modelled mechanistically.

Interactions between plants and herbivores

GUTIERREZ and coworkers (GUTIERREZ *et al.*, 1975,1979a,1979b, 1987a,1988b; WANG *et al.*, 1977) developed demographic models for plant growth and development. Reproductive organs, roots, stems and leaves are considered to have population characteristics, such as numbers, mass, age structures, and birth/death rates. In the analysis of the cotton *Gossypium hirsutum* ecosystem for example, they studied the attacks of the cabbage looper*Trichoplusia ni* (Hübner), the beet armyworm *Spodoptera exigua* (Hübner), the pink bollworm *Pectinophora gossypiella* (Saunders), the Lygus bug *Lygus hesperus* Knight, the boll worms *Heliothis* spp. and the boll weevil *Anthonomus grandis* Boh., as well as infestations of the pathogen verticillium wilt on the cotton crops. The knowledge obtained with this model provided insight into plant-herbivore interactions with implications for management strategies. In some cases the interactions could be reduced to a simple graph representing the dynamics of herbivore-plant interactions (Fig. 12) verified in the field. In other cases simplifications were made permitting economic optimization work that addressed management problems as well as evolutionary aspects in plant-herbivore interactions (GUTIERREZ & REGEV, 1983). A model of cotton and boll weevil *A. grandis* population interactions was built by CURRY *et al.* (1980) and used to evaluate several production strategies. Similar methods were applied to other systems, such as alfalfa and soybean (HUFFAKER, 1980).

The model of GUTIERREZ *et al.* (1975) on cotton growth and development is able to represent leaf age at any point in time. If we accept leaf age as a surrogate for food quality, the model is particularly useful to study plant effects on herbivore dynamics. ZAHNER & BAUMGÄRTNER (1988) used this notion to model the effect of apple leaf age on spider mite population dynamics. The growth rate of shoots predicted by the same model has a profound influence on aphid infestations (GRAF *et al.*, 1985). Leaf quality in terms of nitrogen concentration has recently been used to examine its effect on mite population growth rates in a cassava *M. esculenta* system (GUTIERREZ *et al.*, 1987b,1988a). The properties of the model permit the addition of herbivore feeding as an additional sink for carbohydrates. In addition, these models provide a solid ground for studying herbivore-plant and higher trophic level interactions.

Explicative models on plant-herbivore interactions enable us to study plant influences on herbivore population dynamics. Furthermore, they are valuable instruments to assess the pest status of herbivore populations and to search for optimum pest management strategies. However, they do not comprise explicative models on natural enemies and on their impact on herbivore populations which is a serious limitation in most cases.

Multitrophic population models

Many systems have been studied (HUFFAKER, 1980), but few multispecies population models have been constructed. We summarize here only the recent work on the cassava system (GUTIERREZ *et al.*, 1987b,1988a,1988b,1988c). The systems model for cassava is represented in Fig. 13. In that system, the growth of the plant depends on weather (temperature, solar radiation, rainfall, wind) as well as on soil nitrogen. The factors limiting the growth rates of plant parts are the supply-demand ratios for carbohydrates, nitrogen and water. In addition, the uptake rates of nitrogen are affected by the dynamics of water uptake. The realized photosynthetic rate of the plant affects not only plant growth but also cassava mealybug *P. manihoti* and cassava green mite *Mononychellus tanajoa* (Bondar)*(sensu latu)* growth rates. The stresses within the plant set the limit for herbivore growth and herbivore abundance which further affects plant growth. The dynamics of *P. manihoti* (EM) and *M. tanajoa (sensu latu)* (CGM) and their effects on tuber growth compared with non stress conditions are depicted in Fig. 14. The effects of the exotic parasitoid *Epidinocarsis lopezi* (DeSantis) and native coccinellid beetle of the genus *Exochomus* and *Hyperaspis* on *P. manihoti* are not shown in Fig. 14, but are discussed in full in GUTIERREZ *et al.* (1988a,1988b,1988c). In brief, the parasitoid was found to be an active regulator of *P. manihoti* densities, while the effects of the predators were largely dispensible. The effect of weather could also be assessed. The model is currently being used to estimate the benefits of biological control due to the introduction of *E. lopezi.*

Such comprehensive models have wide areas of application and are a promising tool to overcome some of the lmitations (PLATT & DENMAN, 1975) of the reductionistic approach in ecological research. The cassava model, for example, can now be used in economic analyses and as a building block for evaluating polycultures used in sustainable agriculture (GUTIERREZ, 1987).

5 CONCLUSIONS

The simulation models used in agroecosystem design and management usually address specific problems in pest management systems. Subjective preferences of a researcher too often dictate the level of complexity incorporated into the model. The review, however, suggests that this is not a satisfactory approach, and gives some indications as to the level of complexity required in model building to achieve useful results. Agroecosystem design and management deals primarily with populations interacting with each other under field conditions. Population attributes, such as intrinsic parameters, density dependent and abiotic factors are essential attributes that should be put into such models.

Admittedly our research interests are in animal demography, but from this review it is obvious that the state of the art in agroecosystem design and management differs between disciplines. Entomologists appear more interested in multispecies interactions than are crop physiologists, weed scientists or plant pathologists. This is unfortunate, because the design and management of agroecosystems requires system related multidisciplinary work at all levels.

We have also shown ::ne important contributions of the simulation approach to improvements in agroecosystem de.ign and management. The different model types can easily be arranged on a scale representing biologically and mathematically increasing levels of complexity. With increasing complexity, the applications shift from tactical decision making to planning production strategies and undertaking fundamental research work. Concomittantly there is an increasing need to represent the models in simplified forms for application purposes. Two areas of application for simplified models have been identified: The models can be simplified to fit into an economic decision making environment, or they can be summarized in a convenient form to facilitate tactical decision making. In general we conclude that development of realistic management models for policy making has now become a realistic objective.

6 ACKNOWLEDGEMENTS

The authors thank Prof. Dr. V. Delucchi, Mr. J. Casas (Institute of Plant Sciences, ETH, Zurich) and Dr. W. Van der Werf (Department of Theoretical Production Ecology, Agricultural University, Wageningen) for making helpful suggestions.

7 REFERENCES

ALLEN, 1978. - A modified sine wave for calculating degree days. - Environ. Entomol. 5: 388-396.

BARFIELD, C. S., CARDELLI, D. J. & BOGGESS, W. G. - 1987. Major problems with evaluating multiple stress factors in agriculture. - Tropical pest management 33: 109-118.

BAUMGÄRTNER, J. & DELUCCHI, V. - 1988. Vom integrierten Pflanzenschutz zur optimalen Bewirtschaftung von landwirtschaftlichen Kulturen. - Schweiz. Landw. Fo. 27: 77-90.

BAUMGÄRTNER, J. & SEVERINI, M. - 1987. Microclimate and arthropod phenologies: the leaf miner *Phyllonorycter blancardella* F. (Lep.) as an example. - In: PRODI, F., ROSSI, F. & CRISTOFERI (eds.), Agrometeorology. Int. Conf. on Agrometeorology, Cesena, Fondazione Cesena Agricultura Publ., 498 pp.

BAUMGÄRTNER, J. & YANO, E. - 1988. Aleyrodid population dynamics and modelling. - Proc. XVIII Intern. Congr. of Entomology, Vancouver, July 3 to 9, 1988.

BAUMGÄRTNER, J., BIERI, M. & DELUCCHI, V. - 1987. Growth and development of *Propylaea 14-punctata* L. and *Coccinella 7-punctata* L. (Col.: Coccinellidae) simulated by the metabolic pool model.- Entomophaga 32: 415-423.

BAUMGÄRTNER, J., FAVRE, J.-J. & SCHMID, A. - 1988a. On the use of a time-varying distributed delay model for simulating the flight phenology of the summerfruit tortrix *Adoxophyes orana* F. v. R. in the Valais, Switzerland. Bull. SROP/WPRS (in press).

BAUMGÄRTNER, J., GUTIERREZ, A. P. & KLAY, A. - 1988b. Elements for modelling tritrophic population interactions. - Exp. Appl. Acarol. (in press).

BAUMGÄRTNER, J., REGEV, U., RAHALIVAVOLOLONA, N., GRAF, B., ZAHNER, Ph. & DELUCCHI, V. - 1988c. Rice production in Madagascar: regression analysis with particular reference to pest control. - Agric. Ecosystems Environ. (subm. 1988).

BEDDINGTON, J. R., HASSSELL, M. P. & LAWTON, J. H. - 1976. The components of arthropod predation. II. The predator rate of increase. - J. Anim. Ecol. 45: 165-185.

BIERI, M., DELUCCHI, V., JAGER, P., BAUMGÄRTNER, J. & ROTH, O. - 1987. Belastbarkeit der Erbsenfelder mit der Blattlaus *Acyrthosiphon pisum* HARRIS in der Ostschweiz. - Schweiz. Landw. Fo. 26:161-172.

BOWER, C. C. & KALDOR, J. - 1980. Selectivity of five insecticides for codling moth control: effects on the twospotted spider mite and its predators. - Environ. Entomol. 9: 128-132.

BROWN, A. W. A.. - 1978. Ecology of pesticides. - Wiley, New York, 525 pp.

CAMPBELL, A., FRAZER, D. B., GILBERT, N., GUTIERREZ, A. P. & MACKAUER, M. - 1974. Temperature requirements of some aphids and their parasites. - J. appl. Ecol. 11: 431-438.

CARTER, N., DIXON, A. F. G. & RABBINGE, R. - 1982. Cereal aphid populations: biology, simulation and prediction. - Pudoc, Wageningen, 91 pp.

CHARMILLOT, P. J. - 1980. Développement d'un système de prévision et de lutte contre le carpocapse (*Laspeyresia pomonella* L.) en Suisse romande: rôle du service régional d'avertissement et de l'arboriculture. - Bull. OEPP 10: 231-239.

CROFT, B. A. & HOYT, B. A. - 1983. Integrated management of insects of pome and stone fruits. - Wiley, New York, 454 pp.

CURRY, G. L. & FELDMAN, R. M. - 1987. Mathematical foundations of population dynamics. - Texas A&M University Press, College Station, 246 pp.

CURRY, G. L., SHARPE, P. J. H., DEMICHELE, D. W. & CATE, J. R. - 1980. Towards a management model of the cotton-boll weevil ecosystem. - J. Environ. Management 11: 187-223.

DELUCCHI, V. - 1987. La protection intégrée des cultures. - Parasitis, Genève, 411pp.

DE WIT, C. T. & GOUDRIAAN, J. - 1978. Simulation of ecological processes. - Pudoc, Wageningen, 175 pp.

DE WIT, C. T. & PENNING DE VRIES, F. W. T. - 1985. Predictive models in agricultural production. - Phil. Trans. R. Soc. Lond. B 310: 309-315.

DIAMOND, J. & CASE, T. J. - 1986. Community ecology, Harper & Row, Cambridge, 665 pp.

DOVER, M. J., CROFT, B. A., WELCH, S. M. & TUMMALA, R. L. - 1979. Biological control of *Panonychus ulmi* (Acarina: Tetranychidae) by *Amblyseius fallacis* (Acarina: Phytoseiidae):

a predator-prey model. - Environ. Entomol. 8: 282-292.

DÖRFEL, H. & NEUMANN, V. - 1973. Untersuchung eines Systems der Trieb- und Ertragsbildung bei Apfelbäumen mit Hilfe von Pfadkoeffizienten. - Biom. Z. 15: 527-543.

ENTWISTLE, J. C. & DIXON, A. F. G. - 1987. Short-term forecasting of wheat yield loss caused by the grain aphid (*Sitobion avenae*) in summer. Ann. appl. Biol. 111: 489-508.

FLINT, M. L. & VAN DEN BOSCH, R. - 1981. Introduction to integrated pest management. - Plenum Press, New York, 240 pp.

FRAZER, B. D. & GILBERT, N. - 1976. Coccinellids and aphids: A quantitative study of the impact of adult ladybirds (Coleoptera, Coccinellidae) preying on field populations of pea aphids (Homoptera: Aphididae). - J. ent. Soc. Brit. Columb. 73: 33-56.

GESSLER, C. & BLAISE, Ph. - 1988. The extended progeny/parent ratio model: concepts and practical applications. - Abstract of papers, 5th Intern. Congr. Plant Pathology, Kyoto, August 20-27, 1988.

GETZ, W. M. & GUTIERREZ, A. P. -1982. A perspective of systems analysis in crop production and insect pest management. - Ann. Rev. Entomol. 27: 447-466.

GILBERT, N., GUTIERREZ, A. P., FRAZER, B. D. & JONES, R. E. - 1976. Ecological relationships. - Freeman, Reading, 157 pp.

GRAF, B., BAUMGÄRTNER, J. & DELUCCHI, V. - 1985. Simulation models for the dynamics of three apple aphids, *Dysaphis plantaginea*, *Rhopalosiphum insertum*, and *Aphis pomi* (Homoptera: Aphididae) in a Swiss apple orchard. - Z. ang. Ent. 99: 453-465.

GUTIERREZ, A. P. - 1987. Systems analysis in integrated pest management. - Parasitis, Genève, 411 pp.

GUTIERREZ, A. P. - 1988. Quantitative evaluation of natural enemies. - Proc. XVIII Intern. Congr. of Entomology, Vancouver, July 3 to 9, 1988.

GUTIERREZ, A. P. & WANG, Y. - 1977. Applied population ecology: models for crop production and pest management. - in: NORTON, G. A. & HOLLING, C. S. (eds.), Proc. conf. pest management, Laxenburg, October 25 to 29, 1976, 352 pp.

GUTIERREZ, A. P. & REGEV, U. - 1983. The economics of fitness and adaptedness: the interaction of sylvan cotton (*Gossypium hirsutum* L.) and the boll weevil (*Anthonomus grandis* Boh.). - Acta Oecologica, Oecol. Gener. 4: 271-287.

GUTIERREZ, A. P., BAUMGÄRTNER, J. & HAGEN, K. S. - 1981a. A conceptual model for growth, development, and reproduction in the Ladybird beetle, *Hippodamia convergens* G.-M. - Can. Ent. 113: 21-33.

GUTIERREZ, A. P., BAUMGÄRTNER, J. & SUMMERS, C. - 1984. Multitrophic models of predator-prey energetics. - Can. Ent. 116: 923-963.

GUTIERREZ, A. P., WANG, Y. & DAXL, R. - 1979a. The interaction of cotton and cotton boll weevil (Coleoptera: Curculionidae) - a study of co-adaptation. - Can. Ent. 111: 357-366.

GUTIERREZ, A. P., WANG, Y. & REGEV, U. - 1979b. An optimization model for *Lygus hesperus* (Heteroptera: Miridae) damage in cotton: the economic threshold revisited. - Can. Ent. 111: 41-54.

GUTIERREZ, A. P., DAXL, R., LEON QUANT, G. & FALCON, L. A. - 1981b. Estimating economic thresholds for bollworm, *Heliothis zeae* Boddie, and boll weevil, *Anthonomus grandis* Boh., damage in Nicaraguan cotton *Gossypium hirsutum* L. - Environ. Entomol. 10: 872-879.

GUTIERREZ, A. P., FALCON, L. A., LOEW, W., LEIPZIG, P. A. & VAN DEN BOSCH, R. - 1975. An analysis of cotton production in California: a model for acala cotton and the effects of defoliators on its yield. - Environ. Entomol. 4: 125-136.

GUTIERREZ, A. P., YANINEK, J. S., WERMELINGER, B., HERREN, H. R. & ELLIS, C. K. - 1988a. Analysis of biological control of cassava pests in Africa. III. Cassava green mite *Mononychellus tanajoa*. - J. appl. Ecol. 25 (in press).

GUTIERREZ, A. P., SCHULTHESS, F, WILSON, L. T., VILLACORTA, A. M., ELLIS, C. K. & BAUMGÄRTNER, J. -1987a. Energy acquisition and allocation in plants and insects: a hypothesis for the possible role of hormones in insect feeding patterns. - Can. Ent. 119: 109-129.

GUTIERREZ, A. P., WERMELINGER, B., SCHULTHESS, F., BAUMGÄRTNER, J., HERREN, H. R., ELLIS, C. K. & YANINEK, J. S. - 1988b. Analysis of biological control of cassava pests in Africa: I. Simulation of carbon, nitrogen and water dynamics in cassava. - J. appl. Ecol. 25 (in press).

GUTIERREZ, A. P., WERMELINGER, B., SCHULTHESS, F., BAUMGÄRTNER, J., HERREN, H. R., ELLIS, C. K. & YANINEK, J. S. - 1988c. Analysis of biological control of cassava pests in Africa: II. Cassava mealybug *Phenacoccus manihoti*. - J. appl. Ecol. 25 (in press).

GUTIERREZ, A. P., WERMELINGER, B., SCHULTHESS, F., BAUMGÄRTNER, J., YANINEK, J. S., HERREN, H. R., NEUENSCHWANDER, P., LÖHR, B., HAMMOND, W. N. O. & ELLIS, C. K. - 1987b. An overview of a systems model of cassava and cassava pests in Africa. - Insect Sci. Applic. 8: 919-924.

HARCOURT, D. G. - 1970. Crop life tables as a pest management tool. - Can. Ent. 102: 950-955.

HEADLEY, J. C. - 1972. Defining the economic threshold. - In: Pest control strategies for the future, National Academy of Sciences, Washington, 376pp.

HUFFAKER, C. B. - 1980. New technology of pest control. - Wiley, New York, 500 pp.

HUFFAKER , C. B. & CROFT, B. A. - 1976. Integrated pest management in the U.S.: progress and promise. - Environ. Health Perspectives 14: 167-183.

HUGHES, R. D. - 1963. Population dynamics of cabbage aphid, *Brevicoryne brassicae* (L.). - J. Anim. Ecol. 32: 393-424.

KLAY, A. - 1987. Ecosystème verger de pommier: Enquête faunistique sur les Phytoséiides et étude de leurs interactions avec l'acarien rouge *Panonychus ulmi* (KOCH) en laboratoire. - Thèse EPFZ No. 8386, Zurich, 110 pp.

KREBS, C. J. - 1972. Ecology, the experimental analysis of distribution and abundance. - Harper & Row, New York, 694pp.

LAUGA, J. & ALAUZET, C. - 1983. Dynamique des populations d'un ravageur du Pin: *Pissodes notatus* (Coléoptère, Curculionidae): Optimum écologique de ponte et modèle de fécondité. Acta Oecologica, Oecol. Applic. 4: 151-161.

LIETH, H. - 1974. Purposes of a phenology book. - In: LIETH, H. (ed.), Phenology and seasonality modelling. Springer Verlag, Berlin, 444 pp.

LOGAN, J. A., WOLLKIND, D. J., HOYT, S. C. & TANIGOSHI, L. K. - 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. - Environ. Entomol. 5:1133-1140.

MANETSCH, T. J. - 1976. Time-varying distributed delays and their use in aggregative models of large systems. - IEEE Trans. Sys. Man Cybern. 6: 547-553.

METCALF, R. L. & LUCKMANN, W. H. - 1975. Introduction to insect pest management. - Wiley, New York, 587 pp.

NISBET, R. M. & GURNEY, W. S. C. - Modelling fluctuating populations. - Wiley, New York, 379 pp.

PEDIGO, L. P., HUTCHINS, S. H. & HIGHLEY, L. G. - 1986. Economic injury levels in theory and practice. - Ann. Rev. Entomol. 31: 241-368.

PIELOU, E. C. - 1980. The interpretation of ecological data. - Wiley, New York, 263 pp.

PLATT, T. & DENMAN, K. L. - 1975. Spectral analysis in ecology. Ann. Rev. Ecol. System 6: 189-250.

RABBINGE, R. & MANTEL, W. P. - 1981. Monitoring for cereal aphids in winter wheat. - Neth. J. Pl. Path. 87: 25-29.

RABBINGE, R. & RIJSDIJK, F. H. - 1983. EPIPRE: a disease and pest management system for winter wheat, taking account of micrometeorological factors. - EPPO Bull. 13: 297-305.

REDDY, V. R., BAKER, D. N., WHISLER, F. D. & FYE, R. E. - 1987. Application of GOSSYM to yield decline in cotton. I. Systems analysis of effects of herbicides on growth, development, and yield. - Agron. J. 79: 42-47.

REGEV, U., GUTIERREZ, A. P., DE VAY, J. E. & ELLIS, K. E. - 1988. Optimal strategies for management of verticillium wilt.- (in prep).

RUESINK, W. - 1975. Analysis and modeling in pest management. - In: METCALF, R. L. &

LUCKMANN, W. H. (eds.), Introduction to insect pest management. - Wiley, New York, 587 pp.

SAWYER, A. J. & FICK, G. W. - 1987. Potential for injury to alfalfa by alfalfa blotch leafminer (Diptera: Agromyzidae): simulations with a plant model. - Environ. Entomol. 16: 575-585.

SCHAUB, L., BAUMGÄRTNER, J. & DELUCCHI, V. - 1987. Anwendung multivariater Verfahren auf die Darstellung der Heteropterenfauna unter dem Einfluss der Intensivierung des Apfelanbaus. - Mitt. Schweiz. Ent. Ges. 60: 15-24.

SCHAUB, L., STAHEL, W. A., BAUMGÄRTNER, J. & DELUCCHI, V. - 1988. Elements for assessing mirid (Heteroptera: Miridae) damage threshold on apple fruits. - Crop Protection 7: 118-124.

SCHULTHESS, F., BAUMGÄRTNER, J. & HERREN, H. R. - 1987. Factors influencing the life table statistics of the cassava mealybug *Phenacoccus manihoti*. Insect Sci. Applic. 8: 851-856.

SCHULTHESS, F., BAUMGÄRTNER, J., DELUCCHI, V. & GUTIERREZ, A. P. - 1988. The influence of cassava mealybug, *Phenacoccus manihoti* Mat.-Fer., on yield formation of cassava, *Manihot esculenta* Crantz. - (in prep.).

SHOEMAKER, C. A. - 1980. The role of systems analysis in integrated pest management.- In: HUFFAKER, C. B. (ed.), New Technology of Pest Control. - Wiley, New York, 500 pp.

SILVERTOWN, J. W. - 1982. Introduction to plant population ecology. - Longman, London, 209 pp.

STERN, V. M., SMITH, R. F., VAN DEN BOSCH, R. & HAGEN, K. S. - 1959. The integration of chemical and biological control of the spotted alfalfa aphid. Part I. The integrated control concept. - Hilgardia 29: 81-101.

STINNER, R. E., GUTIERREZ, A. P. & BUTLER, G. D., Jr. - 1974. An algorithm for temperature-dependent growth rate simulation. Can. Ent. 106: 519-524.

TAYLOR, R. A, J. - 1986. Time series analysis of numbers of Lepidoptera caught at light traps in East Africa, and the effect of moonlight on trap efficiency. - Bull. ent. Res. 76: 593-606.

TOFT, C. A. - 1986. Communities of species with parasitic life-styles. - In: DIAMOND, J. & CASE, T. J. (eds.), Community ecology, Harper & Row, Cambridge, 665 pp.

VAN DEN BOSCH, R. - 1978. The pesticide conspiracy. - New York, Doubleday, 223 pp.

VANSICKLE, J. - 1977. Attrition in time distributed delay models. - IEEE Trans. Sys. Man Cybern. 9: 635-638.

VARLEY, G. C., GRADWELL, G. R. & HASSELL, M. P. - 1973. Insect population ecology, an analytical approach. - Blackwell, Oxford, 212 pp.

VON FOERSTER, H. - 1959. Some remarks on changing populations. - In: STOHLMAN, F. (ed.), The kinetics of cellular proliferation, Grune and Stratton, New York.

WAAGE, J. K., HASSELL, M. P. & GODFRAY, H. C. J. - 1985. The dynamics of pest-parasitoid-insecticide interactions. - J. appl. Ecol. 22: 825-838.

WAGNER, T. L., WU, HSIN-I, SHARPE, P. J. H., SCHOOLFIELD, R. M. & COULSON, R. N. - 1984. Modeling insect development rates: a literature review and application of a biophysical model. - Ann. Entomol. Soc. Am. 77: 208-225.

WAGGONER, P. E. - 1974. Using models of seasonality. - In: LIETH, H. (ed.), Phenology and seasonality modelling. Springer Verlag, Berlin, 444 pp.

WANG, Y., GUTIERREZ, A. P., OSTER, G. & DAXL, R. - 1977. A population model for plant growth and development: coupling cotton-herbivore interaction. - Can. Ent. 109: 1359-1374.

WELCH, S. M., CROFT, B. A., BRUNNER, J. F. & MICHELS, M. F. - 1978. PETE: an extension phenology modeling system for management of multi-species pest complex. - Environ. Entomol. 7: 487-494.

WHALON, M. E. & CROFT, B. A. - 1984. Apple IPM in North America. - Ann. Rev. Entomol. 29: 435-470.

WINTER, F. - 1978. Biologisch-ökologische Simulationsmodelle zur Berechnung der Wirtschaftlichkeit von Obstkulturen unter verschiedenen natürlichen und wirtschaftlichen Bedingungen. Mediz. Informatik und Statistik 8: 120-126.

WOODS, A. - 1974. Pest control: a survey. - McGraw-Hill, Maidenhead, 407 pp.

ZADOKS, J. C. & SCHEIN, R. D. - 1979. Epidemiology and plant disease management. - Oxford University Press, New York, 427 pp.

ZAHNER, Ph. & BAUMGÄRTNER, J. - 1988. Analyse des interactions plante-tétranyques-phytoseiides. I. Modèles de population pour la dynamique de *Panonychus ulmi* et *Tetranychus urticae* en vergers de pommier. - Acta Oecologica, Oecol. Applic. 9 (in press).

Tab. 1. Example for a controlled field experiment: testing the selectivity of insecticides in an apple orchard ecosystem (BOWER & KALDOR, 1980).

topics	selectivity of five insecticides for codling moth, *Cydia pomonella* (L.), control: effects on the twospotted spider mite, *Tetranychus urticae* Koch and its predators *Stethorus* spp.
authors	Bower and Kaldor, in Environ. Entomol. 9: 128-132 (1980)
design	experimental layout: randomized complete block design with variable number of replicates in each of three apple varieties treatments: 5 insecticides (7 applications) and control assessments: 1) % damaged fruits (in windfalls and in crop at harvest) 2) mite numbers (in 8 samples during the season) 3) predator numbers (cumulative number in 7 samples)

results	Insecticide	fruit number	damage [in %]	mites[1] [Febr.9]	predator adults	predator larvae
	Azinphos-methyl	665	28.9a	32.8a	10ab	3
	Leptophos	2375	19.5b	19.1ab	4b	0
	Permethrin	2964	25.1a	4.1bc	0	0
	Diflobenzuron	1128	59.6b	2.9cd	119c	19a
	Chlordimeform	2252	74.0b	0.58d	46abd	81b
	Unsprayed (control)	1012	94.0c	0.73cd	85cd	186c

comments: numbers followed by the same letter are not sgnificantly different ($P > 0.05$), numbers not followed by letters were excluded from the analysis, [1] = on one variety only

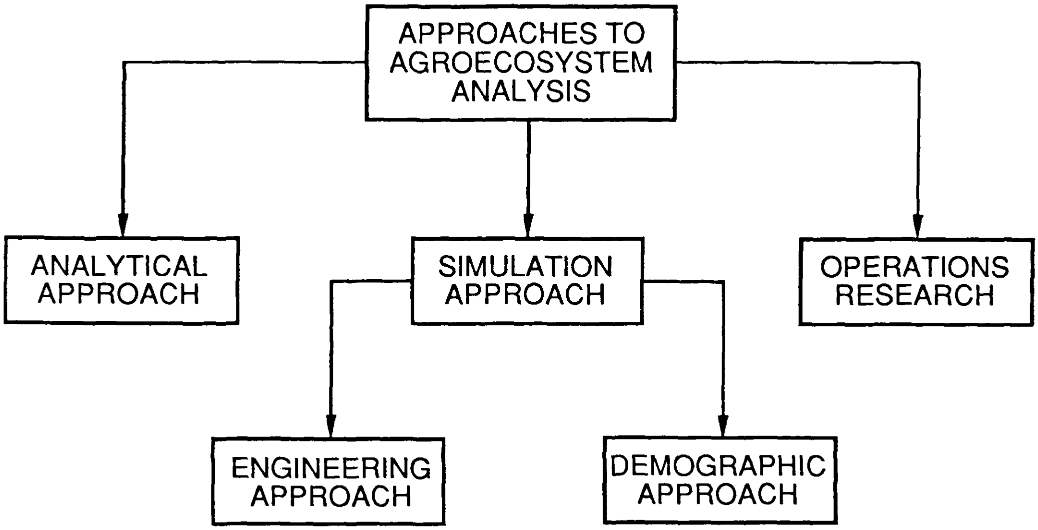


Fig. 1. The different approaches used in agroecosystems analysis (GETZ & GUTIERREZ, 1982, modified).

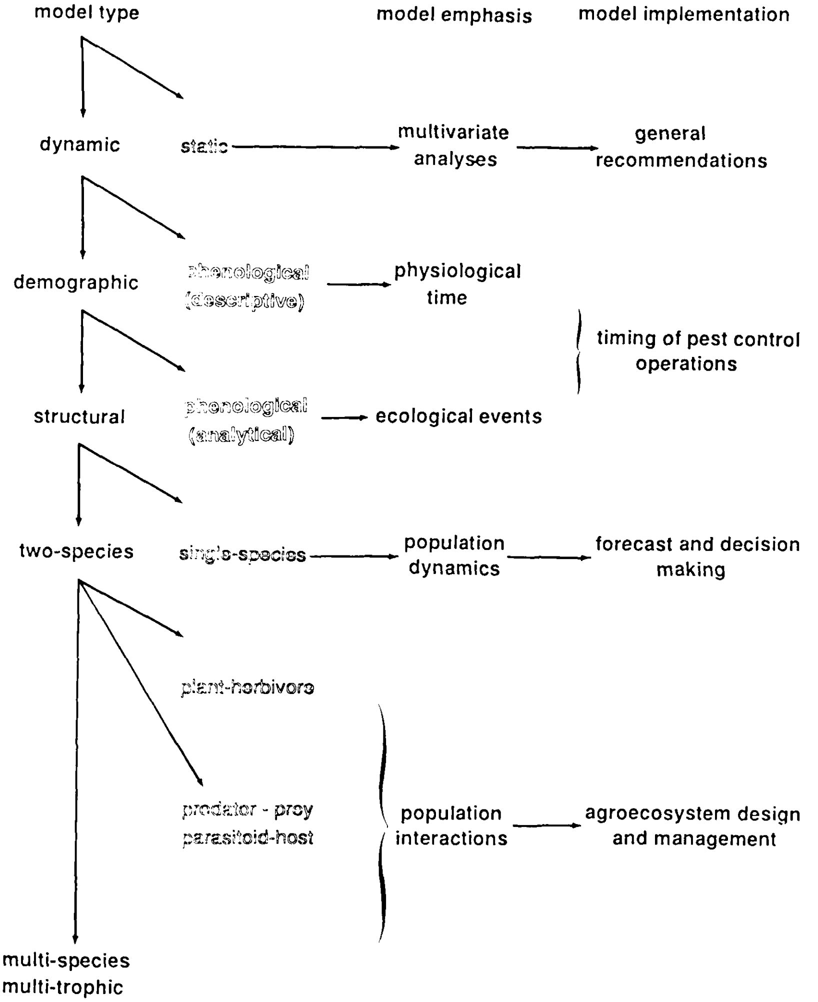


Fig. 2. The simulation approach: overview on model types, the elements they are emphasizing, and their areas of application.

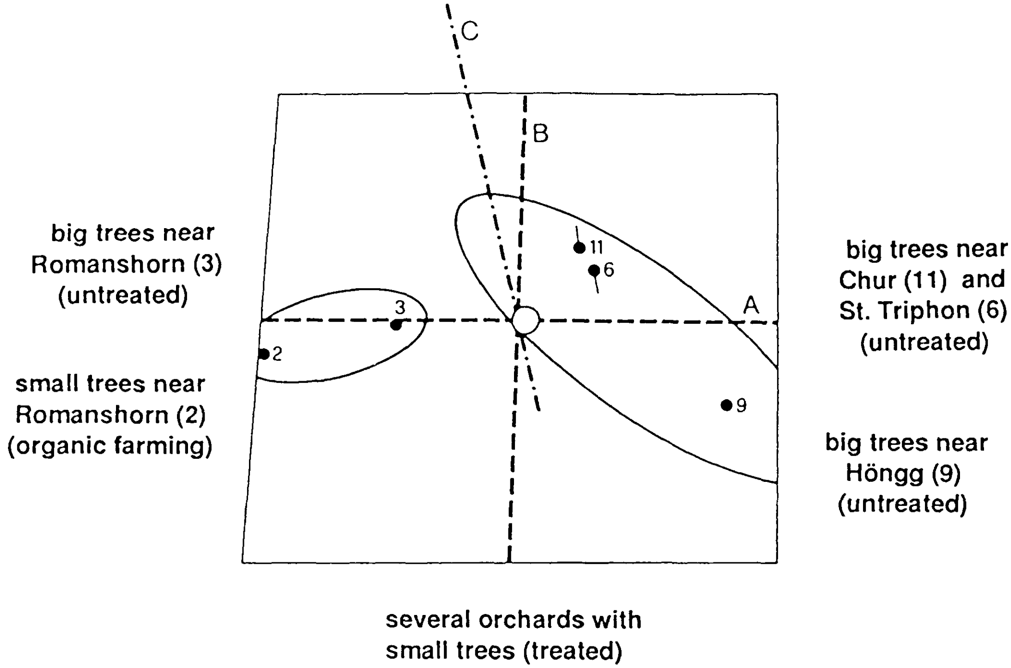


Fig. 3. Statistical model: ordination (correspondence analysis, axes A, B, C) and classification (cluster analysis, ellipses) procedures applied to the heteropteran fauna living in Swiss apple orchards. The procedures separates orchards with a different fauna and different management practices (SCHAUB *et al.*, 1987).

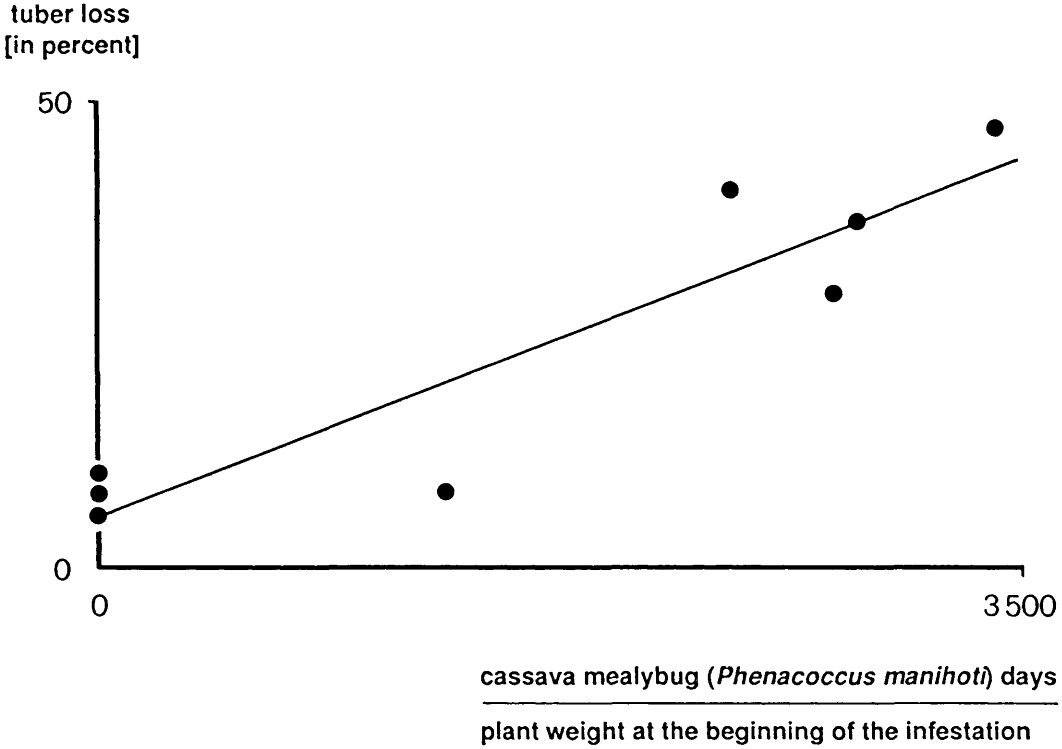


Fig. 4. Regression model: tuber yield loss of cassava *Manihot esculenta* during the dry season as a function of the initial plant size and mealybug *Phenacoccus manihoti* infestations, with data collected in a Nigerian cassava field (SCHULTHESS *et al.*, 1988).

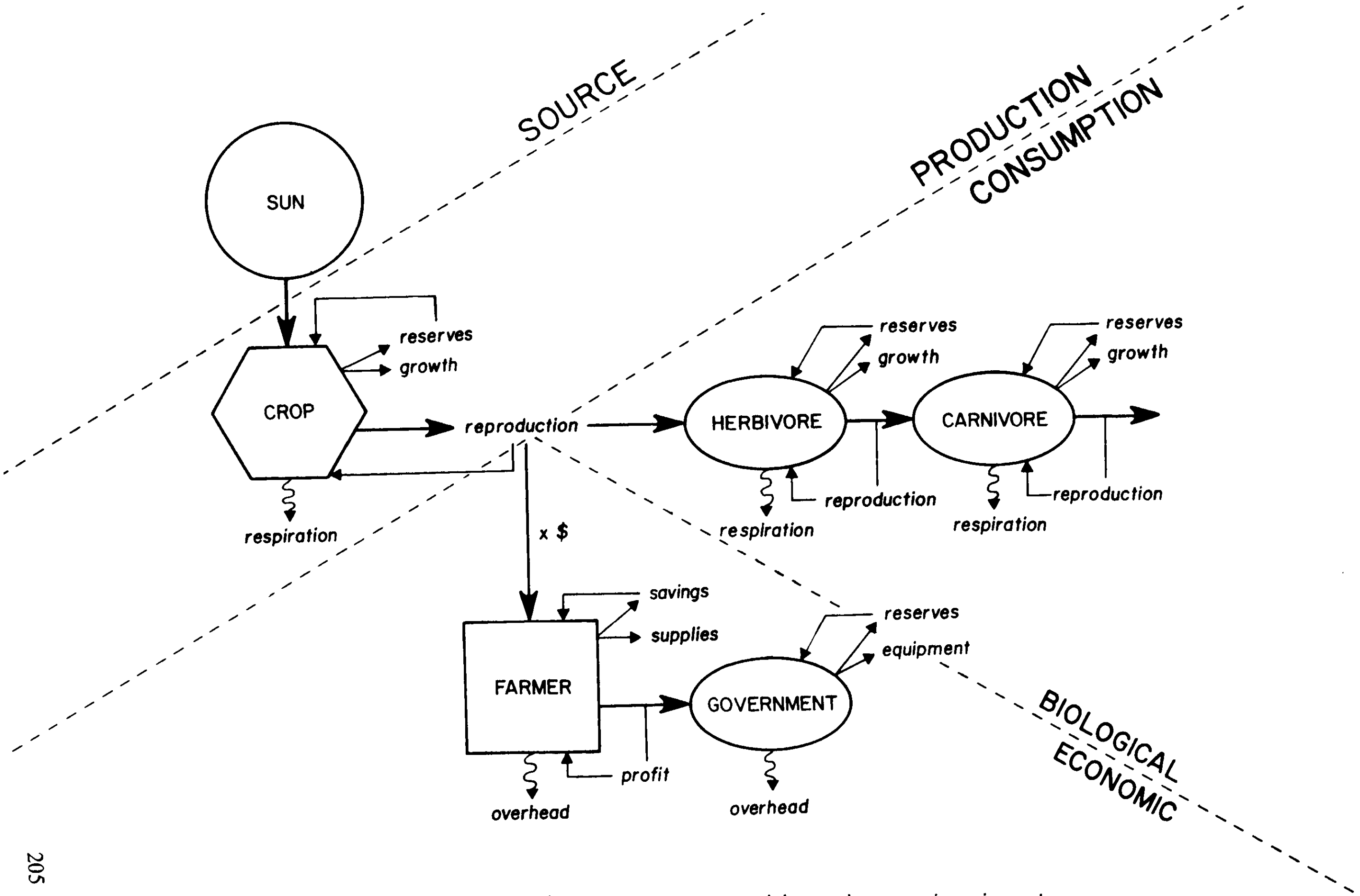


Fig. 5. A comprehensive concept for agroecosystem research in a socio-economic enviroment. The metabolic pool model discussed in the text provides a coherent framework to link ecological and economical processes (GUTIERREZ, 1988).

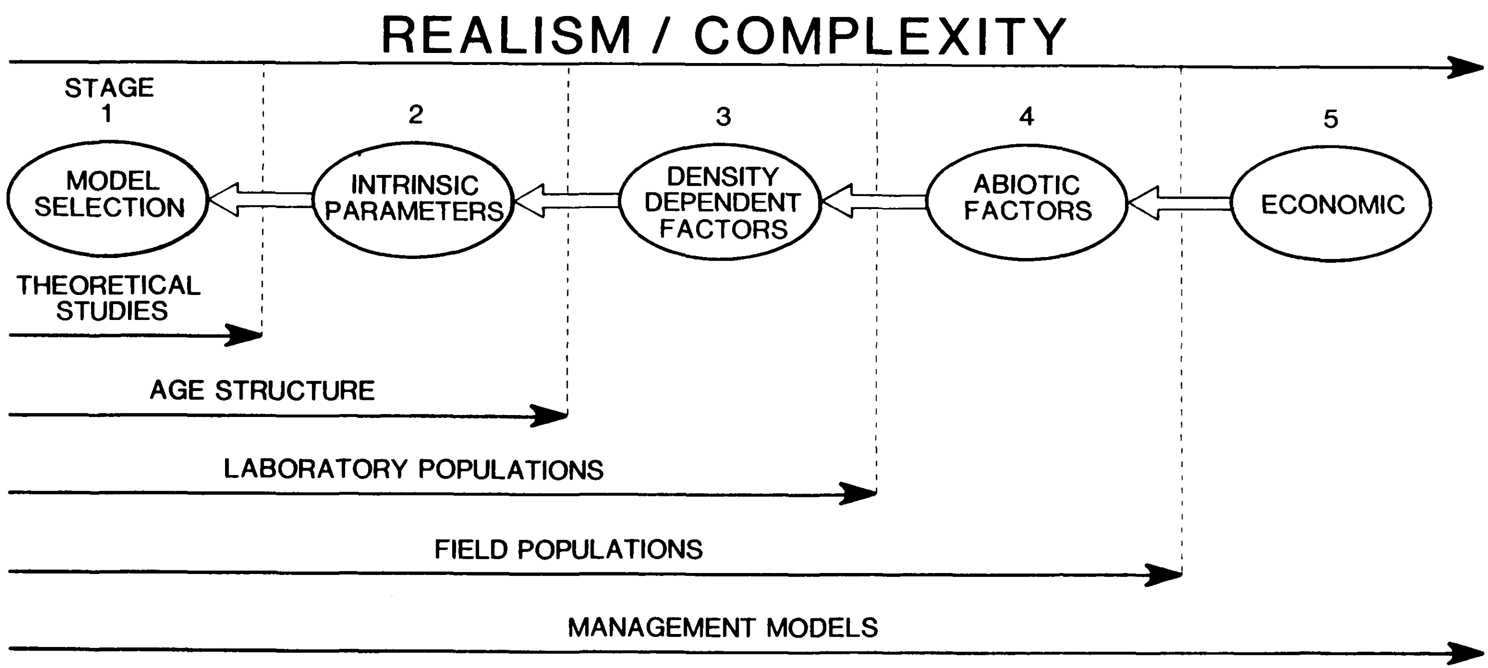


Fig. 6. Basic steps for construction of a simulation model for field populations that can be used to adress management problems in the economic environment. The incorporation of additional stages increases not only the realism of the model but also its complexity. Therefore, simplified forms are often required for application purposes.

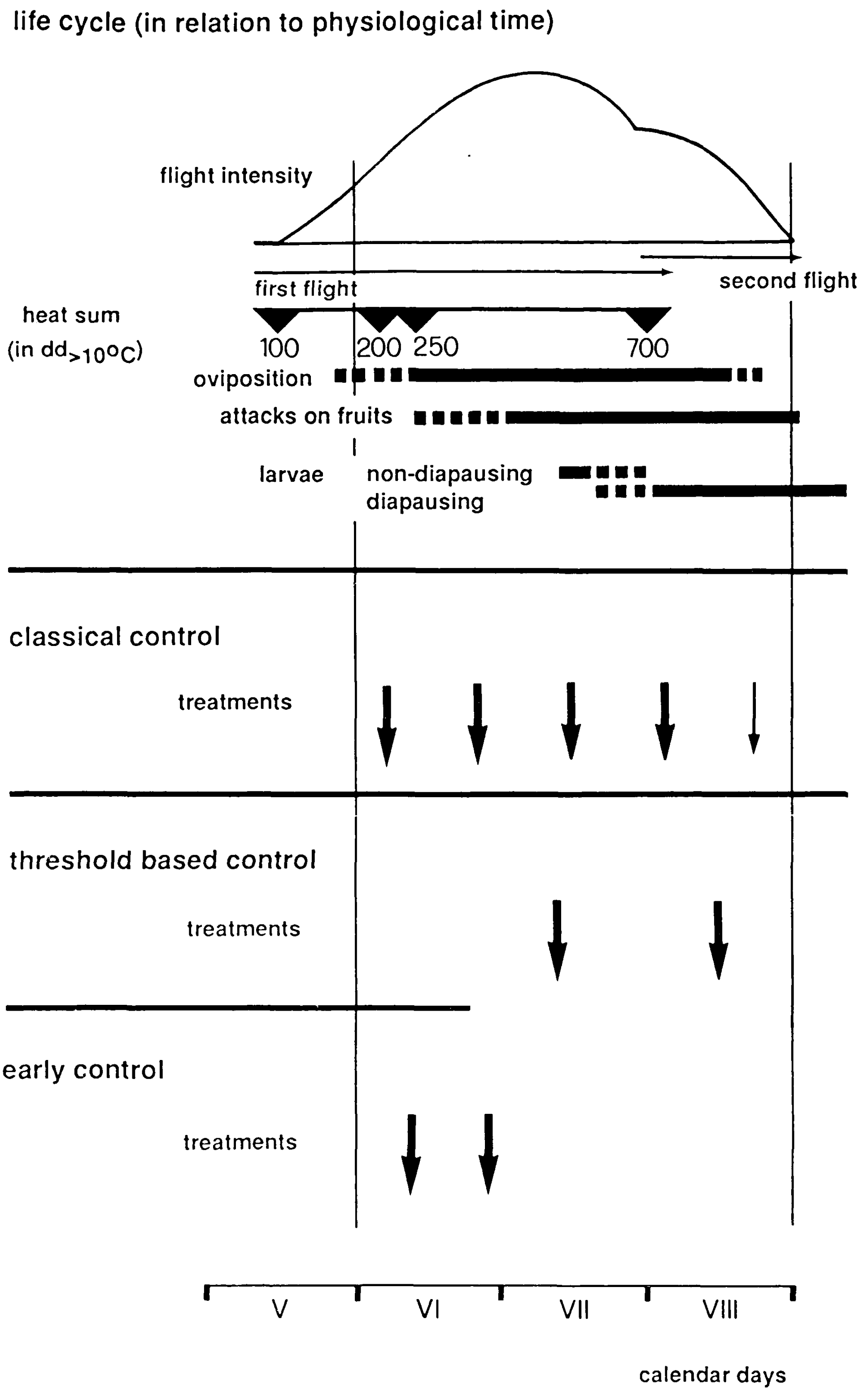


Fig. 7. Example of a descriptive phenological model: three control strategies against codling moth *Cydia pomonella* (dd=degree days; CHARMILLOT, 1980).

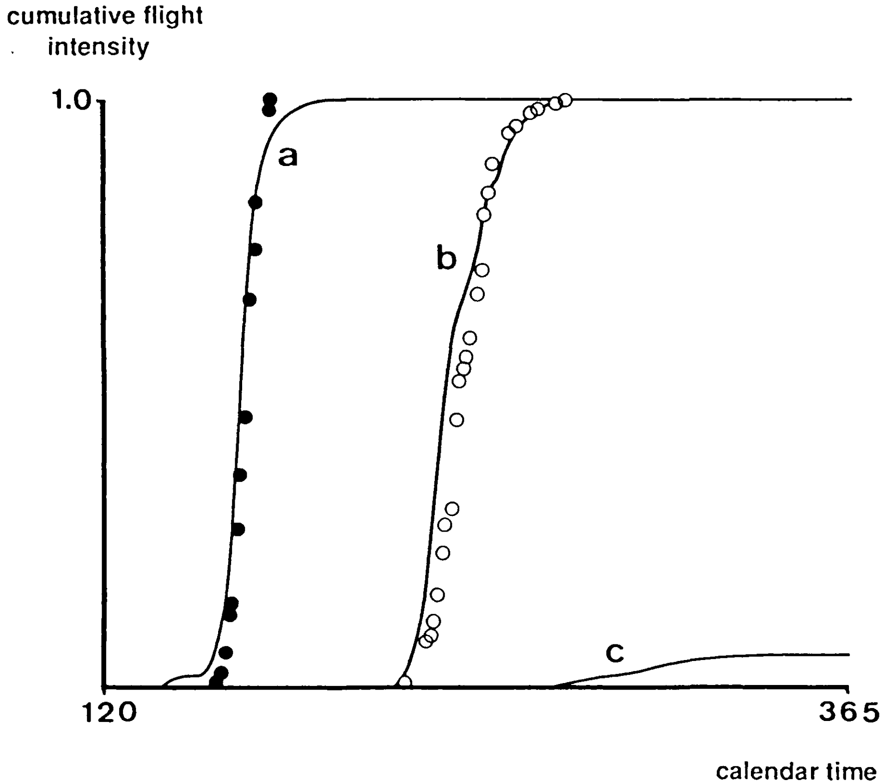


Fig. 8. Example of an analytical phenological model for pest monitoring: the observed (insects caught in pheromone traps) and simulated cumulative flight intensity of *Adoxophyes orana* (a=first, b=second and c=third flight, BAUMGÄRTNER *et al.*, 1988a).

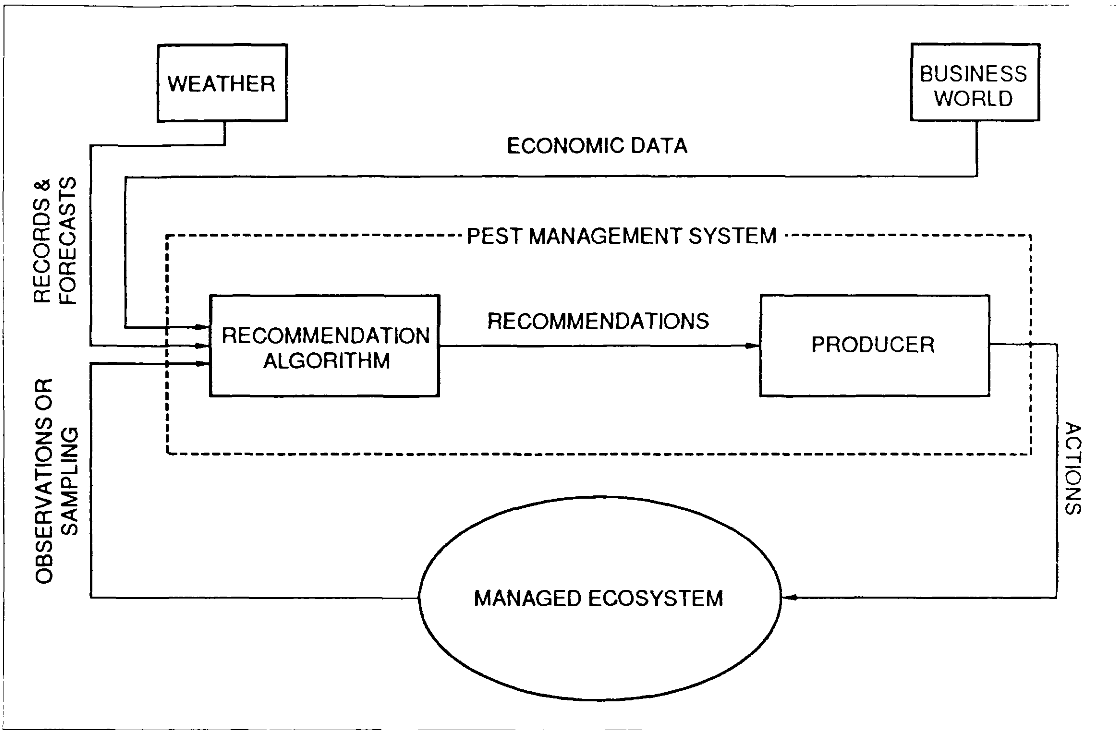


Fig. 9. Representation of a computer-based pest management advisory system, the key of the system is the recommendation algorithm that interpretes the different kinds of information to come to a pest management recommendation (FLINT & VAN DEN BOSCH, 1981 after RUESINK, 1975).

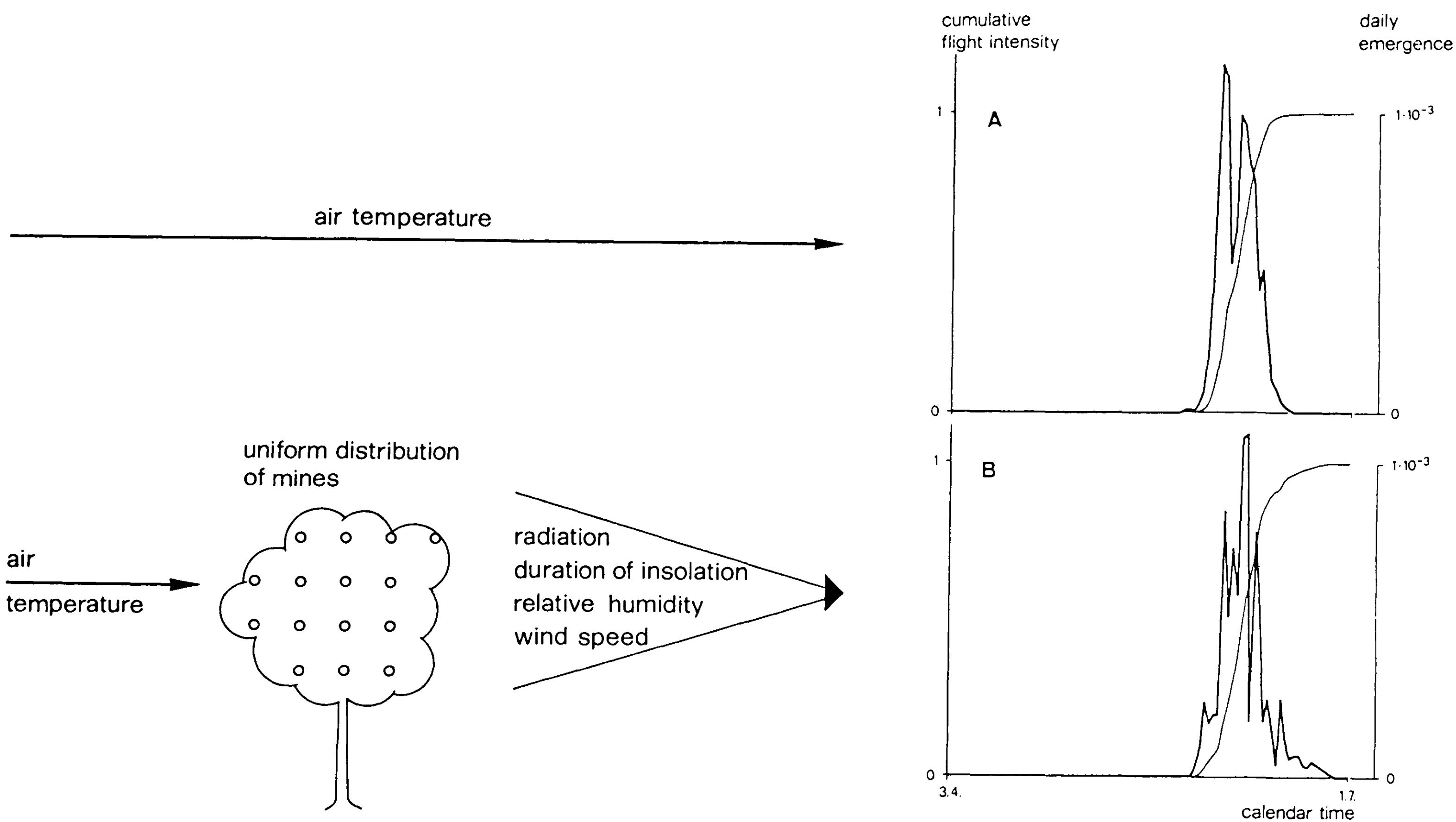


Fig. 10. Example of an analytical phenological model for research on pests: the flight phenology of the apple leaf miner *Phyllonorycter blancardella*, as affected by spatial distributions and microclimatic conditions (BAUMGÄRTNER & SEVERINI, 1987).

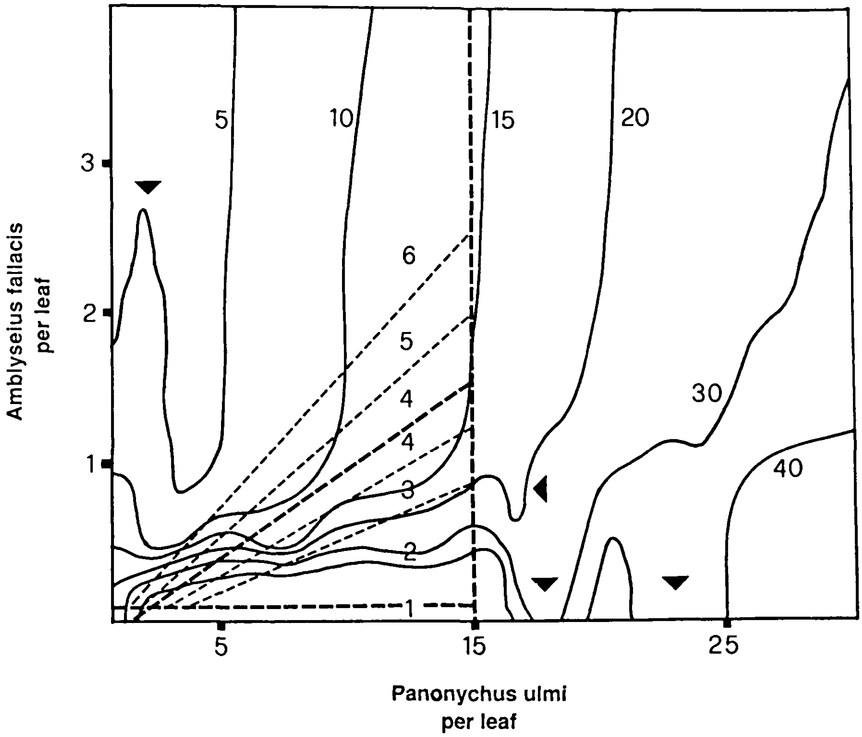


Fig. 11. Application of a predator-prey population model: the contour lines (5, 10, 20, 30, 40) are the simulated maximum prey density (*Panonychus ulmi*) as a function of initial predator (*Amblyseius fallacis*)-prey ratios, while the straight lines (1,2,3,4,5,6) separate the graphs into into regions corresponding to the various mite control recommendations in Michigan (DOVER *et al.*, 1979; CROFT & HOYT, 1983) (▾= model anomalies explained in the original papers).

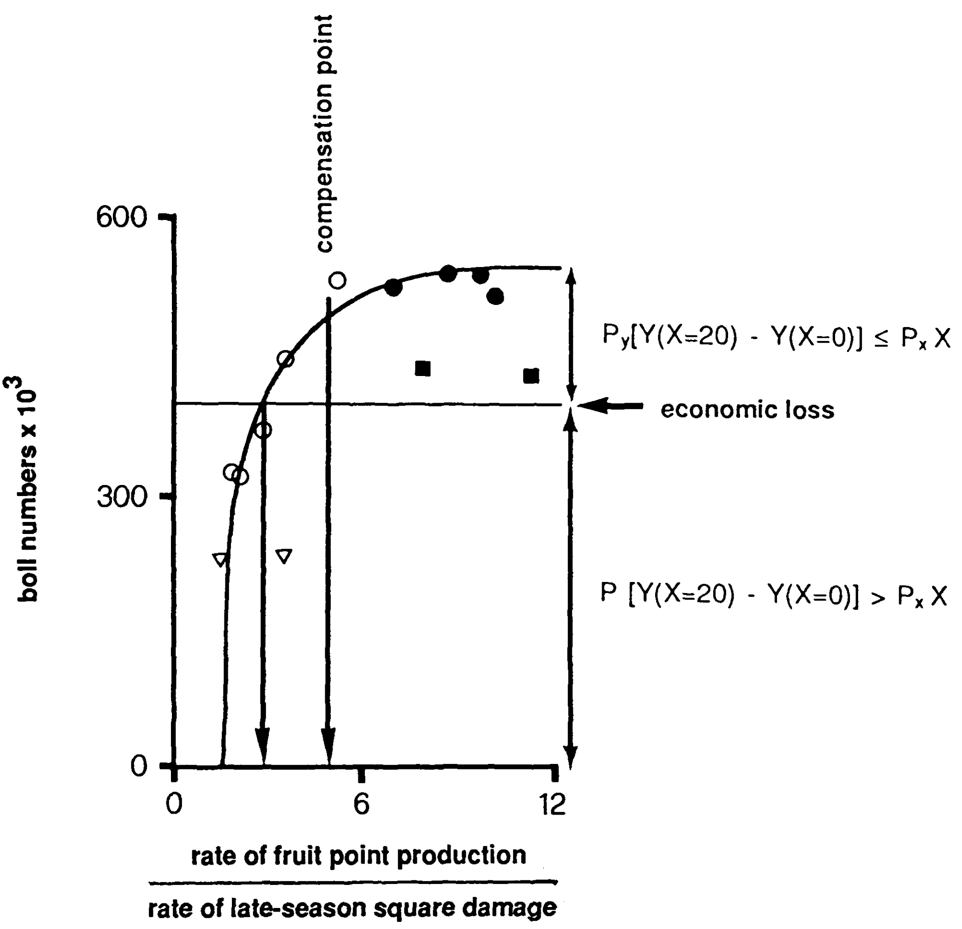


Fig. 12. Application of a herbivore-plant population model: relationship of final boll yield of cotton on the ratio of the rate of fruit point production to the rate of late-season square damage (P_Y = price of the crop (Y), P_X = the cost of each insecticide application, X = number of insecticide applications (X=0 or X=20); o,▽= untreated, and o,■= treated fields), after GUTIERREZ *et al.*, 1981b).

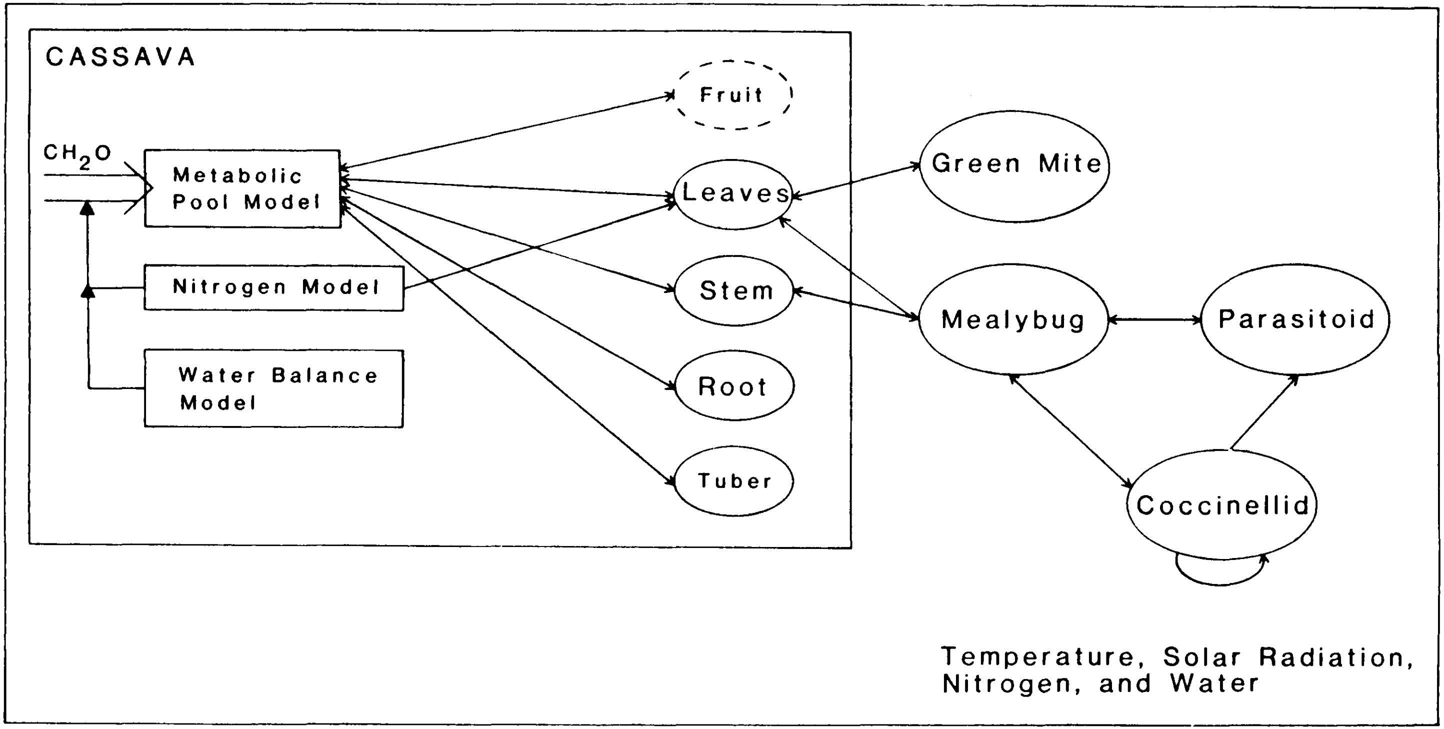


FIG. 13. Exemple of a comprehensive agroecosystem model: the components of the cassava model (plant and its subunits, the cassava green mite *Mononychellus tanajoa* (sensu latu), the cassava mealybug *Phenacoccus manihoti* attacked by the wasp *Epidinocarsis lopezi* and coccinellids) and their interactions as influenced by temperature, solar radiation, nitrogen and water (GUTIERREZ *et al.*, 1987b).

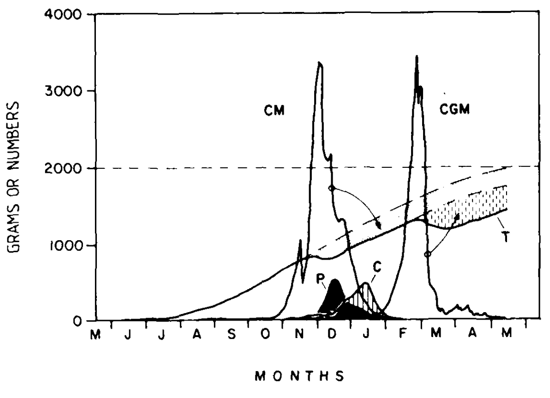


FIG. 14. Exemple of a comprehensive agroecosystem model, including the plant population *Manihot esculenta* (T=tuber yield in g dry matter per plant), the cassava mealybug *Phenacoccus manihoti* (x10^{-2}, CM) and its natural enemies *Epidinocarsis lopezi* (P) and *Hyperaspis* (x10, C) the cassava green mite *Mononychellus tanajoa* (sensu latu) (CGM). The systems model comprises balance equations for water and nitrogen; the reduction in yield due to the attack of the two herbivores is indicated by arrows (GUTIERREZ *et al.*, 1987b).

TRAITEMENT AUTOMATIQUE DES DONNES METEOROLOGIQUES ET PREVISION DES RISQUES PHYTOSANITAIRES

A. Fougeroux, E. Chaisse & L. Fricot
ACTA, Association de Coordination Technique Agricole-Paris, France

RESUME

Le développement de la protection intégrée des cultures nécessite la prise en compte de seuil d'intervention. L'estimation de ces seuils est généralement fastidieuse et de ce fait, est peu pratiquée par les agriculteurs. Les progrès de l'informatique et de l'électronique permettent d'utiliser les modèles d'estimation des risques phytosanitaires en temps réel à l'échelle de la petite région voire de l'exploitation agricole.
L'emploi de stations météorologiques automatiques fournissant les mesures de température, pluie, humidité et humectation devrait permettre l'utilisation de la plupart des modèles et fournir un véritable système d'alerte phytosanitaire.

INTRODUCTION

La Protection Intégrée des cultures est reconnue comme étant la seule stratégie de lutte utilisable à long terme contre les ennemis des plantes cultivées (Delucchi 1986). Celle-ci repose sur le constat qu'il est illusoire de vouloir résoudre un problème phytosanitaire par une seule technique de lutte. Elle consiste donc en l'emploi combiné et raisonné de toutes les méthodes dont on dispose contre les différents ennemis d'une culture de façon à maintenir leur nocivité à un niveau assez bas pour que les dégâts occasionnés soient économiquement tolérables (Brader 1975).

La mise en oeuvre de la Protection Intégrée nécessite :

- la prise en compte de seuils d'intervention,
- l'aménagement du système cultural pour réduire les risques phytosanitaires,
- l'emploi de techniques perturbant le moins possible les équilibres naturels.

(1) ACTA - 149, rue de Bercy - 75595 PARIS CEDEX 12

Les seuils d'intervention font appel à des méthodes d'estimation de l'importance des ennemis des cultures (piégeages, observations, prélèvements...). Il faut reconnaître que dans la plupart des cas, ces estimations sont fastidieuses et plus souvent adaptées à l'expérimentation qu'à une véritable gestion technique d'une exploitation agricole. De plus, ces seuils d'intervention n'intègrent que rarement les données économiques qui jouent pourtant un rôle déterminant dans la décision d'intervention.
Pour ces raisons, et sans contester le bien-fondé de ces seuils, la plupart des agriculteurs n'ont pas adopté ces méthodes. Afin de simplifier ce travail d'estimation, l'emploi de modèles d'estimation ou de prévision paraît une solution élégante. Comme beaucoup de phénomènes biologiques, le développement et la croissance de populations de champignons, de bactéries phytopathogènes ou de ravageurs des cultures sont liés au climat.

Les variables climatiques telles que la température, la pluie, l'humidité expliquent en grande partie le développement de maladies ou de populations de ravageurs. A partir de ce constat, il est tentant de vouloir les représenter par des formules mettant en relation la population estimée avec des variables climatiques.

Cette technique à plusieurs avantages :

- les variables utilisées sont facilement mesurables, en tout cas plus simples à obtenir qu'un dénombrement de population ou qu'une quantification des dégâts ;
- ces variables sont quantitatives et donc aisément utilisables dans des formules mathématiques.

En revanche, certains inconvénients doivent être gardés à l'esprit :

- les données météorologiques n'expliquent pas tout le phénomène biologique étudié et l'adjonction de variables biologiques peut être nécessaire au fonctionnement du modèle ;
- les modèles ne sont qu'une approche de la réalité et les résultats obtenus doivent être vérifiés. De ce fait, il est préférable de considérer les modèles comme des systèmes d'alerte incitant l'exploitant agricole à vérifier l'état sanitaire de ses cultures par des contrôles au champ.

I - LES STATIONS METEOROLOGIQUES AUTOMATIQUES

Pour assurer un fonctionnement correct des modèles élaborés, il faut pouvoir disposer de données météorologiques complètes et fiables. De plus, si ces modèles doivent être utilisés comme outils d'aide à la décision, il est indispensable que ces variables météorologiques puissent être obtenues et traitées en temps réel. A cet égard, les progrès simultanés de l'informatique et de l'électronique permettent désormais de collecter ces données par le biais de Stations Météorologiques Automatiques (SMA), d'en constituer des fichiers immédiatement utilisables par des moyens informatiques appropriés. Très rapidement, les estimations ainsi obtenues peuvent être rediffusées notamment via réseau Télétel (schéma 1).

Parmi les nombreuses SMA disponibles actuellement seules les stations proposées par Cimel répondent aux spécifications de l'INRA et des Instituts et Centres techniques agricoles. Néanmoins d'autres constructeurs proposent des stations susceptibles d'être employées en Agriculture (Vicon, CAA, SIIS, Unidata...). Suivant les modèles ces stations sont plus ou moins adaptées au besoin de la prévision des risques. La possibilité de récupérer et de traiter facilement les données météorologiques compte pour beaucoup dans l'intérêt des SMA. Pour ce faire, les stations nécessitent des installations particulières : câble entre la station et le microordinateur, branchement sur le réseau téléphonique. Dans d'autres cas, il faut se rendre régulièrement sur la station pour récupérer les données météorologiques grâce notamment à un microordinateur portable.

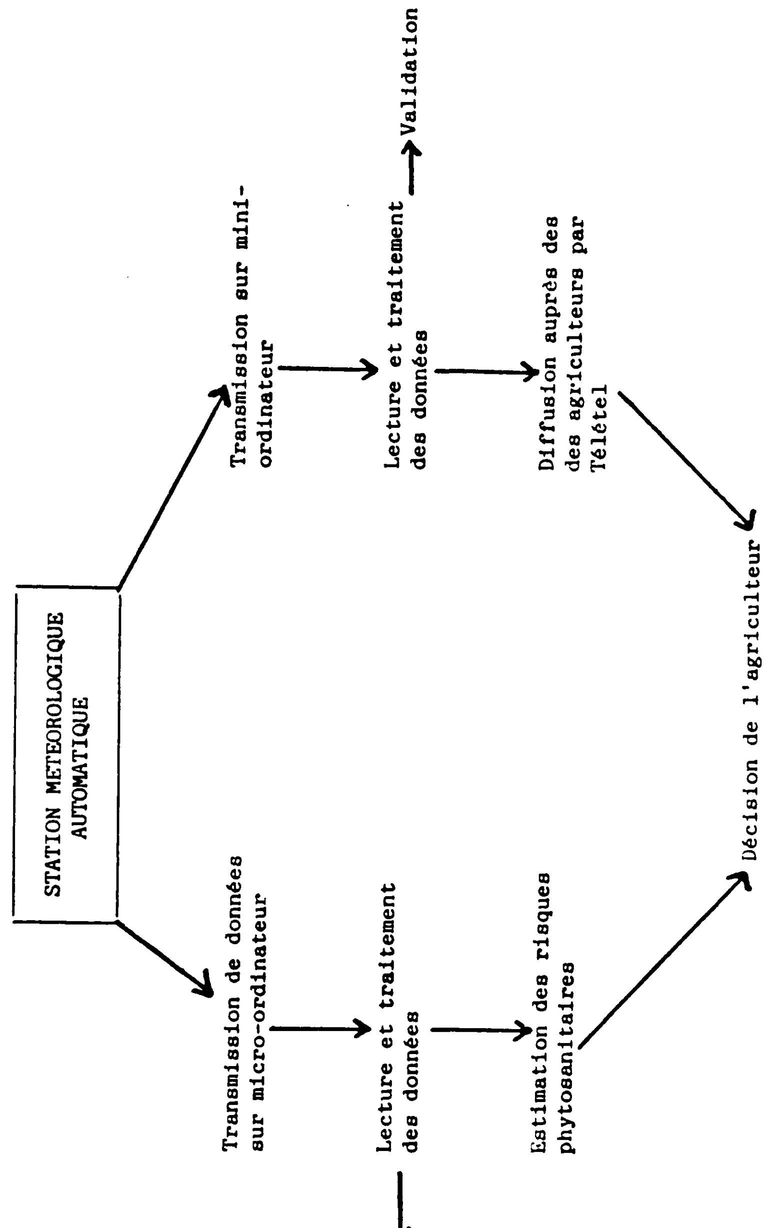
Les capteurs nécessaires à la prise de décision en protection des cultures et dans l'état actuel de développement des modèles sont :

- un capteur de température,
- un capteur de pluie,
- un capteur d'humectation,
- un capteur d'humidité.

Pour certains modèles, des capteurs supplémentaires sont proposés (mesure du rayonnement, indices actinothermiques, températures au sol...). Toutefois, l'ajout de tels capteurs augmente le coût de la station et il semble préférable, pour ce type d'information servant par exemple à calculer l'Evapotranspiration Potentielle (ETP), d'utiliser les valeurs régionales fournies par la Météorologie Nationale ou les organismes de développement agricoles.

II - LES MODELES D'ESTIMATION DES RISQUES PHYTOSANITAIRES

Certains modèles d'évaluation du risque existent aussi bien pour des maladies que pour des ravageurs. Ils ont en commun d'utiliser des données météorologiques parfois complétées de données biologiques (cas des modèles Lépidoptères nécessitant des informations sur les vols d'adultes par exemple.
Ce sont pour la plupart des modèles qualitatifs plus que quantitatifs. Ils permettent de situer un traitement dans le temps. C'est le cas du modèle Tavelure du pommier (Mills et Laplace 1951), du modèle Black rot (Spotts 1977) qui permettent de positionner une intervention avec un fongicide curatif en fonction des dates de contamination. Ceci apparaît actuellement intéressant dans le cas de fongicides triazoles qui expriment mieux leur


STATION METEOROLOGIQUE AUTOMATIQUE
Transmission sur mini-ordinateur
Lecture et traitement des données
Validation
Diffusion auprès des agriculteurs par Télétel
Transmission de données sur micro-ordinateur
Lecture et traitement des données
Validation
Estimation des risques phytosanitaires
Décision de l'agriculteur

action curative deux à trois jours après la contamination (Gendrier, Maurin comm. pers.). C'est aussi le cas des modèles "Lépidoptères" comme le Carpocapse des pommes et des poires (Audemard 1980) ou de l'Eudemis de la vigne (Touzeau 1980) qui situent dans le temps l'éclosion des oeufs et donc la date optimale d'application d'un larvicide.
Les connaissances biologiques sur l'ennemi, dont on cherche à modéliser le comportement, permettent dans une première approche de sélectionner les données météorologiques utiles. Les insectes ou les acariens ; animaux poikilothermes n'ont pas la possibilité de réguler leur température interne et nécessitent pour leur développement une certaine quantité de chaleur. C'est donc la température qui sera prépondérante dans les modèles employés.
Force est de constater que la plupart des modèles proposés pour évaluer un développement d'insectes sont basés sur des sommations de température : Carpocapse des pommes et des poires (Audemard 1980, 1981) ; Tordeuse de la pelure (Staübli et al. 1985) ; Eudemis de la vigne (Touzeau 1980) ; Mouche grise des céréales (Baker comm. pers.).
Les insectes ont comme beaucoup d'autres organismes des températures limités de développement en deçà ou en dessus desquelles le développement ne peut se poursuivre. La connaissance et l'intégration de ces limites sont indispensables au bon fonctionnement du modèle. C'est ainsi que le modèle Carpocapse ne tient compte que des températures supérieures à 10°C (seuil d'activité). Certains de ces modèles pour insectes intègrent en plus la pluie, comme c'est le cas pour les pucerons des épis (Dedryver et al. 1987). Ce facteur peut, en effet, soit perturber les vols (cas des pucerons vecteurs de jaunisse nanisante de l'orge - Bayon et Ayrault 1986) soit agir en favorisant le développement d'agents naturels de régulation. C'est une hypothèse avancée pour le modèle pucerons des épis Ouest de la France (Dedryver et al. 1987).

Bon nombre d'insectes ravageurs ont un cycle de développement comportant une phase de diapause (certains lépidoptères, coléoptères, diptères...). La levée de diapause reste un phénomène complexe et les essais entrepris pour expliquer cette levée de diapause par des données météorologiques simples sont décevants. C'est le cas du modèle Pyrale du maîs (Lechapt et al. 1987).
Pour les champignons phytopathogènes responsables de maladies foliaires, si la température joue un rôle important, l'humectation du feuillage est une condition nécessaire à la contamination. Aussi cette donnée est-elle généralement nécessaire au bon fonctionnement des modèles. Cependant la mesure d'humectation est difficile à obtenir du fait en particulier de la fragilité du capteur utilisé et notamment dans les régions où l'humidité reste élevée (bordure maritime par exemple).
Pour cette raison, il est tentant d'essayer d'établir une relation entre l'humidité relative et l'humectation du feuillage. Malheureusement cette relation ne peut être généralisée en raison de la spécificité entre l'humectation et les caractéristiques du feuillage (densité, port de la plante, nature des cirrhes cuticulaires...). Pour certains champignons phytopathogènes, les projections de spores sur le feuillage, et donc les possibilités de dissémination de la maladie dépendent de la pluie (septoriose des céréales par exemple). Cette mesure est donc nécessaire au

fonctionnement des modèles correspondant à ce type de pathogène. De la même façon, d'après le modèle de Billings, les infections par Erwinia amylovora Burr ne sont possibles que si la quantité de pluie dépasse 2,5 mm.

Enfin certains ennemis des cultures se conservent et accomplissent tout ou partie de leur cycle dans le sol (champignons pathogènes telluriques, ravageurs du sol, nématodes...). Dans ces cas, il est probable que ces modèles d'estimations des risques nécessiteront des mesures de températures et probablement d'humidité dans la zone superficielle du sol (10 cm de profondeur).

Les modèles actuellement opérationnels
De nombreux modèles phytosanitaires sont à l'étude dans les organismes de recherche (INRA, Service de la protection des végétaux, ACTA, Instituts et Centres techniques agricoles...). Toutefois, ces modèles nécessitent pour être élaborés puis validés (encore qu'un modèle agroclimatique ne puisse jamais être complètement validé) de nombreuses observations et mesures. Aussi n'est-il pas étonnant que les nouveaux modèles n'apparaissent pas aussi vite que le souhaiteraient les agriculteurs et leurs conseillers.
Il faut préciser que pour les chercheurs, les fichiers de données météorologiques facilement disponibles devraient simplifier le travail de mise en relation avec des données biologiques.

Certains modèles sont actuellement utilisables. Ils concernent plutôt les plantes pérennes, arbres fruitiers, vigne :

sur pommiers-poiriers :
- Tavelure (Mills et La Plante 1951)
- Carpocapse (Audemard 1980, Audemard 1981)
- Tordeuse de la pelure (Capua) (Staübli et al. 1983).

sur vigne :
- Tordeuse de la grappe (Eudemis) (Touzeau 1980)
- Botrytis (Strizyk 1981, 1982, 1983, 1984)
- Mildiou (Strizyk 1981, 1982, 1983)
- Black rot (Spotts 1977).

En grandes cultures, les travaux de modèlisation sont moins avancés. Des modèles à usage de travaux de recherche existent (modèle Septoriose des céréales - Rapilly 1987).
Pour la pratique agricole, de nombreuses études sont en cours pour mettre au point des modèles : Rouille brune, Septoriose, Pucerons des épis, Puceron vecteur de la jaunisse nanisante de l'orge, Mouche grise sur céréales, Mildiou de la pomme de terre, Pyrale du maïs, Sclerotinia du colza et du tournesol.

Ces modèles nécessitent toutes ou partie des données météorologiques citées précédemment : température, pluie, humidité et humectation. On peut donc raisonnablement penser qu'une station météorologique automatique fournissant ces quatre valeurs permettra de traiter l'ensemble des modèles et on peut s'attendre à une certaine harmonisation dans ce domaine. Harmonisation qui devrait s'étendre à la structure des fichiers nécessaires et par voie de conséquence aux programmes mis en oeuvre.
Toutefois, si elle simplifie la diffusion et l'utilisation des modèles, cette harmonisation se heurte à la nécessité des adaptations locales. En effet, ces modèles sont généralement établis dans une région donnée et leur extension à d'autres secteurs ou d'autres conditions agronomiques des cultures doit être envisagée avec prudence.
Cette difficulté d'extension signalée par Touzeau en 1987 pour le modèle Eudemis établi dans le Sud-Ouest a pu être vérifié. Les simulations réalisées à Bordeaux (L. Fricot 1985) donnent de bons résultats (Fig. 1). Au contraire dans le Sud-Est les estimations bien que très intéressantes sont légérement moins bonnes notamment pour le troisième vol Tableau 1 (Blanc et Chaisse 1987).

CONCLUSION

Le développement d'outils informatiques et de SMA devrait permettre une utilisation simple et donc courante des modèles d'estimation des risques phytosanitaires.
Cette évolution probable conduit bon nombre d'organismes soucieux d'une gestion rationnelle des interventions phytosanitaires à s'intéresser à ces techniques et au développement des SMA. Si certaines exploitations agricoles vont s'équiper de matériels de mesures météorologiques et de microordinateurs pouvant traiter ces données. Dans d'autres cas, on peut penser que des SMA seront utilisées pour un ensemble d'exploitations (coopérative, CUMA, groupement de producteurs...). Dans ce cas le traitement des données pourra se faire soit individuellement sur l'exploitation au moyen de microordinateurs personnels soit collectivement avec une rediffusion vers un microordinateur ou sur le réseau télétel (schéma 1).
L'installation de stations météorologiques automatiques nécessite de connaître le zonage agroclimatique. Ces travaux de zonage sont en cours dans certaines régions (Midi-Pyrénées par exemple).

On peut penser que le développement de ces travaux portant sur le recueil de données météorologiques et leur traitement par l'utilisation de modèles d'estimation de risque phytosanitaire, avec parallèlement l'amélioration du matériel disponible (simplicité d'emploi et d'installation, réduction du coût...) devraient conduire à une utilisation par les agriculteurs et leur conseillers de ces techniques.
Par ce moyen, on peut envisager dans un avenir proche une accélération dans l'utilisation de modèles d'évaluation du risque phytosanitaire, et par là même, pour l'agriculteur, une simplification quant à l'emploi des méthodes de protection intégrée.

Figure 1 : Evolution de l'Eudemis de la vigne selon modèle Touzeau - Blanquefort 1985

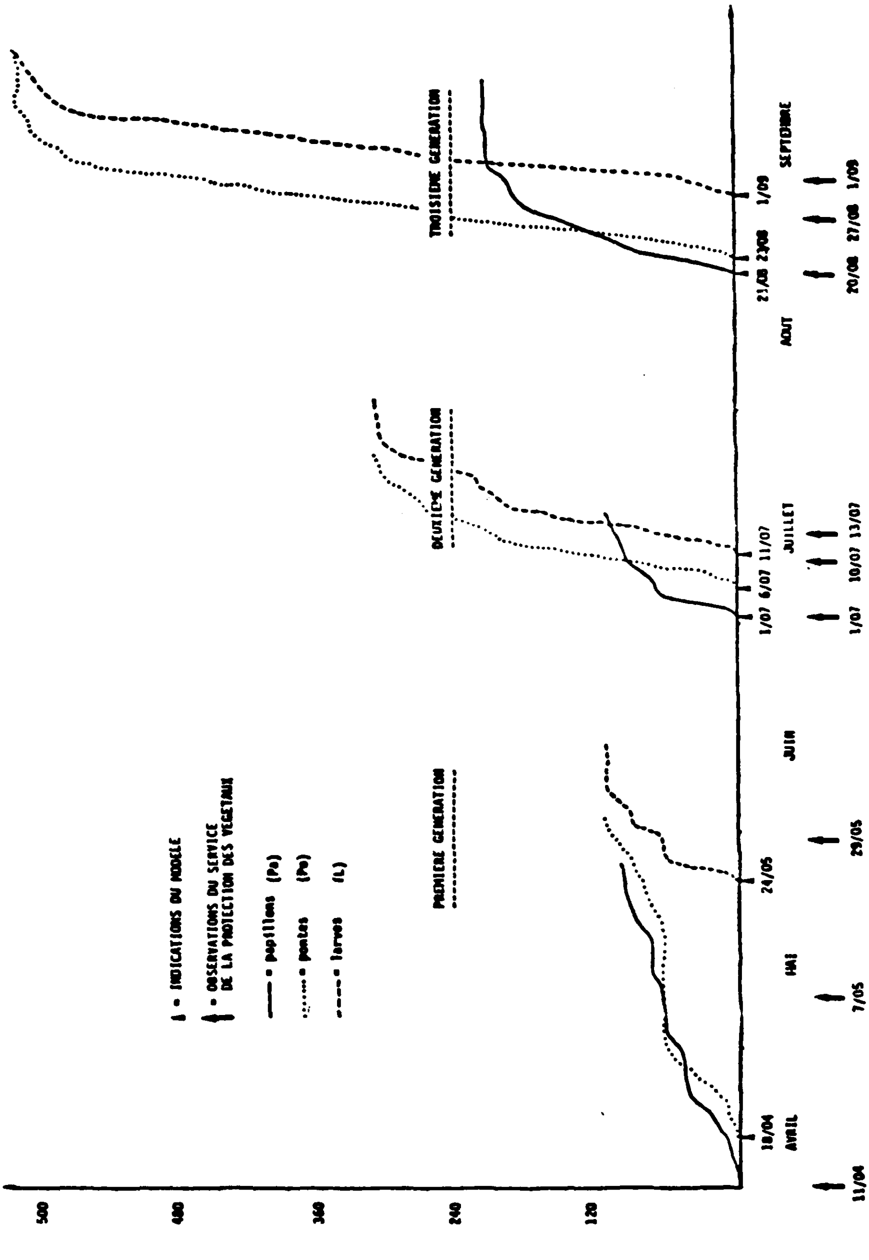


Tableau 1.

	MAZAN		LA TOUR D'AIGUES	
	Evénement prévu	Evénement observé	Evénement prévu	Evénement observé
DEBUT VOL 2	18-06	18-06	20-06	20-06
PONTES :				
- début	27-06	2-07 (8 oeufs pour 20 grappes)	5-07	11-07
- intensification	à partir du 3-07	6-07 (8 oeufs par grappe)	à partir du 8-07	faibles pontes
- fin	18-07		27-07	
ECLOSIONS :				
- début	2-07		10-07	
- intensification	à partir du 8-07 jusqu'au 18-07	15-07 observation de pénétrations	à partir du 13-07 jusqu'au 23-07	21-07 observation de pénétrations
- fin	25-07		2-08	
VOL 3	4-08	15-08	13-08	(prises très faibles à nulles dans pièges sexuels)
PONTES :			à partir du 18-08	
- début	8-08	17-08 (10 oeufs stade "jaune" pour 20 grappes)	18-08	20-08 observation de quelques oeufs
- intensification	à partir du 18-08		à partir du 18-08	
- fin	31-08	19-08 pas encore d'oeufs au stade "tête noire"	10-09	8-09 observation oeufs stade "jaune"-"tête noire"-"éclos"

SUMMARY

Development of Integrated Crop Protection needs to take in account tresholds. Actually, it is difficult to estimate these tresholds and for this reason very few farmers use them. Progress in computer science and electronic allows to use forecasting models in small regions or in farms. The use of automatical meteorological stations giving measures of temperatures, rainfall, humidity and moisture would permit the use of almost all phytosanitary models and provide a real phytosanitary warning system.

BIBLIOGRAPHIE

AUDEMARD H., 1980 - Possibilité d'aménagement de la lutte contre le Carpocapse en verger de poirier. Bulletin OILB-XROP ; III/7, p 29-31.

AUDEMARD H., 1981 - Les tordeuses des arbres fruitiers. Arboriculture fruitière n° 325, p 33-41.

BAYON F., AYRAULT JP., 1986 - Compte rendu d'expériementation ACTA 1986-1987.

BLANC M., CHAISSE E., 1987 - Adaptation et test du modèle Eudemis dans deux régions viticoles. Compte rendu d'expérimentation ACTA, p 17.

BRADER L., 1975 - Integrated Control : a new approach in crop protection. CR 5 e Symp. Lutte intégrée en vergers, OILB/SROP, Bolzans 3-7 septembre 1974, 9-16.

DEDRYVER C.A, FOUGEROUX A., DE LA MESSELIERE C., PIERRE J.S., TAUPIN P., 1987 - Typologie des courbes de fluctuation des populations de Pucerons des céréales sur le blé d'hiver dans le Bassin Parisien et élaboration d'un modèle de prévision des pullulations de Sitobion avenae. ANPP, compte rendu de la Conférence internationale sur les Ravageurs en agriculture, n° 6 Vol II/III, p 93

DEDRYVER C.A., GAMON A., GILLET H., HENRY M., 1987 - Epidemiologie de la Jaunisse nanisante de l'orge dans le Bassin de Rennes. Application à la prévision des contaminations primaires des céréales à l'automne. ANPP, compte rendu de la Conférence internationale sur les Ravageurs en agriculture n° 6, Vol II/III, p 3.

DELUCCHI V., 1987 - La protection intégrée des cultures. V. Delucchi (ed). Protection intégrée : quo vadis ? Parasitis 86, p 7-22.

FRICOT L., 1985 - Contribution à l'étude de l'automatisation de la saisie des données météorologiques pour leur utilisation dans différents modèles de prévision. Mémoire ISA-ACTA, p 95.

MAURIN G., 1983 - Application d'un modèle d'état potentiel d'infection à Plasmopara viticola. Bulletin OEPP, vol 13, n° 2.

MAURIN G., 1984 - Compte rendu d'essais modélisation de la Pourriture grise de la vigne. Compte rendu d'expérimentation ACTA, 1983-1984.

MAURIN G., 1985 - Résultats de quatre années d'essais d'un modèle simulant le comportement épidémique de Plasmopara viticola (B. et C. Berl. et de Toni, agent du Mildiou de la vigne. ANPP, Premières journées d'étude sur les maladies des plantes, p 283-290.

MILLS W.D. et LA PLANTE A.A., 1951 - Control of diseases and insects in the orchards. N.Y. Agric. Exp. Stat. (Ithaca) Ext., Bull. 711, 21-27.

RAPILLY F., 1987 - CR Acad. Agriculture

STAUBLI A., BAILLOD M., CHARMILLOT P.J., GUIGNARD E. et BLASER Ch. - Guide de protection phytosanitaire en viticulture, arboriculture et en cultures de petits fruits, 1983-1984. Revue suisse de viticulture, arboriculture, horticulture, vol. 15, n° 1, p 73.

STRIZYK S., 1981, 1982 et 1983 - Modèle d'état potentiel d'infection : application au Botrytis cinerea de la vigne. Doc. ACTA.

STRIZYK S., 1981, 1982 et 1983 - Etude d'un modèle de développement du Mildiou de la vigne (Plasmopara viticola (B. et C.) Berl. et de Toni). Doc. ACTA.

STRIZYK S., 1984 - Modèle d'état potentiel d'infection : pratique de vulgarisation pourriture grise de la vigne. Doc. ACTA.

TOUZEAU J., 1980 - Etude des principaux paramètres biotiques et abiotiques nécessaires à l'établissement d'un modèle d'évolution de l'Eudémis de la vigne (Lobesia botrana Schiff.) pour la région Midi-Pyrénées. Avertissements agricoles et Lutte intégrée.

TOUZEAU J., 1987 - Modélisation et avertissements agricoles. Colloque Modélisation et Protection des cultures. Académie d'Agriculture, ANPP, INRA, Paris, octobre 1987.

A COMPUTER-BASED ADVISORY SYSTEM FOR CEREAL APHID AND OILSEED RAPE-PEST CONTROL

B. P. Mann & S. D. Wratten
Department of Biology, Agrochemical Evaluation Unit, University-Southampton, Great Britain

SUMMARY

The development of research on cereal aphid damage to winter wheat is described, leading via a research simulation model to the development of a computer-based Viewdata advisory system for cereal aphid control. Aphid control decisións in the UK have frequently been irrational and uneconomic in the past and appear still to be based on inadequate information. A dynamic advisory package based on the economics of insecticide application and the aphid-induced yield loss/growth stage relationship has been developed. A similar system for pests of oilseed rape is also briefly described.

1. INTRODUCTION

As modern agriculture has developed, efficiency has been replacing maximum production as a major goal (ONSTAD & RABBINGE, 1985). To improve the efficiency of crop protection methods, STERN et al. (1959) developed the concept of the economic injury level and the economic threshold which focused attention on both the costs and benefits of pest control. They defined the economic threshold as "the density at which control measures should be determined to prevent an increasing pest population from reaching the economic injury level", and the economic injury level as "the lowest population density that will cause economic damage". The economic threshold is always lower than the economic injury level to permit sufficient time for the initiation of control measures and for these measures to take effect before the population reaches the economic-injury level.

However the economic threshold for a particular pest is more complicated than a simple cost/benefit relationship. The economic threshold cannot be given just as a number of insects per unit of crop but must be qualified by reference to other factors such as growth stage of crop, cost of chemical, and potential marketable yield (CARLSON, 1971). Yet despite these drawbacks, there now exist a large number of simple fixed thresholds for a variety of pests in the UK and elsewhere.

With the increasing use of computers in the 1970's, the terms dynamic economic injury level and dynamic threshold level were being recognised (ONSTAD & RABBINGE, 1985). These levels vary with time and allow decisions to be made on a field by field basis. The Agrochemical Evaluation Unit at Southampton University has developed two interactive pest control packages. This paper describes in detail the structure of an advisory system for cereal aphids on winter wheat, and then moves on to describe briefly a similar system for winter oilseed rape.

2. CEREAL APHIDS IN THE U.K.

Since at least the 1960's the grain aphid, Sitobion avenae (F.) and to a lesser extent, the rose-grain aphid, Metopolophium dirhodum (Walk.) have occurred in sporadic pest outbreaks on winter wheat in the U.K. (VICKERMAN, 1977; VICKERMAN & WRATTEN, 1979). The aphids colonize cereals in May and June and can become sufficiently numerous to cause damage in June and July (ANON., 1986). The irregular occurrence of these outbreaks but high average yield loss (often reaching 12.5 percent in outbreak years, (GEORGE & GAIR, 1979)) has led farmers to control cereal aphids prophylactically (WATT et al., 1984).

A.D.A.S. (M.A.F.F.) advice has changed little since its inception in 1975, although some significant changes were made in 1984 (ANON., 1984a) and 1988 (ANON., 1988). Until 1984, A.D.A.S. advised spraying against S. avenae when, at the beginning of flowering, an average of 5 or more aphids per ear are present and weather favours aphid increase; and for M. dirhodum, to spray when there is on average more than 30 aphids per flag-leaf between flowering and milky-ripe stages (ANON., 1984b). In 1984, A.D.A.S. advice for S. avenae was changed to 5 or more aphids per ear at any time between early flowering and milky-ripe stages. In 1988 further changes were made. The most recent advice is to spray if aphids can be found on approximately 66 percent of ears at any stage between the beginning of flowering and milky ripe stages (ANON., 1988). The advice for M. dirhodum has not changed.

However static spray thresholds such as these often require supplementary advice to make them function properly: delays in the provision of aircraft for contract spraying may require a decision to be made at a date later than that required for the threshold; significant aphid infestations can begin before or after the given growth stages (LEE et al., 1981); the two aphid species often appear together, although one species tends to predominate. Finally, the thresholds do not take into account the economics of the situation; the economics will often vary from field to field and from year to year. Such problems may lead the farmer to spray prophylactically, particularly since broad spectrum insecticides are cheap compared with the cost of possible aphid damage.

There is evidence from farmer surveys that spraying against cereal aphids is frequently irrational economically, with large proportions of the cereal acreage receiving unnecessary pesticide inputs (WATT et al., 1984; WRATTEN et al., 1984, 1989). This is due to a variety of factors including: A.D.A.S. advice being interpreted incorrectly; the low cost of broad spectrum insecticides (e.g. dimethoate); and the increasing use of insecticide/fungicide "tank mixes" resulting in late ineffective spraying after economic damage has already occurred (WRATTEN et al., 1984). A survey carried out in 1984 showed that 80 percent of the surveyed area of 60,000ha of winter wheat received aphicide, although 1984 was not considered to be an aphid outbreak year (WRATTEN, et al., 1989). A similar survey carried out as recently as 1988 depicted a similar story (WRATTEN & MANN, 1988).

Largely as a result of such problems, a research programme was initiated in the mid-1970's, involving Southampton University and A.D.A.S., to look at the effect of aphid damage on winter wheat (WRATTEN & GEORGE, 1985). This work involved a comprehensive series of relationships between aphid populations levels, crop growth stage and yield loss. These results later appeared as simulation models (WATT et al., 1984; WRATTEN et al., 1984) and were eventually convert into a computer-based advisory package for farmers (MANN et al. 1986).

3. FORECASTING METHODS USED FOR CEREAL APHIDS.

Several attempts have been made to forecast cereal aphid outbreaks. These can be classified into long-term or short-term forecasts. Suction traps give warnings of infestations in France (MOUCHART, 1984), Belgium (LATTEUR & NICOLAS, 1984) and the U.K. (TAYLOR, 1977) but have a variable relationship with field counts, and so can only complement field observations. Relationships between aphid infestations and weather (VICKERMAN, 1977; WATSON & CARTER, 1983) show that although a cold winter reduces the number of migrant aphids, a cold spring is more likely to lead to an outbreak, possibly via the effect on aphid reproductive activity and hence on the ability of predators to build up at a time when aphid populations are increasing slowly. Such a relationship is used in forecasting aphid outbreaks in Switzerland (SUTER, 1980). There is potential for using weather data in forecasting aphid outbreaks in the U.K., and a decision tree, based on some of these weather factors has been produced for S. avenae and M. dirhodum (DEWAR & CARTER, 1984). Multiple regressions between weather data and aphid peak numbers have also been developed (PIERRE & DEDRYVER, 1984).

Several short-term methods of forecasting have been devised. The Dutch EPIPRE system uses models that predict population growth and peak numbers (RABBINGE et al., 1979). Although these models have a variable economic threshold level, they require reliable data on immigration and population densities in early crop stages. A similar, though more complex model, has been developed by Carter and coworkers (CARTER et al., 1982). However this is a temperature dependent model with no forecasting aspect. Multiple regression models in which peak aphid numbers are predicted from the rate of increase per tiller per day and per tiller at a given time (ENTWISTLE & DIXON, 1987) may prove to be worthwhile, but their reliability is still uncertain (BURN, 1987).

4. DEVELOPMENT OF A SIMULATION MODEL

The advisory model was based on a research simulation model. The simulation model was developed from data on aphid damage, control costs, grain prices and expected yield (WATT et al., 1984). Aphid populations were fed directly in, rather than simulated from reproductive and developmental data. The model used data on yield loss in relation to growth stage and aphid number and aimed to quantify the economic value of control at different stages of outbreaks of various intensities. Although the model described by WATT *et al.* (1984) was for *S. avenae* only, a second model was developed by Holt (HOLT, J., pers. comm.), based on this model for *M. dirhodum*. The following describes a combined model for both species.

The model calculated: the aphid population density (*A*) for *S. avenae* (*Aa*) and for *M. dirhodum* (*Am*) for each day (*j*) of the infestation (by interpolation between the aphid counts on particular sample dates); the daily (*d*) and cumulative (*dj*) yield losses:

$$dj = \sum_{i=1}^{i=j} (Aa_i Ea_i + Am_i Em_i) \qquad (1)$$

together with the amount of damage saved by an insecticide spray (*sj*):

$$sj = (1\text{-}dt) - (1\text{-}dj)(1\text{-}W) \qquad (2)$$

where *E* was the proportional yield loss per aphid per day for *S. avenae* (*Ea*) and *M. dirhodum* (*Em*) on day *i* (the values of *Ea* and *Em* varying according to the growth stage of the crop), *dt*, the total calculated yield loss and *W* the proportional wheeling loss incurred during spraying. The latter, set at 0.03, was removed if the use of tramlines is simulated. The model also calculated the cumulative, total and avoidable damage in monetary terms (*Dj*, *Dt* and *Sj* respectively):

$$Dj = TGdj \qquad (3)$$

$$Dt = TGdt \qquad (4)$$

$$Sj = TGsj \qquad (5)$$

where *T* is the expected yield (T/ha) and *G* the grain price (£/T).

The simulation model was then able to calculate the long-term profitability of three available strategies: no control, prophylactic control and control based on following pest forecasts over a range of accuracies (WATT, 1983). The analysis at this stage was theoretical in that the model itself did not include a forecasting element but was used to reveal the forecasting accuracy necessary to improve upon prophylaxis and "no control" strategies.

5. CONVERSION OF THE MODEL TO A WORKING ADVISORY SYSTEM

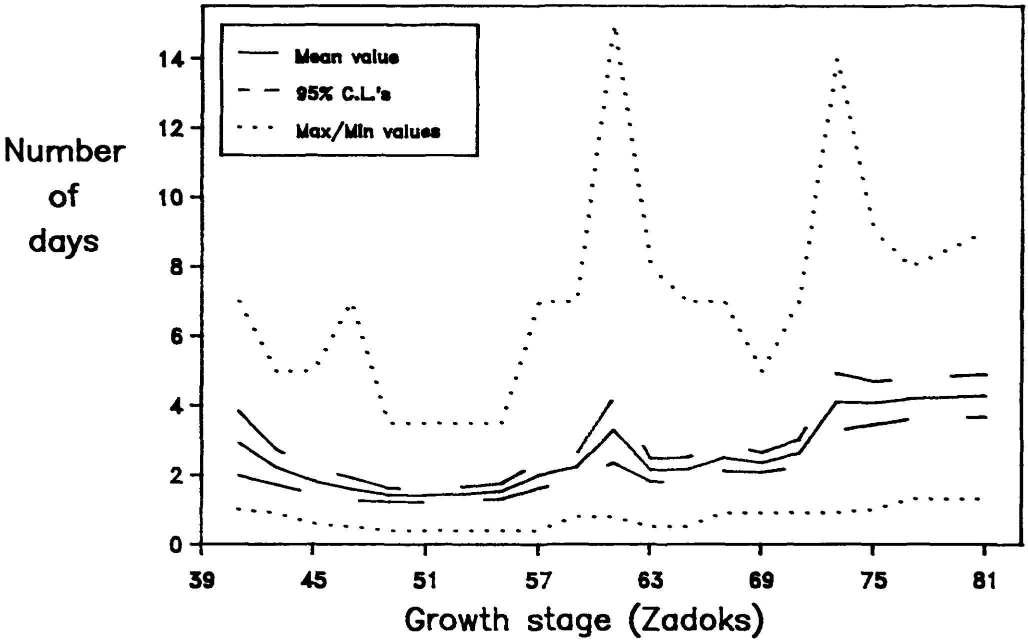
The model did not include an aphid forecasting element, since it uses actual aphid populations as recorded, with interpolations. As such it can only indicate that economic damage had begun, rather than was about to begin. In this respect, two forecasting elements were introduced into the model: a crop development submodel and an aphid population submodel. With these two additions, the model became a working advisory system.

5.1. THE CROP DEVELOPMENT SUBMODEL

Few models have been used to describe crop development. MAAS and ARKIN (1980) developed a general model of wheat development whereas SELIGMAN and VAN KEULEN (1981) considered the effect of water shortage and nitrogen balance on the rate of development of winter wheat. However both can only be used as simulation rather than forecasting models since variables within each model, including day-degrees, radiation levels and nutrient availability, are difficult, if not impossible, to predict. CARTER *et al.* (1982) used a simple algorithm developed by FRAZER and GILBERT (1976) assuming that crop growth was not a limiting factor for the build-up of aphid populations; the development stage is calculated using a polynomial equation based on the accumulated number of day degrees. However models such as these are weather dependent and would be difficult at present to use in a forecasting system since it is difficult to simulate and predict weather conditions beyond a few days.

For the advisory model a much simpler method was developed. Published and unpublished data covering the last 10 years from over 50 fields all over England were collated. For each set of data, the length of time between growth stages was calculated. From this the mean length of each growth stage together with 95% confidence limits, could be calculated (Fig. 1.). The model is concerned only with growth stages 41 to 81 (ZADOKS *et al.*, 1974), covering the period when aphids will be present. Although aphids may be present beyond GS 81, they cause no yield loss (LEE *et al.*, 1981).

Fig. 1. Length of crop growth stages of winter wheat.



5.2. THE APHID POPULATION SUBMODEL

HUGHES and GILBERT (HUGHES & GILBERT, 1968; GILBERT & HUGHES, 1971) developed one of the first simulation models for aphids (the cabbage aphid, Brevicoryne brassicae L.) which concentrated on the inter-relationships between the aphid and its natural enemies. The model used discrete time steps to simulate continuous processes and integrated the effects of time and temperature into "physiological or thermal time", the accumulation of temperature sums above a threshold value. Such processes were used in later models developed by CARTER and RABBINGE (CARTER & RABBINGE, 1980; CARTER et al., 1982). In these models aphid development on cereals was dependent on temperature which precluded their use in a forecasting system.

A different approach was taken by ENTWISTLE and DIXON (1986, 1987). They relied on multiple regression analysis to predict the timeing of peak aphid numbers and peak population size based on samples taken at earlier growth stages, with the population development between the growth stages being based on logarithmic growth. Hence there was no direct dependence on weather data. However the monitoring method used was very labour-intensive (ENTWISTLE & DIXON, 1987) and would probably cost in labour more than the actual spray application. Also, the method did not forecast M. dirhodum populations, which although less frequent, can nevertheless be very important in some years.

For the advisory model the approach was to use simple population growth rates keyed in with a simple sampling method. Published and unpublished data covering the last 10 years from over 50 fields all over England were collated. For each set of data the population growth rate (PGR) was calculated for each growth stage using the equation:

$$PGR = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

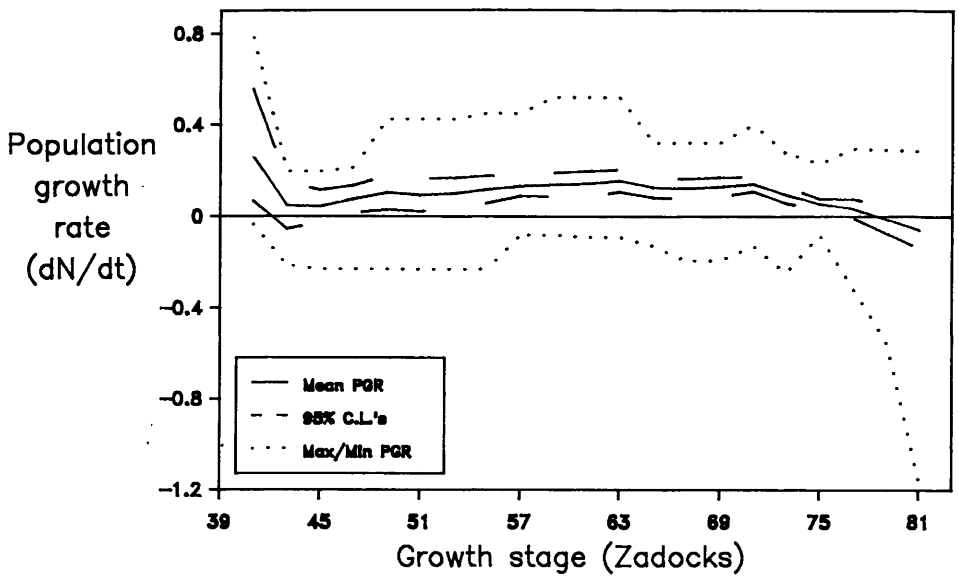
where N_1 is the population at time t_1 and N_2 at time t_2, where t_2 is greater than t_1. From this the mean PGR and 95% confidence limits were calculated for each growth stage (Fig. 2).

6. THE USE OF THE CROP DEVELOPMENT AND POPULATION SUBMODELS IN THE FORECASTING MODEL

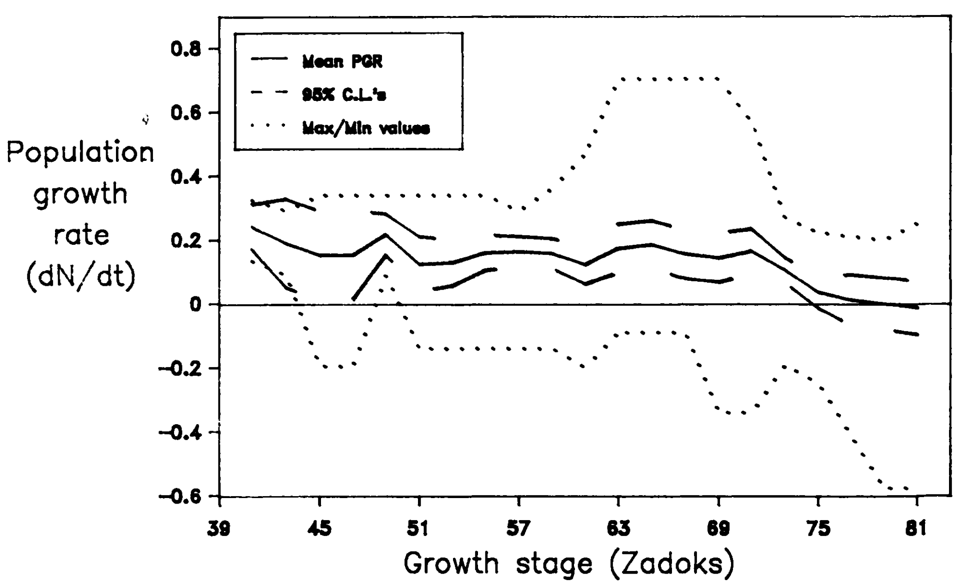
To estimate population development, the forecasting model calculates a growth curve based on the current population and growth stage, using stored values of population growth rate and growth stage length. The model initially used maximum values for these variables. This often resulted in an overestimate of aphid damage, but never an underestimate. To have underestimated would have resulted in occasions when the model would have advised not to spray when a spray should have been carried out. This would have resulted in loss of confidence in the model. However validation of the model by comparing its outputs with known population curves derived from experimental and published data showed that maximum rates often overestimated damage by several magnitudes but the use of 95 percent upper confidence limits (i.e. values derived from mean ± 1.96 estimated standard deviations) instead reduced this to more reasonable levels.

Fig. 2. Aphid population growth rates.

a. *Sitobion avenae*



b. *Metopolophium dirhodum*



7. MONITORING AND SAMPLING FOR CEREAL APHIDS

For any forecasting model a suitable method of pest sampling must be used. Several methods of sampling have been developed for cereal aphids. In 1964, work on insect dispersal at Rothamsted led to the development of the suction trap. However this provides only data on general rather than local aphid populations (HEATHCOTE et al., 1969). More localized methods in the form of sticky traps and water traps have been used. However these only give a measure of activity i.e. the numbers of aphids flying over or within the crop, producing peaks during the migration periods, and do not give a true estimate of aphid density (HEATHCOTE, 1957). D-vac suction samplers provide an easy way of obtaining a population estimate, but the efficiency of a D-vac drops as the crop ripens and there are also large differences between machines (CARTER et al., 1982). Also the method can be time-consuming requiring a bulky, heavy piece of machinery to be manoeuvred between samples, and the aphids to be counted, a task which can be very time consuming.

The only implicitly accurate form of aphid sampling to obtain field densities, is by actual field counts. Again, here, several methods have been used, including a replicated count of the number of aphids in a 0.3m row (DEAN & LURING, 1970), counting a small number of tillers at a given number of sites (DEWAR et al.,1982) and counting individual tillers chosen randomly along a diagonal transverse (DEWAR et al., 1982). In each case the sample size required for statistical reasons can be calculated using simple formulae (WARD et al., 1985).

However counting aphids directly is very time consuming and may be more costly in the time taken to do than the cost of the insecticide. For a forecasting model of cereal aphids to be economically viable the method of sampling must be simple yet cost-effective. To simplify the procedure RABBINGE and MANTEL (1981) found a relationship between the number of aphids per stem and the proportion of stems infested:

$$\text{Probit}(P) = 1.51 \log \mu + 4.63 \qquad (r=0.93, n=229)$$

where μ is the mean density and P the proportion of stems infested. This simple relationship is used in the advisory model. Thus all that is required to be done when sampling for aphids is to count the proportion of stems infested with one or more aphids for both of the species. The advisory model then uses this relationship to calculate the number of aphids per stem.

8. FORECASTING YIELD LOSS AND TIME OF SPRAYING

The advisory model requires the following to be input:

a) Expected yield. This is the yield that would be expected to be obtained if there was no aphid damage.

b) Selling price. This can be obtained form futures prices.

c) Spraying cost. This can be based on the chemical cost alone or on application plus chemical costs. Often aphicides on winter wheat are applied as tank-mixes with fungicides; in this case the chemical cost alone may well be given.

d) Wheeling losses. In those cases where tramlines were not being used and the insecticide was not being applied as a tank-mix, then wheeling losses would be included.

e) Growth stage. The amount of damage per aphid and the total forecasted damage depends on the growth stage of the crop.

f) Aphid infestation. For each aphid species this is given as the percentage of tillers infested.

From this data the model calculates a population growth curve based on the upper confidence interval values for PGR and growth stage. Using this curve the model predicts the daily and total (Dm) damage in monetary terms using equations 1-5. The model also predicts the damage that has already occurred (Dp) by extrapolation using negative population growth rates. The type of advice then offered depends on these values.

Once the aphid population has reached a level such that:

$$Dm > C + WTG$$

where C is the total cost of spraying, the model advises to spray providing that:

$$Dp >= 0.005TG$$

This small amount of damage is an arbitrary value used to prevent a spray being applied too early.

If $Dp < 0.005TG$

then the model calculates how many days, n, it will take to reach this value. The model would then advise to return in n days time with 7 days being the maximum value.

However if

$$Dm < C + WTG$$

then the model advises to return in 7 days time unless the crop is at or beyond G.S.75 when the advice is not to spray.

Thus one of three types of advice would be given:

1) Spray immediately
2) Return in n days' time and reassess the situation
3) Take no action. Do not spray. Do not reassess the situation.

9. EVALUATION OF THE ADVISORY MODEL.

Field trials were conducted from 1985 to 1987 to evaluate the model. These trials were principally carried out to ensure that the models were giving the correct advice. In each year the trials also compared the models' advice with the most recent A.D.A.S. advice (post-1984 advice), the older type of A.D.A.S. advice, i.e. pre-1984 advice (yet still available in A.D.A.S. publications), and not spraying. Obviously the changes made in 1988 could not be tested.

Unmanipulated field trials were used in 1985 and 1986. In 1985 and 1986 there was no yield-reducing aphid outbreak and so in 1987, an artificial aphid outbreak was produced with the use of field cages. These improve the microclimate within the crop, favoring aphid population growth. The following describes the method and results from the 1987 trials.

9.1. MATERIALS AND METHOD

Sixteen mesh-covered cages (2m x 2m x 2m; 1mm mesh) were erected in an 18 hectare field of winter wheat (cultivar Moulin). During ear-emergence and again at mid-flowering, 16 of the cages were infested with a mixed culture of the two aphid species collected from the field. Four of these cages were allocated to each of the following treatments.

A: Control, no aphids. These plots were sprayed every week to keep aphid numbers very low.

B: Aphids present, no spray treatment.

C: Spray according to the pre-1984 A.D.A.S. advice.

D: Spray according to the post-1984 A.D.A.S. advice.

E: Spray according to the models' advice.

The percentage of stems infested by aphids and the number of aphids per stem were counted for each aphid species, according to the requirements of the models' advice and on a twice weekly basis. If a spray treatment was required, the insecticide pirimicarb ("Aphox") was applied at normal field dilution and rate of liquid/ha.

At harvest, 500 ears were removed from the centre of each cage. Five subsamples each of 100 ears were then taken and for each ear the weight of grains and number of grains recorded.

9.2. RESULTS

Aphids were introduced into the field cages at the end of ear emergence and again at mid-flowering. Due to poor weather conditions, aphid numbers remained low until after mid-flowering (G.S. 65). M. dirhodum failed to establish in the cages. However S. avenae rose quickly, reaching c. 9 aphids per stem (83% of stems infested) by early milky-ripe (G.S. 73). At this stage both the model and the post-1984 A.D.A.S. advice, advised spraying. For unsprayed controls and pre-1984 A.D.A.S. advice treatments, no spray was applied; in these cages the population of S. avenae continued to increase, reaching a peak of c. 30 aphids per stem (100%) at G.S. 81 (Fig. 3).

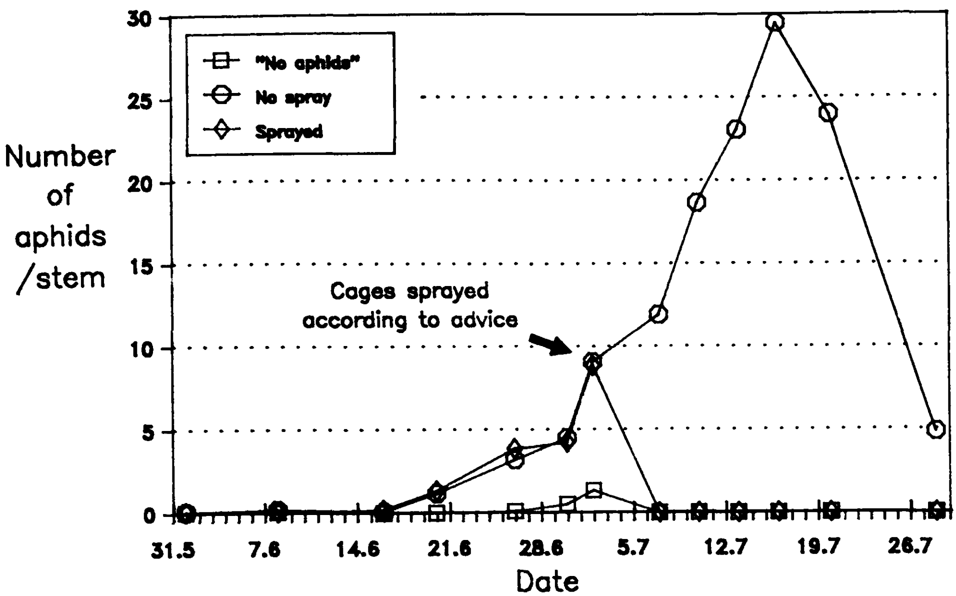
9.3. ANALYSIS OF RESULTS

The results were analysed using a two-way analysis of variance with replicates. Where any significance was found, a Tukey comparison of mean test employing Hartley sequential method of testing was performed; from these, confidence intervals could also be calculated.

Significance was found between treatments in the number of grains per ear, weight of grains per ear and 1000 grain weight (5 samples of 50 ears per replicate; F = 12.963, 23.519, 17.980 respectively) (Fig. 4). A significant difference was found in the number of grains per ear and weight of grains per ear between treatments A+D+E and B+C. Differences were found in the 1000 grain weight between treatment A, D+E and B+C.

Fig. 3. Development of populations of *Sitobion avenae* in 1987 field trials.

a. Number of aphids per stem



b. Proportion of stems infested

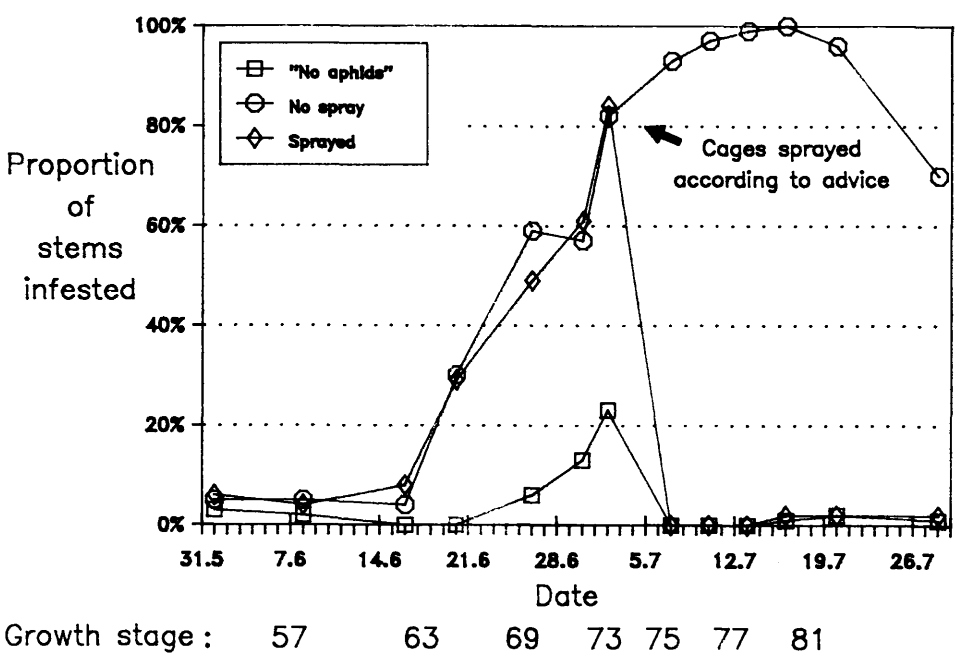
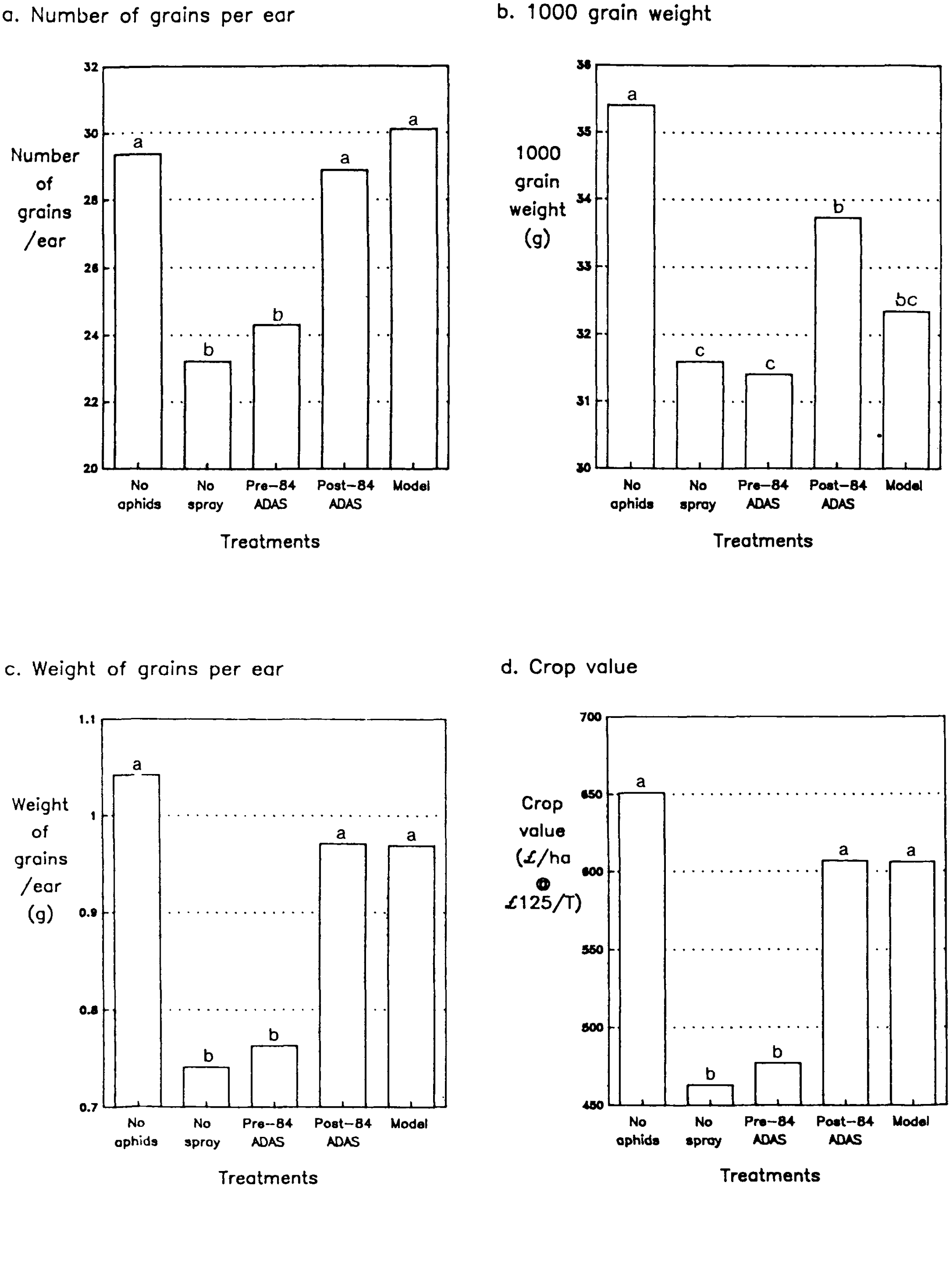


Fig. 4. Effect of different treatments on winter wheat in 1987.



10. DISCUSSION

Although only the 1987 trials are presented here, the model has functioned well in other years, resulting in an overall profit over prophylaxis in 1985 and 1986. Further validation of the model, comparing its output with published and unpublished data obtained from various sources, have shown the model to result in a net deficit over not spraying in only 10% of cases. In these, the model advised a spray treatment to be applied when in hindsight a spray should not have been applied because the aphid populations reduced naturally soon after the spray date. The model has never missed an occasion when a spray should have been applied.

The use of field cages in the 1987 trials resulted in an artificial situation in which the crop within the cage was probably different from that outside, making extrapolation between cage results and field results difficult. Field cages affect the microclimate of a crop by reducing wind speed, light intensity and daily fluctuations in temperature, although they have no effect on maximum temperatures and humidity (WOODFORD, 1973). This would make it difficult to extrapolate between cage results and the field situation, in the same year. However summers vary greatly between years and so the conditions within the cages in 1987 would have simply reflected a year which was less windy and had greater cloud cover (this causes a reduction in light intensity and in daily fluctuations in temperature).

Although the cages create favourable conditions for aphids, they may also prevent adult alates emigrating. However the proportion of aphids that were adult alates remained below 8 percent until G.S. 81 when the proportion rose to over 10 percent. It was not until this later growth stage that a large number of alates were observed flying within the cage.

The model was compared with two types of A.D.A.S. advice. Despite the changes made in 1984, the older advice was still available upto and including 1988 in the form of booklets and leaflets available from M.A.F.F. publications, and on the A.D.A.S. database available through Prestel Farmlink. A survey carried out in 1988 showed that this older type of advice was still being used in a few cases (WRATTEN & MANN, unpublished data).

11. AN ADVISORY MODEL FOR PESTS OF OILSEED RAPE

Winter oilseed rape is now the third most frequently grown crop in the U.K., following wheat and barley (LANE, 1984). During the spring and summer months the crop is attacked by three pests: the pollen beetle (Meligethes spp.), the cabbage seed weevil (Ceutorhynchus assimilis (Payk.)) and the brassica pod midge (Dasineura brassicae (Winn.)).

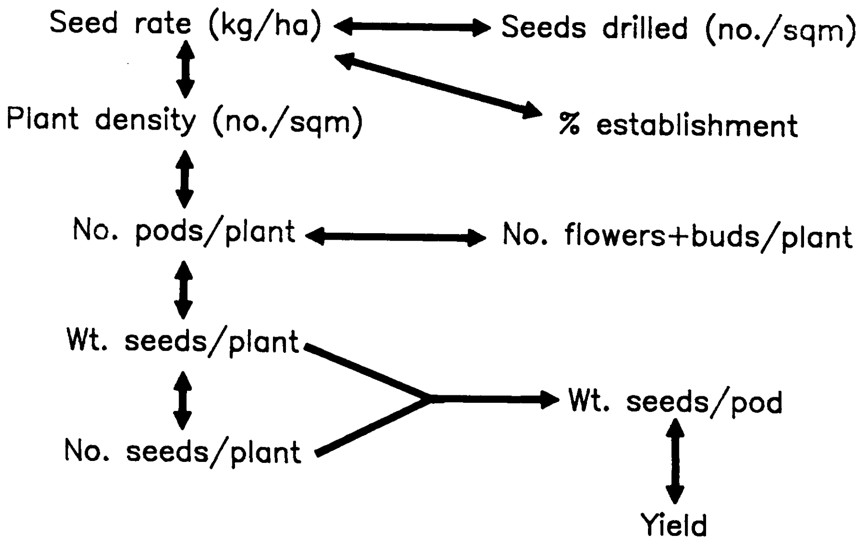
Both adults and larvae of the pollen beetles attack buds and flowers although only adults cause any significant yield loss (WINFIELD, 1962; WILLIAMS & FREE, 1978). Damaged buds wither and die, and as a consequence the potential number of pods is decreased. However rape plants have considerable powers of compensatory growth. The positive correlation between the number of flowers and of podless stalks per plant found by WILLIAMS and FREE (1979) suggests that plants either shed naturally some of the flowers they produce or compensate for setting insufficient pods by producing more flowers. Any podless stalks resulting from pollen beetle injury may, therefore, merely decrease the amount of 'physiological bud shedding' that occurs naturally and yield loss would result only if bud loss caused by pollen beetles prevented a plant from achieving its carrying capacity.

The adults of the seed weevils and pod midges cause no significant yield loss, it is the larave that are important.

Seed weevil adults oviposit in young developing pods. Once hatched, the larvae feed on the seeds within the pod causing about 22% of the seeds to be lost (DMOCH, 1965; LERIN, 1984).

The ovipositor of pod midge adults is too weak to penetrate the wall of a seed pod (STEVENSON, 1955) and so it gains access to pods mainly through feeding or oviposition punctures made by the seed weevil (NIJELDT, 1973; SYLVEN & SVENSON, 1975). FREE and WILLIAMS (1979) found a correlation between the proportion of pods infested with seed weevil larvae and the proportion infested by pod midge larvae. The larave of the pod midge feed on the pod wall causing the pod to swell and split prematurely (SYLVEN & SVENSON, 1975), so shedding its seeds.

Fig. 5. The relationship between the different parameters that determine yield of winter oilseed rape.



Compensation to damage by either seed weevil or pod midge larvae is unlikely owing to the late stage in the plant development at which it occurs. This series of events described has been converted into a model. Using inputs which include sowing information, plant density, density of adult pollen beetles and seed weevils, and economics, the model is able to forecast damage by these pests and the economics of a crop protection measure. The model is similar to the cereal aphid model except that the crop development model is more complex since the number of buds and pods per plant and the number of seeds and weight of seeds per pod needs to be predicted. Significant relationships have been found between different crop characteristics (Fig. 5). Upon this system, crop compensation also works.

The model was evaluated in 1986 and 1987 using field trials. The results showed that the model produced a profit better than prophylaxis and no spraying in both years, and better than ADAS in 1987 (in 1986 the model did as well as ADAS). The model will be described more fully in a later paper.

12. DEVELOPMENT AND MARKETING OF THE ADVISORY SYSTEMS

The models were originally run on Prestel-Framlink, part of British Telecommunications' Viewdata service. By 1988, the models were available to over 2000 farmers who subscribed to Farmlink. However in August 1988 the company was liquidated. The intention now is to develop the models further into a disc-based expert system, the models forming only a part of a database system.

References

ANON. - 1984a. Cereal pest control in autumn sown cereals. Ministry of Agriculture, Fisheries and Food (ADAS), leaflet 858.

ANON. - 1984b. Use of fungicides and insecticides on cereals. Ministry of Agriculture, Fisheries and Food (ADAS), booklet 2257(84).

ANON. - 1986. Use of fungicides and insecticides on cereals. Ministry of Agriculture, Fisheries and Food (ADAS), booklet 2257(86).

ANON. - 1988. Grain aphid control. A.D.A.S. divisional bulletin No. 144, June 1988.

BURN, A.J. - 1987. Cereal Crops. In: "Integrated pest Management". Eds A.J. BURN, T.H. COAKER & P.C. JEPSON. Academic Press, London.

CARLSON, G.A. - 1971. Economic aspects of crop loss control at the farm level. In: Crop loss assessment methods. FAO, Rome 2.3, 1-6.

CARTER, N., DIXON, A.F.G. & RABBINGE, R. - 1982. Cereal aphid populations: biology, simulation and prediction. Simulation Monograph, Pudoc, The Netherlands. 91pp.

CARTER, N. & RABBINGE, R. - 1980. Simulation models of the population development of Sitobion avenae. IOBC/WRPS Bulletin III (4), 93-98.

DEAN, G.J.W. & LURING, B.B. - 1970. Distribution of aphids in cereal crops. Ann. Appl. Biol. 66, 485-496.

DEWAR, A.M. & CARTER, N. - 1984. Decision trees to assess the risk of cereal aphid (Hemiptera: Aphididae) outbreaks in summer in England. Bull. Ent. Res. 74, 387-398.

DEWAR, A.M., DEAN, G.J. & CANNON, R. - 1982. Assessment of methods for estimating the numbers of aphids (Hemiptera: Aphididae) in cereals. Bull. Ent. Res. 72, 675-685.

DMOCH, J. - 1965. The dynamics of a population of the cabbage seed pod weevil (Ceutorhynchus assimilis Payk.) and the development of winter rape. Part I. Ekol. Polska A 8, 249-287.

ENTWISTLE, J.C. & DIXON, A.F.G. - 1986. Short-term forecasting of peak population density of the grain aphid (Sitobion avenae) on wheat. Ann. Appl. Biol. 109, 215-222.

ENTWISTLE, J.C. & DIXON, A.F.G. - 1987. Short-term forecasting of wheat yield loss caused by the grain aphid (Sitobion avenae) in summer. Ann. App. Biol. 111, 489-508.

FREE, J.B. & WILLIAMS, I.H. - 1979. The infestation of crops of oilseed rape (Brassicae napus) by insect pests. Jn Agric. Sci. 92, 203-218.

GEORGE, K.S. & GAIR, R. - 1979. Crop loss assessment on winter wheat attacked by the grain aphid, Sitobion avenae (F.), 1974-77. Plant Path. 28, 143-149.

GILBERT, N. & HUGHES, R.D. - 1971. A model of an aphid population-three adventures. Jn. Animal Ecol. 40, 525-534.

HEATHCOTE, G.D. - 1957. The comparison of yellow cylindrical, flat and water traps, and of Johnson suction traps, for sampling aphids. Ann. Appl. Biol. 45, 133-139.

HEATHCOTE, G.D., PALMER, J.M.P. & TAYLOR, L.R. - 1969. Sampling for aphids and by crop inspection. Ann. Appl. Biol. 63, 155-166.

HUGHES, R.D. & GILBERT, N. - 1968. A model of an aphid population - a general statement. Jn. Animal Ecol. 37, 553-563.

LANE, A. - 1984. The oilseed rape handbook. Open University Press.

LATTEUR, G. & NICOLAS, J. - 1984. Reduction of insecticides against cereal aphids by improving observation and prognosis methods. CEC Programme on Integrated and Biological Control, Final Report 1979/1983, 391-406.

LEE, G., WRATTEN, S.D. & KENYI, K.B.L. - 1981. The effects of growth stage in cereals on yield reductions caused by aphids. Proc. 1981 BCPC, 449-456.

LERIN, J. - 1984. Assessment of the effect of the seed pod weevil (Ceutorhynchus assimilis) on the yield of winter rape. II. Evaluation of losses in cage experiments. Agron. 4, 147-154.

MAAS, S.J. & ARKIN, G.F. - 1980. TAMW: a wheat growth and development simulation model. A publication of The Texas Agricultural Experiment Station, College Station, Texas, United States, Program and Documentation No. 80-83.

MANN, B.P. & WRATTEN, S.D. - 1986. A computer-based advisory system for cereal aphid control. Comp. Elec. Agric. 1, 263-270.

MOUCHART, D.G. - 1984. Five years trapping of cereal aphids with suction traps in France. C.E.C. programme on integrated and biological control, Final report 1979/1983, 353-366.

NIJVELDT, W. - 1973. Een analyse van de toegenomen aantasting van koolzaad door de koolzaadgalmug, Dasineura brassicae (Winn.). Gewasbescherming 4, 1-4.

ONSTAD, D.W. & RABBINGE, R. - 1985. Dynamic programming and the computation of economic injury levels for crop disease control. Agric. Systems 18, 207-226

PIERRE, J.S. & DEDRYVER, C.A. - 1984. A multiple regression model applied to forecast outbreaks of a cereal aphid, Sitobion avenae F., on winter wheat. Acta. Oecol. Applic. 5, 153-172.

RABBINGE, R. & MANTLE, W.P. - 1981. Monitoring for cereal aphids in winter wheat. Neth. J. Plant Path. 87, 25-29.

SELIGMAN, N.G. & AVN KEULEN, H. - 1981. PAPRAN: a simulation model of annual pasture production limited by rainfall and nitrogen. In: M.J. Frissel & J.A. van Veen (Eds): Simulation of nitrogen behaviour of soil-plant systems. Pudoc, Wageningen, pp. 192-221.

STERN, V.M., SMITH, R.F., VAN DEN BOSCH, R. & HAGEN, K.S. - 1959. The integrated control concept. Hilgardia, 29, 81-101.

STEVENSON, J.H. - 1955. Observations on the oviposition of the seed pod midge Dasineura brassicae Winn. on colza. Tijdschrift over Plantenzieten 61, 81-87.

SUTER, H. - 1980. Forecasting aphids in field crops (Swiss model). EPPO Bulletin 10, 143-146.

SYLVEN, E. & SVENSON G. - 1975. Relationship between density of Ceutorhynchus assimilis Payk. (Col.) and damage by Dasineura brassicae Winn. (Cec.) in a cage experiment in summer turnip rape. Meddn St. VaxtskAnst 16, 53-60.

TAYLOR, L.R. - 1977. Aphid forecasting and the Rothamsted Insect Survey. Journal RASE 138, 75-97.

VICKERMAN, G.P. - 1977. Monitoring and forecasting insect pests of cereals. Proc. 1977 BCPC 1, 227-234.

VICKERMAN, G.P. & WRATTEN, S.D. - 1979. Monitoring and forecasting insect pests of cereals. Proc. 1977 BCPC 1, 227-234.

WARD, S.A., RABBINGE, R. & MANTEL, W.P. - 1985. The use of incidence counts for estimation of aphid populations - I. Minimum sample size for required accuracy. Neth. J. Plant Path. 87, 25-29.

WATSON, S.J. & CARTER, N. - 1983. Weather and modelling cereal aphid populations in Norfolk. EPPO Bull. 13, 223-227.

WATT, A.D. - 1983. The influence of forecasting on cereal aphid control strategies. Crop Prot. 2, 417-429.

WATT A.D., VICKERMAN, G.P. & WRATTEN, S.D. - 1984. The effect of the grain aphid, Sitobion avenae (F.) on winter wheat in England; an analysis of the economics of control practice and forecasting systems. Crop Prot. 3, 209-222.

WILLIAMS, I.H. & FREE, J.B. - 1978. The feeding and mating behaviour of pollen beetles (Meligethes aeneus Fab.) and seed weevils (Ceutorhynchus assimilis Payk.) on oilseed rape (Brassica napus L.). Jn. Agric. Sci. 92, 53-59.

WILLIAMS , I.H. & FREE, J.B. - 1979. Compensation of oilseed rape (Brassica napus L.) plants after damage to their buds and pods. Jn. Agric. Sci. 92, 53-59.

WINFIELD, A.L. - 1962. Effect of early flower-bud removal on subsequent development of some Brassica seed plants. Plant Path. 11, 17-22.

WOODFORD, J.A.T. - 1973. The climate within a large aphid proof field cage. Ent. Exper. Appl. 16, 313-321.

WRATTEN, S.D. & GEORGE, K.S. - 1985. Effects of cereal aphids on yield and quality of wheat and barley. Bull. SROP/WRPS Bull. 1985/8/3, 10-35.

WRATTEN, S.D., HOLT, J. & WATT, A.D. - 1984. Conversion of research models to micro-computer advice on cereal aphid control. Proc. 1984 BCPC, 609-614.

WRATTEN, S.D. & MANN, B.P. - 1988. A survey of aphicide use on winter wheat in the summer of 1988. Proc. 1988 BCPC (in press).

WRATTEN, S.D., WATT, A.D., CARTER, N. & ENTWISTLE, J.C. - 1989. Economic consequences of pesticide use for grain aphid control on winter wheat in 1984 in England. Crop Prot. (in press).

ZADOCKS, J.C., CHANG, T.T. & KONZAK, C.F. - 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415-421.

AGRICULTURE ET NOUVEAUX MEDIAS

A. Olivier
PDG, Agence Digitalis-Paris, France

L'expérience de quelques années passées à m'occuper de communication agricole m'a amené à une constatation: la communication agricole n'existe pas!

La règle péjorative qui a longtemps consisté à considérer "les ruraux" comme une cible quelque peu à part, à laquelle il fallait s'adresser d'une façon particulière, est absolument infondée. L'agriculteur "arrièré" vers qui il faut "communiquer simple" n'existe plus que dans l'esprit de quelques technocrates citadins qui ne se sont jamais aventurés au-delà des boulevards périphériques de leur ville. Les cibles rurales et agricoles sont maintenant touchées par les mêmes médias que les citadins, elles reçoivent les mêmes messages, les analysent et y réagissent de la même façon.

Il est par contre incontestable qu'il existe, en agriculture comme dans tous les autres domaines, une communication professionnelle... mais rien là de différent de ce que l'on observe dans l'industrie ou la finance. Il s'agit de la diffusion de messages techniques accessibles aux seuls professionnels et qui correspond, par opposition à la communication "grand public", à ce que les publicitaires appellent le "business to business".

En résumé, la communication destinée aux agriculteurs, qu'elle s'adresse au professionel technicien ou au consommateur, ne diffère en rien dans sa conception et dans sa construction de celle qui s'adresse au reste de la population. Le classement des agriculteurs, et des ruraux au sens large, en "socio-styles", montre d'ailleurs que, comme tous, ils se répartissent en diverses catégories comportementales mais qu'en aucun cas ils ne forment un groupe à part.

Il est indispensable, lorsque l'on doit s'adresser à cette cible, qui n'en est plus vraiment une, disons plûtot à cette catégorie socio-professionnelle de bien avoir cette réalité à l'esprit: dans nos civilisations dites évoluées, elle ne se distingue plus en rien, sociologiquement au moins, de l'ensemble de la population. Il suffit pour s'en convaincre d'analyser les documents publicitaires diffusés par les grandes firmes de l'agro-fourniture. Ceux-ci, élaborés au vu des résultats d'études marketing et d'analyses de cibles très précises, sont, dans leur forme et dans leur esprit conformes à ce qui se fait dans les autres activité industrielles ou commerciales. Ces grandes firmes confient d'ailleurs généralement leurs budgets publicitaires à de grandes agences généralistes que elles sélectionnent sur leurs qualités créatives et le sérieux de leurs études et de plus en plus rarement à des agences spécialisées en agriculture qui continuent à mettre en avant leur "connaissance du milieu" qui n'interesse plus personne. Il s'agit là également d'un signe qui ne trompe pas.

L'ère est peut-être venue où dans l'esprit de chacun, un chef d'entreprise sera considéré comme un chef d'entreprise qu'il fabrique des ordinateurs ou qu'il cultive la terre. Si la communication ne fait déjà plus de distinguo... ce sera un grand pas vers l'indispensable reconnaissance d'un métier qui reste essentiel.

Cette constatation faite revenons au sujet de cette communication: "Agriculture et nouveaux médias", pour laquelle j'ai choisi de retenir 3 médias, la télévision dont je vais parler en détail, puis la radio et la télématique que je traiterai plus rapidement.

La télévision

Ce n'est pas à proprement parler un nouveau média mais il est peu utilisé par l'agriculture. Essayons de voir pourquoi en prenant l'exemple de ce qui se passe en France.

Il y a 6 chaînes de télévision qui diffusent globalement entre 850 et 900 heures de programmes par semaine dont... 1/2 heure d'émission agricole.

Déséquilibre flagrant qui répond à une toute simple logique de marché. Les responsables des chaînes vivent en permanence avec un oeil inquiet sur l'AUDIMAT, indice qui mesure l'audience des émissions en France, car c'est précisément cet indice qui permet de fixer le prix de vente de l'espace publicitaire et, qu'elles soient publiques ou privées, les chaînes vivent en grande partie des recettes publicitaires; une audience élevée et régulière est donc indispensable à leur bon fonctionnement, voire à leur survie.

Or, par nature, un programme agricole intéresse potentiellement 1 à 2% de l'auditoire global d'un média grand public comme la télévision. Dans la logique économique d'un responsable de programme, diffuser une telle émission, c'est voir au moins 9 télespectateurs sur 10 changer de chaîne pour retrouver un jeu ou une série américaine.

Le seul et unique magazine agricole français est d'ailleurs diffusé par une chaîne publique qui a une audience moyenne, le dimanche à 13 heures, à une heure où cette chaîne ne peut, de toute façon, rivaliser avec les grands journaux d'information diffusés par les 2 principales chaînes concurentes. Or, malgré cela et malgré sa qualité indéniable, chacun sait que ce magazine sera, à plus moins brève échéance, condamné sur l'autel de l'AUDIMAT.

Dans toutes les réflexions qui sont menées pour rechercher les façons les plus efficaces de diffuser de l'information agricole et en particulier de l'information technique, on se demande souvent si la télévision est intéressante pour l'agriculture... Cela me semble être un faux problème. La véritable question est de savoir si l'agriculture est intéressante pour la télévision, et là, la réponse est très claire: non! Un média qui, par nature, s'adresse au grand public et qu'un système contraint à des résultats performants ne peut s'adresser à une cible spécifique aussi réduite. L'apparition du cable qui permettra une multiplication des chaînes privées, et notamment de chaînes thématiques, ne résoudra en rien le problème. Si des chaînes musicales, sportives peuvent espérer vivre un jour, envisager une chaîne agricole est totalement illusoire: elle ne trouvera jamais les ressources nécessaires à son fonctionnement.

Ce problème, les publicitaires et les annonceurs qu'ils représentent l'ont bien compris et ils ne diffusent pratiquement pas d'annonces agricoles par ce support. En effet, la télévision est le média le plus économique en valeur absolue, lorsque l'on ramène le coût du spot au nombre de consommateurs potentiels touchés; mais, pour cela, il est bien sûr indispensable de n'y diffuser que des messages qui intéressent potentiellement la plus grande partie de l'auditoire. Acheter trente secondes d'antenne à une heure de grande écoute est inenvisageable si le spot que vous diffusez concerne 1 à 2% des millions de télespectateurs qui le voient.

Entre 1983 et 1985, les investissements publicitaires agricoles à la télévision ont diminué de près de 50% alors que durant la même période, les budgets confiés à la presse ont augmenté de 25%. Et cette tendance ne fait que s'amplifier: actuellement, la télévision ne recueille plus guère qu'1% des budgets publicitaires agricoles.

Quand on connaît l'ampleur de ces dépenses et le sérieux avec lequel sont menées les études conduisant au choix des médias, cela ne fait que confirmer la "quasi-incompatibilité" de fait qui existe, dans le système actuel, entre l'agriculture et la télévision.

Cela ne veut pas dire que cette situation est figée définitivement: un concept nouveau est en train de naître pour permettre à des professions spécifiques de pouvoir utiliser ce média: il combine le marketing direct, la publicité et les relations publiques. Une societé achète du temps d'antenne à une heure d'écoute habituellement très faible, pour arriver à un coût abordable, elle réalise une petite émission de 10 à 20 minutes et prévient par courrier, téléphone ou télex, ses clients et partenaires de cette diffusion. Ainsi même si l'auditoire global est assez faible, il est parfaitement ciblé car les partenaires intéressés et donc intéressant suivent cette émission que l'on peut réaliser dans le cadre d'un budget raisonnable.

Ce concept, testé en France sous le nom de "Régie française d'espace" a donné des résultats qui ont, semble-t-il, satisfait les entreprises qui y ont eu recours. C'est, à mon avis, dans l'avenir la seule solution pour l'agriculture d'utiliser la télévision pour diffuser de l'information.

La radio

Le constat est extrêmement simple: mêmes causes, même effets, même punition! La radio est un média qui s'adresse au grand public et c'est un média cher, même si le coût de la minute d'antenne est sans commune mesure avec celui des chaînes de télévision. L'émission agricole est un luxe qu'aucune station ne peut s'offrir.

L'autorisation d'émettre, accordée il y a 7 ans, en France, à des radios locales privées a fait germer des projets de stations rurales consacrant une petite partie de leurs programmes à des émissions agricoles. Les quelques tentatives dans ce domaine se sont soldées par de cuisants échecs: ces stations se sont heurtées aux dures réalités du marché et elles sont bien vite revenues aux jeux et autres hit-parades.

Dans le domaine de la communication publicitaire, les résultats ne sont guère plus brillants que pour la télévision: moins de 2% des budgets sont affectés à ce média et la tendance est à la baisse.

La télématique

Déjà fortement implanté en France, ce mariage entre l'informatique et le téléphone permet à tout possesseur et utilisateur de minitel d'avoir immédiat accès à de multiples bases de données et messageries professionnelles et à des programmes d'aide à la décision. Consulter au moment où on en a besoin, un système qui permet d'accéder à l'information nécessaire et suffisante, c'est le rêve. Plus de documentation à stocker, plus de temps perdu dans des recherches fastidieuses...

La solution idéale? La réalité n'est pas aussi rose car la première nécessité pour qu'un service de ce type dure dans le temps et s'améliore, est qu'il s'autofinance, ce qui, pour les services à vocation professionnelle, est encore loin d'être le cas actuellement. Là encore, les services agricoles souffrent de l'étroitesse de la cible à laquelle ils s'adressent et qui ne peut pas générer des recettes permettant de rentabiliser les frais de conception et de mise en oeuvre des programmes et du matériel technique.

Par ailleurs, ce média est tout nouveau et les erreurs de jeunesse sont bien sûr nombreuses; les hommes de communication n'ont pas été associés à la conception des produits et même s'ils l'avaient été, il est si difficile de concevoir des messages et un langage pour un support pas encore testé, que le résultat n'aurait sans doute pas été parfait. Il faut simplement espérer que rapidement les services professionnels s'améliorent et fassent un gros effort pour se faire connaître et atteindre le succès des messageries et programmes destinés au grand public.

Ce constat peut sembler pessimiste voire désabusé... et il l'est quelque peu. La presse agricole a, en fait, de beaux jours devant elle, elle recueille, actuellement plus de 90% des quelques 400 millions de dépenses publicitaires agricoles françaises et elle reste la seule et unique source d'information technique et économique pour de nombreux agriculteurs.

Or, la tendance, loin d'être à la diversification des titres, évolue bien au contraire vers une concentration dans des grands groupes. L'agriculture est prise dans un système où un média domine la communication jusqu'à truster toute l'information et les moyens et où quelques groupes contrôlent ce média. A chacun de mesurer les inconvénients et les dangers d'une telle situation.

PEST CONTROL DECISION MAKING UNDER UNCERTAINTY

C. Calvi Parisetti & G. Di Cola
Dipartimento di Matematica, Università-Parma, Italy

In pest control decision making problems, due to the uncertainty of the relevant control variables, the state variables, such as pest density, crop losses, effectiveness of control methods, are to be considered random variables with some distribution function.

The decision maker can modify the probability of the state outcomes to this advantage by means of suitable interventions.

The economic treshold method model developed by entomologists (Stern, 1973), as a working decision rule, gives the level of pest presence at which the expected benefit is at least as great at the cost control (Mumford and Norton, 1986).

From the practical point of view, the treshold method allowed to save a lot of chemical interventions (Briolini e Crovetti, 1986), newerthless it needs informations concerning the complex relations between pest density crop loss, economic loss and it is affected by uncertainty of experimental data (Delucchi, 1986).

For these reasons the method is feasible only in a few cases; it becomes unfeasible when more than one intervention may be applied and when valid control methods, alternative to chemical ones, do exist such as biological, biotechnological control and/or different cultural practices.

This approach is based on the assumption of the certainty about the outcomes, while the decision maker is to be faced with considerable uncertainty when he is deciding on pest control.

Since uncertainty has been hypothesized as a factor inducing pesticide use it needs to investigate the impact of uncertainty on decision taken by farmers rather than to ignore it.

From a general point of view it is possible to consider the problem of choosing the control action, more profitable in order to reach a given goal, as a problem of the decision theory under uncertainty; the rule of expected utility (EUR) is the most frequently used (Roberts, 1979).

This rule is based on choosing the action which maximizes the expected value of an utility function, which takes into account economic and social objectives.

In order to define an utility function, we refer to a set O of random nature states, to a set A of possible actions and to a function $c(\theta,a)$ defined on the set $\theta \times A$, which relates a random consequence C to each pair nature state-action.

Consequences may be expressed in quantitative (profit, revenue, etc.) or qualitative (crop goodness, envïronnemental fallout, etc.) terms.

An utility function U(C) defined on the set of the consequences, represents the decision maker degree of satisfation. U(C) is an increasing and concave function where concavity implies risk aversion of the decision maker (Hadar and Russel, 1969).

When two actions a_1 e a_2 are considered, the action which maximizes the corresponding expected values $E(U(C_1))$ e $E(U(C_2))$ is chosen.

If the consequences are indipendent, the EUR is a Bayesian rule and when a sample data x is present such a criterion has been used by Carlson (1970) and Calvi Parisetti _et al._ (1987).

In this cases, the action a* is chosen such that

$$E^{p(\theta|x)}\, U(\theta,a^*) = \mathrm{MAX}_{a \in A}\, E^{p(\theta|x)}\, U(o,a)$$

where $p(\theta|x)$ is the posterior density function of θ, given the sample x.

When the consequences are not independent a generalized expected utility rule (GEUR), based on the regret criterion, can be used.

In this case the choice criterion based on expected regret refers to an utility function depending on two arguments, rather than one argument; the first argument represents the consequence of the chosen action, the second argument represents the consequence of the forgone one.

Let $U(C_1,C_2)$ be the utility function, according to the GEUR, C_1 is preferred to C_2 (therefore a_1 is preferred to a_2) if

$$E(U(C_1,C_2)) = E(U(C_2,C_1))$$

As an example, consider two possible actions

a_1: chemical intervention

a_2: biological intervention or no-intervention

Let C_1, C_2 be the following consequences

C_1: pest reduction

C_2: crop goodness

The consequences due to the action choice are not independent then a generalized criterion has to be applied.

REMARKS

1) GEUR approach seems to be very interesting when applied to the evaluation and comparison of the performance of different agricultural pest control methods in order to improve techniques of biological control and to reduce pesticide use, taking into account the randomness of pest-crop system.

2) The generalized utility function takes into account the dependence of the states differently from the classical decision theory approach which emphasizes the marginal distributions.

3) EUR and GEUR need the assessment of the utility function which is, in general, an hard and subjective problem. Stochastic dominance rules permit the comparison of alternative pest control method which are based on expected utility. The comparisons are valid for broad classes of utility function but do not requires specific knowledge of an utility function.

BIBLIOGRAPHY

Briolini G., Crovetti A. (1986): Soglie economiche nella difesa delle colture agrarie
La Difesa delle Piante, 9 (2): 67-84

Calvi Parisetti C., Di Cola G., Vacante V. (1987): Decision making in protected crops
Proceedings of a meeting of the E.C Experts group: Integrated biological control in protected crops - Barcellona, Spain, May 1987

Carlson G.A. (1970): A decision theoretic approach to crop disease prediction and control
Am J. Agric. Econ. 52: 216-223

Delucchi V. (1986): La protection intégrée des cultures
Integrated pest management Quo Vadis? - Parasitis 86

Mumford J.D., Norton G.A. (1986): Economic aspects of integrated pest management
Integrated pest management Quo Vadis? - Parasitis 86

Roberts F.S. (1979): Measurement theory with applications to decisionmaking, utility, and the social sciences
Encyclopedia of Mathematics and its applications, Vol. 7

Stern V.M. (1973): Economic threshold
Ann. Rev. Entom. 18: 259-280

ETUDE STATISTIQUE DES CRITERES DE QUALITE DES HUILES D'OLIVE EN VUE DE GESTION DES STOCKS

S. Kmieciak
Centre Universitaire Tizi-Ouzou-Oued Aissi, Algérie

Les valeurs nutritionnelles et organoleptiques exceptionnelles de l'huile d'olive, le caractère saisonnier de la production, une grande variété de la qualité et de la stabilité pendant le stockage engendrent une nécessité de classification de l'huile brut en catégories:

- destiné à la consommation en forme de l'huile vierge;
- destiné au raffinage.

Dans les deux cas il se pose le problème de la durée du stockage, dans des conditions habituelles, qui permettrait d'assurer la stabilité de qualité de l'huile stocké.

Afin de répondre à ces questions il est nécessaire d'avoir la connaissance des relations entre les caractéristiques d'un huile brut fraîchement produit et de la qualité de l'huile correspondant après le raffinage ou/et le stockage prolongé.

L'altération des huiles au cours du stockage consiste, en général, en deux groupes de processus:

- l'hydrolyse qui conduit à l'augmentation de l'acidité;
- les phénoménes d'oxydation et de dégradation oxydative.

Les altérations chimiques des huiles sont reliées avec la détérioration organoleptique.

Contrairement à l'estimation du degré d'acidification des huiles qui se fait d'une façon simple et sans ambiguité, les méthodes actuelles d'évaluation de l'état d'oxydation sont toujours discutées et mises en question. Parmi les critères de l'altération oxydative le plus souvant utilisée semble être l'indice de peroxyde.

Les corrélations entre les caractéristiques physico-chimiques des huiles ainsi que leur rapport avec les notes de dégustation ne sont pas, non plus, suffisamment clairs.

Dans cette approche on essayera d'appliquer les méthodes d'analyse statistique sous forme d'analyse factorielle et de régression linéaire pour trouver des solutions aux problèmes posés, dans le premier temps, en ce qui concerne des huiles d'olive bruts. On prendra la teneur en acidité et l'indice de peroxyde comme les variables dépendantes des autres paramètres inclus dans les normes de qualité.

Ultérieurement, on prévoit la recherche des relations entre les caractéristiques analytiques et les notes de dégustation de l'huile d'olive brut et celles après le stockage et/ou le raffinage.

L'étude de relations entre les critères de qualité pourra améliorer l'objectivité de l'évaluation des effets d'oxydation et, probablement, permettra de suggérer un nouveau critère synthétique de cette évaluation qui rendra possible une meilleure gestion des stocks.

Report on the Session 3: APPLICATION, USE AND TRANSMISSION OF KNOWLEDGE

J. Baumgärtner
ETH, Eidgenössische Technische Hochschule, Institut für Phytomedizin-Zürich, Switzerland

Rational and environmentally sensitive pest control usually relies on quantitative information acquired by remote sensing, synoptic pest monitoring, field sampling or a combination of all three. Experiences with remote sensors have stressed the importance of data quality. Only reliable data collected in crops or in the athmosphere or in the acquatic medium can be used effectively. In any case it is neccessary to combine such information with the information obtained directly in the field. Research on spatial distributions has greatly facilitated sampling work undertaken to obtain reliable estimates of pest population densities.

There are different areas of application for such information but administrators, researchers, field advisers and farmers may be particularly interested in regional crop inventories and in the monitoring of pest populations, as well as in the follow-up of crop yield formation. This information can be complemented with extensive synoptic monitoring schemes such as the Rothamstead insect survey which can be helpful in predicting pest outbreaks. Experiences with this method show furthermore that the information needs to be analyzed and summarized in a very concise and expressive way, for example, by using color graphics, before it is transmitted to decision makers.

Such procedures greatly facilitate understanding. Weather is among the most important driving variables for the development of pests and crops, hence decision making in pest management is greatly influenced by actual weather data and weather forecasts. Therefore, the acquisition of weather data is important and appropriate equipment should be available to record the data useful for monitoring pest and crop development. However, the limitations of weather forecasts still jeopardize our attempts to make predictions of pest numbers.

Nevertheless, we can envisage a greater integration of all these methods which would probably permit more reliable and more efficient decision making. The usefulness of instruments and information in pest management, however, will probably depend less on our ability to integrat than on our knowledge of the economical importance of the target pest. Investments in research and implementation are only justified if the pest status has been satisfactorily assessed.

Newspapers, specialized magazines, television and computer systems are the most widely used media to transmit relevant information to decision makers. Television and radio are the best, but experience in France has shown that information has to be delivered by state channels. Commercial stations do not make time available for transmitting information to the farming community, because the target audience is too small.

Computer systems for real-time management of pests, often combined with telephone links, are particularly suited, with the aid of simulation models, to the acquisition and the processing of the above information. Many computer systems for real-time management of pests are in operation today and the development of computer hardware and software is likely to make these systems more effective and applicable for a wide area of agronomic decision making in the future. As stated above, the assessment of the economic importance of the target pest should be a prerequisite to further investment in pest management decision aids.

Most of the ongoing research work in integrated pest management adresses tactical decision making, however, the importance of strategic and policy decisions for pest management undoubtedly justifies more investment in relevant research and implementation. In particular, such studies should help to elucidate causes for pest occurrences and lead to a comprehensive concept of agroecosystem design and management which is beyond the narrow tactical approach favoured today by many scientists.

SESSION 4

PUBLIC HEALTH AND HYGIENE: HOUSEHOLD PEST CONTROL

Chairman: B. Sánchez Murias

SECURITE D'EMPLOI DES PESTICIDES DANS LA LUTTE CONTRE LES ESPECES NUISIBLES DOMESTIQUES

G. Quelennec
Organisation Mondiale de la Santé-Genève, Switzerland

Résumé

Dans les programmes de lutte intégrée contre les espèces nuisibles, les pesticides chimiques continueront à jouer un rôle majeur dans les années à venir. Bien que par définition les pesticides soient considérés comme des poisons, le danger pour la santé humaine doit être mis en balance avec le bénéfice pouvant découler de leur utilisation dans de bonnes conditions. La toxicité d'un pesticide est un des facteurs dont on doit tenir compte dans le choix d'un pesticide mais il ne doit pas être déterminant. Certaines procèdures permettent de diminuer cette toxicité. Les matières actives utilisées pour la lutte contre les espèces domestiques nuisibles ont généralement une faible toxicité pour les mammifères et sont formulées de manière appropriée. Il n'en reste pas moins que les traitements pesticides intra-domiciliaires impliquent une exposition potentielle maximum des opérateurs et des habitants ce qui contribue au danger représenté par ces traitements.

Ce document passe en revue plusieurs principes de base pouvant contribuer à réduire le danger des pesticides appliqués à l'intérieur des habitations.

1. INTRODUCTION

En matière de lutte contre les vecteurs de maladies et les espèces nuisibles pour l'homme, l'accent est actuellement mis sur la lutte intégrée. Ce type de lutte utilise toutes les techniques disponibles pouvant assurer un degré satisfaisant de destruction de ces espèces.

Il s'en suit que la lutte intégrée,appliquée aux espèces domestiques nuisibles comprend l'emploi de grillages moustiquaires, de répulsifs, d'agents biologiques, des modifications des lieux d'habitation et de l'environnement, ainsi que l'emploi d'agents chimiques.

Alors que les agents chimiques devraient, dans la plupart des cas, jouer un rôle secondaire, on doit constater qu'ils continuent de constituer l'arme de prédilection dans la lutte intradomiciliaire. Cette situation est essentiellement due au fait que les autres méthodes, quoique généralement plus prometteuses à long terme, nécessitent des investissements que les personnes ou les gouvernements ne sont pas en mesure de supporter financièrement ou ne sont pas prêts à consentir.

Les pesticides sont donc appelés à continuer de jouer un rôle primordial, dans les années à venir, en matière de lutte contre les espèces domestiques nuisibles.

Il convient peut-être d'insister, ici, sur le fait que cette communication à pour but de signaler les dangers potentiels de la lutte contre les espèces domestiques et non pas les dangers présentés par les formulations pesticides à usage domestique.

2. DANGER DES PESTICIDES

Bien que, par définition, les pesticides soient des produits toxiques, le danger pour la santé humaine doit être mis en balance avec le bénéfice que l'on peut retirer de leur emploi à condition de les utiliser dans de bonnes conditions de sécurité.

La toxicité aigue d'un produit est un facteur important que doit être pris en considération dans le choix d'un pesticides, mais ce facteur n'est pas déterminant, car le danger pour la santé des opérateurs et des habitants des locaux traités peut être réduit par un certain nombre de techniques.

Il semble important de faire une distinction terminologique entre la toxicité qui est la capacité d'une substance à provoquer des dommages pour la santé des êtres vivants et le danger qui est le risque d'empoisonnement pouvant survenir dans la pratique. Ainsi les pesticides les plus toxiques peuvent être manipulés dans la pratique avec un risque minimum si l'on adhère pleinement à des règles bien établies concernant leur utilisation. En conséquence toxicité et danger ne sont pas synonymes car le danger est fonction de deux autres variables en plus de la toxicité, ce sont la contamination et le temps d'exposition à la substance toxique[2].

Danger = Toxicité x Contamination x Temps

3. CONTRIBUTION DE L'OMS A LA SECURITE D'EMPLOI DES PESTICIDES

Afin d'aider les Etats membres dans leur choix des pesticides actifs, des formulations les plus appropriées et des dosages optima, l'OMS a préparé un certain nombre de documents et de publications dont les plus importants sont:

3.1 La Classification OMS Recommandée des Pesticides en Fonction des Risques qu'ils présentent[4] dont l'objet est d'aider les utilisateurs de pesticides à sélectionner les produits les plus appropriés. Cette classification a été approuvée par l'Assemblée mondiale de la Santé en 1975 et publiée pour la première fois en 1978. Depuis des directives pour la classification des pesticides ont été revues et republiées tous les deux ans. La classification est basée au départ sur la toxicité aigue orale et cutanée pour le rat. Elle fait la distinction entre les formes qui sont plus ou moins dangereuses. Par exemple, les pesticides présentés sous forme solide sont en générale moins dangereux que les liquides. Le classement des produits se fait également selon leur toxicité par voie orale ou par voie cutanée.

3.2 Les Méthodes Chimiques de Lutte contre les Arthropodes Vecteurs et Nuisibles importants en Santé Publique[3]

Cet ouvrage donne une liste des méthodes de lutte, avec quelques détails sur les techniques à employer. Il énumère également les composés et formulations qui se sont montrés actifs, dans des conditions bien définies, contre les arthropodes cibles, ainsi que les dosages les plus appropriés.

3.3 Les Normes pour les Pesticides Utilisés en Santé Publique[1]

Ce manuel décrit les normes elles mêmes, c'est à dire la teneur en produit actif du produit technique et des formulations, la teneur en impuretés, les caractéristiques physiques et chimiques des produits. Il décrit également les méthodes physiques et chimiques permettant de déterminer les normes et inclue des recommandations pour l'étiquetage et sur les précautions à prendre pour l'utilisation des produits. Ces normes peuvent être utilisées pour l'achat et le contrôle de qualité des pesticides.

3.4 Les Fiches d'Information FAO/OMS sur les Pesticides[5]

En tout 75 fiches ont été publiées, 19 sont en préparation. Ces fiches sont diffusées en français et en anglais et adressées à plus de 1500 personnes. Chaque fiches comprend cinq parties:

1 Renseignements généraux
2 Toxicologie et Risques
3 A l'Usage des Autorités chargées des Contrôles - Recommandations relatives à la Règlementation du Composé
4 Prévention de l'Intoxication chez l'Homme et Premiers Soins
5 A l'Usage du Personnel médical et de Laboratoire

4. DANGER DES PESTICIDES DANS LA LUTTE CONTRE LES ESPECES DOMESTIQUES NUISIBLES

Lorsqu'il s'agit de lutter contre des espèces domestiques, le contact entre le pesticide, les opérateurs et les populations est relativement étroit. Le problème est donc de limiter le danger en agissant sur les autres facteurs de l'équation figurant au paragraphe 2:

Toxicité - D'une façon générale on choisira des insecticides dont la toxicité est minimale et des formulations pouvant encore dinimuer cette toxicité. Dans le choix des insecticides il faudra tenir compte de leur toxicité cutanée s'il sont destinés à être pulvérisés.

Dans certains cas, magasins de stockage, soutes de navires infestés de rongeurs il se peut qu'il soit indispensable d'employer des poisons violents tels que le bromure de méthyle, le phosphure d'hydrogène et l'acide cyanhydrique. Les opérations doivent alors être confiées à des équipes très spécialisées, parfaitement instruites du danger et équipées en conséquence.

Contamination - La contamination sera limitée au maximum par le port de vêtements protecteurs, y compris des masques de différents types selon la toxicité du produit. La contamination sera réduite en évitant le contact avec le produit. Dans cette optique, une importance grandissante est donnée à l'emploi d'emballages spéciaux, sachets scéllés ou sacs hydrosolubles contenant la quantité juste nécessaire de formulation correspondant au volume de l'appareil d'application. La contamination sera également minimisée si l'opérateur est bien entrainé dans la technique de traitement, d'où l'importance d'une bonne formation.

Temps - Le temps de travail prescrit ne devra pas être excèdé. La peau contaminée devra être lavée à l'eau et au savon sans délai, les vêtements protecteurs changés et lavés après chaque séance de travail.

Les espèces nuisibles domestiques se rencontrent dans les habitations. Ce sont parfois des vecteurs de maladies le plus souvent dans les pays chauds. Ces nuisibles se rencontrent également dans les entrepôts, sur les navires qui peuvent être considérés soit comme des hôtels soit comme des entrepôts flottants, dans les avions qui peuvent transporter des moustiques adultes vivants infectés ou non, des puces infectées ou non, des blattes, des mouches, des rongeurs, aussi bien que des ravageurs des cultures. Dans tous ces cas, la désinfection est le plus souvent pratiquée par des équipes expérimentées.

Cependant, les occupants des habitations peuvent eux-mêmes assurer la salubrité de leurs locaux. Il est alors conseillé d'utiliser des pesticides à usage domestique tels que les spirales insecticides qui par combustion libèrent des fumées toxiques pour les arthropodes, des pastilles fumigantes chauffées électriquement, des aérosols, des poudres, des appâts pour rongeurs, des shampooings, lotions, crèmes à utiliser contre les poux. Tous ces produits, à conditions d'être employés selon les directives, sont inoffensifs. Ils ne doivent toutefois pas être laissés à la porter des enfants.

5. CONCLUSION

Chemical pesticides will continue to play a major role in household pest control. It is important to make a clear distinction between the toxicity of pesticides and the hazard they create for humans. Hazard is in fact function of the toxicity of the product, the contamination and the time of exposure. WHO contributes to the safe use of pesticides in providing a number of documents, to assist users in their choice of effective pesticides, of appropriate techniques to control household pests, and to provide them with instructions on the precautions to be taken for the safe use of these materials. Hazard in using pesticides for the control of household pests can be reduced provided that certain procedures and techniques are applied. Householders can use household pesticides such as mosquito coils, electric mats, aerosol sprays, powders, baits, shampoos, lotions, etc. which are almost hazardless but which have nevertheless to be kept out of the reach of children.

- - - - - - - -

ANONYME, 1985 - Specifications for Pesticides Used in Public Health, World Health Organization, Geneva, 384 pages

PLESTINA, (R), 1986 - Intoxication par les Insecticides, Prévention Diagnostic et Traitement, Organisation mondiale de la Santé, document non publié, WHO/VBC/84.889, 72 pages

ANONYME, 1988 - Méthodes Chimiques de Lutte contre les Arthropodes Vecteurs et Nuisibles Important en Santé Publique, Organisation mondiale de la Santé, Genève, 119 pages

ANONYME, 1988 - The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1988-1989, World Health Organization, document non publié, WHO/VBC/88.953, 39 pages

ANONYME - Fiches d'Information FAO/OMS sur les Pesticides, Organisation mondiale de la Santé, série de documents non publiés - Genève.

DEVELOPPING THE INSECTS GROWTH REGULATORS

R. Rudolph
Zoecon Corporation-Dallas, USA

Methoprene and hydroprene are potent insect growth regulators, more specifically juvenoids. As such, they target an endocrine system specific to invertebrates and are thereby much less toxic to vertebrate life forms than most conventional insecticides.

These juvenoids have found many applications in animal health and premise insect control.

Methoprene was developed to provide extensive residual protection against indoor flea infestations by preventing successful development of adult insects. Low level application of this compound (3.16 mg/m^2) provides extended (40 weeks) flea control.

Hydroprene is more active on cockroaches than methoprene. It was developed as an adjutant to conventional insecticides in an effort to control this insect. Application of hydroprene alone successfully controls cockroaches in four to five months, as its principal mode of action is to prevent the ability to reproduce in the developing insects. The addition of a good roach killing agent such as Safrotin® accelerates the elimination of the pest population. Re-application of the hydroprene treatment every 4 months is sufficient to maintain a pest-free environment.

Insect Growth Regulators "IGR" are synthetic analogues of naturally occuring insect juvenile hormones (JH). Their action is triggered only at certain life stages, ie egg, larva, pupa or adult. The effect is observed at a later stage.

To explain how IGR's work a basic understanding of how JH-I works is needed :

At the proper time and under the proper circumstances, in an immature insect's life, the brain (see illustration 1) releases a neuro hormone which activates the prothoracic gland. The prothoracic gland is stimulated by the presence of the brain hormone to product a molting hormone, ecdyson. In order for insects to grow they must shed their exoskeleton, this shedding process is called molting and is controlled by ecdyson.

During immature molts (molts between larva stages), an additional hormone is produced by the brain, JH-I or juvenile hormone. As each developmental stage is passed toward a more mature insect, the level of juvenile hormone present at the time of molt is less. When the insect reaches the final molt stage, this hormone is completely absent.

The action of methoprene and hydroprene is to mimic the activity of JH-I. Those tissues sensitive to controlled levels of JH-I are susceptible to methoprene and hydroprene, eg late larva/pre-pupal stages.

As the development of the insect approaches a stage when low levels of JH-I are required to express the proper characteristics, exposure to methoprene or hydroprene artificially elevates the JH activity level in the insect's tissues. Those susceptible tissues are directed toward immature characters, and less or unsusceptible tissues express mature characters. Frequently in this mixed state of development the insect cannot survive and dies.

Since invertebrates utilize a different class of compounds to control this development process, as opposed to higher vertebrate life forms, these chemicals have no application in the vertebrate system. They are highly active against insects and inactive against vertebrates. This makes them extremely effective and safe to use around man and in his living environment.

Many commercial applications have been discovered for methoprene and hydroprene as can be seen from Table I.

FLEAS

The use of Precor® for indoor flea control was developed in early 1980.

Flea infestations develop in homes from the flea eggs that fall from infested pets, not from adults hopping off the pets or coming in from the outside. One adult female flea may lay as many as 600 eggs during her life. These eggs are deposited on the pet's skin where they readily fall from the pet as he moves about.The eggs hatch into tiny worm-like larva and feed on protenacious debris found in the environment. The larva pass through three steps before spinning a tiny cocoon. Pupation occurs within the cocoon and adult fleas emerge. The entire cycle can take as little as 3 weeks under ideal conditions.

If methoprene has been applied to the interior of the home where flea development is taking place all of the immature fleas are prevented from becoming mature biting fleas (see illustration 2).

Field tests performed with methoprene applied to carpet at 3.2 mg/m^2 revealed excellent inhibition of adult flea development for 41 weeks. These studies were performed in homes on carpet that was subject to normal cleaning practices of sweeping and vacuuming (see Table 2).

Since methoprene does not have a toxic effect on the adult stage of the flea it was necessary to combine methoprene with a conventional insecticide to "clean-up" the initial adult problem.

The first combination products contained dichlorovos and propoxur with methoprene. Since those first products many other conventional insecticides have been combined with methoprene ; permethrin, chlorpyrifos, pyrethrin to name a few. In each case the methoprene is applied at the same low rate of 3.2 mg/m^2 and provides extended residual against reinfestation.

COCKROACHES

Next Zoecon turned its attention to an even larger insect problem. Cockroaches are perhaps the most common household pest worldwide. It was discovered that hydroprene was much more active on cockroaches than methoprene.

Hydroprene, like methoprene, is a chemical analog of the naturally occurring insect juvenile hormone. It has also been called ZR-512, Altozar IGR, Gencor and now Mator.

The german cockroach, Blattella germanica, is perhaps the most cosmopolitan of the cockroach species. It was selected for this reason and because of its ease in rearing. This species has six immature nymphal instars. The female lays eggs in a capsule called an oothica, up to 40 at a time. The life cycle, egg to adult, can be as short as 43 days.

The effect of hydroprene on these hemimetamorphic insects is different from the effect of methoprene on fleas. Hydroprene does not prevent the development of the adult cockroach. Adult insects are formed but they are unable to reproduce. Both the male and female roach are affected in this way. Once affected the roach is permanently sterile, even if the roach wanders into an untreated environment it still cannot reproduce.

In 1982 and 1983 active development of hydroprene for cockroach control took place. Laboratory trials were initiated to determine rates and specific effects on small closely monitored cockroach populations. These trials were conducted by the U.S. Navy, Zoecon Product Development Dallas, Texas, and Zoecon Research Palo Alto, California. Results of these tests (see Table 3) revealed under carefully controlled conditions hydroprene can dramatically affect cockroach populations.

During this same period larger scale chamber testing, to simulate field applications, was undertaken. The objective of the chamber trials was to assess the effect of a single hydroprene treatment on a large cockroach population.

Results of this testing, presented in Table 4, confirm the results of the laboratory trials. Under simulated field conditions, hydroprene prevents cockroach population explosion.

Data from the laboratory correlated with chamber testing, these data allowed the design of field trials. Initially, during the 1982-1983 period, a single treatment of hydroprene only was compared to a single treatment of dichlorovos and propoxur applied to single family units (houses).

Results of this testing are presented in Table 5. Under actual field conditions hydroprene can control cockroach populations in individual family dwellings.

Further testing in apartments (multiple family dwellings) pointed out the need for a retreatment at four month intervals.

Again it was necessary to combine hydroprene with an effective conventional insecticide to provide the homeowner with short term relief while eradication and prevention of reinfestation was brought about by the IGR treatment.

The best insecticide for combination with hydroprene turned out to be Safrotin®. Testing done during 1984-1985 in apartments revealed excellent results with this combination of products (see Table 6). Retreatment occurred at month four.

In conclusion :

- o Conventional insecticides are important and provide immediate, but short-term, population reduction.
- o Hydroprene reduces populations.
- o Hydroprene plus conventionalm insecticides gives the benefit of short-term reduction and long-term eradication.
- o Hydroprene plus Safrotin® delivers exceptional activity.

TABLE I

IGR COMMERCIAL APPLICATIONS

Altosid® SR-10	Controls Mosquitos
Altosid® Briquets	Controls Mosquitos
Altosid® CP-10	Controls Hornflies
Apex™	Controls Mushroom Flies
Manta™	Increases Silk Production
House Plant Mist	Controls Major Houseplant Pests
Pharorid™	Controls Pharoah's Ants
Diacon™	Controls Stored Commodity Insects
Dianex™	Controls Insects in Warehouses and Processing Facilities
Kabat®	Controls Cigarette Beetles
Precor®	Controls Fleas
Mator®	Controls Roaches

TABLE 2

EFFICACY TEST RESULTS

0.15% Methoprene Total Release Aerosol (Fogger)

Weeks Post Treatment	Average % Inhibition of Adult Flea Emergence
4	97.6
6	95.9
7	99.4
8	96.4
10	98.9
12	92.5
14	93.6
16	97.6
18	95.0
19	99.5
20	61.5
22	100.0
23	100.0
25	100.0
26	93.2
31	100.0
40	99.1
41	100.0
60	60.0
61	29.9

TABLE 3

Hydroprene Roach Testing
Laboratory Trials

0.15% Hydroprene Total Release Aerosols (Foggers)

Time in Months	Untreated Control # of Roaches	Hydroprene # of Roaches
0	10	10
1	103	98
2	96	83
3	84	65
4	197	22
5	797	5

TABLE 4

Chamber Trials

1.2% Hydroprene Total Release Aerosol (Foggers)

Time (Days)	Control Chamber # of Roaches	Hydroprene Chamber # of Roaches
0	697	670
14	702	1096
29	620	937
43	1595	460
57	1160	456
78	1997	106
92	1528	356
120	4538	630
148	22,557	1327

TABLE 5

House Testing

0.6% Hydroprene Total Release Aerosols (Foggers)

0.5% DDVP, 1% Propoxur Total Release Aerosols (Foggers)
Applied at 2X Label Rate

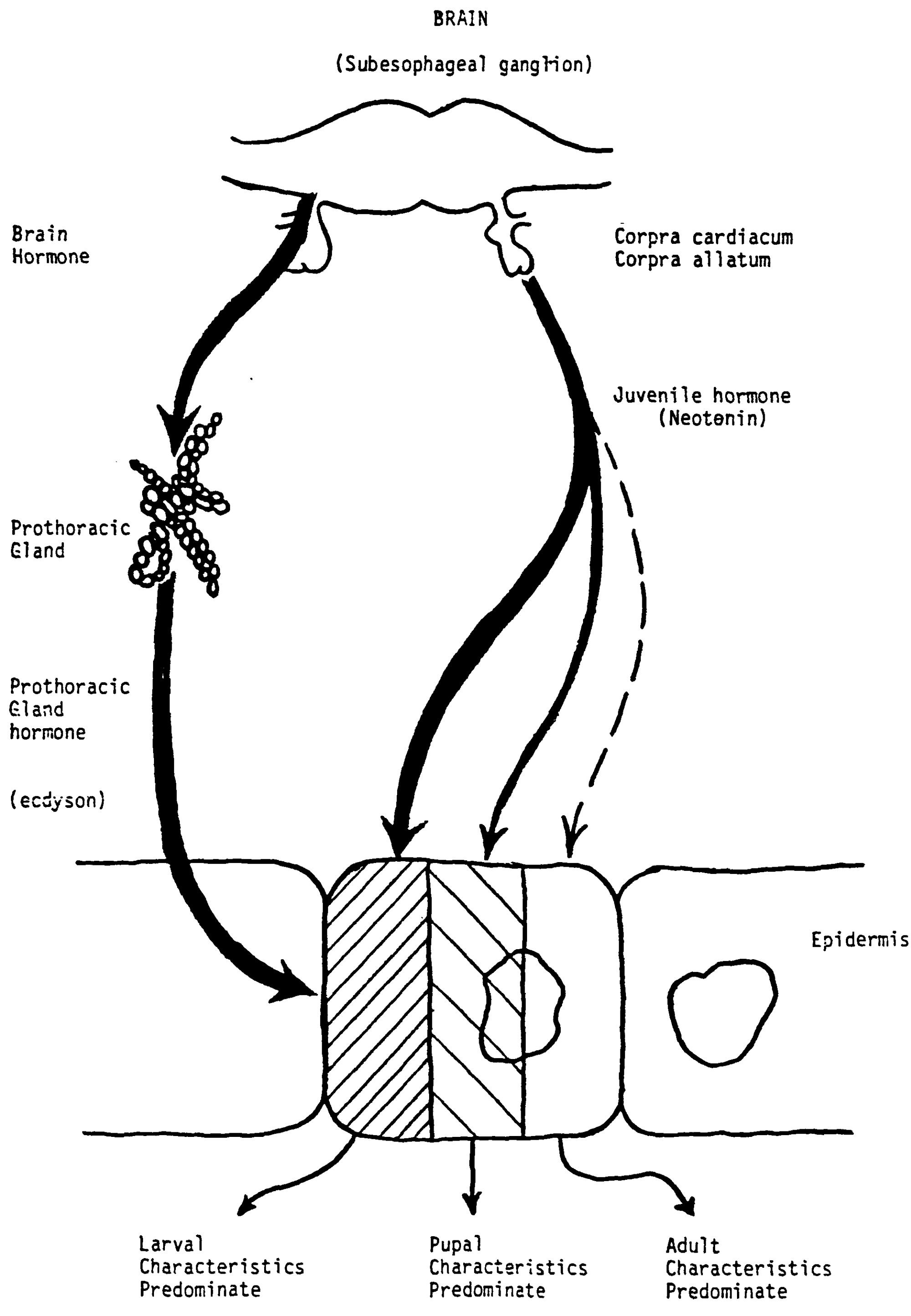
Time (Months)	DDVP/Propoxur % Reduction Roaches	Hydroprene % Reduction Roaches
1	44%	0%
2	45%	0%
3	31%	29%
4	8%	80%
5	0%	94%
6	0%	98%
7	0%	99%

TABLE 6

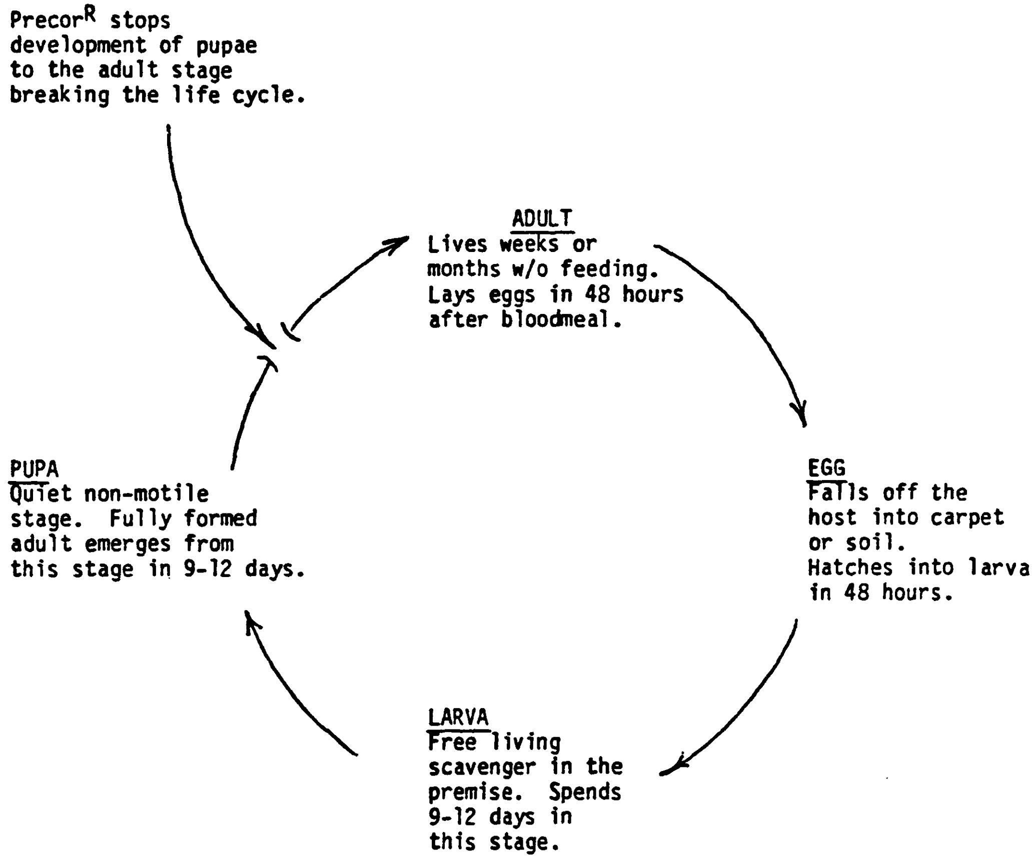
1984/1985 Apartment Testing Aerosol

0.6% Hydroprene Aerosol
1.0% Safrotin E.C.

Time (Months)	1% Safrotin	1% Safrotin + .6% Hydroprene
1	53%	91%
2	80%	96%
3	78%	97%
4	61%	99%
5	67%	99%
6	22%	98%
7	0%	-
8	-	99%
9	-	100%


BRAIN
(Subesophageal ganglion)
Brain
Hormone
Corpra cardiacum
Corpra allatum
Juvenile hormone
(Neotenin)
Prothoracic
Gland
Prothoracic
Gland
hormone
(ecdyson)
Epidermis
Larval
Characteristics
Predominate
Pupal
Characteristics
Predominate
Adult
Characteristics
Predominate

DOG OR CAT FLEA LIFE CYCLE



Doc #0058J

PHOTOSTABLE SYNTHETIC PYRETHROIDS IN VECTOR CONTROL

V. Dartigues
Roussel-Uclaf-Paris, France

1/INTRODUCTION

Since the XIXth century, some arthropods have been recognized to be able to transmit pathogenic agents from one infected host to an other and have been called from that time vectors. Their control became part of the prevention of certain pathogenic diseases and therefore a public health activity.

In 1939, the discovery of the insecticidal effect of DDT led to a revolution in the control of malaria and other endemic diseases; for year the DDT has been the main if not the only tool in the control of many parasitic diseases with a particular emphasis on malaria. 50 years later, the situation has largely evolved: various strains or species of vectors have become resistant to DDT; the impact of DDT on the environment through its accumulation in soil or in the trophic chain of wild animals cannot be minimized; and last this product cannot be easily used at the community level in the Primary Health Care programmes advocated by WHO in their new policy in vector control.

Therefore we think that it has become of utmost importance to find and develop insecticides of a different chemical family to replace DDT. Synthetic pyrethroids are compounds possessing all qualities for being an excellent replacement as a result of their insecticidal characteristics, their lack of effects on the environment and their high utilization safety margin.

2/BASIC IDEAS ON THE PYRETHROID CHEMICAL STRUCTURE

The synthetic pyrethroids used in Public Health are in their large majority from the so-called second generation of pyrethroids because, by contrast with the first generation, they are characterized by their great stability to light, making them particularly useful for applications where a high persistency is required (agriculture but also vector control).

Pyrethroids basically are carboxylic acid esters. Their insecticidal activity depends upon their stereo chemistry, in other words on the structure of their molecule in space. This spacial structure will be given by no more than three chiral centres, located at carbon 1 and 3 of the cyclopropane ring and at the α-carbon of the alcohol moiety. With 3 asymmetric carbons, a maximum of 8 isomers are therefore possible, depending on the configuration around these chiral centres.

One of the main features of the pyrethroids is that not all of the isomers of a given compound display the same activity. In the photostable pyrethroid serie used in vector control, only 2 isomers (out of 8) are active, one surpassing the other in potency. The active isomer shows the perfect chemical conformation in which all groups responsible for the toxic action are properly orientated to match the chiral receptors of the insects.

The pyrethroids used in Public Health exhibit different composition of isomers. Deltamethrin for example is a pure single isomer product, the most active selected from 8 possible. In contrast, the other pyrethroids (permethrin, cypermethrin, alpha-cypermethrin, cyfluthrin) are composed of mixtures, leading to a dilution of the active isomer in the commercial products. This aspect is of a certain importance because the overall quantities of product sprayed will be less in a single-isomer compound and as such minimal will be the impact on the environment.

3/ INTRINSIC ACTIVITY OF PYRETHROIDS AGAINST INSECTS OF PUBLIC HEALTH IMPORTANCE

The discovery of photostable synthetic pyrethroids has been a real breakthrough in the chemical control of vectors. This is due above all to their high insecticidal activity against vector insects.

Against Anopheles sp., vector of malaria, deltamethrin for example is 700 times more active than DDT. Against Aedes sp., vector of yellow and dengue fever, the same product displays an efficacy 40 times higher than malathion, the product of reference for that use. Against the tse-tse fly and the Triatomine bugs, vectors of the african and american trypanosomiasis (the sleeping sickness and Chagas disease), pyrethroids are also very active: deltamethrin and cypermethrin are respectively 87.5 and 10 times more active than endosulfan against tse-tse fly and 106.7 and 20 times more active than malathion against Triatomine bugs the 2 products of reference on these 2 vectors.

The insecticidal effectiveness of many pyrethroids have been evaluated by WHO through its Pesticide Evaluation Scheme. In many circumstances, their use in space sprays and residual applications is recommended by WHO along with other compounds from other chemical families.

4/ PRESENT AND FUTURE USE OF PHOTOSTABLE PYRETHROIDS IN PUBLIC HEALTH

4.1/ Control of Chagas disease

As efficient treatments and vaccination still do not exist against this disease, elimination of the vectors which are blood-sucking reduviidae bugs (sub-family: Triatominae) is the only way to control it. In Brazil and Argentina, the 2 main countries affected by this disease and that have a good control programme, the products of choice are pyrethroids: cypermethrin and deltamethrin in Brazil and deltamethrin in Argentina. Residual treatments are applied in the interieur and in the peridomestic area of the houses. Houses after the application of pyrethroids are free of Triatomine bugs for at least one year and in certain circumstances up to 2 years.

In Argentina, a special formulation of deltamethrin is in development: it concerns a fumigant canister which is the perfect individual means for cleaning a house of its Triatomine bugs.

4.2/ Control of tse-tse fly

2 new concepts have been developed in the control of tse-tse flies these last years: the impregnation with pyrethroids of either screens or traps and the sequential aerial sprays.

The first concept consists in impregnating screens or trap with pyrethroid, in most cases with deltamethrin. The screen is a square blue or black heavy-duty cotton sheet (100 cm X 100cm) mounted in a frame of metal tubing, pivoting on a pole driven into the ground. This target must be placed in a clear area to enable it to turn into the wind and to be visible by the flies. The blue or black color attracts the tse-tse flies that land on the screen and collect, by a single contact, the lethal dose of insecticide.

With deltamethrin, 4 targets per square kilometre of bush are sufficient to achieve temporary eradication from an area of 1000 square kilometres.

The second concept consists in 5 sequential applications of ULV aerial sprays, applied at intervals dictated by the development of the female flies.

No residual effect is required: only the flies present during the period of treatment will be eradicated. An other application is performed when a new generation of fly has taken over.

5 applications for example of deltamethrin at 0.25 g/ha with a 10-15 day time interval achieves in most cases a total eradication of the tse-tse flies in the treated area.

4.3/ Malaria control

The goal of malaria control is, at first, to decrease and if possible to suppress malaria mortality and morbidity as defined by WHO. Vector control along with an adequate chemioprophylaxis is an important tool for keeping malaria under control. Pyrethroids are used through 2 different types of applications: the indoor residual sprays and the impregnation of mosquito nets.

Pyrethroids are already used in various countries through residual applications. Deltamethrin for example has been used now for 5 years in Guatemala with succes. 2 indoor residual applications every year have been performed. The area treated with deltamethrin experienced a very sharp decrease in its parasitological indexes: incidence and prevalence. In India, in an area covering 20 000 people, the results were also quite satisfactory. The same in Nicaragua or in Congo. Pyrethroids are for the time being replacing DDT in areas where the Anopheles sp. are developing resistance. In the future, we expect the pyrethroids to play a more decisive role in malaria control and displace the DDT thanks to their high efficacy and safety.

Pyrethroids are also used in impregnation of mosquito nets. This technics requires very little expertise and can be easily used at the community level in Primary Health Care programmes. Deltamethrin and permethrin are the 2 pyrethroids at the present time used on a large scale. We can evaluate that 2 millions people sleep in China under mosquito nets impregnated with deltamethrin.

5/ CONCLUSIONS

Pyrethroids are meant to play a leading part in the future in Public Health. Along with the discovery of new drugs and vaccines, the use of the pyrethroids will certainly be extended in the vector control and be part of more integrated programme of control of endemic diseases. Their high efficacy combined with their safety makes them a valuable tool in vector control.

NEW RODENT REPELLENT «CYCLOHEXIMIDE (NARAMYCIN) MICROCAPSULE»
PROTECTION OF ELECTRIC INSTALLATIONS AND FACILITIES BY NARAMYCIN MICROCAPSULE AGAINST RAT

N. Fujiki, S. Oshima, O. Ueda
Tanabe Seiyaku Co., Ltd.-Osaka, Japan

INTRODUCTION

Recently, in spite of progress in environmental arrangements, it has been reported that the number of rodenticide-registant rat living in the buildings has increased.

At the same time, the progress of environmental arrangements in cities brings rapid increases in electric wires, communication cables and wiring for computer. In other words, overcrowding and complicated networks of electric wires and cables have caused expantion of area where rats are quite active behind our scenes.

Damages of electric facilities and installations by rats are now becoming a big social and economic problem throughout the world.

A wide variety of rat repellents have been studied in the world, however, no compound being superiour to CYCLOHEXIMIDE has so far been found out. CYCLOHEXIMIDE, still has unfavorable chemical properties in stability to heat, water and alkali, and also it is fairly toxic, which causes difficulty in its use from a safety standpoint.

In an effort to make up for these disadvantages of CYCLOHEXIMIDE and to enhance its safety as well as efficacy, we have successfully developed through intense studies a microcapsulated material, NARAMYCIN MICROCAPSULE.

Owing to the microcapsulation, the product now can more properly used for processing of paints, tapes and sealing materials for rat repelling purposes. Also due to improved heat stability, NARAMYCIN MICROCAPSULE can be incorporated into polyvinylchloride (PVC) for wire and cable sheath.

We, therefore, would like to report the measures for protecting electric facilities and installations by using NARAMYCIN MICROCAPSULE and its preparations.

We hear that the number of rats inhabiting cities have decreased recently because of progress in environmental arrangements, probably. However, it is nonsense to believe such rumor.

Actual situation is quite different. It is not too much to say that urbanization leaves cities in a defenseless state against rats. The progress of arrangements in cities brings rapid increases in electric wires and cables including electric wiring, telephone lines and computer wiring.
In other words, over-crowding and complication of networks of electric wires and cables have caused expansion of areas where rats are quite active behind our scenes. When urbanization progresses, the environment provides a better inhabiting and reproduction place for rats, and they make mischief at places, invisible for us.

Recently, one of the largest Japanese newspapers spent great deal of space with such headlines stating that the number of rodenticide-resistant rat have increased in buildings. Rodenticide-resistant rats have been damaging wiring and cables as well as computer wiring in buildings.

As the result, electric functions have increasingly been disturbed. Damages by rats are similarly increasing in large cities of European countries and USA. For example, destructions by rats have been reported in London, Paris, New York and so on.

Damage of electric facilities and installations by rats are now becoming a big social and economic problem throughout the world.
We would like to approach this damage and what we can do against it from three standpoints.

Firstly, the damage of electric facilities and installations by rats. Secondly, the rat repellent CYCLOHEXIMIDE, and thirdly, the protective measures for electric facilities and installations by NARAMYCIN MICROCAPSULE.

As a first approach, We would like to roughly divide the damage by rats into three categories.
(1) The first type of damage by rats is wire and cable cutting. Both leak and abnormal operation due to exposed core after biting damage.
(2) The second type of damage is short-circuit accident due to rats, entered in a power recieving installation and making earth-contact with the electrical circuits.
(3) Thirdly, nesting, urination and feaces cause deterioration of material quality, breaking of wire, contact abnormalities, abnormal operation by the secondary corrosion, and damage or contamination of data and stored electric information.

Among these three categories, the first type of damage is the most devastating, causing paralysis of many public services and arrangements such as interruption and confusion of telephone networks, computer networks and systems, electrical power station, railway signal syatems and so on. Damage which we believe is occuring in large cities in Europe.

We would like to take an example a recent accident in Japan. On February of this year, the largest fish market in Tokyo caughta big fire. The next morning paper said that an electricity failure had often occurred in this rat-infested fish market and, therefore, the cause of this fire would be attributed to leak after biting damage of wire and cable. Although the fire department at this district did not announce officially the real cause of the big fire, many people are convinces that the most probable cause had been an electrical power leak due to rat biting.

This example of damage is very obvious; a total fish market was devastate by fire. But how about the economic impact of the hidden wiring and cable damage by rat biting ? Although it is very difficult to obtain hard figures, we are convinced that a very large amount of man hours is spent annually by public and private organizations to restore and repair wiring and cables.

Apart from the economic impact caused by rat biting damage, paralysing numerous public services, the social life in a big city can be thrown into big confusion.

For this reason, Japanese Ministry of International Trade and Industry established "Safety Measure Standards for Computer System" ten years ago. Namely, the government has recommended and promoted rat repellent measures such as application of a rat repellent CYCLOHEXIMIDE on wiring and structural provisions to stop invasion of rat into computer instrallations.

Rat repellent cycloheximide :

We would like to discuss about the second topic, a rat repellent CYCLOHEXIMIDE.
Repellents do not kill rats. Rats refuse approach to them. Rat repellents do not reduce directly the number of rats, but in difficult places like large cities, a combination use of a rat repellent with a rodenticide is the most effective method for eradication of rats.

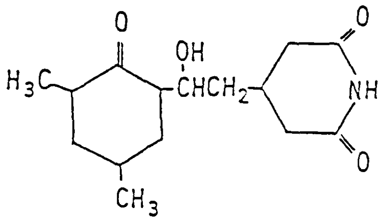
A wide variety of rat repellents had been studied in the world, however, no compound being superiour to CYCLOHEXIMIDE has been found until present.

The research group of TANABE SEIYAKU CO.,LTD., discovered an Actinomyces antibiotic from the soil of the Nara Prefecture of Japan in 1951, and designated as NARAMYCIN.
This antibiotic which has a rat repellent effect was identified later to be CYCLOHEXIMIDE. The chemical properties will be illustrated by the next table.

NARAMYCIN-Cycloheximide-

General neme: Cycloheximide

Chemical structure:



$C_{15}H_{23}NO_4$
m.w. 281.4

Description: White or colorless needle crystals

Melting point: 115–117°C

Solubility: Soluble in organic solvent, except in petrolium ether. Soluble 2% in water (room temperature)

Stability: Unstable against water, high temperature, alkali, acid and chloride

The mechanism of rat repellent action of NARAMYCIN is attributed to the sense of taste and smell. According to the experience gained by physiologists, when rats taste NARAMYCIN once, immediately rats feel a very disagreeable and astringent bitter taste at the root of tongue. Simultaneously, rats memorize the characteristic odor of NARAMYCIN. Rats, who once experienced the taste and odor of NARAMYCIN, avoid NARAMYCIN by the odor alone from the next time.

The repelling pattern of rats to NARAMYCIN varies with the application, but the product shows a rat repellent effect at a very small dose. When NARAMYCIN is mixed in drinking water, 0.0025% or 25 mg/l or higher doses the rat repellent effect is evident. When it is mixed with feed, 0.002% or 20 mg/kg shows the effect.

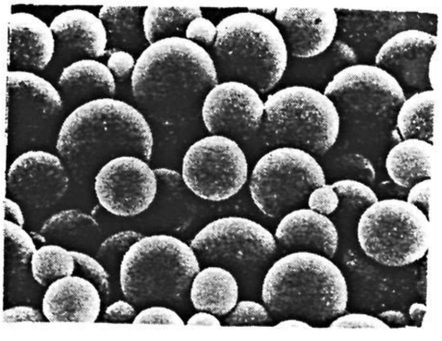
Unfortunately, NARAMYCIN has unfavorable chemical properties in the registance to heat, water and alkali. If we use NARAMYCIN outdoors, it is decomposed rapidly by rain water and sun light and loses its efficacy within a short period of time. In addition, when it is incorporated with cable coating Polyvinyl chloride (PVC) as one of the important applications of NARAMYCIN, it is also decomposed by high temperature during incorporation and excluding process of PVC and by hydrochloric acid which is formed by the thermal decomposition of PVC. Therefore, it has been used for indoor application only. Furthermore, NARAMYCIN is fairly toxic which causes difficulty in its use from a safety stand-point.

In order to overcome these shortcomings and to enhance its safety as well as its workability and stability, micro-encapsulation of NARAMYCIN was investigated extensively. Eventually, NARAMYCIN MICROCAPSULE was successfully prepared and has now become available on a commercial production scale.

The next shows the electron micrograph, physico- chemical property, method of use, and applications of NARAMYCIN MICROCAPSULE. Microencapsulation of NARAMYCIN has enhanced the safety and stability because this formulation intercepts the direct contact of NARAMYCIN with external deteriorating parameters and human body. Owing to this formulation, outdoor use of NARAMYCIN become possible and has opened the door for many applications.

NARAMYCIN MICROCAPSULE

Naramycin content:	8%
Description:	White powder like microcapsule
Wall material:	Melamine resin
Particle size:	
Prim. distribution:	Smaller than 10 µm
Sec. Particle (average):	Smaller than 60 µm
Water:	Not more than 3%
Specific gravity:	1.2
Density:	0.3 g/cm^3



Toxity of Naramycin microcapsule

A. Acute toxity of Naramycin microcapsule (LD_{50})

	Naramycin	Naramycin microcapsule
Rat (p.o.) (♂)	2.3 mg/kg	>132 mg/kg
Mouse (p.o.) (♂)	181 mg/kg	>2300 mg/kg

B. Rabbit skin inrritation of Naramycin microcapsule

	0.3 g/m²		1.5 g/m²		3.0 g/m²	
	(A)	(B)	(A)	(B)	(A)	(B)
Naramycin	0.5	0.56	1.19	1.12	1.50	1.44
Naramycin Microcapsule	0	0	0.19	0.13	0.50	0.19

Notes: (A): Normal skin; (B): Stratum corneums removed
Draiz's skin reaction score <1: slight irritation
1.1–2.9: light irritation
3.0–5.9: medium irritation
>6: severe irritation

Prospective measures for electric facilities and installations by NARAMYCIN MICROCAPSULE.

As to the third topic, We like to summarize the protective measures of electric installations by NARAMYCIN MICROCAPSULE against rats in three view points.

(1) The first measure is to block the invasion of rats into the electric installations and facilities.
(2) The second measure is to stop rat biting on wire and cable.(3) The third measure is to get rid of rats inhabiting around electric installations and facilities for protecting against invasion.

As to the first measure, the complete electric facilities and installations should be constructed in a rat-barrier structure. Because young rats can pass through any opening of 8 mm, it is practically impossible to construct a perfect rat-barrier structure. Such small openings can be completely obstructed by the NARAMYCIN MICROCAPSULE-incorporated or NARAMYCIN MICROCAPSULE PAINT-applied sealing materials or NARAMYCIN MICROCAPSULE treated rat-repellent tape.

The second measure is the most desirable rat extermination technique. Let us take the high performance computerlized intelligent buildings which have been constructed more and more in recent years. Advanced information processing brought the change in electric installations of these buildings. Equipments with advanced functions and their networks are installed in such buildings. If these facilities and installations are obstacled in these intelligent buildings themselves will be greatly affected but also the end users connected to these network.

It is, therefore, essential to take protective measures for electric facilities, especially wire and cable, against rat biting. Wiring and cables have been and are still mainly protected against rats,by metals such as stainless steel tape and metal corrugation tube notwithstanding the disadvantages in price and operation. However, rat repellent cable, which is prepared by incorporation of NARAMYCIN MICROCAPSULE with the cable sheath at 2.5% concentration, has an excellent rat repellent effect and stability.

Stability of Naramycin microcapsule incorporated into polyvinylchloride (PVC)

	Test period					
	Initial		6 months		12 months	
	CHI content	Ratio	CHI content	Ratio	CHI content	Ratio
A	1220	100%	1132	93%	1060	87%
B	1028	100%	991	96%	955	93%
C	1146	100%	1099	96%	1014	88%
D	1264	100%	1290	102%	1243	98%
Average	1165	100%	1228	97%	1068	92%

CHI: Cycloheximide-Naramycin

The cost of the treated cable is not so different from that of the non-treated cable, and operation for the treated cable is similar to that for the non-treated one.

Our estimation on the operation period of the rat repellent effect based on the currently available data, it is considered to be more than 10 years. And so, protection of electric facilities by metals against rats will surely be replaced with NARAMYCIN MICROCAPSULE treatment in future.

If a paint incorporating 2% NARAMYCIN MICROCAPSULE is applied on the already installed wire, a rat repellent effect can be expected for more than 1 year.

Concerning the third measure, rats inhabiting around and in electric installations must be stamped out, followed by rat protective treatment and obstruction of re-invasion of rats into installations.

Subsequently, we must rely on experts for PCO. In such case, sealing agent incorporated with NARAMYCIN MICROCAPSULE, NARAMYCIN MICROCAPSULE PAINT and/or Aerosol are applicable.

Apart from the use for electric facilities and installations the applications field of the preparations is broad. For example, NARAMYCIN MICROCAPSULE preparations are applicable to the field where protection of the packing materials and textile products against rats are required.

As a matter of fact, NARAMYCIN MICROCAPSULE-treated flexible containers and sheets for transport use have been already developed and are now available.

We shouldn't underestimate injurious rats. It is too late to rush for taking rat repellent measures after facilities and installations have been damaged by rats. We must fully recognize a fear of damage by rats, and the attitude of serious tackle on rat repellent measures is necessary.

LOS SERVICIOS DE DESINFECCION, DESINSECTACION Y DESRATIZACION EN EL MUNICIPIO DE BARCELONA

MUNI-

igiene

ıız, Encargado del

D. G. Monzón Fernández-Peña
Unidad Operativa de Higiene de los Alimentos y Zoonosis-Barcelona, Spain

D. E. Barahona Ruiz
Servicio de Desinfección, Desinsectación y Desratización-Barcelona, Spain

En el año 1888 aparece en Barcelona la necesidad de controlar la población de insectos y roedores, ya que la civilización ha alterado las condiciones de vida de muchas especies animales, pero en muchos casos estos cambios han sido beneficiosos.
Se hace una reseña histórica de la creación de los Servicios de Desinfección, Desinsectación y Desratización del Excelentísimo Ayuntamiento de Barcelona y se describen las actuaciones actuales para el control de los insectos y múridos en prevención de enfermedades transmisibles y en aras de una mejor calidad de vida ciudadana.

ANTECEDENTES DEL SERVICIO

La actuación del Ayuntamiento de Barcelona en materia de desinfección y desinsectación se remonta a las actuaciones sanitarias del antiguo "Consell de Cent" y que a través de los tiempos tuvo su continuidad junto con el Cuerpo Directivo y Administrativo del antiguo "Hospital de la Santa Cruz".
La repercusión que tuvieron en todo el mundo los descubrimientos de Pasteur hicieron eco en Barcelona en la persona del bacteriólogo Ferrán y la atención del Ayuntamiento hacia los problemas sanitarios, culminó con la creación del Laboratorio Microbiológico Municipal hacia el año 1888 (Alcalde: Rius i Taulet).
En el año 1890 se fundó el "Instituto y Laboratorio de Higiene Urbana" dirigido por el Dr. L. Comenge. Destacan en estos tiempos además de los Maestros Ferrán y Turró, higienistas de gran prestigio como Comenge, Viñas, Grau..., que estudian problemas sanitarios de España y del extranjero comisionados por el Ayuntamiento de Barcelona.
El Instituto Municipal de Higiene se funda en 1890 y cuenta con los servicios de "Desinfección" y del llamado "Policía Sanitaria". Este último encargado de instruir denuncias y asesorar al ciudadano.
Con ocasión de los focos epidémicos de peste bubónica en los años 1905 a 1906, localizada en patios y almacenes de desperdicios de basuras, el Instituto Munici-

pal de Higiene intervino eliminando las basuras por incineración y practicando riegos con desinfectantes fenólicos, desratizaciones y aplicaciones de sueros y vacunas a la población. Con esta ocasión se establece un Hospital constituído por dos pabellones, uno de madera de tipo "Docker" y otro de mampostería que se instalan en la barriada de "Casa Antunez" cerca del cementerio del Sudoeste y a los que se dota de los elementos necesarios para el cuidado, tratamiento y control de los enfermos afectados.

Simultáneamente se establece un lazareto de observación en tres edificios y en el antiguo local llamado "Museo Zootécnico" que existía en la "Sección Marítima del Parque de la Ciudadela" y que fué el rudimento del actual Hospital de Infecciosos (Hospital de Nuestra Señora del Mar) que una vez ampliado y reformado sigue hoy ubicado en el mismo lugar de su primer emplazamiento.

En Septiembre de 1914 aparecen casos de fiebre tifoidea que duran los meses de Octubre y Noviembre, causando más de 2.000 defunciones. El brote fué debido a la contaminación de la galería de conducción de agua de Montcada, que en estas fechas llegaba hasta el repartidor sin tuberías y al edificar los solares por donde discurría la mina, dieron origen a la contaminación de las aguas.

Esta catástrofe ciudadana hizo reaccionar a las autoridades municipales que procedieron a aislar y proteger la mina hasta la Trinidad, donde se instaló una estación elevadora con depósito regulador y, desde allí, conducida por una tubería aislada en una galería, hasta la calle Xifré, lugar en el que finalmente se distribuyó a la parte baja de la ciudad en tuberías de hierro fundido, evitando así recidivas en la contaminación.

El Instituto Municipal de Higiene intervino este momento en desinfecciones y aislamientos en el Hospital de la "Sección Marítima".

Todas estas incidencias sanitarias pusieron en evidencia la necesidad de una mayor coordinación y organización sanitaria municipales, creándose en 1917 el "Instituto Municipal de Higiene" que amparaba la organización sanitaria municipal, incluyendo en él, las actuaciones de desinfección, desinsectación y Policía Sanitaria, creándose simultáneamente la sección de Epidemiología que tenía a su cargo la supervisión del Hospital de Infecciosos.

Se estableció un servicio de profilaxis antivariólica que además de practicar las vacunaciones en los convivientes y vecinos de los variolosos (aún existía viruela en Barcelona y por esta profilaxis desapareció a partir del año 1922). Se llevaba un registro general de nacimientos y como soporte se ordenaba la vacunación de los nacidos a los 6 meses de edad por comunicación cursada a sus respectivos padres.

Existía también un rudimento de estadística y de padrones sanitarios cuidando de remitir a los facultativos de la ciudad los formularios de declaración obligatoria de las enfermedades infecto-contagiosas.

En otoño de 1918 sufrió Barcelona una epidemia de gripe, iniciada de forma benigna en la primavera anterior y que causó una gran morbilidad y también mortalidad, principalmente a causa de las complicaciones bronco-pulmonares. Las medidas preventivas que pudieron tomarse fueron escasa e ineficaces.

El Ayuntamiento de Barcelona considerando cada día más la importancia de realizar medicina preventiva recopilada en bases aprobadas en 25 de abril de 1921 por las que el Instituto Municipal de Higiene quedaba bién delimitado con sus servicios propios y con las instituciones anexas de Laboratorio Municipal, Hospital de Infecciosos y Servicios de Veterinaria Municipal.

En estas bases al Director del Instituto Municipal de Higiene se le daba el nombre de "Jefe de Salubridad de la Urbe".

En el año 1922 apareció un brote de peste bubónica de 28 casos, detectado originariamente en la fábrica de harinas "Callarda" en la Carretera de Ribas y en la Estación del Norte. Intervino intensamente el Instituto Municipal de Higiene con vacunaciones, desinfecciones y desinsectaciones en los domicilios y en los locales afectados, aislando a los convivientes y hospitalizando a los enfermos en el Hospital de Infecciosos.

Se hizo una intensa campaña de desratización, principalmente en la zona afectada y vecina al puerto, con cebos envenenados (Pasta fosfórica) y se efectuaron sulfuraciones de locales y cloacas mediante aislamientos parciales estancos.

Visitaron Barcelona los Doctores Martín Salazar y F. Murillo, "Director General de Sanidad" y de "Instituciones Sanitarias" respectivamente, que comprobaron la organización sanitaria municipal delegando en élla toda la actuación sanitaria y manifestando que la tendrían presente y contarían con dicha organización en lo sucesivo.

En 1930 quedó terminado el Centro de Desinfección, Desinsectación y Desratización en la calle Francisco Aranda, 60 que funciona con nueva estructura en la actualidad. Se terminó también por esta fecha la instalación del Laboratorio Municipal con amplias secciones y un pabellón especial para el examen de ratas y quedó terminado el Hospital de Infecciosos, situado en el mismo emplazamiento, pero formado por pabellones de una sola planta, con habitaciones de aislamiento individual dotados de servicio de lavabos, W.C. en cada habitación y los servicios anexos correspondientes.

El día 5 de agosto de 1931 al Instituto Municipal de Higiene se le notificó un caso sospechoso de peste bubónica, que se confirmó, siendo el número total de casos que se produjeron de 26 (17 en la barriada de Hospitalet, 5 en el Torrente de la Guineu de la barriada de San Martín y 4 dispersos por Sans, Carretera de la Bordeta, Carretera del Puerto y Casa Antunez). Los casos se localizaron en zonas donde era más densa la presencia de ratas. Se tomaron las medidas de aislamiento oportunas y se intensificaron las actuaciones de desratización, creándose para éllo una brigada de 60 individuos dotados de los medios adecuados para destrucción de múridos y transporte de los mismos en forma apropiada al laboratorio de la rata para la ulterior determinación de su estado de infección. Se establecieron cuatro zonas de desratización con cuatro locales, uno en San Martín, otro en el Palacio de Agricultura de Montjuich, el tercero en Sarriá y el cuarto en la calle Martí de la barriada de Gracia.

La Dirección General de Sanidad encargó al Instituto Municipal de Higiene la dirección de la campaña, que tuvo feliz éxito y, además por la Comisión Permanente de Investigación Sanitaria se designó a un facultativo sanitario para que intervi- niera en la campaña, el cual se encargó especialmente del laboratorio de la rata o pabellón de la peste, fué comisionado posteriormente para que visitara Londres y estudiara las técnicas de desratización y todo lo relacionado con la campaña antipestosa.

Con anterioridad al año 1976, la desratización en nuestra ciudad se efectuaba de una forma esporádica, actuando en las distintas zonas a partir de las denuncias que se recibían del ciudadano, recogiendo el mayor número de datos posibles para la planificación de una campaña intensiva en los meses de mayo y junio de cada año.

Se solicitaba la colaboración personal de "Fomento de Obras" con la contratación durante estos dos meses de 12 personas y la prestación por parte del "Parque Móvil Municipal" de 2 vehículos con sus correspondientes conductores, que unidos a los tres coches y 15 funcionarios de este Departamento constituían el equipo de actuación de la citada campaña. Los resultados que se obtenían eran de momento positivos, pero poco duraderos ya que en los primeros meses de octubre o noviembre comenzaban a detectarse la presencia de roedores por las evidencias observadas y por las denuncias que se recibían.

A partir del año 1976 se empezó a estudiar la forma de que este Servicio efectuase una campaña de desratización sistemática y continuada a lo largo de todo el año, para poder hacer un estudio comparativo a la vista de los resultados que pudieran derivarse de la misma; se pudo estimar a su término que el balance había sido positivo, puesto que disminuyeron el número de denuncias y la evidencia

de presencia de ratas tanto en superficie como en la red de alcantarillado.
Dado el resultado positivo del ensayo efectuado el Servicio de Desinfección, Desinsectación y Desratización comienza a concretar de una forma seria la elaboración de una campaña sistemática continuada, que tiene como objetivo principal el fomentar un sistema de lucha contra las ratas, siguiendo pautas de actuación conjuntas en superficie y profundidad, que puedan garantizarnos resultados satisfactorios, estadísticamente valorables y rentables. Es un plan de actuación sin interrupción a lo largo de todo el año, al mismo tiempo que se van realizando los servicios correspondientes a las denuncias que se reciban.

La ineludible necesidad de la existencia de los servicios de desinsectación, desinfección y desratización en la organización sanitaria, deriva de la existencia de un gran número de enfermedades infecciosas cuya transmisión tiene lugar por diferentes vías de inoculación y contagio. Entre estas enfermedades las hay que a través de los tiempos han ocasionado a la humanidad gravísimas epidemias y algunas de éllas persisten aún en determinadas zonas o países del mundo como una de las causas de morbilidad y mortalidad importantes.

La transmisión del agente etiológico de la enfermedad puede tener lugar desde el reservorio de virus a hombre sano por medio de artrópodos que pueden ejercer su acción parasitaria indistintamente sobre ambos o bien puede ser solamente interhumana, mediante vectores parásitos exclusivos del hombre. En el primer caso puede tener un doble origen, el del animal reservorio o bien el de otro hombre enfermo.

También el papel del huesped vector es muy distinto según las diferentes enfermedades, unas, las que entran en el grupo cuyo mecanismo de actuación es denominado de transmisión activa o biológica y en el que el agente etiológico (bacteria, protozoo, virus...) vive y se reproduce en el organismo del vector, mientras que en los casos en los que la transmisión es pasiva el vector actua solamente por un contagio mecánico y fortuíto.

Así pués existen enfermedades en las que la transmisión se cumple a merced de complicados fenómenos de parasitismo obligado, que implican el que tengan lugar en el organismo del artrópodo un ciclo biológico del parásito (ciclo completo en determinadas especies o incompleto) sin el cual la transmisión no puede tener lugar. En otras enfermedades en cambio, el paso del agente etiológico por el huesped vector no va ligado a ningún ciclo vital y sí solo a una reproducción y proliferación del agente causal, que si no llega a producir la muerte del artrópodo, facilita extraordinariamente la transmisión ulterior.

En la lucha contra las enfermedades cuyo mecanismo epidemiológico de propagación es la de los huéspedes vectores, las prácticas de desinsectación han sido y continuan siendo las actuaciones fundamentales y básicas. En la mayoría de estas enfermedades la eliminación del eslabón obligado de su cadena epidemiológica constituye el mejor procedimiento de erradicación.

Aparte de estas circunstancias en las que la desinsectación es una técnica sanitaria fundamental e imprescindible, es innegable que en toda sociedad civilizada, deben constituir una norma higiénica y elemental todas aquellas medidas activas o pasivas que conducen a que el hombre en comunidad esté siempre libre de parasitación, por constituir ésta un peligro en potencia para la salubridad humana.

Por consiguiente las técnicas de desinsectación no deben considerarse como una medida de emergencia sino como normas de higiene general de aplicación contínua y periódica.

Bajo el nombre de desinsectación se comprenden todas aquellas medidas activas o pasivas destinadas a la lucha contra los insectos y contra los artrópodos, vectores y reservorios en general.

Es fácil deducir la gran importancia de la desinsectación en epidemiología humana, en medicina preventiva y en la aplicación en la agricultura y ganadería con un vastísimo campo de acción, ya que son enormes las pérdidas económicas que los insectos ocasionan en todos los órdenes.

SITUACION ACTUAL

Para facilitar nuestro trabajo diversificamos el servicio a lo largo de los diez distritos de la ciudad, cada uno de los cuales lo está a su vez en zonas de unas 40 hectáreas aproximadamente cada una, con un total de 176 zonas (7.120 hectáreas). Las zonas quedan ubicadas en completo dentro de cada distrito, con la finalidad de la descentralización administrativa y de poder facilitar la información directa con los servicios técnicos de los distritos.

Marcamos unas zonas para cada uno de los equipos que disponemos, procurando que a lo largo de todo el año actue siempre el mismo equipo sobre las mismas zonas, por la eficacia que representa el peculiar conocimiento de cada una de éllas.

A cada zona se le abre una carpeta-dossier donde el encargado del equipo correspondiente anota: estado, incidencias y problemática sobre la presencia de ratas en solares, almacenes, colegios, casas en construcción, edificios abandonados,

descampados, rieras... Se reflejan también las denuncias cumplimentando su servicio, exponiendo la causa del problema de la denuncia y efectuando un informe sobre la manera de proceder para subsanar las deficiencias. Trimestralmente se procede a efectuar un simil-encuesta sobre presencia de roedores en la zona. Con la misma periodicidad se colocan rateras metálicas en superficie y red de alcantarillado, para capturas de ejemplares que nos dan idea aproximada del número de ejemplares existentes y que nos sirven al mismo tiempo para poder efectuar pruebas de comprobación de los distintos rodenticidas empleados. Se realizan pruebas biológicas y pruebas químicas en el Laboratorio Municipal.
Para poder evaluar el grado de infestación de una zona, combinamos los siguientes parámetros: denuncias - informe del equipo que verifica la zona - signos objetivos de la presencia de roedores (pasos, nidos, excrementos, roces, materiales roídos...) - encuestas y capturas.
Conjugados todos estos parámetros a cada zona se le coloca un indicativo de clasificación referente a su problemática de infestación que al mismo tiempo nos refleja la atención que debemos prestarle.

- Sector "NORMAL" △
- Sector "MEDIA ATENCION" ◡
- Sector "ATENCION ESPECIAL" ○

Se sobreentiende que por diversas circunstancias puede alterarse dicha calificación que implica la modificación inmediata de la zona correspondiente.
A la vista de todo éllo podemos conjugar la actuación de los servicios, intensificando su actuación en los lugares más comprometidos.
Esta actuación sistemática y continuada se desarrolla: En Profundidad, con la colocación de tóxicos en la red de alcantarillado a través de los imbornales o descendiendo a las mismas alcantarillas a través de sus bocas de acceso (el tóxico empleado es fosfuro de zinc, cuya elaboración se efectua en este Centro por el personal propio de este servicio). De los 1.250 Kms. de alcantarillado de la ciudad de Barcelona, 970 Kms. de éllos son visitables, es decir, puede entrar el hombre.
En superficie, colocando rodenticidas anticoagulantes en solares, rieras, escuelas, mercados, casas deshabitadas..., amoldándose la colocación del producto raticida a la problemática y necesidades de cada zona.
Una vez terminada la aplicación de los productos, se colocan carteles indicativos de zona desratizada y al mismo tiempo se reparten hojas informativas con el número de teléfono del Servicio, para evacuar consultas o suministrar información a los ciudadanos que lo soliciten.

CONSIDERACIONES

Catalogando el número de denuncias en los diferentes Distritos observamos un aumento progresivo a lo largo de los tres primeros trimestres del año, con un notable descenso en el cuarto, en concordancia con la tesis mantenida del aumento de mayor proliferación y aparición de múridos en los meses comprendidos entre abril y octubre, con los signos favorables de -aumento de temperatura, menor pluviosidad, mejor adaptación al medio ambiente-, siendo bien diferenciados el número de denuncias en los diferentes distritos, no indicando éllo el que haya mayor o menor número de roedores, puesto que la determinación de este parámetro debería acomodarse al número de habitantes, número de hectáreas, status del distrito y concienciación ciudadana del mismo.
El mayor número de denuncias corresponde generalmente a la proliferación de solares sucios, con maleza o escombros, a casas derruídas o en construcción, que son lugares ideales para la anidación y procreación de ratas. A partir de cualquier denuncia, se efectua la correspondiente visita de inspección y comprobado si hay estado de abandono, suciedad... en el solar, casa abandonada o en construcción, se conmina al dueño del mismo a que proceda a su limpieza

y a pesar de que ésta se realiza en el 85% de las veces que se procede a sus desratizaciones, colocando carteles indicativos de zona desratizada y la recomendación de que se mantenga limpio en beneficio del ciudadano. La mayoría de los lugares mencionados vueltos a reinspeccionar a los tres meses siguientes, se encuentran casi en idénticas condiciones de abandono y suciedad, creada por el propio vecindario, encerrándonos por tanto en un círculo vicioso al no contribuir el ciudadano a la desratización pasiva.

Estas zonas mejorarían sensiblemente si la respuesta ciudadana en la desratización pasiva, manteniendo la limpieza y no poniendo alimentos al alcance de los roedores. Si las pequeñas deficiencias técnicas del entorno se subsanasen y se complementasen con la lucha activa de este Servicio, quedarían incluídas automáticamente en sectores de mínima atención, con un máximo beneficio para la ciudad y sus habitantes.

La irrupción de los rodenticidas anticoagulantes en la lucha contra las ratas tuvo lugar a partir del año 1948, con la introducción en el mercado de productos a base de warfarina.

El efecto de los productos anticoagulantes se basa en un principio idéntico, al administrar sustancias anticoagulantes se obtiene un efecto similar al de la deficiencia en vitamina K en el organismo, ya que se desplaza la vitamina K de la enzima formadora de la protrombina, colocándose en su lugar. Si estas sustancias activas se encuentran a un nivel suficiente en la sangre, compite con éxito contra la vitamina K, actuando como pseudovitamina. La función antagonista de los anticoagulantes frente a la vitamina K, deriva de la semejanza de su estructura molecular. Las pseudovitaminas engañan al organismo tanto que éste incluso tiene más apetencia por sus moléculas a las de las vitaminas naturales. La enzima sintetizada de esta manera es sin embargo inactiva y no puede producir protrombina, de tal manera que, el contenido de ésta en la sangre alcanza bajos niveles, con todas las consecuencias para la coagulación. A la par comienzan a desarrollarse lesiones en las paredes de los vasos sanguíneos. El paso siguiente serán las graves hemorragias y por último la muerte sin trauma.

El proceso tóxico dura generalmente tres o cuatro días y el efecto es por tanto solapado. El periodo latente entre la ingesta del veneno y la aparición de los síntomas es por tanto largo y en gran medida dependiente de la dosis y de la vía de administración, hoy día superados con la administración de rodenticidas de una sola dosis.

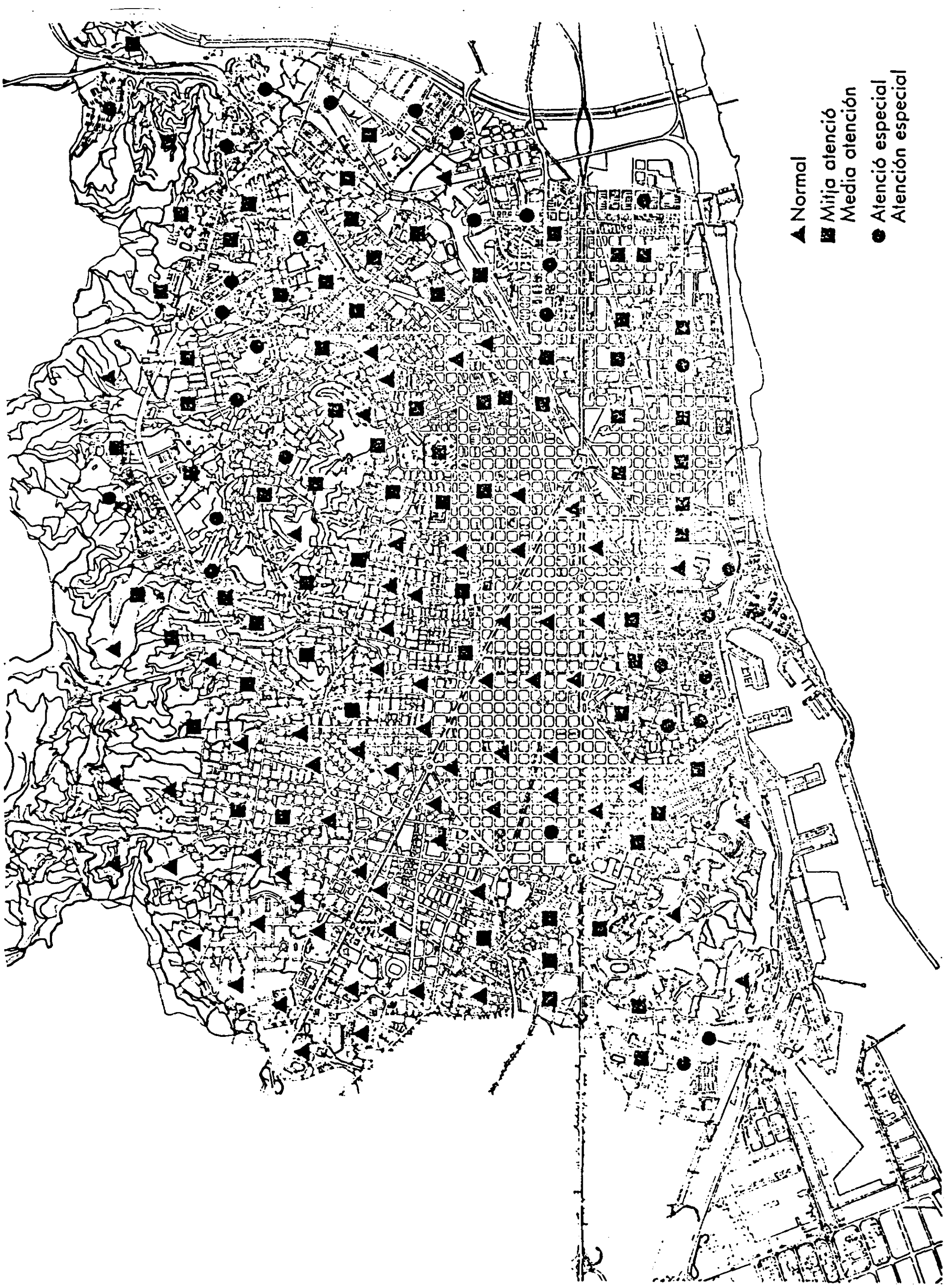
Los diversos productos anticoagulantes se diferencian mucho en lo que concierne a la dosis letal, al cuadro temporal de efectos y a su aceptación por las ratas,

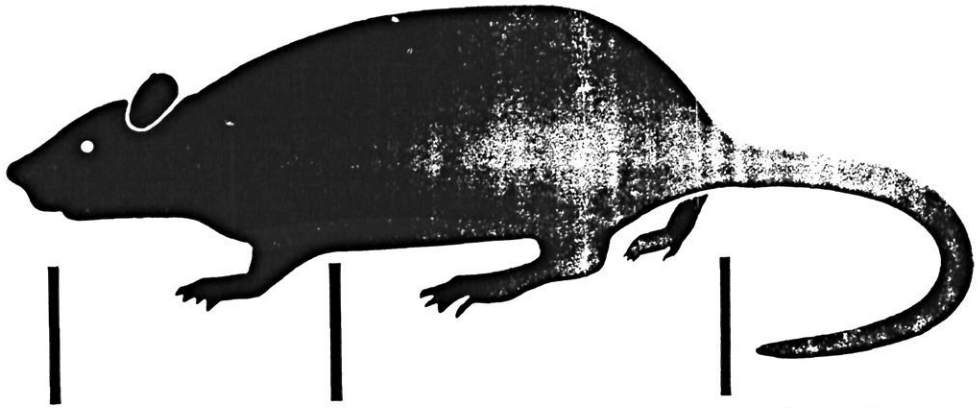
pudiendo variar estas propiedades según las distintas especies.
La sintomatología inicial de envenenamiento en las ratas se caracteriza por anorexia, cansancio, sed, apatía y desorden motriz. Transcurrido un tiempo, los animales mueren en estado de agotamiento. Este proceso es indoloro y su comportamiento se caracteriza por la calma y la apatía sin presentar convulsiones. Es frecuente que los animales mueran en la posición que se adopta para dormir.
Los primeros síntomas tóxicos visibles pueden aparecer dos días después de la ingesta del producto. La piel no pilosa de las orejas y de las patas se tornan pálidas y los vasos sanguíneos subcutáneos se rompen permeabilizándose. A menudo existe epíxtasis y hematemesis, melenas y hematurias o bien un sangrado lento de la piel recubierta de pelo, especialmente al rascarse la rata, con sus patas. Cuando se producen hemorragias internas, en especial en el tracto intestinal, son raros los signos externos de envenenamiento, sucediendo lo mismo en la uremia causada por el flujo de sangre en las nefronas.
En general los síntomas de envenenamiento no aparecen súbitamente, sino de una manera solapada, culminando el proceso con la muerte del individuo al cabo de 5 a 7 días.
Los anticoagulantes presentan la ventaja de que los animales afectados no notan el inicio del envenenamiento, no apareciendo con éllo sospecha alguna y no causando desconfianza o aversión hacia el cebo que se suministra.
En este capítulo de consideraciones integraremos también el "cálculo de la población de múridos en un medio urbano" procedimiento utilizado por el Servicio de Desratización y que nos permite en todo momento conocer la concentración de roedores en las distintas zonas de la ciudad de Barcelona (Estudios, Informes y Debates; Gaceta Sanitaria Mayo-Junio 1983, nº 9, vol. II, pag. 93).
En la misma línea se llevan a término asesoramientos e información al ciudadano, ya sea sobre las sustancias rodenticidas o bien sobre las técnicas de desratización a emplear.
Integramos en el Servicio los estudios sobre los programas más eficaces para llevar a cabo el exterminio de roedores e insectos, fundamentalmente dirigidos a Organismos Oficiales y vía pública, así como los ensayos de técnicas que nos permitan preveer las tendencias de la población de múridos y, a la par, que evaluamos los resultados de las campañas de lucha que ensayamos.
Son preocupantes las posibilidades de movilidad de la población de ratas, que, como toda sociedad organizada tiende a mejores hábitats. Esto nos obliga a estudiar y planificar nuevos programas de lucha integrada y selectiva.
El estudio de la efectividad de los productos rodenticidas lo controlamos mediante

pruebas biológicas y químicas, utilizando, en base a un programa de economía y efectividad, los productos más adecuados en los lugares más idóneos, a la par que investigamos prácticamente, nuevas formas de control de las ratas pués se producen resistencias a anticoagulantes o rechazos a venenos tóxicos.
Los estudios en equipo conjuntamente con el Museo de Zoología y la Facultat de Farmacia (Cátedra de Parasitología) nos advierten sobre el control que se debe ejercer sobre los endo y los ectoparásitos, problema fundamental en la transmisión de enfermedades. Análogamente acontece con el control de vectores y reservorios así como de agentes patógenos, que nos indican los caminos efectivos para combatir de una manera activa, la población de ratas de nuestra ciudad.

PERSPECTIVAS DE FUTURO. PLAN INFORMATICO

Todo el trabajo manual que se realiza, ya sea en forma de aplicaciones por nuestros equipos o como informaciones y denuncias que se reciben del ciudadano, se informatiza en la actualidad. Otros temas a informatizar en breve son, los stocks de productos, densidades de parasitaciones e influencias climatológicas sobre la aparición de focos parasitarios. También los estudios de costos/actividades y frecuencias de aplicaciones en las distintas zonas de la ciudad, así como todos aquellos parámetros que nos conduzcan a un mejor conocimiento de la situación actual.
Sabiendo que las recomendaciones de la O.M.S., en cuanto al control de la población de ratas, las cumple el Municipio de Barcelona, se pretende estar por debajo de estas indicaciones y eliminar los posibles focos esporádicos que pudieran producirse por alteraciones climáticas, de población, de hábitos... o incluso de inapetencia a los rodenticidas por parte de la población de roedores que tenemos que combatir.


Normal
Mitja atenció
Media atención
Atenció especial
Atención especial



Per mossegada
Por mordedura

Sodoku

Tularemia

Ràbia/rabia

Per ingesta d'altres mamífers
Por ingesta de otros mamíferos

Triquinosi

Brucelosi

Per ectoparàsits
Por ectoparásitos

Pesta bubònica

Tifus exantemàtics

Febre Lassa/
Fiebre Lasa

Per dejeccions, contaminació d'aigües o aliments.
Por deyecciones, orinas, contaminación de aguas o alimentos

Leptospirosi

Salmonellosi

Helmintiasi

Amebiasi

Micosi

Coriomeningitis linfocites

CALCULO DE LA POBLACION MURIDA EN UN MEDIO URBANO

La evaluación del número de ejemplares que forman una población determinada de roedores es, al mismo tiempo que un punto de inicio primordial e imprescindible para intentar cualquier estudio sobre la ecología de una población múrida, una ayuda muy importante en la orientación de los sistemas para combatirlos.
Las evaluaciones relativas basadas en métodos que no comprenden la captura pueden proporcionarnos una indicación aceptable del número de ejemplares ; así no obstante, las estimaciones basadas en la apreciación visual de los signos objetivados están expuestas a interpretaciones tendenciosas a causa de diversos problemas localizados y por tanto, no es fácil catalogar y valorar los signos de evidencia, ni que emprendamos la técnica del "consum d'esquers" (CHITTY y SHORTEN), que es una de las más recomendables para comprobar la exactitud de los medios más subjetivos.

Las evaluaciones relativas basadas solo en las capturas, aún que pueden ser consideradas como el método fundamental de vigilancia, cuando deben hacerse apreciaciones del número de roedores, debe unificarse en cierta manera, su metodología, ya que están sometidas a múltiples factores de variabilidad (método de "captura-eliminación"; técnicas del "índice lineal" de MYLLYMAKI y colaboradores; método de "captura-marcado-recaptura"). Y, en general, se deben efectuar cálculos más o menos complejos a partir de los datos de captura (SMITH y colaboradores), para poder evaluar el número real de roedores existentes.
Los métodos de captura-marcado-recaptura (C.M.R.), permiten hacer apreciaciones mínimas bastante precisas; no obstante, estos métodos también resultan falseados por la variable capacidad de atrapar a los roedores. Por esto TANTON propuso su método de "frecuencia de capturas" i PETRUSEWICZ y ANDREZEJEUSKI introducen los conceptos de "residente" y "transeunte", aunque tampoco contribuyen demasiado a resolver el problema básico con estos razonamientos teóricos de la misma manera que no se puede resolver mediante la aportación de MYLLYMAKI que contempla los desiguales resultados que proporcionan las capturas entre los subgrupos de una población múrida.
Vemos entonces, que el problema que representa el cálculo de una población de múridos, hasta ahora no se ha podido resolver mediante métodos matemáticos, por muy cuidadosos que sean. Hasta algunos investigadores han señalado que el uso de estos métodos puede exagerar el error inicial. Por todo lo dicho, en la actualidad solo pueden recomendarse las estimaciones mínimas basadas en las cifras reales de ejemplares estudiados por las técnicas de CMR, con

las debidas correcciones aplicadas en cada sistema, o sea, condicionando los distintos comportamientos de las especies y las diferentes condiciones locales prevalentes.

Teniendo en cuenta toda la metodología antes expuesta, con los inconvenientes ya mencionados y valorando el hecho de que otros sistemas y métodos no pueden ser aplicables a la ciudad dado que son idóneos para terrenos abiertos, vemos que solamente nos quedan por mencionar algunas otras evaluaciones aproximadas que parten de índices diversos (peso aproximado del producto comido, cantidad de ratas muertas que aparecen en las salidas de los colectores,...) que naturalmente son modificados por múltiples circunstancias y por lo tanto no son fiables, ya que las proporciones fluctuan excesivamente.

Por todos los razonamientos expuestos, en este Servicio, con medios muy escasos e inadecuados, pero con gran entusiasmo, nos hemos dedicado a la búsqueda de unos índices sencillos y que al mismo tiempo nos sirvieran para un cálculo aproximado de la población múrida de nuestra ciudad.. Como fruto de nuestro trabajo y por razón de la valoración de las experiencias adquiridas, podemos ya exponer los resultados obtenidos y lo hacemos seguidamente.

El método de recuento adoptado por nuestro Servicio, lo podríamos haber hecho más complejo, introduciéndole un número más elevado de parámetros de apreciación visual (machos-hembras, adultos-jóvenes...), no obstante lo hemos encasillado en los ya tradicionales y hemos procurado alargar tanto como hemos podido el tiempo de mantener las rateras colocadas , para tratar de convertir las capturas realizadas en uno de los índices apreciables tenidos en cuenta, tanto para las capturas conseguidas en superficie, como por las realizadas en la red de alcantarillado. Al mismo tiempo hemos añadido un dato, el "factor M", que es el resultado de considerar como índice aceptable de presencia de roedores en las grandes ciudades, la equivalencia de una rata por habitante, haciendo valer la pauta establecida derivada de los estudios ecológicos realizados por los especialistas de la O.M.S.

Entre el 1 de septiembre y el 30 de noviembre de 1981, realizando muestreos con recogida de datos en cada uno de los distritos de la ciudad, pudimos elaborar unos índices que nos permitieron llegar a conseguir la fórmula motivo de estos trabajos. Planificamos nuestra actuación dividiendo el mapa de la ciudad en cuatro zonas, cada una de las cuales englobaba un número prefijado de distritos municipales, la actuación se encargará siempre al mismo equipo a fin de que el conocimiento del terreno y de su problemática se tradujera en más rentabilidad operativa. Dividimos los distritos en sectores de aproximadamente unas 40 hectáreas cada uno de éllos, con un total, en conjunto de 7.119 hectáreas. Los muestreos de cada uno de los distritos fueron realizados a una media de uno por cada 500 hectáreas; también se hizo un muestreo en los distritos que no conseguían la superficie antes mencionada. Los componentes de cada uno de los equipos, se dedicaron a su zona, a realizar recorridos lineales dentro de los límites marcados por una plantilla equivalente a 20 hectáreas y que superpuesta al mapa de la ciudad que utilizamos (escala 1x5.000) nos delimita el terreno que nos permitirá extraer los datos para las anotaciones V, SO y E.

V = apreciaciones visuales del número de ratas en 20 hectáreas.

C = número de ratas capturadas con rateras, señaladas con azul de metileno y soltadas otras vez.

RC = número de ejemplares marcados, soltados y vueltos a capturar.

SO = signos objetivos (pasos, nidos...).

E = número aproximado de excrementos (las deposiciones de una rata son aproximamente unas 19/día) (11 contabilizadas).

M = margen aceptable de rata/habitante.

Estos son los signos empleados para la evaluación del número de ejemplares en la superficie. Para considerar los de la red de alcantarillado utilizaremos las mismas letras con una comilla en el lado superior derecho.

Cada viernes colocábamos 50 rateras metálicas, todas con su cebo, en lugares que considerábamos idóneos y las recogíamos el lunes siguiente. Después de haber contado el número de ejemplares capturados y señalarlos con azul de metileno los volvíamos a soltar y teníamos la información necesaria para las anotaciones C.

Al cabo de cuatro días de haber soltado los ejemplares que habíamos capturado y señalado, volvíamos a colocar el mismo número de rateras y precisamente en los mismos lugares donde habían estado colocadas la semana anterior. Y el lunes siguiente recogíamos las rateras y contábamos únicamente los ejemplares señalados; así obteníamos la información RC.

Todo lo que hemos explicado hasta aquí hace referencia a la normativa empleada para considerar el número de ratas en superficie. En cuanto al número de las que quedan en las alcantarillas, se procede igualmente, con la única diferencia de omitir SO, puesto que no podemos contabilizarlo a causa de la dificultad de su apreciación.

RESULTADOS TOTALES SOBRE LA APRECIACION DE ROEDORES EN LA CIUDAD DE BARCELONA, DESDE EL 1 DE SEPTIEMBRE HASTA EL 31 DE NOVIEMBRE DE 1981

No se han contabilizado las zonas de montaña y sin habitantes de los Distritos II, III, IX y XII.

DISTRITO	POBLACION	HECT.	MUESTR.	NUM. RATAS
I	80.000	246	1	121.527
II	137.913	624	2	123.565
III	151.237	1.300	3	131.312
IV	99.840	257	1	71.551
V	73.000	93	1	84.437
VI	105.235	201	1	63.821
VII	131.247	290	1	104.769
VIII	176.428	592	2	152.468
IX	432.215	1.268	3	479.607
X	186.952	818	2	202.311
XI	114.914	552	2	109.153
XII	257.350	878	2	259.924
TOTAL	1.964.331	7.119	21	1.904.445

La fórmula elaborada y utilizada por este Servicio para calcular el número de ratas en un medio urbano fue:

V'= 1 x 50

C'= 1 x 50 x 3

R'C'= 1 x 50 x 9

S'O'= no se contabiliza

E'= 1/11 x 50

$$\frac{V' + C' + R'C' + E'}{4} = \text{Número ratas alcantarillado (NRC)}$$

V = 1 x 100

C = 1 x 100 x 3

RC = 1 x 100 x 9

SO = 1 x 100

E = 1/11 x 100

$$\frac{V + C + RC + SO + E}{5} = \text{Número ratas superficie (NRS)}$$

$$\frac{NRS + NRC + M}{3} = \text{Número apreciación ratas (NAR)}$$

El hecho de haver podido determinar esta estimación nos permite establecer conclusiones de actuación, de previsión y de perspectiva muy valiosas:

A - Conocer las zonas de más densidad múrida y en consecuencia donde deben realizarse actuaciones de una manera más intensa.

B - Mostrar que donde la recogida de basuras es más deficiente (zonas periféricas), también es más alto el grado de infestación de roedores.

C - Reafirmar la gran importancia que tiene en la aparición de focos, la existencia de solares y la falta de medidas de control en las obras de nueva construcción.

D - Planificar razonablemente las campañas de desratización.

E - Cálculo previo del coste aproximado de la próxima campaña.

F - En los distritos de más densidad humana, comprobación de un notable incremento de la población múrida en la red de alcantarillado muy por encima de la existente en superficie (por ejemplo, Distrito V). Por otra parte, en los distritos con abundancia de solares y zonas con campos abiertos, muchos de éllos llenos de vegetación (por ejemplo, Distritos IX y XII), gran predominio de ratas en superficie.

BIBLIOGRAFIA

- Ayuntamiento de Barcelona. Coordinación de Servicios de Asistencia Sanitaria: Dispensarios Municipales y Otros Centros Asistenciales. Memoria de Actuación, 1981.

Hospitales Municipales. Memoria de Actuación, 1981.

Delegación de Servicios de Sanidad. Servicios Hospitalarios Municipales. Memoria de Actuación, 1980.

- Diputación de Barcelona (1977). Una nueva perspectiva 1973-1977.

- Diputación de Barcelona. Presupuesto Económico-Funcional de Gastos para el Ejercicio de 1980.

- Durán Heras, A. La financiación de los gastos de Salud. "Primeras Jornadas sobre Economía de la Salud".

- Durán Heras, A. Financiación de los servicios de salud. Ponencia presentada al Simposium Internacional Economía y Salud. Madrid, 17 al 19 de mayo de 1982.

- "Generalitat de Catalunya. Departament de Sanitat i Seguretat Social". Estudios y dictámenes sobre Sanidad. Servicios de Publicaciones de la Generalitat de Catalunya. Departamento de la Presidencia. Barcelona, abril de 1980.

- I.N.E. Estadística de Establecimientos Sanitarios en Régimen de Internado, 1976.

- Maluquer y Oromí, Roser (1982). Algunas consideraciones respecto a la planificación y costes de la asistencia psiquiátrica y salud mental. Comunicación presentada en las Primeras Jornadas de Economía de los Servicios Sociales. Barcelona, 6 y 7 de mayo de 1982.

- Primeras Jornadas sobre Economía de la Salud. La utilización del análisis económico en los servicios sanitarios. Barcelona, 5 y 6 de diciembre de 1980. Colegio de Economistas de Cataluña. Barcelona, 1981.

Rovira Forns, J. Aspectos económicos de la medicina privada. "Primeras Jornadas sobre Economía de la Salud".

- UNESPA. Estadística de las Mutuas Patronales de Accidentes de Trabajo. Año 1980.

- UNESPA. Estadística de Seguros. Año 1980.

- W.H.O. Financing of health services. Report of a WHO Study Group. Technical Report Series. 625. Geneva, 1978.

SESSION 5

THE SAFETY OF PESTICID'S USE

Chairmen: R. Bailly
J. L. Cervigon

QUELQUES RISQUES ECOLOGIQUES DE L'UTILISATION DES PESTICIDES

A. Fougeroux
ACTA, Association de Coordination Technique Agricole-Paris, France

RESUME

Pour l'agriculture, l'usage des produits phytosanitaires est actuellement, et encore pour de nombreuses années, une nécessité. Dans de très nombreux pays, les pesticides ont contribué à pallier les réductions de main-d'oeuvre agricole, accroître les rendements de nombreuses cultures et maintenir une régularité de la production avec toutes les conséquences socio-économiques que cela implique.

Les pesticides n'agissent pas uniquement contre la cible vis-à-vis de laquelle ils sont employés, mais aussi sur le reste de l'écosystème. Ces constats et une inquiétude du grand public vis-à-vis de ces produits ont conduit de nombreux chercheurs à s'intéresser à l'écotoxicité des pesticides.

Cet exposé est une revue des connaissances et des lacunes en matière d'écotoxicité des produits phytosanitaires, notamment vis-à-vis de la faune et de la flore des sols et des milieux aquatiques, de la faune gibier, des pollinisateurs et de la faune auxiliaire.

L'utilisation rationnelle de produits phytosanitaires implique d'en connaître les répercussions sur l'ensemble de l'écosystème. Après l'emploi d'organochlorés, le constat que ces produits avaient contaminé de vastes zones de la biosphère a conduit les spécialistes de la protection des cultures à développer des programmes de surveillance du devenir des produits phytopharmaceutiques dans l'environnement.
Ces programmes comportent généralement des études dont le but est de connaître :

- les mécanismes responsables de la dispersion,
- les quantités transportées,
- la nature des molécules en cause (métabolites),
- le comportement des pesticides ou de leurs métabolites dans les écosystèmes,
- les perturbations qu'ils peuvent induire.

Ces études correspondent à l'écotoxicologie, elles nécessitent généralement, pour être complètes, de tenir compte des nombreuses interactions qui existent entre les différents compartiments de l'écosystème (figure 1 Jamet 1983).

I - DEFINITION DES RISQUES ECOLOGIQUES D'UN PESTICIDE

Définir les risques écologiques d'un pesticide se heurte d'emblée à la définition de l'environnement auquel on s'intéresse et à la période pendant laquelle l'effet du produit est évalué. Il est possible en effet d'étudier les effets à court terme ou à long terme, de même que l'on peut limiter la recherche des effets sur une seule espèce, sur un couple proie-prédateur, sur une chaîne trophique ou encore sur la structure et/ou le fonctionnement d'une biocénose.
Si ces "découpages" facilitent les recherches d'effets, ils sont arbitraires et tôt ou tard, il est nécessaire d'aborder simultanément l'ensemble des effets et leurs interactions. Cependant, il faut bien reconnaître que l'analyse globale du comportement d'un xénobiotique au sein d'un écosystème reste, pour des problèmes méthodologiques, complexe.
Sur le plan écologique, un pesticide est un produit étranger introduit dans un écosystème induisant des modifications dans les structures des populations et parfois des modifications fonctionnelles. Ces modifications peuvent s'effectuer de manière réversible ou irréversible.
M. Lebrun (1985) a dressé une liste des effets écologiques des pesticides au niveau des populations (Tableau 1).

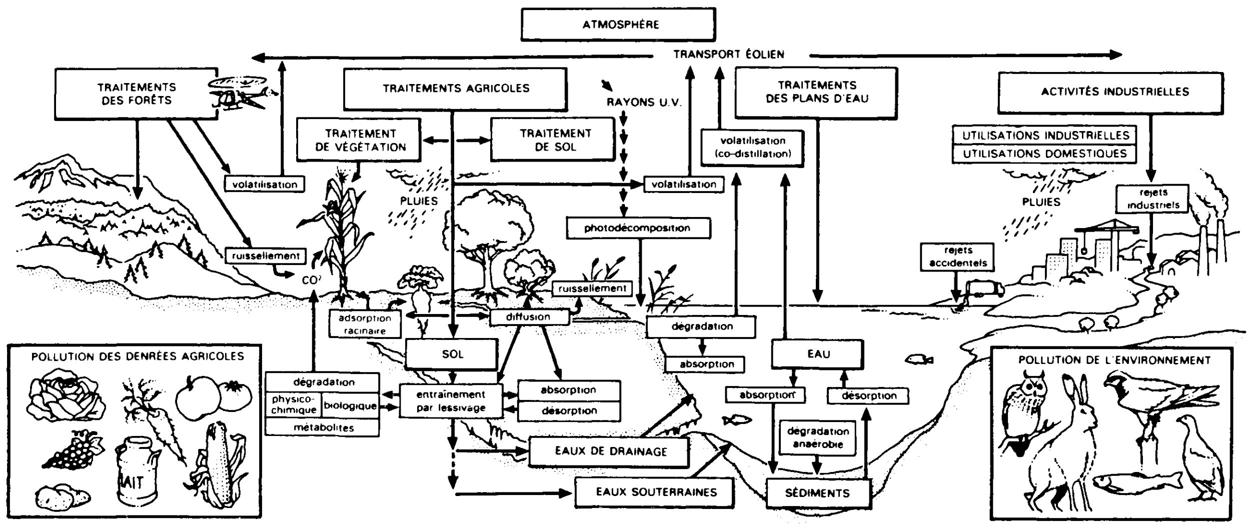


Figure 4 : Les pesticides sont introduits dans l'environnement au cours d'opérations bien connues, traitements agricoles, forestiers et aquatiques, utilisation domestique, activité industrielle) et le plus souvent parfaitement contrôlables ; cependant le devenir de ces molécules dans l'air, le sol et l'eau est pratiquement incontrôlable. Les phénomènes responsables du devenir des pesticides dans l'environnement sont représentés sur ce schéma ; selon les conditions et le pesticide utilisé, certains phénomènes joueront un rôle déterminant dans la dispersion des résidus ; après avoir porté essentiellement sur la pollution des denrées agricoles, les recherches s'intensifient actuellement pour comprendre le rôle des différents phénomènes mentionnés ici, dans le but de prévoir et de limiter la dispersion des pesticides dans l'environnement. (D'après Jamet, 1983).

Tableau 1 : Effets écologiques des pesticides au niveau des populations (une seule espèce ou nombre limité d'espèces)

ESPECES CIBLES	ESPECES NON CIBLES
Augmentation de la mortalité	
- Régression ou extinction de la population. - Possibilité de substitution d'espèce.	- Régression ou extinction de la population. - Perte de diversité génétique - Perte de fonction écologique
Augmentation de la mortalité spécifique (sexe, âge..)	
- Régression ou extinction de la population. - Possibilité de remplacement d'espèce.	- Régression ou extinction de la population. - Perte de diversité génétique - Perte de fonction écologique
Changement de fécondité	
- Régression ou extinction ou augmentation de la population. - Possibilité de substitution d'espèce. - Possibilité de résurgence d'espèce.	- Régression ou extinction ou augmentation de la population. - Perte de diversité génétique - Perte de fonction écologique - Banalisation des flores et faunes.
Changement génotypique	
- Résistance. - Possibilité de résurgence d'espèce.	- Résistance.
Bioconcentration	
- Contamination des chaînes trophiques.	- Contamination des chaînes trophiques.

Ces effets ont pu être observés sur des cas particuliers, cependant leurs conséquences sont plus difficiles à mesurer et bien souvent les méthodologies font défaut. C'est pourquoi, en raison de la complexité, des phénomènes mis en jeu obligent les spécialistes à utiliser des indicateurs biologiques servant de modèles. La connaissance du comportement de ces indicateurs en présence de pesticides permet de prévoir, au moins partiellement, les répercussions liées à l'emploi de produits agropharmaceutiques. Certains modèles font l'objet d'attentions particulières :

- le milieu aquatique,
- le sol et son activité biologique,
- la faune sauvage,
- l'entomofaune épigée dont les pollinisateurs,
- l'apparition d'espèces résistantes.

Quel que soit le modèle étudié, la connaissance du comportement physico-chimique du produit est un préalable indispensable aux études. A cet égard, les substances introduites dans le milieu naturel vont évoluer par Adsorption, Volatilisation, Oxydation, Hydrolyse, Biodégradation ou Métabolisme. Les paramètres qui influencent la dispersion des pesticides dans l'environnement sont résumés dans le Tableau 2.

Tableau 2 : Paramètres influençant la dispersion des pesticides dans l'environnement (Lefeuvre 1985)

PARAMETRES			
Climatiques	Biologiques	Phénomène Physico-Chimique	Propriétés du produit
Température	Biodégradation	Ruissellement Lessivage	Tension de vapeur Solubilité
Pluviomètrie	Absorption par les êtres vivants	Adsorption-Désorption - Volatilisation	Chaleur latente de vaporisation
Vent	 Accumulation dans les réseaux trophiques.	Diffusion Photodégradation	Stabilité (U.V. θ, pH..) Coefficient de distribution (eau/lipides..) Type de formulation.

II - LES PESTICIDES ET LE MILIEU AQUATIQUE

L'eau est considérée, à juste titre, comme le symbole de la vie aussi est-il légitime de chercher à maintenir sa qualité, et, les problèmes de la pollution de l'eau constituent un point particulièrement sensible. L'utilisation de pesticides et leurs possibilités de transport par l'eau représente une des nombreuses sources potentielles de pollution des milieux aquatiques. Par ailleurs, l'exemple de la large dispersion des produits organohalogénés dans la biosphère, et notamment les milieux marins et limniques, reste présent dans tous les esprits. Ces différents points ont fait du milieu aquatique un indicateur important du devenir des produits agropharmaceutiques dans l'environnement et aucun pays n'homologue actuellement des produits phytosanitaires sans disposer d'informations précises sur leur écotoxicité vis-à-vis de ce milieu.
Cependant si les intentions sont louables, leur mise en application est difficile. La circulation d'eau dans l'environnement et les équilibres biologiques qui lui sont associés sont eux-mêmes complexes.
Dans les travaux conduits il est possible de distinguer quatre types d'expérimentations :

- les études du comportement physico-chimique du produit dans l'eau ;
- les études de laboratoire sur des espèces végétales ou animales jouant le rôle d'indicateurs biologiques ;
- les études sur des modèles de chaînes trophiques (mesure de bioaccumulation) ;
- les études plus globales sur l'écosystème aquatique.

Ces quatre types de travaux se complètent mutuellement.

1) Les études de comportement physico-chimique

A part quelques cas particuliers (démoustication, désherbage des zones aquatiques, traitement des rizières ou lutte contre certaines endemies) l'application de produits phytosanitaires est réalisée sur le milieu terrestre et les risques de pollution du milieu aquatique sont fonction de l'efficacité des voies de transfert entre le lieu d'application et l'eau (Hascoet et Jamet 1987).
Trois voies de transfert coexistent :

- le lessivage,
- le ruissellement,
- la dérive des embruns de traitement.

Le lessivage constitue un risque qui ne peut être négligé car ce phénomène entraîne directement la pollution des nappes phréatiques et donc des eaux de consommation. Ce lessivage semble peu important dans nos régions tempérées.

C'est ainsi que les niveaux de pollution des eaux souterraines du bassin parisien sont faibles : pas de détection d'organophosphorés ni de carbamates, des triazines sont détectées suite aux épandages de printemps, parmi les organochlorés seul le lindane est encore détecté à des teneurs de 2 à 10 mg/l (S. Rauzy 1987). Cet exemple ne doit pas faire oublier que les risques de lessivages sont plus importants dans des sols plus fragiles en raison d'une dégradation de la matière organique, d'une activité microbienne plus faible, ou encore en présence d'un régime de pluies plus violentes (climats tropicaux, sub-tropicaux, équatoriaux ou méditerranéens).
Le ruissellement peut être la cause d'entraînement de pesticides vers les rivières. En France, ce phénomène est généralement considéré comme mineur. Il est fonction de la pente des terrains sur lesquels sont réalisés les pulvérisations, le couvert végétal, le régime des pluies, les façons culturales et l'état du sol en surface. Certaines études réalisées sur des vignobles en côteaux ont montré cependant que ce ruissellement pouvait être la cause de pollutions ponctuelles par certains herbicides ou fongicides (Belamie 1986).Là aussi, sous des climats où l'intensité des pluies est plus importante, ce phénomène est loin d'être négligeable.
La dérive par les embruns de traitement peut et doit être évitée lors de l'application des produits en tenant compte du vent ou en réglant correctement son pulvérisateur (taille des gouttes supérieure à 200 microns). Cette précaution vise les points d'eau consommable par l'homme et les animaux ainsi que les périmètres de protection des captages, les bassins de pisciculture, conchyliculture, aquaculture, rivières, marais salants. Elle protège aussi le littoral maritime, les cours d'eau, les canaux, les lacs et les étangs d'eau douce ou saumatre, les fossés d'assainissement (UIPP 1987).

2) Les études de laboratoire sur des espèces végétales ou animales

L'évaluation de la toxicité d'un produit vis-à-vis d'un écosystème aquatique est extrêmement complexe. Aussi est-il nécessaire, par des études de laboratoire, de réaliser une première approche. Celle-ci porte sur des sénarios très simplifiés. Ils mettent en jeu un indicateur biologique généralement une population homogène présentant une sensibilité définie (Cabridenc 1987). Dans ces expérimentations on réalise des tests d'écotoxicité directe sur des individus placés dans un biotope simplifié avec des durées d'exposition réduite (6-96 heures). Par ailleurs, en laboratoire, des essais peuvent aussi être conduits sur des biotopes plus complexes mettant en particulier en oeuvre des chaînes trophiques.
Quatre niveaux trophiques sont principalement étudiés :

- les poissons,
- les algues,
- les microcrustacés,
- les bactéries hétérotrophes.

D'autres travaux complémentaires sont parfois effectués sur limnées, protozoaires.. Vis-à-vis des poissons, les essais peuvent être réalisés sur de nombreuses espèces. Les espèces les plus utilisées sont rappelées en tableaux I, II, III (d'après Cabridenc 1987).

Vis-à-vis des algues de nombreuses normes d'études existent ; elles portent sur l'évaluation de l'inhibition de croissance d'algues telles que Scenedesmus subspicatus, Skeletonema costatus, Chlorella vulgaris, Selenastrum capricornutum.
Les travaux sur microcrustacés portent essentiellement sur l'évaluation des concentrations responsables de l'immobilisation ou de la mort des individus. L'espèce la plus utilisée est Daphnia magma.

Enfin concernant les bactéries hétérotrophes, comme dans le cas des études de microflore tellurique on évalue l'effet du toxique vis-à-vis de la consommation d'oxygène par des bactéries (souches pures ou mélanges de souches) ou dans le cadre de la méthode Microtox sur l'inhibition de la bioluminescence chez une bactérie marine Phytobacterium phosphoreum.
Ces évaluations de laboratoire apportent de nombreuses informations. Cependant leur extrapolation aux écosystèmes aquatiques reste délicate. Envisageons deux possibilités. Si le produit est jugé peu toxique vis-à-vis d'une espèce de poisson, il est nécessaire de poursuivre les travaux pour évaluer l'effet sur les sources alimentaires de cette espèce, l'effet sur la reproduction.. En revanche, si le produit est jugé toxique pour le poisson en question, il est tentant de considérer qu'il sera aussi toxique en pratique agricole. Un exemple de jugement erroné de ce type est fourni par la deltaméthrine. Ce produit présente en laboratoire une très importante toxicité aiguë vis-à-vis des poissons, les CL 50 étant voisines de 1 ppb (L'Hotellier et al. 1987). En revanche, des traitements directs en milieu naturel sur eau stagnante (Tobby et al. 1981), sur rivières (Rawn et al. 1985) ou en rizières (Santosa et al. 1985) ont montré que la deltamethrine n'entraîne qu'un risque très faible pour la faune aquatique. Plusieurs explications ont pu être avancées : hydrolyse de la deltamethrine en milieu aquatique alcalin, biodégradation progressive par les microorganismes, ou encore adsorption de la matière active sur sédiments minéraux ou organiques. Dans ce cas précis, les essais en milieu naturel, par les nombreuses interactions qu'ils mettent en jeu, ont contredit les résultats de laboratoire.

3) Les essais en milieu semi artificiel

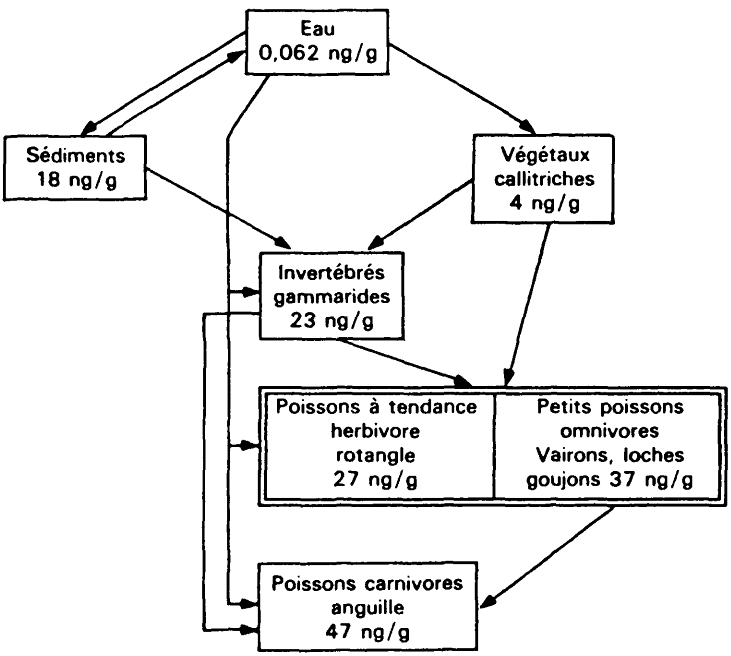
Le principe de ces études revient à reconstituer un milieu aquatique aussi proche que possible du milieu naturel. Pour ce faire, dans un bassin, sont introduits des végétaux aquatiques, des algues, crustacés, mollusques, insectes et poissons. On reconstitue ainsi des chaînes trophiques relativement stables dans le temps ce qui permet d'envisager des études d'écotoxicité à moyen terme. Ces systèmes présentant peu d'échanges avec l'extérieur permettent un bon contrôle des populations et donc des fluctuations différentielles que peuvent engendrer les pesticides par rapport à un bassin non traité.

4) **Les travaux en milieu naturel**

Etant les plus complexes, ils sont peu nombreux. Ils ne permettent pas de comparer différents traitements entre eux mais plutôt l'incidence d'un ensemble de pratiques agricoles réalisées à proximité de l'écosystème aquatique étudié. Un exemple de ce type d'évaluation est fourni par des comparaisons de biotopes lentiques situés dans des zones d'agriculture intensive et d'agriculture n'utilisant pas ou très peu d'intrants chimiques (Echaubard 1987).

Les prélèvements réalisés sur le phytoplancton et les macroinvertébrés permettent d'évaluer les biomasses, les richesses respectives des peuplements et de calculer des indices de diversité. Ces méthodes mises en relation avec l'utilisation de pesticides et l'analyse de résidus fournissent des indications précieuses quant aux risques qu'entraînent ces produits sur ces biotopes. C'est ainsi que les effets toxiques sur le phytoplancton d'urées substituées ou d'azine apparaissent. Le phytoplancton, constitué d'organismes autotrophes producteurs primaires, étant affecté, on peut penser que l'ensemble de la chaîne trophique est perturbée.

Ces suivis en milieu naturel permettent, de plus par l'analyse de résidus, de déceler des problèmes de bioaccumulation des pesticides, comme ce fut le cas pour les insecticides organochlorés. Le lindane fait encore à cet égard l'objet de surveillance comme le rappelle la figure 1 (d'après Ombredane et al. 1980).



Comme on peut donc le constater, les travaux réalisés pour mesurer l'impact de pesticides vis-à-vis de la faune et la flore aquatique sont nombreux, variés et souvent complexes. Ils fournissent suffisamment d'indications pour permettre d'écarter les pesticides trop toxiques en pratique pour les biotopes aquatiques. Il en résulte qu'à part le cas d'application directe, les risques de pollution de l'eau par les produits agropharmaceutiques sont peu importants qu'il s'agisse de lessivage ou de ruissellement, dans nos régions. De nombreux essais, réalisés en France sur vigne ont montré que l'aldicarbe (et ses métabolites actifs) utilisés en traitement de sol à 20 kg/ha n'ont jamais été retrouvés à plus de 70 cm de profondeur. Des risques modérés ont pu être détectés avec les triazines détectées (à moins de 2 % des doses d'emploi) dans les eaux de drainage. Ils ont été confirmé par la mise en évidence de ces herbicides dans les nappes phréatiques en Italie conduisant à une réduction des doses autorisées en désherbage du maîs (1 kg/ha).
Les travaux de pré-homologation complétés par les suivis de post-homologation permettent ainsi de préserver la qualité de l'eau vis-à-vis des pesticides et il faut reconnaître que les accidents liés à l'application de pesticides en milieu agricole restent très exceptionnels.

III - LES PESTICIDES ET LES ORGANISMES VIVANTS DANS LE SOL

Le sol représente l'outil de production agricole par excellence, et le maintien de sa "fertilité" reste la base d'une bonne pratique agricole. Toutefois, pendant longtemps, le sol a été considéré comme un support physique aux cultures, lieu d'échanges chimiques avec la plante cultivée.
La prise en compte de l'activité biologique du sol et de l'importance de celle-ci pour les bonnes relations entre le sol et la plante est récente.
Le sol constitue une cible "privilégiée" lors de l'application des produits antiparasitaires (Hascoet 1984). De même qu'en milieu aquatique, le comportement physico-chimique de la molécule (mobilité, persistance, métabolisme) et ses effets sur le biotope doivent être étudiés. Malheureusement, pour ce dernier point les indicateurs biologiques et les méthodologies d'études ne sont pas toujours bien définies essentiellement en raison des difficultés d'estimation des populations d'organismes telluriques et des interactions entre celles-ci.
L'un des premiers points à considérer pour l'évaluation des risques qu'un pesticide peut faire courir à une biocénose tellurique est sa durée de vie dans le sol. La persistance des pesticides dans le sol dépend de plusieurs facteurs (d'après Edwards 86). Les pesticides organochlorés présentent des demi-vies dans le sol de plusieurs années, et il faut reconnaître que les nouvelles familles d'insecticides ont vu leurs demi-vies décroître au fur et à mesure de leur mise en marché conduisant de ce fait à une moindre écotoxicité de ces produits.

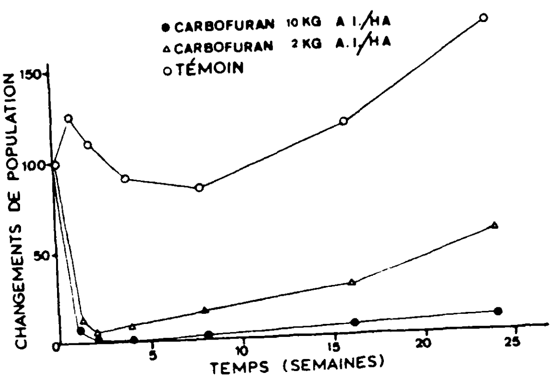
La tendance est donc bien dans le sens d'une réduction des risques. Toutefois la surveillance est de rigueur et certains produits, herbicides notamment, méritent encore une surveillance dans la mesure où leur persistance dans le sol excède une année (triazines carbamates). Enfin, les liaisons chimiques entre molécules phytosanitaires et constituants du sol (acides humiques, fubriques ou colloïdes) sont encore mal connues et des phénomènes de blocage de ces molécules conduisent à sous-estimer la persistance de ces produits dans le sol :

- la structure chimique du produit,
- le type de sol sur lequel le produit est appliqué notamment, le taux de matière organique, l'humidité du sol et sa température ;
- la population microbienne de ce sol.

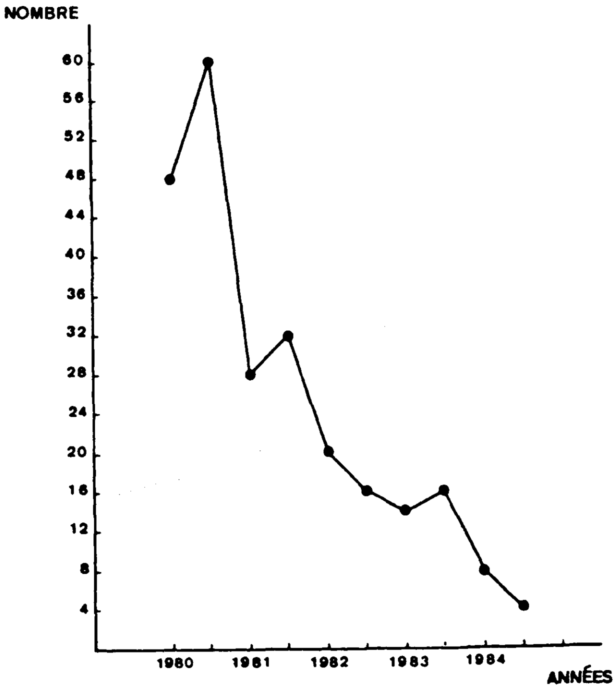
Sur les organismes vivants dans le sol ou en surface, les effets des pesticides sont analogues à ceux enregistrés en milieu aquatique ou sur les biocénoses épigées : modification de la structure et de la diversité des communautés, apparition de résitances, changement des fonctions écologiques.

1) Modification de la structure et de la diversité des communautés.

De nombreux travaux ont été conduits pour évaluer les variations de structures des communautés édaphiques. La plupart mettent clairement en évidence les sensibilités différentielles des espèces et des familles selon leur position trophique (Lebrun 1984). Les organismes prédateurs ou sapophages s'avèrent souvent plus sensibles que les phytophages (Lebrun 1977). Les populations d'organismes indicateurs tels que les Collemboles ou les Lombriciens, connus pour leurs rôles dans les cycles de l'azote et du carbone peuvent se trouver suite à certains traitements très perturbées :



Evolution relative de la densité d'une population d'un Collembole (Folsonia candida) soumise à un traitement insecticide : moyenne de deux parcelles (Thirumurthi et Lebrun 1977).



Evolution d'un peuplement de Lombriciens soumis à deux traitements insecticides annuels (aldicarbe 0,2 g m.a./m^2) (d'après Lebrun 1984).

Notons que dans ce cas la recolonisation d'un sol est beaucoup plus lente qu'en milieu aérien puisqu'il faut plusieurs années, pour un sol traité avec un insecticide du sol, pour voir la population de Lombriciens se reconstituer.

2) Modification dans les fonctions écologiques

D'un point de vue anthropocentrique, deux fonctions exercées au niveau du sol sont importantes, la prédation des ravageurs et la dégradation biologique de la matière organique. Pour ce qui concerne la prédation, des travaux réalisés par Edwards (1984) montrent que des perturbations peuvent apparaître liées à une destabilisation des équilibres trophiques (voir figure ci-après).

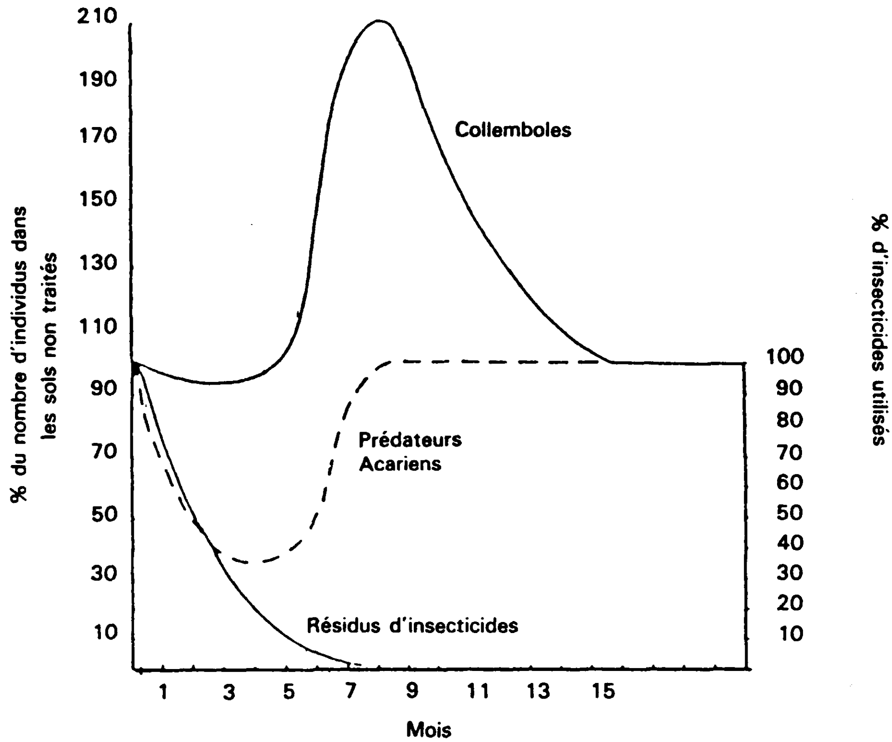


Figure : Relations entre les résidus d'un insecticide organophosphoré, le nombre d'acariens prédateurs et de collemboles. (D'après Edwards, 1984).

La dégradation de la matière organique, les cycles de l'azote et du carbone peuvent aussi être affectés par l'action de produits agissants sur les organismes responsables de cette biodégradation. C'est ainsi que la quantité de matière organique décomposée se trouve réduite du fait de l'appauvrissement des populations de microorganismes du sol suite à un traitement

Couverture morte d'une forêt d'erables rouges (Ontario, Canada)		
	Parcelle témoin	*Parcelle traitée (carbofuran à 285 g a.i./ha)*
Taux de décomposition	1·5–2·2 g m^{-2} $jour^{-1}$	1·0–1·3 g m^{-2} $jour^{-1}$
Quantite décomposée par année (Mai à Novembre)	315–460 g (100)	210–270 g (60)

$\Delta = 40\%$.

Décomposition de litière forestière sous l'action d'insecticide (Weary et Merriam 1978).

Les problèmes de décomposition des pailles sont maintenant bien connus des agriculteurs utilisant de nombreux fongicides sur céréales.
Ces risques sont bien connus, il est donc nécessaire de les évaluer pour les nouveaux produits apparaissant sur le marché. A cet égard, force est de constater que les méthodes d'appréciation ne sont pas clairement définies au niveau européen. Ces méthodes sur la faune et la flore du sol peuvent porter sur :

- des organismes indicateurs de la mésofaune tellurique : Carabiques, Staphylinides, Lombriciens, Mollusques...
- l'activité microbienne du sol par l'évaluation de la consommation d'oxygène, de dégagement de gaz carbonique, l'activité enzymatique (phosphatases) ou le taux de dégradation de matière organique.

Malheureusement, dans de nombreux cas, ces éléments ne sont pas disponibles et dans la pratique, le respect de l'activité biologique du sol influe peu sur le choix de produits appliqués.
Ceci est regrettable dans la mesure où les surfaces recevant des traitements de sol sont loin d'être négligeables. En France, la surface recevant un insecticide du sol varie annuellement entre 3 et 4 millions d'hectares (1/4 à 1/3 de la surface cultivée). De même l'application de molluscicide représente environ 3 millions d'hectares. Dans ces conditions, il est exclu que l'on puisse se désintéresser de l'effet de ces produits sur le comportement du sol.

3) La biodégradation accélérée

Le "monotraitement" de certains sols avec des insecticides peut conduire à une biodégradation accélérée de produit et donc à une perte de leur efficacité. Ce phénomène a pu être mis en évidence dans plusieurs situations de monoculture, de maîs recevant régulièremnt un insecticide du sol de la famille des carbamates (Naîbo et Sombrun 1987).
Ceci n'est pas totalement expliqué. Dans ces sols la multiplication de certains microorganismes (bactéries du genre Pseudomonas ou Achromobacter) entrainerait une dégradation rapide de ces insecticides. Dans la pratique, il reste à savoir si ce phénomène peut apparaître quel que soit le type de sol et si l'emploi d'autres matières actives issues d'autres familles chimiques constitue une alternative.

IV - LES EFFETS SUR L'ENTOMOFAUNE EPIGEE

Parmi les arthropodes vivants dans la végétation cultivée, certains assurent une régulation des populations de déprédateurs des cultures : ce sont les auxiliaires.
Ce rôle régulateur doit être préservé, de même certains organismes participent à la production par la pollinisation qu'ils assurent. Toujours d'un point de vue anthropocentrique ces deux fonctions écologiques et économiques ne doivent pas subir exagérément des effets négatifs de traitements phytosanitaires. C'est pourquoi de nombreux travaux sont conduits pour évaluer l'effet de pesticides sur ces arthropodes auxiliaires ou sur certains pollinisateurs (abeilles et mégachiles).

1) effets sur les auxiliaires

Les études sur les arthropodes auxiliaires font l'objet de discussions au sein d'un groupe de travail de l'OILB. Le principe d'évaluation "en cascade" a été retenu (Hassan et al. 1985). Ces évaluations portent sur l'étude de l'effet d'un pesticide en laboratoire, puis lorsqu'il est toxique en plein champ à court terme enfin, si il est à nouveau reconnu toxique, en plein champ à moyen terme.
Cette méthode permet ainsi de diminuer le coût des travaux dans la mesure où des produits non toxiques en laboratoire seront à fortiori non toxiques au champ. Celle-ci tient aussi compte du fait que l'appréciation des produits au champ à moyen terme devient plus délicate. Pour les évaluations à moyen terme l'ACTA a mis au point une méthode en verger (Reboulet 1987) et en arboriculture la plupart des produits insecticides et acaricides ont aussi pu être testés.

En céréale des travaux similaires ont été réalisés par l'ITCF et l'ACTA pour définir les effets d'aphicides (Taupin et al. 1987).

- L'effet des insecticides sur les espèces utiles					
Arthropodes visés	Produit				
	phosalone	deltamétrine	endosulfan + thiometon	lambda-cyhalotrine	fluvalinate
Coccinelles	++	+++	?	?	+++
Chrysopes	?	++	+	++	+
Syrphes	?	?	?	?	+
Carabiques	+	++	+	++	+
Staphylins	+	++	+	++	+
Larves de coléoptères	+	+	+	+	+
Araignées	+	+++	+	+++	+++
Hyménoptères totaux	+	+	?	?	+
Efficacité : +++ forte toxicité; ++ toxicité moyenne; + toxicité faible; ? pas d'information ou à confirmer.					

Les effets à moyen terme restent quant à eux plus difficiles à interpréter. Ils portent généralement sur le comportement global d'une biocénose (Chambon). Toutefois dans ces études, il reste délicat de faire la part des choses entre l'effet toxique d'un produit et l'effet trophique lié à la destruction de l'espèce phytophage servant de proie à la faune auxiliaire. Afin de distinguer ces deux effets, Reboulet (1987) propose d'étudier l'effet du pesticide non pas directement sur les populations mais sur le rôle régulateur des auxiliaires.

Cette approche se développe puisque ce type d'étude a été repris par divers auteurs (Inglesfield 1987 ; Villaronga et Garcia - mari 1987). Deux modèles sont alors retenus, les pucerons des céréales et leur cortège d'auxiliaires ou les acariens des arbres fruitiers et leurs prédateurs.
La synthèse de ces travaux permet d'évaluer l'intérêt de produit dans le cadre d'une lutte intégrée n'employant que des pesticides perturbant le moins possible la régulation naturelle des ravageurs.

V - LES PESTICIDES ET LES POLLINISATEURS

Parmi les animaux pollinisateurs, l'abeille est la plus étudiée, d'une part en raison de la production de miel, d'autre part pour l'activité pollinisatrice. Cette position en fait un bio-indicateur de choix à l'interface entre agriculture et environnement. Par ailleurs, son comportement social en fait un modèle intéressant pour évaluer les répercussions d'insecticides.

Dans la plupart des pays, l'abeille est protégée par une règlementation classant les produits en toxiques ou peu toxiques pour les abeilles. De ce fait, les organismes officiels et les firmes sont amenés à tester les produits en laboratoire et dans des conditions proches de la pratique agricole. Selon les pays, ces derniers tests sont effectués en plein champ (USA par exemple) ou en tunnels (Debray 1987). Dans les deux cas, il est possible d'évaluer ainsi la toxicité à court terme des produits, en la comparant avec une référence (phosalone par exemple) et un témoin non traité. Tout produit appliqué en période de butinage entraînant une mortalité d'abeille comprise entre celle du témoin et de la phosalone est classé peu toxique. Toute mortalité supérieure à la phosalone entraîne un classement interdisant l'emploi du produit en période de butinage (floraison ou exudation de miellat par les pucerons, psylles...).
Cette méthode apparemment simple est en fait, délicate d'interprétation car :

- les abeilles réagissent différemment au stress provoqué par la pulvérisation de produit selon les conditions climatiques ou suivant l'état général de la ruche,
- le test est lourd et la répétition est surtout possible dans le temps.

Cette classification est pratique car elle complète utilement les travaux de laboratoire. Par cette technique, la relative innocuité des pyréthrinoîdes a pu être montrée. Les résultats des essais de laboratoire mettaient en effet, en évidence une toxicité de ces produits par contact et par ingestion. En test de plein, l'effet répulsif mis en évidence réduit ce risque d'intoxication.
En revanche, ces tests en tunnels présentent une lacune de taille. Ils ne permettent pas de déceler aisément les effets à moyen terme de produits et à cet égard, les connaissances sur les produits restent insuffisantes. Enfin, la législation pour la protection des abeilles ne prend en compte que les spécialités phytosanitaires employées isolément. Hors des expériences sous tunnels ont montré que des mélanges mettant en oeuvre un fongicide inhibiteur de la biosynthèse des stérols et un insecticide pyréthrinoîdes est toxique alors que les deux composants pris isolément sont peu toxiques. Il y a là un effet de synergie que la législation française n'a pas encore prévu. Hors ce risque pour les abeilles devrait s'amplifier dans la mesure où les mélanges sont de plus en plus pratiques, dans un souci d'économie de passage.

VI - LES PESTICIDES ET LA FAUNE SAUVAGE

L'emploi massif des organochlorés par les phénomènes de bio-magnifications et de bio-amplification a été responsable de la mort de nombreux vertébrés menaçant certaines espèces de disparition. Depuis de nombreux travaux ont été conduits pour évaluer les risques que pouvaient encourir le gibier suite à l'emploi de pesticides. Certains produits présentent encore un risque important sur le gibier.

INSECTICIDES DU SOL	INSECTICIDES ET ACARICIDES
Aldicarbe (B) Carbofuran (B) Carbosulfan ((B) Chlorfenvinphos (B) Chlorméphos (B) Chlorpyriphos éthyl (A) Chlorpyriphos éthyl + Lindane (A) Chlorthiophos + Lindane (A) Diazinon (A) Disulfoton (B) Ethoprophos (B) Fonofos (B) Parathion (B) Terbuphos (B) Thiofanos (B) Thrichloronate (B)	Azinphos éthyl et méthyl (B) Chlorfenvinphos (A) Déméton-5 méthyl sulfone + Azinphos (B) D.N.O.C. (B) D.N.O.C. + huiles (= huiles jaunes) (B) Endosulfan + Parathion éthyl (A) Formétanate (B) Méthomyl (B) Névinphos (B) Nicotine (B) Oléoparathion (B) Parathion éthyl (B) Parathion méthyl (A) Parathion méthyl + Carbophénothion (A) Parathion + huiles (B) Prothoate (A) Toxaphène (A)

Insecticides ayant une action toxique sur le gibier (ONC 1982)

Toutefois, il faut nuancer ces mentions car, force est de constater que les accidents sont surtout le fait de mauvais emploi des produits (dépôts de granulés insecticides ou molluscicides, rodenticides). De plus, les intoxications enregistrées en France par le Centre antipoisons vétérinaires ne sont constituées que pour 2,8 % par les animaux sauvages (Lorgue 1987), toutefois les données pour cette faune restent plus imprécises que pour la faune domestique.
Enfin l'apparition d'insecticides, tels que les pyréthrinoîdes, inoffensifs pour les animaux à sang chaud devrait encore minimiser les risques pour la faune sauvage. Le réseau de surveillance mis en place a d'ailleurs montré la prédominance d'affection parasitaire dans les causes de mortalité (Mallet et al. 1987).

VII - LES RESISTANCES AUX PESTICIDES

La résistance des ennemis des cultures aux pesticides est l'un des aspects les plus préoccupants des effets des produits sur l'environnement. Le nombre de cas de résistance s'accroît de façon exponentielle avec l'accroîssement du marché phytosanitaire. Jusqu'à présent l'apparition de souches résistantes aux produits n'a eu qu'un impact limité dans la mesure où les sociétés phytosanitaires ont régulièrement mis au point de nouvelles molécules contournant ces résistances. Cette stratégie permettant l'alternance ou l'association de matières actives ayant des sites d'action différentes continue d'être utilisée avec succès : alternance morpholines/ triazoles en céréales, mélanges de fongicides systèmiques et de contact contre le mildiou de la vigne... Toutefois cette méthode pour retarder ou contourner l'apparition de résistances est intéressante lorsque celle-ci correspond à une modification du site d'action du produit (triazines, benzimidazoles, imides cycliques...). En revanche, elle voit ses limites lorsque des mécanismes de détoxication plus généraux sont mis en oeuvre. La résistance de Myzus persicae aux insecticides constitue l'exemple le plus gênant pour la pratique agricole puisque le système enzymatique de détoxication est très polyvalent et inhibe l'activité d'insecticides aussi divers que les carbamates, les organochlorés, les organophosphorés et les pyréthrinoîdes.

On voit mal dans ces conditions comment proposer aux producteurs une alternative.

CONCLUSION

Les effets de pesticides sur l'environnement sont évidemment plus variés que le simple "tour d'horizon" réalisé ici. Toutefois les études menées sur les différents compartiments de l'écosystème, souvent pour répondre à des exigences réglementaires, permettent d'avoir une vue assez précise des risques que peuvent induire l'application de pesticides. Ces travaux menés sur des bio-indicateurs sont complétés d'études de résidus à postériori dans l'air, l'eau et le sol. De plus de nombreux pays disposent d'organismes chargés de surveiller les "accidents" sur le gibier, les animaux domestiques, le cheptel apicole, l'apparition de souches résistantes... L'association d'études sur l'évaluation des risques à priori et cette surveillance reste indispensable, car malheureusement les travaux faisant intervenir l'ensemble des interactions au sein d'un écosystème restent difficiles à mener et à interpréter. A cet égard, la modélisation devrait devenir un outil commode pour connaître le comportement des produits dans les différents compartiments d'un écosystème. Quoiqu'il en soit, les résultats obtenus au travers de ces différentes démarches sont encourageants pour la protection de l'environnement. En effet, on assiste régulièrement au retrait de substances toxiques : produits mécaniques, arsénicaux, organochlorés,... D'autres sont encore préoccupants et sont étroitement surveillés : paraquat, aldicarbe...
La disparition progressive de produits peu sélectifs, présentant des risques pour l'environnement est une bonne chose, elle doit se faire en connaissance de cause et au sein d'un dialogue entre les organismes de protection de l'environnement et les organismes de développement agricole.
Enfin, une protection phytosanitaire moins toxique pour l'environnement suppose aussi de mieux raisonner les traitements et de soigner les techniques d'application dans le respect des méthodes de protection intégrée.

SUMMARY

In agriculture, use of phytosanitary compounds is actually, and for a long time, necessary. In numerous countries, pesticides are useful to increase or to maintain yields. Pesticides act against pests and diseases but also on other parts of ecosystem. For this reason, numerous studies are set up to try to appreciate pesticides ecotoxicity.
This report is a review of knowledge and lacks concerning ecotoxicity of phytosanitary compounds especially on fauna and flora of soils, aquatic organisms, wildlife and beneficial.

BIBLIOGRAPHIE

BELAMIE R. - GIROUD S. (1986) : Aperçu des pollutions liées à l'utilisation des pesticides par l'Agriculture en France n° 38 CTGREF 31 p.

CABRIDENC 1987 : Intérêt et limite des méthodologies d'évaluation des effets toxiques des pesticides dans les eaux. Colloque ANPP "Pollution des eaux par les pesticides et les nitrates n° 5 p7-15.

ECHAUBARD M. (1987) : Contamination des milieux lentiques et conséquences biocenotiques en milieu agricole : un exemple, le cas de quelques mares de la région d'Orsay. Colloque ANPP "Pollution des eaux par les pesticides et les nitrates" n° 5 p 57-76.

HASCOET M. 1984 : Evaluation et procédés de mise sur le marché des pesticides agricoles. Environment and chemicals in Agriculture ed Winteringham 229-241.

HASCOET M. - JAMET P. (1987) : Evaluation du risque de contamination des eaux par les pesticides utilisés en agriculture. Colloque ANPP "Pollution des eaux par les pesticides et les nitrates" n° 5 7-15.

INGLESFIELD C. 1987 : Effects of cascade on predatory mites field studies. ANPP Conférence internationale sur les ravageurs en agriculture n°6 vol I/III 75-82.

L'HOTELLIER M. - VINCENT P. (1987) : Les facteurs contribuant à l'absence d'Ichtyotoxicité de la deltaméthrine en milieu naturel. Colloque ANPP "Pollution des eaux par les pesticides et les nitrates" n° 5 231-243.

LORGUE G. : Intoxication des animaux domestiques et sauvages par les pesticides. ANPP Conférence internationale sur les ravageurs en agriculture n° 6 Vol I/III 211-219.

MALLET C. - ARTOIS M. - FRANCOIS JM. (1987) : Surveillance sanitaire nationale de la faune sauvage en France. ANPP Conférence internationale sur les ravageurs en agriculture n° 6 Vol I/III 221-226.

NAIBO B - SOMBRUN F. (1987) : Perte d'efficacité d'insecticides du sol vis-à-vis des larves de Taupins Agriotes sp en monoculture de maîs ANPP Conférence internationale sur les Ravageurs en agriculture n°6 Vol II/III 237-244.

RAUZY (1987) : Les résidus de pesticides dans les eaux souterraines. Colloque ANPP n°5 95-105.

RAWN G.P., MUIR D.O.G., GRIFT P.G. (1985) : Fate of the pyrethroid insecticide deltamethrine in small ponds, a mass balance study. Journal of Agricultural Food Chemistry 33-603-609.

REBOULET (1987) : Etude de plein champ des effets des pesticides sur la faune auxiliaire. ANPP Conférence internationale sur les Ravageurs en Agriculture n°6 Vol I/III 83-90.

SANTOSA, KOESOEMADINATE, SUSTRINO (1985) : Fiels experiment on the effect of the insecticide Decis 5.42 Flow and Decis 0,42 Flow to fish culture in rice paddies.

TAUPIN, FOUGEROUX, DUVERNET (1987) : Effets de quelques insecticides sur les ravageurs et la faune utile du blé au printemps. ANPP Conférence internationale sur les Ravageurs en Agriculture n°6 Vol II/III 149-167.

TOBBY T.E., THOMPSON A.N., RYCROFT R.J., BLACK I.A., HEWSON R.T. (1981) : A pond study to investigate the effects on fish and aquatic invertebrates of deltamethrin applied directly on to wathr. Rapport non publié. Fisheries Lab. Ministry of Agriculture, Fisheries and Food - GB.
Agency for Agricultural Research and Developpement, Research institute for fresh water fisheries, Boger, Indonesia.

UIPP 1987 : Protection des plantes et qualité des eaux 12p.

VILLARONGA, GARCIA MARI (1987) : Influence of deltamethrin carbaryl and captan on phytoseiid and tetranychid populations in Tarragona hazelnuts. ANPP Conférence internationale sur les Ravageurs en Agriculture n°6 Vol I/III 119-125.

SECURITE DES UTILISATEURS DES PRODUITS PHYTOSANITAIRES

J. Fages
Médecin National Adjoint, Caisses Centrales de Mutualité Sociale Agricole-Paris, France

RESUME

La protection des utilisateurs de pesticides, dans la plupart des pays les plus anciennement concernés par les traitements phytosanitaires systématiques, repose sur un double dispositif réglementaire qui s'adresse, d'une part, aux fabricants et importateurs, d'autre part, aux utilisateurs eux-mêmes. Des tentatives de "surveillance médicale" adaptée ont été faites ; difficile à mettre en oeuvre, cette surveillance peut s'avérer décevante en pratique courante. Il semble bien que l'essentiel de l'effort à consentir soit d'ordre éducatif. S'appuyant sur la réglementation et les structures médicales spécialisées lorsqu'elles existent, cette action éducative doit mobiliser tous les partenaires "de terrain" de formation différente pour amener chaque utilisateur à prendre en charge, en toute clarté, sa sécurité personnelle et celle de son entourage.

INTRODUCTION

Le programme de cette session consacrée à la sécurité dans l'utilisation des pesticides semble tout droit sorti du "Discours de la méthode" tant il obéit à la logique du deuxième précepte de DESCARTES : diviser chacune des "difficultés" examinées..."en autant de parcelles qu'il se pourrait et qu'il serait requis pour les mieux résoudre".

Après avoir vu les mesures à prendre pour la sécurité de la plante-cible, objet de nos soins, puis pour le milieu dans lequel nous vivons avec elle, nos réflexions et nos échanges se portent maintenant sur la "parcelle" qui concerne la sécurité de l'applicateur.
Le fait de confier à un médecin, fut-il spécialisé en prévention, la tâche de conduire cette réflexion signifie-t-il que la santé des personnes qui utilisent des substances toxiques, est du seul ressort d'une surveillance médicale ? Il n'en est rien :
la sécurité de chaque applicateur ne saurait être mieux assurée que par lui-même.

Quand on observe, notamment dans les pays dits "industrialisés", sur quoi repose la sécurité des utilisateurs, on constate qu'un double dispositif est partout présent :

. un dispositif législatif et réglementaire qui concerne à la fois la mise sur le marché et les modalités d'utilisation des produits de traitement,

. un dispositif de "surveillance médicale" des personnes exposées.

Certes, selon le pays concerné, ces dispositions sont plus ou moins développées et se répartissent de façon très différentes, mais on les retrouve sous des formes assez voisines.

Cependant, si le but à atteindre consiste bien à induire un comportement individuel plus responsable et plus pertinent, nul doute qu'il faille aller au-delà de mesures obligatoires passivement vécues. Pour susciter non seulement une prise de conscience réaliste des risques encourus, mais parvenir aussi à une véritable maîtrise de "l'outil de travail", il faut entreprendre une démarche éducative en profondeur, persévérante, adaptée à la situation des utilisateurs, qui nécessite la mobilisation de tous les partenaires "de terrain".

1. : **Place de la réglementation dans la sécurité de l'utilisateur**

Dans la plupart des pays industrialisés, où l'utilisation des produits antiparasitaires à usage agricole s'est largement développée au lendemain de la deuxième guerre mondiale, les pouvoirs publics ont dû se préoccuper de règlementer leur mise sur le marché et la manière de les utiliser. De ce fait, on peut distinguer deux ordres de mesures : celles qui concernent les fabricants ou les importateurs de pesticides, celles qui s'adressent aux utilisateurs.

Tous les pays n'ayant pas encore atteint le même niveau de "compétences techniques spécialisées" et tous les gouvernements n'ayant pas encore mis en oeuvre des processus efficaces de réglementation et de contrôle de la distribution et de l'utilisation des produits de traitement, l'Organisation des Nations Unies pour l'alimentation et l'agriculture (F.A.O.) a publié en 1986 un "Code international de conduite pour la distribution et l'utilisation des pesticides". Si tous les pays voulaient bien s'y conformer, un pas important serait fait pour améliorer la sécurité des utilisateurs...et des autres.

1.1. Les dispositions réglementaires concernant fabricants et importateurs

L'objectif de ces dispositions peut se résumer simplement : ne pas laisser mettre sur le marché n'importe quel produit dangereux, pour servir à n'importe quoi, en l'utilisant n'importe comment.

1.1.1. C'est le fabricant (ou l'importateur) du produit qui doit saisir les autorités compétentes d'une demande d'homologation des spécialités qu'il souhaite commercialiser ("Directives et plan modèle pour la création d'organisations nationales chargées de l'homologation et du contrôle des pesticides" - ROME FAO 1985). Il importe que soient précisés les végétaux auxquels la spécialité est destinée, les parasites visés, le type de traitement, le mode d'application, la ou les concentrations d'utilisation.

1.1.2. Efficacité et innocuité du produit sont examinées par les instances chargées de l'homologation. Si les expérimentations nécessaires pour démontrer ces propriétés sont à la charge du demandeur, l'autorité compétente doit conserver la possibilité de faire pratiquer des "essais de contrôle" par un laboratoire neutre si elle le juge nécessaire. L'innocuité est démontrée par une recherche de la toxicité directe et indirecte sur l'animal (FOULHOUX P., FOURNEX R. 1988). L'importance du risque doit prendre en compte le type de formulation et le mode d'application ; d'éventuelles restrictions d'emploi peuvent en résulter : l'accessibilité de certains produits particulièrement difficiles à utiliser sans danger peut être limitée à certaines catégories d'utilisateurs (équipes spécialisées ayant reçu une formation spécifique : emploi des fumigants par exemple). L'homologation doit avoir une durée limitée, une dizaine d'années en général, elle donne lieu à l'octroi d'un numéro d'enregistrement qui permet de suivre la spécialité sur le marché.

1.1.3. L'instance compétente pour l'homologation définit le contenu de l'étiquette qui sera apposée sur les conditionnements du produit (Directives pour le conditionnement et le stockage des pesticides, ROME FAO 1985 - Directives FAO sur les bonnes pratiques d'étiquetage des pesticides, ROME FAO 1985). L'étiquette porte directement à la connaissance des utilisateurs les informations élémentaires indispensables pour apprécier les risques encourus lors de l'emploi des produits. La catégorie de risque à laquelle expose la substance doit être aisément repérée par un symbole ou pictogramme très apparent sur l'étiquette en plus des instructions et mises en garde écrites.

1.1.4. Dans le cadre de la Communauté Economique Européenne, une directive (67/548/CEE.1967, 87/432/CEE.1987) définit "la classification, l'emballage, l'étiquetage" des préparations dangereuses. Les symboles de danger (Tabl. 1) : toxique et très toxique (T et T+), nocif (Xn), irritant (Xi), corrosif (C), inflammable (I), comburant (O), explosif (E), sont complétés par des phrases de risque R (par exemple : "nocif par inhalation"...) et des conseils de prudence S (par exemple : "ne pas respirer les aérosols"...).

L'étiquette doit en outre comporter :

- le nom chimique ou commun de la substance, la formulation,

- les nom et adresse du vendeur, le n° d'inscription au registre de sortie,

- la destination du produit (quelle culture ? quel parasite ?),

- l'époque du traitement autorisée,

- la durée de la période d'interdiction d'emploi avant la récolte.

1.1.5. Quelques pays exigent aujourd'hui que les fabricants mettent à la disposition des revendeurs et des utilisateurs des "fiches de sécurité" établies pour chaque spécialité, donnant des informations plus détaillées sur les matières actives qu'elle contient et sur les risques et précautions qui en découlent.

1.1.6. L'addition de matières odorantes ou colorantes aux substances dangereuses (voire d'un émétique dans les préparations commerciales : paraquat en France), peut rendre le risque plus repérable dans certaines préparations : appâts empoisonnés à destination des rongeurs ou des oiseaux, par exemple.

Ces mesures réglementaires s'avèrent tout à fait indispensables pour limiter les dangers auxquels les utilisateurs sont exposés. Pour s'en convaincre, s'il en est besoin, il suffit de regarder ce qui se passe dans les pays dépourvus de ce type de législation, pays en voie de développement en particulier. Les accidents y sont nombreux, graves, affectent parfois la quasi totalité d'une population rurale (BOUGUERRA M.L., 1986), au point de poser de véritables problèmes de Santé Publique et non plus seulement d'intoxications liées à l'activité professionnelle.

1.2. Les dispositifs réglementaires concernant les utilisateurs eux-mêmes

Il s'agit de prolonger les effets protecteurs des réglementations précédentes de portée collective, par une action plus individualisée s'adressant aux personnes qui utilisent les pesticides pour réaliser les traitements. Ces mesures concernent le stockage des produits sur l'exploitation ou dans l'entreprise, leur préparation et leur dispersion, l'élimination des surplus et des emballages vides.

1.2.1. Pour rendre les produits dangereux inaccessibles aux enfants et aux animaux, mais aussi aux adultes non avertis, pour les maintenir dans un bon état de conservation et éviter des émanations gazeuses, il faut disposer d'un local de stockage, frais et ventilé, dépourvu d'humidité, fermant à clef, éloigné autant que possible de l'habitation et des étables.

1.2.2. Pour la préparation des épandages et leur réalisation, des moyens individuels de protection sont préconisés ou rendus obligatoires : vêtements spéciaux imperméables aux produits de traitement, gants, bottes, coiffure, masque filtrant avec éventuellement des lunettes si le masque ne couvre pas toute la face. Ne pas manger, boire, fumer pendant les manipulations et sur les lieux de travail. Se laver soigneusement après le travail, prendre une douche si possible.

1.2.3. Les emballages vides ne doivent en aucun cas être abandonnés sur le terrain ou dans une cour de ferme, pas même dans une décharge publique. Ils constituent un risque grave de contamination accidentelle des personnes, des animaux et de l'environnement. Dans les pays pauvres, leur récupération pour le stockage de denrées alimentaires est une des causes préoccupantes des accidents graves (BOUGUERRA M.L., 1986). Après un lavage soigneux, l'eau de rinçage étant versée dans la cuve d'épandage, les récipients doivent être rendus inutilisables : brûlés s'ils sont combustibles sans danger pour l'environnement (les récipients en PVC ne doivent pas être brûlés), cassés ou écrasés s'ils sont incombustibles. Leur collecte, comme le ramassage des restes inemployés, leur destruction dans des incinérateurs à très haute température, qui apporteraient une bonne réponse à ces questions, s'avèrent difficiles à mettre en oeuvre et le coût de telles opérations est tellement élevé qu'on ne peut, jusque là, envisager d'en faire une mesure générale.

1.2.4. Le port des dispositifs individuels de protection énumérés ci-dessus rencontre encore de vives résistances chez de nombreux utilisateurs (PORTOS J.L. et al. 1987). Lorsque l'activité physique est intense, ou que la température extérieure est élevée, ils peuvent entraîner une gêne réelle et un grand inconfort. En particulier, les appareils de protection respiratoire sont souvent mal acceptés. Il est vrai que quelques obscurités subsistent quant à leur emploi : choix du dispositif le mieux adapté, durée de l'efficacité des cartouches filtrantes, précautions pour l'entretien. Les plus confortables, à ventilation assistée s'avèrent parfois les moins performants quant à la durée de vie des filtres en raison de l'important débit d'air qui les traverse. Sauf, bien entendu, pour l'utilisation des fumigants qui nécessite le port d'appareils respiratoires autonomes, de nombreuses études semblent montrer que l'exposition respiratoire n'excède pas habituellement 1 % de l'exposition cutanée (W.H.O.,V.B.C/82.1) et que pour des conducteurs de tracteur avec "cabines" assurant une pulvérisation sur des citronniers, l'exposition respiratoire ne représente que 0,029 % de l'exposition totale (U.S. Environmental Protection Agency, 1987, Tabl. 2). Sans doute faut-il tenir grand compte de ces constatations pour sensibiliser les utilisateurs à l'intérêt des protections individuelles et leur apprendre à s'en servir intelligemment en fonction du degré de risque qu'il faut leur apprendre à évaluer.

2 : Place d'une "surveillance médicale" dans la sécurité de l'utilisateur

L'Organisation Mondiale de la Santé propose une classification des "risques" dus aux pesticides qui s'appuie sur la dose létale 50 propre à chaque matière active ("Guidelines to classification 1988-1989).

Quatre grandes classes sont ainsi distinguées : produits extrêmement dangereux, classe Ia (61 molécules) - hautement dangereux, classe Ib (92 molécules) - modérément dangereux, classe II (162 molécules) - légèrement dangereux, classe III (157 molécules). Une liste de 263 matières actives qu'il est "peu probable" de voir causer un risque aigu en usage normal complète ces classes.

On dispose ainsi de documents qui permettent de hiérarchiser les risques et donc, d'exercer une éventuelle surveillance des personnes qui manipulent les substances les plus dangereuses. A l'instar de ce qui est réalisé dans l'industrie, on a pu espérer appliquer à l'agriculture les notions de "valeurs limites d'exposition", ou "indices biologiques d'exposition" qui permettent de contrôler les risques d'intoxication. Dans la pratique, cet espoir se réduit singulièrement : le dosage dans l'air ambiant, lorsqu'on travaille à l'extérieur n'a pas grande signification et les "indices biologiques" sont souvent difficiles à définir et à mettre en évidence. S'il est possible d'envisager une stratégie précise de surveillance pour les travailleurs qui appartiennent à des équipes spécialisées de désinsectisation, de désinfection ou d'aéroculture (effectifs bien circonscrits, produits bien connus, modes d'intervention bien codifiés,...), c'est beaucoup plus aléatoire quand il s'agit de suivre une population dispersée dans les exploitations de production, notamment les travailleurs indépendants que sont les exploitants agricoles. Nous verrons que sur le strict plan médical, les choses ne sont pas simples non plus.

Schématiquement, en Europe, deux types de stratégie de surveillance sont mises en oeuvre : une surveillance systématique régulière des travailleurs, des études ponctuelles de "situations à risques" selon des protocoles strictement établis.

2.1. Surveillance médicale systématique

Les pays qui disposent d'une "médecine du travail" obligatoire (Belgique, France,...) font examiner au moins une fois l'an tous les salariés. Le risque toxique, selon les produits concernés, peut même donner lieu à une "surveillance spéciale" qui comporte un rythme d'examen et un contenu le plus souvent laissés à la convenance et à la compétence du médecin du travail spécialisé territorialement concerné.

2.1.1. L'expérience montre qu'il est très difficile d'adapter les examens aux périodes d'exposition : variations en fonction des productions, des aléas climatiques, voire des caprices des attaques parasitaires. Par ailleurs, les examens de laboratoire disponibles à caractère spécifique sont très peu nombreux hormis le dosage des produits eux-mêmes ou de leurs métabolites dans les liquides organiques (sang, urine surtout) voire dans l'air expiré. Ces examens, qui ne sont pas "de routine", ne sont guère réalisables que dans de rares laboratoires très spécialisés et pourvus de l'appareillage perfectionné nécessaire (chromatographes en phase gazeuse, spectographes de masse, etc). Dans ces conditions, la surveillance est essentiellement assurée par un questionnaire centré sur les manifestations d'allure banale éventuellement observées par l'utilisateur à l'occasion des traitements (PORTOS J.L., 1987) et par un examen clinique approfondi (BONDERF J. et al., 1988). Les examens de laboratoires destinés à s'assurer de l'intégrité des fonctions hépatiques et rénales, de l'absence d'agression sur les lignées sanguines, qui sont parfois pratiqués (SHAPIRO S. 1980 - FOURNIER E. 1983 - DUBRISAY J. 1986), ne permettent pas d'affirmer qu'il n'y a aucun risque à long terme.

2.1.2. L'utilisation habituelle des insecticides organo-phosphorés (parathion, malathion, dichlorvos...) et des carbamates insecticides peut conduire à surveiller les cholinestérases plasmatiques et globulaires des utilisateurs (EFTHYMIOU M.L., 1978 - DALLY S., 1983). Ceci requiert un dosage préalable à toute exposition pour "étalonner" chaque individu, et une prise de sang systématique pour tout contrôle ce qui n'est pas facilement accepté, sans compter les erreurs de dosages qui peuvent résulter de délais trop longs d'acheminement des échantillons vers le laboratoire (TEULON F. et al. 1982 - W.H.O., 1982). Pour pallier ces inconvénients, le dosage urinaire des dialkylphosphates par chromatographie a été tenté (SHAFICK et Miss BRADWAY, U.S.A. CHALABREYSSE J., PIERRELATTE, FRANCE), mais sans aboutir aux résultats escomptés.

2.1.3. Au total donc, cette surveillance "systématique" s'avère assez décevante, elle ne peut d'ailleurs prétendre qu'à une action de dépistage, fusse au stade infraclinique d'imprégnation, c'est-à-dire à une opération de prévention secondaire (DUBRISAY J., 1986). On tend actuellement à privilégier la prévention primaire qui anticipe sur la notion de risque.

2.2. Etudes ponctuelles de "situations à risques"

Ces études sont essentiellement de deux ordres : études du niveau d'exposition par "dosimétrie passive", qui ne constituent pas à proprement parler une surveillance médicale,
études rejoignant la préoccupation ci-dessus exposée de surveillance infraclinique : "monitorage biologique" (biological monitoring).

Ces études se déroulent selon des protocoles bien codifiés ("Pesticides assessment guidelines" de l'U.S. Environmental Agency - 1987 -"Protocole standard de surveillance "sur le terrain" de l'exposition aux pesticides" de l'Organisation mondiale de la santé - 1982).

2.2.1. La "dosimétrie passive" tente d'évaluer la quantité de produit qui atteint la surface de la peau de l'utilisateur pendant son travail, ou la quantité susceptible d'être inhalée, en utilisant des capteurs adaptés, le plus souvent réalisés sous forme de "compresses" d'alpha-cellulose et de gaze hydrophile qui sont fixées sur les vêtements selon une cartographie bien établie. Ces compresses sont ensuite traitées par le laboratoire pour doser le produit capté et sa répartition sur le corps.

A cette méthode des "compresses" on tend aujourd'hui à préférer l'usage de vêtements de protection jetables en matériau "non tissé" qui sont, après exposition, découpés en segments traités séparément au laboratoire.

L'avantage majeur de cette méthode tient à ce qu'elle permet de séparer et de comparer les régions exposées, donc d'adapter les moyens de prévention à la réalité de l'exposition, y compris pendant une activité réduite.

2.2.2. Le "monitorage biologique" évalue la dose interne :

- par le dosage de la quantité du pesticide ou de ses métabolites éliminée,

- ou bien, de manière plus agressive, en mesurant la quantité de produit stockée dans des tissus ou dans des fluides organiques connus pour leur avidité vis-à-vis du produit.

L'essentiel de ces dosages s'effectue selon les techniques de laboratoire très perfectionnées ci-dessus mentionnées, et donc peu disponibles en tous lieux. Le document Tabl. 3, établit la liste des 25 matières actives et de leurs métabolites qu'il est aujourd'hui possible de rechercher en chromatographie gazeuse, selon une technique mise au point pour chaque substance (ce qui souligne le caractère non exhaustif du monitorage actuellement réalisable et donc les limites de cette stratégie). Elle n'est réellement applicable qu'à des études de terrain bien délimitées et non à une surveillance continue d'une population agricole toute entière.

3. : **Nécessité d'une action éducative "en profondeur"**

Ce qui précède montre à l'évidence que si les contraintes législatives et réglementaires qui pèsent sur les fabricants et les importateurs de pesticides ont un intérêt majeur pour la sécurité des plantes cultivées, de l'environnement et des utilisateurs de produits, les mesures de prévention prévues pour les personnes exposées ne trouveront leur plein effet que si elles sont intelligemment appliquées. Certes, là où elle peut s'exercer, une surveillance médicale peut aider à la prise de conscience des risques, mais elle ne peut pas, à elle seule, garantir la sécurité des manipulateurs de substances dangereuses, ses ambitions sont limitées.

Utiliser des pesticides pour lutter contre les agresseurs des plantes cultivées c'est, en somme, leur administrer des "médicaments" pour lutter contre leurs "maladies". Cela ne peut pas s'improviser, il faut donc acquérir une réelle compétence dans son métier d'agriculteur, mesures préventives incluses.

3.1. Cette acquisition de compétence commence avec la formation professionnelle. Elle suppose donc des enseignants non seulement experts en "bonnes recettes" pour produire mais n'ignorant rien des propriétés nocives des substances utilisées pour améliorer la production et de leurs éventuels effets pervers sur l'homme, la faune sauvage ou domestique, l'environnement. Il faut qu'ils puissent attirer l'attention de leurs élèves, dès les premières leçons, sur les précautions à prendre pour éviter de nuire.

3.2. Il faut ensuite que tous les techniciens concernés par le "développement agricole" acceptent de jouer le jeu de la clarté dans leurs conseils techniques sans omettre d'expliquer les erreurs de comportement à éviter. Il va de soi que les vendeurs de produits phytosanitaires ont aussi, comme relai privilégié de l'information préventive, un rôle de premier plan à jouer, il faut assurer leur formation pour qu'ils sachent pleinement tenir ce rôle.

3.3. Il est de toute première importance que les mesures de prévention préconisées ou rendues obligatoires, ne soient plus vécues par les utilisateurs comme des contraintes artificielles, imaginées par des fonctionnaires bien à l'aise dans leurs bureaux, mais qu'elles apparaissent au contraire comme la marque d'un professionnalisme éclairé et qu'elles soient synonymes de performance. Les protections individuelles (ZELLER H., 1980), intelligemment et judicieusement choisies, portées aux moments opportuns, pourraient apparaître alors comme les signes extérieurs de la maîtrise de "l'outil pesticide" et donc comme un attribut de compétence, et non plus comme l'aveu d'une vulnérabilité et l'expression d'une peur... L'instauration de cet état d'esprit nécessite évidemment que tous ceux qui, sur le terrain, rencontrent les utilisateurs, partagent les mêmes convictions et tiennent le même langage. C'est l'effort persévérant de tous les intervenants qui doit permettre à chacun des utilisateurs d'assurer pleinement sa propre sécurité, en s'appuyant sur les prescriptions réglementaires et sur les données médicales pour parfaire et entretenir ses connaissances.

CONCLUSION

Dans l'activité professionnelle, l'accident, l'intoxication, la maladie induite par le métier, ne sont pas des fatalités inéluctables, le tribut qu'il faudrait payer au progrès technologique, à l'amélioration des rendements... Les "risques du métier" sont pour la plupart mesurables et, fort heureusement, il est possible d'en limiter un grand nombre, voire d'en supprimer beaucoup.

Une formation technique de qualité ne peut, aujourd'hui, méconnaître les règles de sécurité : les outils, les machines, les produits disponibles sur le marché n'atteignent leur niveau élevé de performance que s'ils sont parfaitement maîtrisés, et donc correctement utilisés. Il en va des produits phytosanitaires comme des autres éléments techniques de production : bien les connaître permet de choisir le mieux adapté au traitement nécessaire, d'utiliser le matériel qui convient, dans les meilleures conditions météorologiques et avec le minimum de précautions indispensables. Nul doute que la sécurité se trouve ainsi renforcée, mais c'est aussi constater que rendement et sécurité ne s'opposent pas l'un l'autre, qu'ils peuvent la plupart du temps aller de pair si les formateurs intègrent systématiquement l'un à l'autre dans la formation qu'ils dispensent.

Cette approche pluridisciplinaire de la sécurité, la forme d'insistance que représente alors la répétition d'un même message au travers d'interventions techniques de nature différente auprès des utilisateurs de produits, s'inscrivent dans la perspective de formation continue indispensable pour faire avancer la sécurité, en agriculture plus encore que dans les autres types d'activités pour compenser les effets négatifs liés à la dispersion de la population concernée.

CONCLUSION

El accidente, la intoxicación, la enfermedad inducida por la actividad profesional no estan ineluctables fatalidades, no estan un tributo que pagar en contraparte del progreso tecnologíco, de la mejora de los rendimientos. Los riesgos acarreados por la actividad estan en mayor parte medibles y, por suerte, se puede disminuir su nombre, seguramente suprimir muchos de ellos.

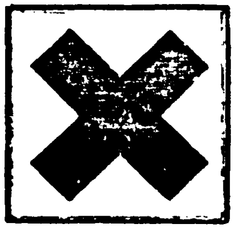
No hay, hoy, formación tecnica de cualidad sin verdadero conocimiento de las normas de securidad : para alcanzar sus mejores resultados, las hérramientas, las maquinas, todas las producciones disponibles se deben de ser perfectamente dominadas, es decir correctamente utilisadas.

Es lo mismo para los productos phytosanitarios como para todos los otros elementos tecnicos de producion : bien conocerlos permitte elegir el más adaptado al tratamiento necesario, utilisar los materiales adecuados en las mejores condiciones meteorologistas y con el minimum de precauciones indispensables. Sin ninguna duda, de esta manera, la securidad esta reforzada ; es también comprobar que rendimiento y securidad no se oponen, que pueden ir juntos si las personas encargadas de formacion integran sistematicámente uno al otra en sus enseñanzas.

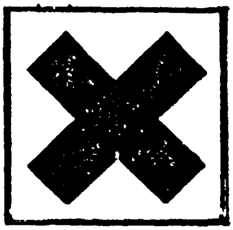
Esta manera de acercar la securitad respecto a una pluralitad de disciplinas, l'insistencia entonces representada por la repetición del mismo mensaje con ayuda d'intervenciones tecnicas de differente natura, todo esto deber ser incluido en la formación continua indispensable para que progresse la securidad en el sector de la agricultura aun más que en los otros sectores de actividad, para compensar las consecuensias negativas de la disperción de la población concenida.

T- Toxique

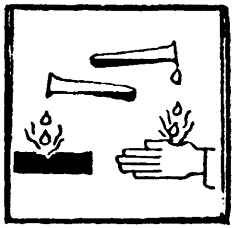
T+-Très toxique



Xn-Nocif



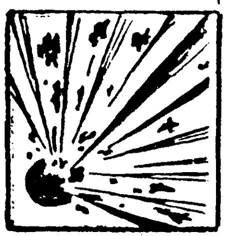
Xi-Irritant



C-Corrosif

F- Inflammable

O-Comburant



E-Explosif

Tableau 1

ACTIVITY	HAND EXPOSURE AS % OF TOTAL DERMAL EXPOSURE	RESPIRATORY EXPOSURE AS % OF TOTAL EXPOSURE
Drivers of tractors equipped whith canopies during air-blast spraying of citrus	41 %	0,029 %*
Bulk spray suppliers for above application	52 %	0,004 %*
Drivers of ordinary tractors during boom spraying of tomatoes	25 %	0,04 %**
Applicators using hand guns to aquatic weeds from airboat	47 %	None detectable**
Drivers of airboats for above application	89 %	None detectable**

D'après "Pesticide Assessment Guidelines"

U.S. Environmental Protection Agency

Subdivision U - WASHINGTON 1987

* WOJECK et al., 1982
** WOJECK et al., 1983

Tableau 2

MONITORAGE BIOLOGIQUE - CHROMATOGRAPHIE GAZEUSE - (Protocole Standard)		
MATIERE ACTIVE		ELEMENT RECHERCHE
<u>INSECTICIDES</u>		
- **Organophosphorés**		
Azinphos-éthyl	Sang	Le produit lui-même
Chlorfinvenphos	Urine	Acide 2,4 dichlorobenzoïque
Dichlorvos	Sang	Acide diméthylphosphorique
"	Urine	Acide diméthylphosphorique
Dicrotophos	Urine	Acide diméthylphosphorique
Diméthoate	Sang	Le produit lui-même
Fénitrothion	Urine	p. nitrophénol
Malathion	Urine	Acide malathion - Alpha-monocarboxylique
Mevinphos	Urine	Acide diméthylphosphorique
Monocrotophos	Urine	Acide diméthylphosphorique
Oxydéméthon-méthyl	Sang et Urine	Le produit lui-même
Parathion-éthyl	Sang	Le produit ou le paraoxon
"	Urine	p. nitrophénol
Parathion-méthyl	Sang	Le produit ou paraoxon-méthyl
"	Urine	p. nitrophénol
Trichlorfon	Sang	Le produit lui-même
- **Carbamates insecticides**		
Carbaryl	Urine	1 - naphtol
Propoxur	Sang	Le produit lui-même
"	Urine	2 - isopropoxiphénol

Tableau 3

- **Organo-chlorés**		
Aldrine	Sang	Dieldrine
Dieldrine	Sang	Le produit lui-même
Endrine	Urine	Anti-12 - hydroxyendrine
- **Pyréthrinoïdes**		
Cyperméthrine	Urine	Acide 2,2 - dichlorovinyl - 3,3 diméthyl cyclopropane - 1 - carboxylique
Perméthrine	Urine	
- HERBICIDES		
2,4 - D	Sang	Le produit lui-même
	Urine	"
Flamprop-isopropyl (propionate)	Urine	Acide N.benzoyl-N-(3 chloro-4.fluorophényl 1) - 2.amino-propionique
Flamprop -méthyl	Urine	
Paraquat	Plasma	Le produit lui-même
- FONGICIDES		
Pentachlorophénol	Plasma	Le produit lui-même
"	Urine	

D'après "Field surveys of exposure to pesticides - Standard protocol" (V.B.C/82.1)

W.H.O. - GENEVE 1982

Tableau 3 (suite)

A.C.T.A. - Index phytosanitaire
Association de Coordination Technique Agricole - PARIS - Edition annuelle

BONDERF J., BRETEAU M., CONSO F., DALLY S., EFTHYMIOU M.L., FURET Y ET JAMET P. - Les prinicipales intoxications aiguës par les pesticides : diagnostic, thérapeutique et prévention
Encycl. Méd. Chir. (PARIS - FRANCE). Intoxications, pathologie du travail - 16 001, L $_{10}$, 7-1988, 12 pages.

BOUGUERRA M.L. - Les pesticides et le tiers-monde
LA RECHERCHE - n° 176, Avril 1986, 546.563

C.E.E. - a) Directive 67/548/CEE du Conseil concernant le rapprochement des dispositions législatives, réglementaires et administratives relatives à la classification, l'emballage et l'étiquetage des substances dangereuses - 27 juin 1967 (J.O.CE n° 196 du 16.8.67), modifiée en dernier lieu par la directive 87/432/CEE du 3 août 1987 (J.O.CE n° 239 du 21.8.87).

b) Directive 80/1107/CEE du Conseil concernant la protection des travailleurs contre les risques liés à une exposition à des agents chimiques, physiques et biologiques pendant le travail. 27 novembre 1980 (J.O.CE n° 327/8 du 3/12/8C

c) Proposition de directives du Conseil relatives aux aux équipements de protection individuelle 88/c 141/03 14 avril 1988 (J.O.CE du 30 5 88 n° 141 14).

d) Proposition de directive du Conseil concernant les prescriptions minimales pour l'utilisation par les travailleurs d'équipements de protection individuelle sur le lieu de travail 88/c 161/01 - 11 mars 1988 (J.O.CE n°NC 161/1 du 20 6 88).

DALLY S. - Le point sur la toxicologie des insecticides
Xè symposium National de Médecine Agricole - TOURS - FRANCE, Avril 1983

DUBRISAY J., FAGES J., DELEMOTTE B. - Pathologie rurale professionnelle
Encycl. Méd. Chir. (PARIS-FRANCE). Intoxications, pathologie du travail - 18 538 A_{10}, 4.1986, 12 pages.

EFTHYMIOU M.L. - Données pratiques sur la toxicité des produits phytosanitaires, PPE Edit. PARIS 1978.

F.A.O. - Code international de conduite pour la distribution l'utilisation des pesticides. Organisation des Nations Unies pour l'alimentation et l'agriculture ROME 1986 - 19 pages.

FOULHOUX P., FOURNEX R. - Les tests biologiques au service de la sécurité chimique ROUSSEL-UCLAF Ed. PARIS 1988 - 19 pages.

FOURNIER E. et BONDERF J. - Les produits antiparasitaires à usage agricole Tech. et doc. LAVOISIER - PARIS 1983, 334 pages.

PORTOS J.L. et coll. - Le risque pesticide en agriculture ; La prévention du risque pesticide. Arch. Mal. Prof. 1987, 48, n° 6, 467-475 et 477-484.

SHAPIRO S. et FOSTER D. - Hazards to agricultural workers - Arizona Center for occupational Safety and Health - TUCSON-ARIZONA sept. 1980.

TEULON F. et CHALABREYSSE J. - Les contrôles toxicologiques en milieu professionnel leur importance et leurs limites. Arch. Mal.Prof. 1982, 43, n° 1, 11-19.

U.S Environmental Protection Agency - Pesticide Assessment Guidelines. Subdivision U. Applicator exposure monitoring - WASHINGTON 1987.

W.H.O. - Exposition aux pesticides : limites recommandées d'exposition professionnelle à visée sanitaire - Rapport n° 677 - GENEVE 1982.

W.H.O. - Field surweys of exposure to pesticides. Standard protocol - Pesticide development and safe use unit - Division of vector biology and control Organisation mondiale de la Santé - GENEVE (V.B.C/82.1)

W.H.O. - The WHO recommanded classification of pesticides by hazard and guidelines to classification - 1988-1989 - Organisation Mondiale de la Santé - GENEVE (V.B.C./88.953).

ZELLER H. - Toxicological studies on pesticides for protection of the users, consumer and the environment. Chem. Landwirtsch - 1980, 85-111.

THE ROLE OF FORMULATION IN THE SAFE USAGE OF PESTICIDES IN CROP PROTECTION

W. T. C. Holden
Formulations Department, Schering Agrochemicals Limited-Saffron Walden, Great Britain

SUMMARY

Formulation is the process of devising and developing compositions of active compounds suitable for use by the farmer or grower. The design of any formulation must take account of activity/crop selectivity, safety, application and registration requirements, cost, suitability for large scale manufacture, marketing requirements (image) and shelf life. The ideal formulation of any compound or mixture of compounds is the one providing the best balance of these factors for its intended use.

The paper will deal with two of these factors safety in use and selectivity to the crop. Safety aspects relate to manufacture, transport and storage, application, disposal of residues and packs plus effects on the environment.

The toxicity of any biologically active molecule cannot be materially altered by formulation. However, by selection of appropriate formulation systems and 'inerts', hazards can be minimised. The major means by which hazards can be reduced include (i) the use of non-flammable compositions, (ii) the development of formulation/pack systems which minimise operator contact and reduce risks which may arise through inhalation or dermal absorption of the compound (iii) the minimisation of residues in emptied packs and in sprayers.

Selectivity to the crop is again dependant on the selection of the formulation system and the inerts. There is often a close relationship between activity and crop effect. With many compounds the choice of solvent and surfactant is a critical one.

Most of the examples given in the paper will relate to formulations intended for application as hydraulic sprays. Current trends to the use of water-based and dust free formulations will be described. Reference will be made to the need to design formulations for use in closed filling systems which minimise operator contact.

More environmentally acceptable formulation/application systems are required in the future. The use of seed-treatments, granules and other non-sprayable systems will be described.

1 INTRODUCTION

Safety is of increasing concern in the usage of pesticides for all purposes. This short paper considers the role of formulation in the development of safe and selective pesticide products for use in crop protection.

2 FORMULATION

First we must define the term 'formulation' which is the process of devising and developing compositions of active compounds suitable for use by the farmer or grower. Whereas the synthetic organic chemist designs biologically active molecules it is the role of the formulation chemist to design or select formulation systems (compositions) for a particular active compound or mixture of active compounds.

Factors which influence the selection of a composition or formulation system include:-

i) the physical and chemical properties of the compound(s)
ii) activity/crop selectivity
iii) preferred means of application
iv) safety
v) registration requirements
vi) cost
vii) suitability for large scale manufacture
viii) shelf life

The important point in the selection and development of a formulation system for a given active compound is to obtain the best 'balance' of all these factors. A formulation which may be perfectly acceptable for use for example on cereals may be unsuitable for use on another crop.

Most products currently used in crop protection are applied by hydraulic spraying. Details of the more commonly used formulation types are given below:-

Emulsifiable concentrate	(EC)
Wettable powder	(WP)
Soluble powder	(SP)
Solution concentrate	(SL)
Suspension concentrate	(SC)
Oil in water emulsion	(EW)
Water dispersible granule	(WG)
Water soluble granule	(SG)

In addition granules and seed treatments are routinely used but only represent a small proportion of total pesticide usage in crop protection.

It can be seen from the summary set out below that in France which is representative of West European practice the majority of formulations currently used are of four well established types, EC's, WP/SP's, SL's and SC's.

Survey of crop protection products sold in France - Various types of formulation used for spray application (fungicides* and herbicides only)

	Number of products Fungicides	Herbicides
EC	27	129
WP/SP	291	106
SL	9	155
SC	89	110
EW	3	0
WG/SG	17/3	7/5
SE	1	2
SC/SL	0	2
SC/EC	1	0

Information taken from:- Index Phytosanitaire 1988

N.B. The list is the sum of the number of products available on the market and hence includes similar formulations of the same active materials from different companies.

* Products for application to the aerial parts of plants.

3 SAFETY ASPECTS

In the handling of pesticide products there are two basic problems. Firstly one must take account of the intrinsic toxicological properties of all the components of the formulation as well as the composition itself and the flammability/ignition hazards produced by these same mixtures.

These problems can be considered in four separate areas:-

1 Manufacture

2 Transport/storage

3 Application/operator exposure

4 Environmental contamination/crop residues

3.1 Manufacture

Manufacturing operations are carried out on specially designed formulation plants under defined conditions where there are appropriate exhaust extraction and decontamination facilities, plus direct access to specialist services. However, these operations involve the handling and storage of large quantities of some raw materials and finished products. It would not be uncommon for liquid formulations to be made in batches up to 10,000 litres in size. Again in the manufacturing situation operators may be working for considerable periods on a given product.

The main hazards relate to the direct toxic/irritation effects of the active materials or other formulants, the flammability of any solvents used and the ignition problems associated with the grinding of dry particulate formulations. These hazards must be considered and appropriate data generated as part of the overall development of the product. An example of a check list used before any formulated product can be manufactured on Schering Agrochemicals plant at Hauxton is given below.

PRODUCT HAZARD CHECKLIST - for use with new products or new raw materials in Production areas.

A. PHYSICAL PROPERTIES

Melting Point	....	Soluble in	....
Boiling Point	....	SG	....
Behaviour to 200°C	....	pH	....

B. FIRE/EXPLOSION/DECOMPOSITION HAZARDS

Liquids		Powders	
Flash Point	....	Flammable/combustible	....
LEL	....	MIE	....
UEL	....	Resistivity	....
Resistivity	....	SADT	....

Products of combustion/decomposition ....

Autoignition temperature ....

Has a summary report on fire/explosion/decontamination hazards for the material been issued?

....

C. TOXICOLOGICAL HAZARDS

Inhalational	LC_{50}	....	Skin Irritancy	....
Oral	LD_{50}	....	Skin Sensitisor	....
Dermal	LD_{50}	....	Mutagenic Effects	....

Has a summary report on toxicological hazards for the material been issued? ....

D. WASTE DISPOSAL

Note any special comments with regard to safe disposal of waste/effluent arising from the new operation :

The use of water based formulations or compositions containing high boiling solvents will reduce any fire risk. Wet grinding of SC formulations rather than dry grinding operations to produce WP's eliminates ignition hazard and reduces operator contamination.

3.2 Transport and storage

Crop protection products are stored on manufacturing sites, distribution stores and on the farm. They may be transferred by road, rail, sea and air. Transfer and storage are controlled by regulations both national and international.

The main hazards presented relate to:-

i) flammability
ii) corrosivity/effect on packaging materials
iii) direct toxic and environmental effects in the event of a fire, spillage or leak

Products may be transferred in bulk, but the product sold to the farmer or grower is the formulation in its final pack. This sales pack not only protects the product from deterioration but is the means by which the product is conveyed safely to the end user.

Exhaustive testing of the formulation and potentially suitable packs are carried out as part of the development process. It is vital that the formulation must not reduce the mechanical strength of the pack, affect the seal or permeate through the pack.

3.3 Application/operator exposure

My comments here relate to formulations intended for application by hydraulic spraying. The main requirement in safety terms is to minimise contamination of the operator in:-

i) the emptying of the pack
ii) filling of the sprayer
iii) and final decontamination of the sprayer and container

Formulations must be easy to reconstitute, measure and transfer to the sprayer. Mobile liquids and free flowing granules are the easiest types of formulation to handle. On dilution the formulation must present no problems of filter or nozzle blockage or leave residues inside the sprayer which are difficult to remove. Minimal effects on the materials of sprayer construction are essential. Formulations which present:-
EMPTYING, MIXING, BLOCKAGE OR DECONTAMINATION PROBLEMS MUST BE AVOIDED.

Dermal penetration of the active compound through the skin is the major route by which pesticides enter the body. Formulations can influence the penetration of both protective clothing and the skin itself. Solvent based products (EC's) tend to penetrate more readily but tend to present fewer dilution or blockage problems.

In the United Kingdom 'A Predictive Model for the Determination of Pesticide Spray Operator Exposure' is being developed. In the future it is proposed that the model should be used in the selection of formulation systems for new products.

The choice of pack is also important in minimising operator exposure. Recently containers constructed both of solvent resistant and non-solvent resistant polymers have been introduced to the market. These packs make for much easier pouring and rinsing than the tinplate and steel containers they have replaced.

Closed filling systems are under development which provide for minimum contact in the emptying and rinsing of the product into the sprayer. In future the properties of formulations must be so designed as to be compatible with this type of system. Mobile readily water miscible formulations are required.

Water soluble packs have been used for some time. Their wide adoption could give wettable powders which can present dust inhalation problems a longer lease of life.

3.4 Environmental contamination/crop residues

The persistence of a pesticide molecule in treated crops or in the environment is a function of the stability of the compound and the means of its application. The selection of the appropriate type of formulation provides for the desired level of biological control at the minimum application rate.

Application techniques other than hydraulic spraying, for example seed treatments and in furrow use of granules, provide for less environmental contamination. However, these techniques may not provide the degree of protection required throughout the life of the crop.

The choice of 'inert' or formulants must be made on the basis of providing for minimum or zero residues. In environment terms these components should either be inert e.g. a clay filler or readily degraded on the crop or in the soil.

The use of surfactants and solvents in pesticide formulations is regarded in some quarters as an area of concern. If we consider typical EC and SC formulations then at a rate of 1 litre per hectare the following rates of surfactant and solvent would be applied.

i) <u>400 g/litre EC</u>

Surfactants (emulsfiers)	100-150 g
Solvents (aromatic hydrocarbon)	500 g

ii) <u>500 g/litre SC</u>

Surfactants (wetting and dispersing agents)	50-70 g

If we were to assume that these surfactants and solvents were distributed to the top 10 cm of soil in the treated 1 hectare area and the wt/ml of the soil was 1.0, then the residue levels would be in the range 0.05 - 0.5 ppm. Solvents are volatile and both aromatic solvents and the commonly used surfactants are biodegradable. Perhaps the issue of ground water contamination is a minor one, as far as the 'inert' components of pesticide formulations are concerned.

Pesticide residues in 'empty' containers present considerable problems and this issue will be addressed in the final section of my paper.

4 SELECTIVITY (CROP SAFETY)

The whole basis of crop protection is one of selective toxicity, the objective is to control pathogens, insects or weeds without significant effect on the crop.

As part of routine formulation development of a new compound the influence of formulation type and formulants on its activity and selectivity is examined in detail. The benefits of using adjuvants are also examined. In the development of prochloraz formulations, for example, several hundred compositions were examined as part of the development process.

Activity and crop effects are related. A change in formulation type may just alter the level of activity/selectivity but in some cases useful improvements can be made in selectivity.

In general terms WP's are often safer to crops than SC's which in turn are safer then EC's. For a given type of formulation the selection of surfactant or solvent may be critical. In the development of amitraz EC for use on top fruit the choice of surfactant is important.

Safety or regulatory consideration may rule out the use of a particular solvent or other inert which could result in the adoption of a less active formulation.

5 FUTURE TRENDS IN THE DEVELOPMENT OF SAFER AND MORE SELECTIVE FORMULATIONS

The major safety problems which can be influenced by formulation are as follows:--

1	flammability or other ignition hazards		
2	toxic/irritant effects	-	permeation of protective clothing and skin
		-	adoption of dust free formulations
3	Environmental issues	-	container disposal selection of biodegradable inerts

These safety requirements must always be considered in the light of providing effective and selective products. Formulation studies will continue to be focussed on the need to provide the most appropriate composition for a given crop/pest situation. As mentioned earlier it is the overall balance of factors that is important in the selection of a formulation system.

Efficiency and safety present conflicting requirements at times. Solvent based formulations may provide a means of using lower doses of active material but at the safety cost of incorporating combustible if not flammable solvents into the composition. This may in turn increase the risk of penetration of the active compound through the skin.

Container disposal is a vexed question. It is not enough to state 'DISPOSE OF SAFELY' on the label. Rigid containers do present problems. However closed fill systems should minimise operator contact, and provided the rinsing system and the formulation are mutually compatible, residue problems in the emptied container would be virtually eliminated.

For the present time the formulation chemist must continue to develop two different types of formulation system:-

1 Liquid systems
- of low or zero solvent content
- low dermal toxicity
- mobile and easily rinsed

SC's, EW and melt dispersions are examples of this type of system. Microemulsions and oil dispersions may be useful.

2 Solid particulate systems - dust free readily dispersible formulations
WG's, tablets.
WP's in soluble packs.

With the liquid products rigid packs will continue to be used in the immediate future. Particular emphasis will be placed on emptying and rinsing in the design of both the formulation and the pack.

For solid formulations other than those in water soluble packs, it is important that the packs can be emptied and rinsed completely. Non-rigid packs can be used and as when emptied they occupy a relatively small volume, the problems of container disposal are reduced.

To avoid problems in handling and application it is important that products of high and consistent quality are provided. The physical properties must be designed to permit safe and easy handling and there should be no significant impairment of the properties on storage.

Where active materials are available which can provide the appropriate degree of control without recourse to the use of hydraulic spraying, then the opportunity must be taken to develop more environmentally friendly formulations and application systems. Here I am thinking particularly about seed coatings and granular treatments.

To conclude, the challenge faced in formulation is to provide safer products and at the same time ensure that they are selective and effective in use. It would appear that there is at least a few more years work ahead for formulation chemists engaged in pesticide work.

GLOSSARY OF TERMS

1 Abbreviations relating to Fire/Explosion/Decomposition Hazards

LEL	-	Lower Explosive Limit
UEL	-	Upper Explosive Limit
SADT	-	Self Accelerating Decomposition Temperature.

2 Description of Formulation Types

Code descriptions taken from 'Catalogue of Pesticide Formulation Types and International Coding System" (GIFAP Technical Monograph No.2).

1 For dilution with water

Aqueous Concentrate (SL)
Water Soluble Powder (SP)
Emulsifiable Concentrate (EC)
Emulsion Concentrate (O/W) (EW)
Wettable Powder (WP)
Water Dispersible Granules (WG)
Suspension Concentrate (SC)
Micro-Emulsion/Miceller Dispersion

Oil Dispersion
Suspo-Emulsion (SE)
EC/SC - dispersion of solid active in EC type system
SC/SL - SC in which a water soluble pesticide is dissolved in the aqueous phase

2 Special spray formulations

ULV systems (UL)
Aerosols (AE)
(+ fogs and mists)

3 Products not applied by spraying techniques

Granules (GR)
Dusts
Baits
Seed Treatments (powders, flowables, solutions, slurries)

Report on the Session 5: THE SAFETY OF PESTICID'S USE

R. Bailly
ACTA, Association de Coordination Technique Agricole-Paris, France

La sécurité d'emploi des pesticides est une nécessité.

Une nécessité qui concerne tous les partenaires du phytosanitaire tant cette utilisation pour être convenable doit faire l'objet de vigilance: au niveau de l'industriel qui fabrique, formule, teste, les produits phytosanitaires, au niveau des médecins qui sont à même de prévenir les risques liés à l'emploi de ces produits, enfin le prescripteur qui doit connaître au mieux (dans la mesure du possible) l'écotoxicité des pesticides qu'il va recommander.

Cette session reflète cette indispensable synergie entre les différentes catégories professionnelles. Chacune à son niveau est susceptible d'informer correctement l'utilisateur et ainsi de le sensibiliser.

Une bonne application de produit phytosanitaire est une application sûre.

Tous les participants à cette session ont insisté sur le besoin de s'interroger sur les risques que l'on peut faire apparaître par des utilisations peu judicieuses.

En effet, il s'est avéré que si des efforts importants de recherche ont été déployés, tant dans les organismes publics que dans les firmes phytosanitaires pour évaluer la toxicité et l'écotoxicité des produits proposés, ces évaluations sont faites à partir de modèles, souvent d'ailleurs avec des coefficients de sécurité. Or les chercheurs savent que les modèles ne sont qu'une représentation partielle des phénomènes biologiques ou écologiques.

Il reste donc nécessaire d'affiner les méthodologies et d'avoir des réseaux de surveillance de l'emploi des produits phytosanitaires: surveillance médicale, surveillance du gibier, des nappes phréatiques, etc.

Autre point abordé au cours de la session, l'élimination des emballages de produits. Il faut reconnaître que rares sont les Pays qui disposent de méthodes satisfaisantes d'élimination de ces emballages.

Enfin, et c'est sûrement le point majeur ressortant des interventions et des discussions, la nécessité d'informer et d'éduquer les utilisateurs dans le cadre de programme de formation ne faisant pas apparaître les consignes de sécurité comme des contraintes mais plutôt comme partie intégrante de l'outil technique performant qu'est devenue la protection des cultures.

SESSION 6

QUALITY ASSESSMENT OF BIOLOGICAL CONTROL AGENTS

Chairmen: J. P. Aeschlimann
D. G. Casanova

ON THE IMPORTANCE OF ASSESSING THE QUALITY OF BENEFICIAL ORGANISMS MASS-PRODUCED FOR USE IN BIOLOGICAL CONTROL PROGRAMMES AGAINST NOXIOUS PLANTS AND ANIMALS

J. P. Aeschlimann
CSIRO, Biological Control Unit-Montpellier, France

SUMMARY

Recent progress in the discipline of biological control against pest species demonstrates that the quality of the beneficial organisms used has to be assessed during the production process. For each biological control agent, all available biotypes should be kept separate, identified biochemically as far as possible, and compared under adequate rearing conditions as to their efficiency. The most promising should then be selected for mass-propagation, based on its potential efficiency in the field.

Recent examples are provided that show the importance of a continuous assessment of the biological control agents mass-reared for field use.

Concern about a possible deterioration in the quality of mass-reared organisms has repeatedly been expressed over the years, especially by entomologists in charge of rearing units. As regards arthropod species, initial steps to develop a set of procedures aimed at measuring the "quality" of insects and mites maintained under artificial conditions were made more than 10 years ago (BOLLER & CHAMBERS, 1977), and during the last decade, the need for some form of quality control in large rearing facilities has been widely recognized. Agreement has been reached on an adequate terminology, and the many variables needed to assess the quality of insect species mass-reared in the laboratory have been precisely identified and described ; as a consequence, a number of tests which measure the overall performance of the cultured species have been incorporated in most important routine rearings (KING & LEPPLA, 1984 ; MOORE *et al.*, 1985).

Experts in mass-producing beneficial organisms for use in the biological control of pest species face similar problems, but somehow seem to have overlooked these recent advances. The importance of thorough quality assessment is best demonstrated *a contrario* by the absence of commercial success for biological control preparations that lacked any decent efficiency. It suffices to remember the experience in the United States for instance with nematode preparations reportedly offered sometimes for sale in a very precarious condition to realize that successful commercialization requires highly effective quality assessment.

As far as microbial products are concerned, the state of the art in the field of quality control is extremely well covered in the review by RIBA (1988). In terms of controlling the quality of macroorganisms mass-produced for biological control purposes, the present situation is basically not different. The first meeting specifically devoted to examining the principles of quality assessment of mass-reared parasitoids and predators of pest species was convened in July 1988 at Vancouver (Canada) by the IOBC Global Working Group on Quality Control. On this occasion, it was noticed with interest that both the official research stations and the private producers of biological control agents had in fact already developed various tests to check the quality of their cultures. Other meetings are now due to follow, that will be open to everyone concerned, and from which adequate and generally acceptable guidelines are expected to emerge.

To date, specialists have established standards for the various traits of mass-reared arthropod species and for each trait, checks are carried out to ensure that the end product conforms to specified standards. Traits have been chosen to adequately reflect the overall success of the production process on the one hand, and the overall performance of the living material produced under the relevant rearing system on the other. The whole set of procedures now constitutes a proper quality assessment that is dealt with for instance by VACANTE (1988) for some natural enemies used against greenhouse pests, whereas WAJNBERG (1988) discusses some of the problems encountered in mass-producing *Trichogramma* spp. (Hym.: Trichogrammatidae) for field release.

In addition to considering overall rearing success and performance, it is also of foremost importance to the research workers involved in biological control of pests to carefully select particular biotypes of a beneficial agent. We would like to make the point here, that the efficiency of a range of biotypes should be compared before release, of which the most efficient and best fitted only will be mass-reared ; isozyme analysis has proved very helpful in characterizing the different biotypes during the selection process, and its use should be generalized. It is felt that this aspect has been somewhat neglected this far, and two recent examples of biological control will serve to illustrate this point.

(a) It has been implicitly suggested by many authors that biological control agents are able to adapt to their target host regardless of their own exact provenance, in particular for a weevil like *Rhinocyllus conicus* FRÖHLICH (Col.: Curculionidae) which attacks a very broad spectrum of thistle species under a large range of climatic conditions. In fact, this species has been used successfully to control introduced carduines in North America and New Zealand, but several biotypes failed to establish when they were released on thistle species that differed from the original host plant, both in North America (ZWÖLFER & PREISS, 1983), and in South Africa.

(b) Other examples of wrongly selected beneficial organisms may be found in the genus *Microctonus* (Hym.: Braconidae), several species of which have been deliberately introduced into

North America in an attempt to control weevils of the genera *Hypera* and *Sitona* noxious to leguminous crops. In several instances the releases were either complete failures or have led to substantial rates of parasitoid eggs being encapsulated in the host hemocoele. Detailed investigations have revealed that in the case of *M. aethiopoides* LOAN, several biotypes may occur sympatrically in slightly different ecological niches and infest closely related host species (AESCHLIMANN, 1983 a). Recent releases in Australia have confirmed that of a range of *M. aethiopoides* biotypes, only one was adapted to the particular host species (*S. discoideus* STEPHENS) and is thriving under the same set of climatic conditions (AESCHLIMANN, 1983 b).

To conclude, research workers dealing with inundative releases of biological control agents would greatly benefit from introducing quality assessment in their rearing process. Also, it is recommended to carefully select between all available biotypes before embarking on mass-culturing a beneficial organism and this step should become part of the current quality assessment practice.

RESUME

De l'importance des contrôles de qualité chez les organismes bénéfiques utilisés en lutte biologique contre les espèces déprédatrices

Des expériences récemment enregistrées dans le cadre de la lutte biologique contre les plantes et animaux nuisibles montrent que la qualité des auxiliaires bénéfiques utilisés doit être évaluée au mieux au cours des élevages pratiqués sous conditions artificielles. Pour chaque espèce bénéfique, il est recommandé de comparer en parallèle une série de biotypes maintenus en élevages séparés. L'analyse approfondie du potentiel biotique des différentes souches, accompagnée d'une différenciation isozymique permettra de choisir la plus appropriée.

L'importance des contrôles de qualité peut être démontrée *a contrario* par l'absence de succès commercial rencontré par certaines préparations biologiques pourtant dûment homologuées. En fait, les responsables d'élevages massifs de diptères par exemple ont défini et introduit depuis longtemps

dans leur pratique courante de très stricts contrôles de qualité. Les producteurs et distributeurs d'agents de lutte biologique devraient s'inspirer de ces récents développements.

ACKNOWLEDGEMENTS

I would like to thank D.T. BRIESE (Montpellier) for his highly valuable comments on early stages of this article.

REFERENCES

AESCHLIMANN, J.P. - 1983 a. Notes on the variability of *Microctonus aethiopoides* LOAN (Hymenoptera: Braconidae: Euphorinae). - Contrib. Amer. Ent. Inst. 20: 329-335.

AESCHLIMANN, J.P. - 1983 b. Sources of importation, establishment and spread in Australia, of *Microctonus aethiopoides* LOAN (Hymenoptera: Braconidae), a parasitoid of *Sitona discoideus* GYLLENHAL (Coleoptera: Curculionidae). J. Aust. ent. Soc. 22: 325-331.

BOLLER, E.F. & CHAMBERS, D.L. (eds.) - 1977. Quality control. An idea book for fruit fly workers. - IOBC/WPRS Bulletin 1977/5: 162 pages.

KING, E.G. & LEPPLA, N.C. (eds.) - 1984. Advances and challenges in insect rearing. - USDA/ARS: 306 pages.

MOORE, R.F., ODELL, T.M. & CALKINS, C.O. - 1985. Quality assessment in laboratory-reared insects. In : SINGH, P. & MOORE, R.F. (eds), Handbook of insect rearing. 1. - Elsevier Sci. Publ., Amsterdam : 107-135.

RIBA, G. - 1988. Les contrôles de qualité des préparations microbiologiques entomopathogènes. - Parasitis (this issue).

VACANTE, V. - 1988. Perspectives et limites des méthodes de protection biologique et intégrée des cultures d'aubergines sous serre en Italie. - Parasitis (this issue).

WAJNBERG, E. - The problem of superparasitism in the production of natural enemies for inundative biological control : A genetical approach. - Parasitis (this issue).

ZWÖLFER, H. & PREISS, M. - 1983. Host selection and oviposition behaviour in West-European ecotypes of *Rhinocyllus conicus* Froel. (Col., Curculionidae). - Zeits. ang. Entomol. 95: 113-122.

LES CONTROLES DE QUALITE DES PREPARATIONS MICROBIOLOGIQUES ENTOMOPATHOGENES

G. Riba
Station de Recherche de Lutte Biologique, INRA-Guyancourt, France

G. Croizier
Station de Recherches de Pathologie Comparée, INRA-CNRS-Saint Christol-les-Alès, France

RESUME

Plusieurs souches de micro-organismes entomopathogènes ont déjà été commercialisées ou sélectionnées à des fins commerciales.

Afin de définir et de comparer les performances de telles préparations biologiques, il convient d'effectuer un certain nombre de contrôles de qualité dans le but de maintenir ou améliorer la stabilité génétique de la souche, la persistance de ses potentiels étiologique et épidémiologique et d'éliminer les éventuelles contaminations microbiennes. A cette fin les souches clonées seront identifiées par des critères pathogéniques et biochimiques faisant appel aux techniques de caractérisation moléculaire du génome. En outre, une attention particulière sera accordée aux risques d'apparition de sous-populations résistantes de l'insecte-cible.

Le développement économique de ces produits dépend de la précision et de la rigueur des contrôles de qualité qui, seuls, garantiront leur efficacité dans des limites agronomiques et environnementales connues.

1 INTRODUCTION

En 1988 le marché des insecticides microbiens reste très largement dominé par les préparations à base de **Bacillus thuringiensis.** Ce germe agit par l'intermédiaire d'une endotoxine cristalline, il n'a pas de potentiel épidémiologique et de ce fait représente un cas très particulier d'entomopathogènes.

Par contre, bien qu'il n'y ait pas encore commercialisation importante de préparations entomopathogènes virales, fongiques ou microsporidiennes, dans chaque cas, plusieurs applications expérimentales sont suffisamment encourageantes pour penser que cet objectif sera atteint dans un proche avenir.

Il est donc urgent de se préoccuper des contrôles de qualité que devront subir ces préparations microbiennes au cours de leur synthèse ou de leur commercialisation. Pour simplifier notre exposé nous considèrerons que le germe a été sélectionné et que ses formulations ont été définies. De plus, nous considèrerons que la préparation insecticide a satisfait aux exigences des Commissions de Toxicologie et d'Homologation.

Les contrôles de qualité d'un produit microbiologique sont rendus nécessaires pour définir sa compétence (par l'établissement d'une relation entre la dose et l'efficacité), pour positionner un produit par rapport aux concurrents. C'est dire que ces contrôles exigent l'établissement de références définies par des normes et estimées par les techniques les plus performantes et les plus appropriées. Ces contrôles auront pour but de vérifier :

- la stabilité génétique de l'agent sélectionné, multiplié puis commercialisé ;

- la persistance de son potentiel étiologique et épidémiologique ;

- les éventuelles contaminations microbiennes de la préparation.

2 STABILITE GENETIQUE

L'identité d'un micro-organisme ne peut être connue aussi précisément que ne l'est la structure atomique d'une molécule. Néanmoins, la découverte récente de marqueurs moléculaires tout en permettant une analyse génétique fine de l'organisme complète utilement les critères systématiques, morphologiques, antigéniques et pathogéniques déjà utilisés.

2.1. Caractérisation des souches bactériennes

B. thuringiensis, B. sphaericus, B. popilliae ou **B. moritai** sont les candidats de choix à la lutte microbiologique contre les insectes ravageurs des cultures ou vecteurs de maladies humaines.

La caractérisation des souches fait appel à des critères pathogéniques et biochimiques.

2.1.1. Critères pathogéniques

Le pouvoir pathogène des souches de **B. thuringiensis**, bactérie entomopathogène la plus commercialisée, repose sur la notion de spectre d'hôtes et surtout sur une estimation de la virulence des souches sélectionnées à l'égard d'insectes-test.

Le spectre d'hôte.

Il fallut attendre 1977 pour que le spectre d'hôte de **B. thuringiensis** ne se limite plus seulement à l'ordre des Lépidoptères. Cette année là, en effet, GOLDBERG et MARGALIT découvrirent une nouvelle souche capable d'infecter des larves de Culicides ou Simulides. Suscitant un extraordinaire engouement pour de nouvelles prospections plus larges, cette découverte fut suivie par celle d'une souche capable de contaminer des larves de Coléoptères (KRIEG et al., 1983) ; en 1980 et 1984, PADUA et al. isolaient de nouvelles souches à partir de Culicides.

Chaque souche, grâce à des tests biologiques de laboratoire peut donc être caractérisée par les espèces d'insecte qu'elle affecte et sa virulence à l'égard de certaines d'entre elles.

Le titrage biologique et la notion d'étalon.

La virulence de chaque souche commercialisée est exprimée lors d'essais biologiques par rapport à un étalon standard. Le titrage biologique limite les variations dues à l'expérimentateur ou provoquées par des modifications génétiques ou épigénétiques de la sensibilité de l'insecte-test à la maladie peut sensiblement varier au cours de l'élevage. De plus, cette technique universalise les données ce qui permet une comparaison des résultats obtenus par différents laboratoires.

L'activité insecticide du produit testé est exprimée par son rapport à l'efficacité du standard selon la formule ci-dessous :

$$\frac{\text{LC 50 standard x titre du standard}}{\text{LC 50 produit}}$$

Le premier standard fut déposé à l'Institut Pasteur en 1961, il est issu du sérotype H1 de **B. thuringiensis.** L'insecte de référence est le pyralide **Ephestia küehniella** (BONNEFOI et al., 1958 ; BURGERJON, 1959). Le titre de l'étalon fut arbitrairement fixé à 1000 unités toxiques internationales. Les collègues américains qui développaient l'utilisation d'une autre souche appartenant au sérotype H3 ont défini un autre standard : HD1-S-1971 qui recommande **Trichoplusia ni** (Lép. Géométride) comme insecte-test. A ce nouveau standard, plus toxique que la référence française, fut affecté le titre de 8000 ITU/mg de produit.

Bien sûr, l'isolement de souches uniquement pathogènes pour des larves de Diptères vecteurs exigea la création de nouveaux standards dont l'IPS 82 actuellement en service en France. Il fut constitué à partir de la souche 1884 du sérotype H14. Son titre est de 15000 ITU/mg sur larves de quatrième stade de **Aedes aegypti** (de BARJAC et LARGET-THIERY, 1984 ; THIERY, 1986).

Des démarches similaires sont à l'origine du standard SPH 84 de **B. sphaericus.** Cet étalon est constitué à partir de la souche 2297 du sérotype H25 qui fut découverte par WICKBEMESINGHE et MENOIS (1980) et dont l'activité culicide est élevée (KALFON et al., 1983). Le titre de ce standard très stable fut fixé à 1500 ITU/mg sur larves de **Culex pipiens** (THIERY, 1986).

Standardisation des méthodes.

La stabilité d'un étalon doit être la plus grande possible ou pour le moins évaluée dans le temps. Elle est le fondement de la reproductibilité des estimations de l'activité des préparations industrielles à base de **B. thuringiensis** ou **B. sphaericus.**

Par ailleurs, le titrage biologique ne prend toute sa valeur que s'il s'accompagne du maintien de la qualité des insectes-tests utilisés. Cette exigence requiert une standardisation de la technique d'élevage qui fournit des populations larvaires homogènes et synchrones, telle que celle proposée par LARGET (1980) pour **Aedes aegypti.**

2.1.2. Critères biochimiques

La classification des souches de bacilles entomopathogènes fut établie sur la base des antigènes flagellaires H (de BARJAC et BONNEFOI, 1962, 1967). On connaît aujourd'hui 25 sérotypes et 33 biotypes de **B. thuringiensis** et une variété **"wuhanensis"** qui ne possède pas de flagelle. Actuellement l'ensemble des souches de **B. sphaericus** se répartissent en 47 sérotypes dont 6 seulement incluent des souches pathogènes d'insecte.

Très pratique, couramment utilisée et universelle, cette classification n'est pas correllée à la pathogénicité des souches. Ainsi, la souche PG14 du sérotype H8 (PADUA et al., 1982) est culicide mais ne peut attaquer les espèces de Coléoptères qui sont par contre sensibles à la souche 256-82 du même sérotype, elle-même incapable d'attaquer une larve de moustique. Cette remarque justifie la recherche de marqueurs biochimiques complémentaires. Ainsi, KRYCH et al. (1980) proposèrent de classer les souches de **B. sphaericus** en fonction d'une estimation des homologies des ADN chromosomiques. De même, il semble que les souches de **B. thuringiensis** soient bien discriminées par leur contenu plasmidique respectif.

Les souches de **B. thuringiensis**, et probablement celles de **B. sphaericus**, tuent les insectes par l'intermédiaire de toxines incluses dans un cristal protéique pro-toxique dont l'analyse peut contribuer à caractériser les souches. Ainsi, par électrophorèse en gels natifs ou dénaturants de la toxine entière ou hydrolysée, on peut obtenir une idée du nombre et de la taille des protéines synthétisées par la souche. Cette analyse peut être approfondie par une approche sérologique. En effet, les entomotoxines de **B. thuringiensis** peuvent être grossièrement réparties en 4 groupes : toxines actives sur les Lépidoptères à l'exclusion des Noctuides ; toxines actives sur les Noctuides ; toxines des Culicides et Simulides et protéines toxiques pour les Coléoptères. Les anticorps monoclonaux permettent aujourd'hui de séparer ces différentes protéines qui, chacune, possède un nombre variable d'épitopes spécifiques.

2.2. Caractérisation de souches virales

Les Baculovirus, strictement inféodés aux Arthropodes et plus précisément aux Lépidoptères, sont, pour l'instant, les candidats de choix pour la lutte microbiologique contre certaines tordeuses ou Noctuelles. Ils sont identifiés et classifiés grâce à des critères morphologiques, pathogéniques et biochimiques.

2.2.1. Systématique.

On distingue généralement 3 sous-genres au sein du genre Baculovirus. Les plus connus d'entre eux sont les virus du groupe A dont les virions, responsables de polyèdroses nucléaires, sont inclus dans un polyèdre protéique. Chaque polyèdre contient plusieurs virions infectieux eux-mêmes entourés individuellement (SNPV de **Bombyx mori**) ou collectivement (MNPV d'**Autographa californica**) d'une enveloppe.

Le groupe B se distingue du précédent par le fait que chaque polyèdre ne contient qu'un seul virion lui-même ne comprenant qu'une seule nucléocapside. Enfin, le groupe C est constitué de virions qui ne sont jamais inclus dans un polyèdre. Le plus célèbre de ces baculovirus non inclus est celui d'**Oryctes rhinoceros** déjà utilisé dans des programmes de lutte biologique.

2.2.2. Critères pathogéniques.

BURGERJON (1977) fut le premier à signaler la spécificité parasitaire de souches de Baculovirus. De ce fait, cette propriété peut servir à classifier et reconnaître différents isolats viraux soit en comparant leur virulence à l'égard de quelques espèces d'insectes-tests, soit en estimant leur spectre d'hôte. L'existence de tests standardisés, par pulvérisation contrôlée (BURGERJON, 1956) ou par dépôt de gouttelettes de suspension virale titrée sur une feuille de téflon (HUGHES et WOOD, 1981) permet une estimation rapide de la virulence des souches. Le spectre d'hôte des souches de Baculovirus est très variable puisque certaines d'entre elles, comme le NPV d'**Autographa californica** attaquent un grand nombre d'espèces d'insectes, alors que d'autres, telle le NPV de **Spodoptera littoralis**, ne sont capables d'infecter qu'un nombre limité d'insectes.

2.2.3. Critères moléculaires.

La caractérisation biochimique des souches de Baculovirus est basée sur le polymorphisme soit des protéines de structure, soit de l'ADN.Les analyses électrophorétiques révèlent jusqu'à 90 polypeptides (SINGH et al., 1983). Performante et utile pour le rapprochement taxonomique de souches, cette technique a des limites définies d'une part par le risque de modifications post-traductionnelles, d'autre part par la relative conservation des protéines des virions ou du corps d'inclusion comme le montrent les études de MERDAN et al. (1977) ou de CROIZIER et CROIZIER (1977). Les cartes peptidiques établies par électrophorèse bidimensionnelle à haut voltage d'hydrolysats trypsiques des protéines du corps d'inclusion sont plus discriminantes (MARUNIAK et SUMMERS, 1978). Les techniques immunochimiques, par leur rapidité et leur spécificité, constituent une alternative d'analyse intéressante (MAZZONE et TIGNOR, 1978) d'autant plus efficace qu'aujourd'hui des anticorps monoclonaux encore plus spécifiques ont été obtenus (ROBERTS et NASER, 1982), des western-blots ont été réussis (S-MITH et SUMMERS, 1981). Cependant, HOMMANN et FAULKNER (1983) ont montré, grâce à la technique ELISA, que des anticorps monoclonaux de différentes souches présentaient des réactions croisées.

Finalement, les cartes de restriction de l'ADN par des endonucléases restent la technique la plus performante. En multipliant les enzymes utilisées (il en existe plus de 200) on peut considérer que chaque souche a un profil unique (NATHANS et SMITH, 1975). Des souches très proches peuvent ainsi être discriminées (SMITH et SUMMERS, 1979) fussent-elles des recombinants (SUMMERS et al., 1980).

2.3. Caractérisation des champignons entomopathogènes

Seule la multiplicité des critères morpho-systématiques, pathogéniques et biochimiques permet une caractérisation suffisamment précise des champignons entomopathogènes.

2.3.1. Systématique

Par définition, l'espèce est bien délimitée pour les organismes dont la sexualité est connue. Par contre, pour les Hyphomycètes entomopathogènes se pose d'une part le problème du fondement biologique d'organismes regroupés sous la même espèce uniquement par des critères onto-morphogéniques, d'autre part celui des relations phylogénétiques entre les différents genres et espèces décrits. Il serait en effet satisfaisant de comprendre l'évolution qui permit à des espèces appartenant à un même genre, par exemple **Verticillium** ou **Paecilomyces fumoso-roseus**, de coloniser saprophytiquement le sol, de parasiter des plantes, des insectes ou des nématodes. Ces interrogations justifient la recherche de critères biochimiques qui contribueront à une meilleure connaissance biosystématique de ces germes.

2.3.2. Critères biochimiques

Le polymorphisme isoenzymatique est suffisamment important pour permettre un regroupement des espèces.

LATGE et BOUCIAS (1984), par une étude comparée de profils électrophorétiques de souches de **Conidiobolus obscurus**, ont distingué 3 groupes majeurs dans cette espèce hétérogène. De même, une étude approfondie de la variabilité isoenzymatique de 86 souches de **M. anisopliae** placées en conditions de culture identiques (RIBA et al., 1986) définit des sous populations même au sein des isolats de la variété **anisopliae.**

Cette technique, suffisamment performante pour être utilisée à l'étude du polymorphisme génétique de populations fongiques (POPRAWSKI et al., 1988), n'est pas suffisante pour une caractérisation précise des souches car beaucoup d'isoenzymes sont influencées par les conditions et antécédents culturaux. Il en est de même pour l'étude comparée des antigènes somatiques ou métaboliques qui contribue néanmoins à la caractérisation de souches de **B. tenella, B. bassiana** ou **M. anisopliae** (FARGUES et al., 1974, 1975).

Aujourd'hui par l'exploitation de la variabilité des RNA ribosomiques S5 (RAKOTONIRAINY, 1988) ou par l'analyse du polymorphisme des RFLP (Restriction Fragment Length Polymorphism) on peut espérer une définition stable et plus précise de chaque souche de champignon entomopathogène.

2.3.3. Critères pathogéniques.

Spécifiques (FERRON et DIOMANDE, 1969 ; FARGUES et REMAUDIERE, 1977) les souches des champignons entomopathogènes peuvent être caractérisées par leur spectre d'hôtes ou (et) leur agressivité à l'égard de quelques espèces-cibles. A cette fin, furent mis au point des tests standardisés de pulvérisation de suspension conidienne à la surface de larves du même âge traitées le lendemain d'une mue pour éviter l'élimination prématurée de l'inoculum lors de l'exuviation (FARGUES et VEY, 1974).

3 PERSISTANCE DU POTENTIEL ETIOLOGIQUE ET EPIDEMIOLOGIQUE

L'efficacité d'un agent microbien entomopathogène peut être perçue à court terme dans le cas d'un traitement inondatif induisant un effet choc, ou à plus long terme si l'on cherche l'induction d'une épizootie. Selon le cas, les performances du germe dépendront de sa virulence, de son adaptabilité à l'environnement et (ou) enfin des réactions des populations de l'insecte-cible. Pour minimiser ces risques il convient donc de s'assurer que l'agent n'a pas subi de modifications génétiques au cours de la multiplication, et, d'autre part, que la population cible reste sensible.

3.1. Les bactéries entomopathogènes

3.1.1. Stabilité des souches.

L'activité insecticide de **B. thuringiensis** ne dépend en fait que de la toxine cristalline dont les gènes sont soit portés par l'ADN chromosomique, soit sur un plasmide. Les conditions standardisées et stériles de fermentation liquide garantissent une production stable et homogène de bactéries infectieuses, sous réserve que l'on se soit au préalable assuré de l'absence de bactériophages dont plusieurs ont déjà été identifiés.

3.1.2. Résistance de la population cible

L'efficacité du traitement ne dépend donc, comme avec un insecticide chimique, que de la sensibilité de la populatoin cible. Difficile à induire (DEVRIENDT et MARTOURET, 1976), la résistance à **B. thuringiensis** fut cependant observée par Mc GAUGHEY (1985) dans la population de **Plodia interpunctella** d'un silo à grains plusieurs fois traité par **B. thuringiensis**, ainsi que dans des populations de moustiques (GOLDMAN et al., 1986).

Ce risque est suffisamment élevé pour inciter à une modification des stratégies de création d'exploitation de plantes transgéniques ayant reçu le gène du peptide toxique de **B. thuringiensis** (VAECK et al., 1986). En effet, on cherche maintenant l'introduction et l'expression simultanée de plusieurs gènes toxiques de façon à réduire la probabilité d'apparition d'une population résistante, tandis que les agronomes et les entomologistes devront définir les meilleures techniques culturales.

3.2. Les virus entomopathogènes

3.2.1. Stabilité de la souche

De nos jours encore la technique la plus prometteuse de production des Baculovirus à des fins agronomiques consiste en la multiplication in vivo du germe à partir d'élevages automatisés de l'insecte-hôte. Au cours de ce processus, il peut y avoir modification du génome viral ou substitution de la souche sélectionnée par une souche virale latente. Dans les deux cas, le contrôle régulier de la carte de restriction de la souche produite permet de repérer toute modification.

HINK et VAIL (1973) découvrirent des variants FP (Few Polyhedra) apparus spontanément au cours de la réplication in vitro du virus de la polyèdrose nucléaire du NPV de **Trichoplusia ni**. La fréquence de ce phénotype est accrue lors de passages successifs et en série sur cultures de cellules (POTTER et al., 1976). Ce n'est qu'en 1982 que MILLER et MILLER, par analyse de cartes de restriction, réussirent à démontrer que ce phénotype résulte de l'insertion de copies d'éléments transposables dérivant du génome de l'insecte-hôte, ce qui permet en outre de comprendre la faible réversion de ce phénotype.

Par 25 passages successifs du NPV de **Mamestra brassicae** sur des larves du 5e stade de ce ravageur, CROIZIER et al. (1985) n'ont pu observer de modifications génomiques importantes. Par contre, BURAND et SUMMERS (1982) ont noté des modifications des cartes de restriction d'une souche de la polyèdrose nucléaire **Autographa californica**. En fait, ces modifications ne résultent pas de mutations mais d'insertions de fragments du DNA viral répétées.

Il faut admettre que la réplication in vivo de fait contre sélectionne tout mutant dont la virulence à l'égard de l'insecte-cible serait réduite. Par contre, l'induction d'une virose latente par l'infection d'une autre souche virale hétérologue a plusieurs fois été décrite chez **Aglaïs urticae**, **Porthetria dispar** (LONGWORTH et CUNNINGHAM, 1968) ou **Spodoptera** et

Heliothis (Mc KINLEY et al., 1981). Il convient donc de minimiser ce risque en plaçant au moment de la sélection la souche d'insecte en conditions de "stress" de façon à favoriser l'expression d'une virose latente éventuelle. En outre, les cultures de cellules peuvent aussi supporter des Baculovirus persistants tels que le système Hz-1V des cellules ou tissus ovariens d'**Heliothis zea** (VAIL et al., 1973). De telles infections latentes ont également été induites dans des lignées cellulaires homologues de **Spodoptera frugiperda** (Mc INTOSH et IGNOFFO, 1981).

Cependant, on doit noter l'influence de facteurs épigénétiques, puisqu'une maturation des polyèdres serait nécessaire pour l'expression totale de leur virulence. IGNOFFO et SHAPIRO (1978) démontrèrent en effet que sans modification génétique observable, il apparaît que les polyèdres issus de cadavres sont plus infectieux que ceux issus de larves moribondes.

3.2.2. Résistance de la population cible

Les facteurs de développement qui affectent beaucoup la sensibilité des chenilles aux Baculovirus (BURGERJON, 1981) peuvent être contrôlés par un choix judicieux des doses et fréquences d'application.

Par contre, les facteurs génétiques qui contrôlent la résistance des populations d'insectes aux Baculovirus sont moins bien connus. BRIES et al. (1980) ont isolé des populations du Tortricide **Epiphyas postvittana** 160 fois plus résistantes que la souche originelle de la polyèdrose nucléaire. Dans ce cas, comme dans celui de la résistance de **Phthorimaea operculella**, l'obtention d'hybrides entre souches résistantes et sensibles, permit de démontrer le contrôle génétique du phénomène. Ainsi, la résistance à la granulose de **P. operculella** ou celle de **B. mori** à la souche d'une virose cytoplasmique, est gouvernée par un seul gène autosomal dominant (BRIESE, 1982 ; WATANABE, 1965), alors que la résistance de **E. postvittana** à sa polyèdrose nucléaire est polygénique. Par ailleurs, on sait que les gènes de résistance aux pyréthrinoïdes de synthèse n'influencent pas la résistance aux polyèdroses nucléaires, puisque deux populations de **Spodoptera frugiperda** diffèrent pour le premier caractère mais sont également sensibles aux virus (CHAUFAUX et FERRON, 1986).

Il conviendra donc d'accompagner les futurs programmes d'utilisation agronomique intensive des virus entomopathogènes d'un contrôle de la résistance des populations d'insectes-cibles de façon à estimer la probabilité de sélection d'une sous-population non sensible.

3.3. Les champignons entomopathogènes

3.3.1. Stabilité de la souche

La difficulté essentielle pour le maintien des caractéristiques biologiques de souches de champignons entomopathogènes réside dans l'importance du déterminisme épigénétique de l'agressivité. KREJZOVA (1977) fut la première à démontrer ce phénomène par réactivation d'une souche de **Conidiobolus coronatus** grâce à des passages successifs sur l'insecte-hôte d'origine. FARGUES et ROBERT (1983) ont montré que la réactivation d'une souche de **M. anisopliae** sur l'espèce d'insecte-hôte originelle pouvait réduire la DL 50 d'un facteur 100. La réversibilité de ce phénomène est tout aussi rapide que son induction puisque parfois un seul passage sur milieu artificiel de culture peut induire une chute sensible d'agressivité. On ignore cependant le déterminisme moléculaire de ce phénomène qui n'a pu être retrouvé avec des isolats de **Verticillium lecanii** (HALL, 1980).

Pour éviter cette trop grande perte d'agressivité, des "réactivations" régulières sont effectuées par réisolement du germe à partir d'un insecte préalablement traité. Cette technique, en conditions de laboratoire, bien que moins efficace (RIBA, non pub.) est préférable au prélèvement d'un insecte mycosé dans un champ préalablement traité avec la souche. En effet, on a pu montrer (RIBA et al., 1987) que plusieurs souches de **M. anisopliae**, cohabitent dans le même champ de canne à sucre attaqué par le Cercopide **Mahanarva posticata**.

3.3.2. La résistance de l'hôte

On ne connaît pas de cas de sélection de résistance d'insectes aux champignons entomopathogènes. Néanmoins, l'étude comparée de la sensibilité de races de **B. mori** à **B. bassiana** en démontra le déterminisme génétique. RIBA et al. (1982) ont mis en évidence que le tégument de plusieurs races chinoises ralentit la pénétration de souches de **B. bassiana** pourtant très agressives à l'égard de races japonaises de cet insecte. Possible, la sélection de sous-populations résistantes parait donc peu probable, puisque plusieurs gènes sont impliqués dans le déterminisme de l'agressivité comme le démontrent les études d'héritabilité (RIBA et al., 1982).

4 CONTROLE DE LA PURETE MICROBIOLOGIQUE

Les bactéries et les champignons entomopathogènes sont produits et récoltés en conditions stériles ou semi-stériles. Les risques de pollution microbienne de telles préparations sont donc très faibles. Néanmoins, dans le cas où la préparation commercialisée est constituée de granules pré-colonisés par la seule phase mycélienne, il convient de conserver une pureté microbiologique suffisante pour ne pas réduire la conidiogenèse qui se déroulera aussitôt après l'application au champ.

Par contre, la production in vivo des virus d'insectes consiste en un élevage non stérile de plusieurs milliers de chenilles. L'obtention d'une préparation insecticide virale exempte de bactéries ne peut être conçue pour des raisons économiques. C'est pourquoi la synthèse du produit s'accompagne d'une précipitation acétonique qui tue les formes végétatives des bactéries et cryptogames, et de l'adjonction d'adjuvants de formulations bactériostatiques. Finalement, les contrôles consistent d'une part en un dénombrement des germes totaux par rapport aux polyèdres infectieux et, d'autre part, à la recherche de germes toxiques tels que **Shigella vibrio** ou **Salmonella**.

5 CONCLUSION

Les agents microbiens sélectionnés pour la lutte microbiologique ont été retenus à la fois pour :

- leur virulence à l'égard d'une espèce de ravageur ;
- leur grande spécificité parasitaire qui confère, entre autres, l'innocuité à l'égard des vertébrés et notamment de l'homme ;
- leur facilité de production ;
- leur efficacité au champ.

Il convient d'apporter une attention toute particulière à cette dernière propriété car seuls les essais en conditions agronomiques permettent d'estimer les limites de l'adaptabilité du germe. Son aptitude à manifester sa pathogénie en diverses conditions climatiques, géographiques, pédologiques, agronomiques et biocoenotiques doit être connue. Insisterons-nous suffisamment sur cette exigence dont le non respect fut à l'origine de déceptions cruelles. Certes **Verticillium lecanii** est parfaitement capable de juguler les populations d'Homoptères ravageurs des cultures de chrysanthèmes. Mais, les conditions hygro-thermiques de cette culture sont toujours favorables à l'expression du germe, ce qui n'est pas le cas pour les cultures protégées de tomates ou concombres pouvant être attaquées par des insectes du même ordre. Une grande rigueur expérimentale doit définir les différents essais de façon à bien connaître les limites tant sont fortes pour le chercheur ou l'industriel les tentations d'élargir rapidement le marché potentiel d'une préparation entomopathogène.

Par ailleurs, un micro-organisme cloné dont le génome est unique est proposé à l'industriel. Dans le cas d'organismes eucaryotiques diploïdes ou plurinuclées il s'agit la plupart du temps de souches homozygotes ou homocaryotiques. Dans le cas des Baculovirus, il peut s'agir d'un mélange de variants d'un même virus différent seulement par 1 ou 2 sites de restriction (KNELL et SUMMERS, 1981). Cette constatation implique des contrôles précoces de la stabilité génotypique des populations de variants proposées à la commercialisation. Des techniques de biologie moléculaire permettent, nous l'avons vu, de telles études (CROIZIER et al., 1980).

A partir du moment où le germe présélectionné a les qualités requises, il suffit de s'assurer de la stabilité génétique au cours des différentes étapes de la production. Autrement dit, et sous réserve d'une sélection correctement effectuée selon les critères précédemment évoqués, nous estimons que les contrôles de laboratoire, selon les techniques précitées de caractérisation pathogénique et biochimique, suffisent pour garantir l'efficacité biologique de la préparation. De plus ces contrôles assurent à l'industriel le bon fonctionnement de la chaine de fabrication du produit. Leurs fréquences seront à définir en fonction des processus de synthèse des conditions de stockage et de distribution. Bien sûr, il va de soi que ces contrôles doivent être effectués par un personnel qualifié travaillant dans des laboratoires d'expertise. Pour chaque germe, les autorités nationales compétentes se doivent de désigner ces laboratoires.

5. CONCLUSION

The microbial agents have been selected for microbial control due to :

- their virulence towards a pest species ;
- their high parasitic specificity and especially their safety towards vertebrates and man ;
- their easy production ;
- their field efficiency.

This last characteristic must be particularly considered as only the field tests allow the evaluation of the germ adaptability. Its capacity to demonstrate its pathogenicity under various climatic, geographic, pedologic, agronomic and biocenotic conditions must be known. This condition, will never be emphasized enough, since when it was not considered, it resulted in a cruel disappointment. Indeed **Verticillium lecanii** can regulate Homopteran insect pests of chrysanthemum crops. But the hygro-thermic conditions of this crop are always favourable to the germ expression ; that is not the case of tomato or cucumber greenhouse crops which can be attacked by insects of the same order. The different tests must be carried out very strictly in order to have a good knowledge of the limits as in research and in industry the temptations are strong to extend quickly the potential market of an entomopathogenic preparation.

Moreover, a cloned micro-organism whose genome is single is proposed to industry. In the case of diploid or plurinucleated eucaryotic organisms, these are most often homozygous or homocaryotic strains. In the case of Baculoviruses, it can be a mixture of variants from the same virus, differing only in 1 or 2 restriction sites (KNELL and SUMMERS, 1981). This statement implies early tests of the genotypical stability of the variants'populations proposed for commercialization. As we have already seen, molecular biology techniques allow such studies (CROIZIER et al., 1980).

From the moment when the preselected germ has the qualities required, it is enough to make sure of the genetic stability through the different steps of the production. That is to say, if the selection according to the criteria above-mentioned has been well carried out, we think that the laboratory tests, according to the above-mentioned techniques of pathogenic and biochemical characterization, are sufficient to ensure the biological efficiency of the preparation. Moreover, these tests are a guarantee for industry of the efficiency of the production chain of the preparation. Their frequency must be defined according to the syntheses processes, the storage and distribution conditions. Of course these tests must be carried out by a qualified personnel working in expert laboratories. For each germ, the national authorities concerned must appoint these laboratories.

BIBLIOGRAPHIE

de BARJAC, H. & BONNEFOI A.- 1962. Essai de classification biochimique et sérologique de 24 souches de **Bacillus** du type **B. thuringiensis**. Entomophaga, 7 (1), 5-31.

de BARJAC, H. & BONNEFOI A.- 1967. Classification des souches de **Bacillus thuringiensis**. C.R. Acad. Sci., D, Paris, 264, 1811-1813.

de BARJAC, H. & LARGET-THIERY I.- 1984. Characteristics of IPS 82 as standard for biological assay of **Bacillus thuringiensis** H-14 preparations. Doc. mimeog. OMS, WHO/VBC/84.892, 1-9.

BONNEFOI, A., BURGERJON, A. & GRISON, P.- 1958. Titrage biologique des préparations de spores de **Bacillus thuringiensis** Berliner. C.R. Acad. Sci.,247,1418-1420.

BRIESE, D.T.- 1982. Genetic basis for resistance to a granulosis virus in the potato moth **Phthorimaea operculella**. J. Invertebr. Pathol., 39, 215-218.

BRIESE, D.T., MENDE, H.A., GRACE, T.O.C. & GEIER P.N.- 1980. Resistance to a nuclear polyhedrosis virus in the light-brown apple moth **E-**

piphyas postvittana (Lepidoptera : Tortricidae). J. Invertebr. Pathol., 36, 211-215.

BURAND, J.P. & SUMMERS, M.D.- 1982. Alteration of **Autographa californica** nuclear polyhedrosis virus DNA upon serial passage in cell culture. Virology, 119, 223-230.

BURGERJON, A.- 1956. Pulvérisation et poudrage au laboratoire des préparations pathogènes insecticides. Ann. Epiph., 4, 677-686.

BURGERJON, A.- 1969. Titrage et définition d'une unité biologique pour les préparations de **Bacillus thuringiensis** Berliner. Entomophaga, 4, 201-206.

BURGERJON, A.- 1977. Use of specificity difference indices for the identification of nuclear polyhedrosis viruses (Baculovirus) of insects. Entomophaga, 22 (2), 187-192.

BURGERJON, A., BIACHE, G., CHAUFAUX, J. & PETRE Z.- 1981. Sensibilité comparée, en fonction de leur âge, des chenilles de **Lymantria dispar**, **Mamestra brassicae** et **Spodoptera littoralis** aux virus de la polyèdrose nucléaire. Entomophaga, 26 (1), 47-58.

CHAUFAUX, J. & FERRON, P., 1986. Sensibilité différente de deux populations de **Spodoptera exigua** Hüb. (Lepid. Noctuidae) aux baculovirus et aux pyréthrinoïdes de synthèse. Agronomie, 6 (1), 99-104.

CROIZIER, G. & CROIZIER L.- 1977. Evaluation du poids moléculaire de la protéine des corps d'inclusion dans divers Baculovirus d'insectes. Arch. Virol., 55, 247-250.

CROIZIER, G., CROIZIER, L., BIACHE, G. & CHAUFAUX, J.- 1985. Evolution de la composition génétique et du pouvoir infectieux du baculovirus de **Mamestra brassicae** L. au cours de 25 multiplications successives sur les larves de la noctuelle du chou. Entomophaga, 30 (4), 365-374.

CROIZIER, G., GODSE, D. & VLAK, J.- 1980. Sélection de types viraux dans les infections doubles à Baculovirus chez les larves de lépidoptères. C.R. Acad. Sci., Paris, D, 290, 579-582.

DEVRIENDT, M. & MARTOURET, D.- 1976. Absence de résistance à **Bacillus thuringiensis** chez la teigne des crucifères, **Plutella maculipennis** (Lep. : Hyponomeutidae). Entomophaga, 21 (2), 189-199.

FARGUES, J., DURIEZ, T., ANDRIEU, S. & POPEYE R.- 1974. Analyse sérologique comparée de deux champignons entomopathogènes **Beauveria bassiana** et **Beauveria tenella**. C.R. Acad. Sci., Paris, Ser. D, 278, 2245-2247.

FARGUES, J., DURIEZ, T., ANDRIEU, S., POPEYE, R. & ROBERT, P.- 1975. Etude immunologique comparée de souches de **Metarrhizium anisopliae** (Delacroix) Siem., champignon Hyphomycète entomopathogène. C.R. Acad. Sci., Paris, Ser.D, 281, 1781-1784.

FARGUES, J. & REMAUDIERE G.- 1977. Considerations on the specificity of entomopathogenic fungi. Mycopathologia, 62, 31-37.

FARGUES, J. & ROBERT, P.H.- 1983. Effects of passaging through scarabeid hosts on virulence and host specificity of two strains of the entomopathogenic hyphomycete **Metarhizium anisopliae.** Can J. Microbiol., 29, 576-583.

FARGUES, J. & VEY, A.- 1974. Modalités d'infection des larves de **Leptinotarsa decemlineata** par **Beauveria bassiana** au cours de la mue. Entomophaga, 19, 311-323.

FERRON, P. & DIOMANDE, T.- 1969. Sur la spécificité à l'égard des insectes de **Metarhizium anisopliae** (Metsch.) Sorokin (**Fungi Imperfecti**) en fonction de l'origine des souches de ce champignon. C.R. Acad. Sci. Paris, D, 268, 331-332.

GOLDBERG, L.J. & MARGALIT, J.- 1977. Bacterial spore demonstrating rapid larvicidal activity against **Anopheles sergentii, Uranotoenia unguiculata, Culex uninittatus, Aedes aegypti** and **Culex pipiens.** Mosquito News, 37, 355-358.

GOLDMAN, I.F., ARNOLD, J. & CARLTON B.C.- 1986. Selection for resistance to **Bacillus thuringiensis** subspecies **israelensis** in field and laboratory populations of the mosquito **Aedes aegypti.** J. Invertebr. Pathol., 47, 317-324.

HALL, R.A.- 1980. Effect of repeated subculturing on agar and passaging through an insect host on pathogenicity, morphology and growth rate of **Verticillium lecanii.** J. Invertebr. Pathol., 36, 216-222.

HINK, W.F. & VAIL, P.V.- 1973. A plaque assay for titration of alfalfa looper nuclear polyhedrosis virus in a cabbage looper (TN-368) cell line. J. Invertebr. Pathol., 22, 168-174.

HOHMANN, A.W. & FAULKNER, P.- 1983. Monoclonal antibodies to Baculovirus structural proteins : determination of specificities by western blot analysis. Virology, 125, 432-444.

HUGHES, P.R. & WOOD, H.A.- 1981. A synchronous peroral technique for the bioassay of insect viruses. J. Invertebr. Pathol, 37, 154-159.

IGNOFFO, C.M. & SHAPIRO, M.- 1978. Characteristics of Baculovirus preparations processed from living and dead larvae. J. Econ. Entomol., 71, 186-188.

KALFON, A., LARGET-THIERY, I. & CHARLES, J.F.- 1983. Growth, sporulation and larvicidal activity of **Bacillus sphaericus.** Eur. J. Appl. Microbiol. Biotechnol., 18, 168-173.

KNELL, J.D. & SUMMERS, M.D.- 1981. Investigations of genetic heterogeneity in wild isolates of **Spodoptera frugiperda** nuclear polyhedrosis virus by restriction endonuclear. Analysis of plaque purified variants. Virology, 112 (1), 190-197.

KREJZOVA, R.- 1977. Enhancement of pathogenicity of the fungus **Conidiobo-**

lus uronatus by means of multiple precultivation insect hosts. Vestn. Cesk. Spol. Zool., 41, 105-113.

KRIEG, A., HUGER, A., LANGENBRUCH, G. & SCHNETTER, W.- 1983. **Bacillus thuringiensis** var. **tenebrionis**, new variety a new pathotype effective against larvae of coleoptera. Z. ang. Ent., 96, 500-508.

KRYCH, V.K., JOHNSON, J.L. & YOUSTEN, A.A.- 1980. Desoxyribonucleic acid homologies among strains of **Bacillus sphaericus**. Int. J. Syst. Bact., 30, 476-482.

LARGET, I.- 1980. Etude du pouvoir entomopathogène de **Bacillus thuringiensis** var. **israelensis** sur les larves de Culicidae (Diptères Nématocères). Mise au point d'un titrage biologique. Thèse de 3e Cycle, Octobre 1980, Université Paris-Sud Orsay, 148 pp.

LATGE, J.P. & BOUCIAS, D.G.- 1984. Intraspecific variability in **Conidiobolus obscurus**. J. Gen. Appl. Microbiol., 30, 135-150.

LONGWORTH, J.F. & CUNNINCHAM, J.C.- 1968. The activation of occult nuclear polyhedrosis viruses by foreign polyhedra. J. Invertebr. Pathol., 10, 361-367.

McGAUGHEY, W.H.- 1985. Insect resistance to the biological insecticide **Bacillus thuringiensis**. Science, 4709, 229, 193-

McINTOSH, A.H. & IGNOFFO C.M.- 1981. Replication and infectivity of the single combedded nuclear polyhedrosis virus, baculovirus **Heliothis** in homologous cell lines. J. Invertebr. Pathol., 37, 258-264.

McKINLEY, D.J., BROWN, D.A., PAYNE, C.C. & HARRAP, K.A- 1981. Cross-infestivity and activation studies with four baculoviruses. Entomophaga, 26, 79-90.

MARUNIAK, J.E. & SUMMERS,M.D.- 1978. Comparative peptide mapping of baculovirus polyhedrins. J. Invertebr. Pathol., 32, 196-201.

MAZZONE, M.M. & TIGNOR, G.H.- 1978. Insect viruses : serological relationsships, in Advances in Virus Research, 20, VAUTTER M.A., BANG F.B., MARAMOROSCH K., SMITH K.M. Eds., Acad. Press, N.Y., 237-420.

MERDAN, A., CROIZIER, L., VEYRUNES, J.C. & CROIZIER G.- 1977. Etude comparée des protéines des polyèdres et des virions de trois isolats de baculovirus de **Spodoptera littoralis**. Entomophaga, 22, 413-420.

MILLER, D.W. & MILLER, L.K.- 1982. A virus mutant with an insertion of a copia-like transposable element. Nature (London), 299, 562.

NATHANS, D. & SMITH, H.O.-, 1975. Restriction endonucleases in the analysis and restructuring of DNA molecules. Annu. Rev. Biochem., 44, 273.

PADUA, L.E., OHBA, M. & AIZAWA K.- 1980. The isolates of **Bacillus thurin-**

giensis serotype 10 with a highly preferential toxicity to mosquito larvae. J. Invertebr. Pathol., 36, 180-186.

PADUA, L.E., OHBA, M. & AIZAWA, K.- 1984. Isolation of a **Bacillus thuringiensis** strain (serotype 8a, 8b) highly and selectively toxic against mosquito larvae. J. Invertebr. Pathol., 44, 12-17.

POPRAWSKI, T.J., RIBA, G., JONES, W.A. & AIOUN, A.- 1988. Variation in isoesterase profiles of geographical populations of **Beauveria bassiana (Deuteromycotina** : Hyphomycetes) isolated from **Sitona weevils** (Coleoptera : Curculionidae). Environ. Entomol., 17 (2), 275-279.

POTTER, K.N., FAULKNER, P. & McKINNON, E.A.- 1976. Strain selection during serial passage of **Trichoplusia ni** nuclear polyhedrosis virus. J. Virol., 18, 1040-1050.

RAKOTONIRAINY, M.S.- 1987. Contribution à l'étude du polymorphisme des RNA ribosomiques de quelques espèces d'Hyphomycètes entomopathogènes. DEA Phytopathologie Univ. Paris XI, 22 p.

RIBA, G., de AZEVEDO, J.L., MESSIAS, C., DIAS DA SILVEIRA, W.& TUVESON R. 1985. Studies of the inheritance of virulence in the entomopathogenic fungus **Metarhizium anisopliae.** J. Invertebr. Pathol., 46, 20-25.

RIBA, G., BOUVIER-FOURCADE, I. & CAUDAL, A.- 1986. Isoenzymes polymorphism in **Metarhizium anisopliae** (Deuteromycotina : Hyphomycetes) entomogenous fungi. Mycopathologia, 96, 161-169.

RIBA, G., KATAGIRI, K. & KAWAKAMI, K.- 1982. Preliminary studies on the susceptibility of the silkworm, **Bombyx mori** (Lepidoptera : Bombycidae), to some entomogenous hyphomycetes. Appl. Entomol. Zool., 17 (2), 238-243.

RIBA, G., LOURDES de AQUINO, M. & RIBEIRO, A.S.- 1987. Polymorphisme chez des souches brésiliennes de l'Hyphomycète entomopathogène **Metarhizium anisopliae** (Metsch.) Sorokin, inféodés à des Cercopidae. Agronomie, 7 (10), 763-768.

ROBERTS, P.L. & NASER, W.- 1982. Characterization of monoclonal antibodies to the **Autographa californica** nuclear polyhedrosis virus. Virology, 122, 424-430.

SINGH, S.P., GUDAUSKAS, R.T. & HARPER, J.D.- 1983. High resolution two-dimensional gel electrophoresis of structural proteins of baculoviruses of **Autographa californica** and **Porthetria (Lymantria) dispar.** Virology, 125-370.

SMITH, G.E. & SUMMERS, M.D.- 1979. Restriction maps of five **Autographa californica** MNPV variants, **Trichoplusia ni** MNPV and **Galleria mellonella** MNPV DNAs with endonucleases. Smal, KpnI, Bam HI, Soc I, Xho I and Eco RI. J. Virol., 30, 828-838.

SMITH, G.E. & SUMMERS, M.D.- 1981. Application of a novel radiommunoassay to identify baculovirus structural proteins that share interspecies antigenic determinants. J. Virol., 39, 125-137.

SUMMERS, M.D., SMITH, G.F., KNELL, J.D. & BURAND, J.P.- 1980. Physical maps of **Autographa californica** and **Rachiplusia** on nuclear polyedrosis virus recombinants. J. Virol., 34 (3), 693-703.

THIERY, I.- 1986. Constraints and value of bioassays for standardization of **Bacillus thuringiensis** var. **israelensis** and **Bacillus sphaericus**. In : Fundamental and applied aspects of Invertebrate Pathology (ICIP, 1986), Veldhoven, 18-22 august, SAMSON, R.A. et al. Eds., 677-681.

VAECK, M., HOEFTE, H., REYNAERTS, A., LEEMANS, J., VAN MONTAGU, M. & ZABEAU, M.- 1986. Engineering of insect resistant plants using a **B.thuringiensis** gene. Molecular strategies for crop protection. Dupont-UCLA Symposium, Steombaat Springs, Colorado.

VAIL, P.V., JAY, D.L. & HINK, W.F.- 1973. Replication and infectivity of the nuclear polyhedrosis virus of the alfalfa looper, **Autographa californica**, produced in cells grown in vitro. J. Invertebr. Pathol., 22, 231-237.

WATANABE, H.- 1965. Resistance to peroral infection by the cytoplasmic-polyhedrose virus in the silkworm, **Bombyx mori** (Linneaus). J. Invertebr. Pathol., 7, 257-270.

WICKREMESINGHE, R.S.B. & MENOIS, C.L.- 1980. **Bacillus sphaericus** spore from Sri Lanka demonstrating rapid larvicidal activity on **Culex quinquefasciatus**. **Mosq. News, 40, 387-389.**

CONTROL DE CALIDAD DE ENTOMOFAGOS CRIADOS EN CAUTIVIDAD: POSIBILIDAD DE APLICACION EN EL CASO DE *CRYPTOLAEMUS MONTROUZIERI* MULSANT

J. L. Ripolles & A. García
Servicio de Protección de los Vegetales-Almazora, Spain

RESUMEN

La existencia en nuestro país de varios insectarios dedicados a la cría de C. montrouzieri, y la posibilidad de que se produzcan cambios en su comportamiento, ha hecho que se aborde el problema del control de su calidad.

Si tenemos en cuenta que en toda cría masiva de insectos, es fundamental conseguir una alta eficacia de producción al mínimo coste posible, y que el comportamiento de un insecto es función de un componente genético y otro ambiental, es fácil pensar que en estas condiciones dichos cambios no solamente se pueden presentar con suma facilidad sino que segun el fin a que se destine el insecto criado, pueden disminuir sensiblemente su eficacia.

En esta comunicación se indican las etapas a seguir para establecer un control de calidad de un entomófago y en especial la situación actual del control de calidad de C. montrouzieri.

INTRODUCCION

Con la utilización de los primeros pesticidas orgánicos de síntesis, se pensó que habían quedado resueltos los problemas que la defensa fitosanitaria de las plantas cultivadas presentaba, pero el uso masivo y en ocasiones indiscriminado de dichas sustancias ha demostrado que no es así, haciendo que la estrategia a seguir sea mucho más complicada.

Son varios los efectos que el uso masivo de dichos pesticidas conlleva; veamos algunos de ellos:

1.- Aparición de resistencias. Fenómeno frecuente en varios hongos e insectos, que puede ser hereditario y en ocasiones afecta a toda una familia de pesticidas.

2.- Cambios fisiológicos en los vegetales; que pueden producir un incremento de la población del organismo nocivo, bien por mejorar su alimento tanto en calidad como en cantidad, bien induciendo a su vez cambios fisiológicos en dicho organismo.

3.- Recurrencia del fitófago; dicho fenómeno consiste en que el organismo a controlar reaparece incluso con mayor intensidad luego de un intento de control, fundamentalmente por la eliminación de competencias tanto intra como interespecíficas.

4.- Aparición de fitófagos secundarios. La ruptura del equilibrio biológico mediante la eliminación de los enemigos naturales de una plaga o de la competencia de otros fitófagos, explica que este sea un fenómeno muy frecuente.

5.- Residuos. Los efectos anteriores, aumentan más aun el problema de residuos, encontrándose cada vez más sustancias químicas en los vegetales, que introducidas en la cadena trófica, pueden acarrear consecuencias mas o menos graves en la fauna y flora así como en la sanidad humana.

6.- Contaminación del medio. Fenómeno que hay que distinguir del anterior ya que este se origina o bien por descuidos, mal uso de los pesticidas o incluso en el proceso de su fabricación, aunque los efectos pueden ser mas graves pero más localizados.

A pesar de todo ello y debido fundamentalmente a la simplicidad del ecosistema agrícola (Agroecosistema) y en ocasiones a la falta de otras soluciones, hoy por hoy no podemos prescindir totalmente de la lucha química, por ello surgen nuevas o viejas estrategias, como la Agricultura Biológica o la Lucha integrada.

En nuestra opinión ambas estrategias persiguen los mismos fines en el campo de la defensa de las plantas cultivadas, es decir ambas pretenden mediante un buen conocimiento del agroecosistema, (del vegetal, de la flora, del clima y de todos los organismos existentes), intervenir integrando todos los sistemas de lucha disponibles, haciendo que esta sea más racional y mejorándola económica, ecológica y sanitariamente.

A consecuencia de todo ello, la utilización de entomófagos (depredadores y parásitoides) o la aplicación de nuevas técnicas como la lucha autocida (uso de machos estériles), uso de semioquímicos (feromonas o kairomonas), o el uso de entomopatógenos (virus, bacterias u hongos), adquiere un gran interés en la actualidad.

En todos los casos citados anteriormente, es necesario la producción mas o menos masiva de microorganismos o de insectos en cautividad, y en algunos casos como el uso de machos estériles, el uso de entomopatógenos o el uso de entomófagos mediante incremento artificial de sus poblaciones, la exigencia de conseguir una alta eficacia de producción al mínimo coste posible, puede conducir a cambios importantes en el comportamiento de dichos organismos. Estos cambios tendrán mayor o menor importancia dependiendo del fin a que se destinen. Así por ejemplo en los casos citados anteriormente en que los organismos utilizados deben mantener una competencia intraespecífica o interespecifica, estos cambios pueden disminuir sensiblemente su eficacia . Por ello el control de su calidad es imprescindible, fundamentalmente para intentar descubrir el inicio de estos cambios y tomar medidas oportunas para corregirlos.

El CONTROL DE CALIDAD

Para entender mejor este concepto nos basaremos en una definición cualquiera del mismo. Así por ejenplo la calidad es el conjunto de propiedades o características inherentes a una cosa, que permiten apreciarla como igual, mejor o peor que las restantes de su especie.

De su definición se deduce que cualquier control de la misma debe tener presente los siguientes términos:

- El conjunto de propiedades, características o rasgos, que será necesario definirlos y jerarquizarlos en cada caso segun el objetivo a que sea destinada su producción.

- El concepto de relatividad, es decir es necesario establecer comparaciones mediante técnicas establecidas con anterioridad, entre los rasgos o propiedades de diferentes poblaciones de la misma especie.

- Población de referencia, que será necesario fijar en primer lugar y posteriormente establecer los parámetros correspondientes a las características seleccionadas, para poder realizar dichas comparaciones.

- Periodicidad, termino que si bien no se refleja en la definición es necesario tener en cuenta, ya que no podemos hablar de control de calidad si este no se realiza con cierta asiduidad para constatar cualquier cambio en sus características.

ETAPAS A SEGUIR

Para establecer un control de calidad de los entomófagos criados en cautividad deben cubrirse las siguientes etapas:

1) Elección de las características que la definan.

El conjunto de características que mejor definen el comportamiento de un organismo, dependerán del objetivo para el que sean destinados dichos organismos.

Segun Boller y Chambers (1.977), es necesario dividir los componentes de la calidad de un organismo en principales, que a su vez se dividen en rasgos de calidad individuales con parámetros fácilmente medibles. Segun ellos en general son los siguientes:

Componentes principales	Rasgos individuales
Adaptabilidad	Supervivencia Búsqueda del alimento Aclimatación
Movilidad	Dispersión Localización del lugar de apareamiento Localización del huésped
Actividad sexual	Búsqueda de la pareja Aceptación de la pareja Capacidad de inseminación
Reproducción	Fertilidad Búsqueda del huésped Aceptación del huésped
Colonización	Oviposición Competitividad

En el caso concreto de los entomófagos, consideran como mas importantes la adaptabilidad, reproducción y colonización, caracteres que coinciden con los requisitos exigidos a cualquier depredador o parásito. Veamos cuales son estos requisitos:

- Tener una buena capacidad de Búsqueda. Este carácter se considera el de mayor importancia.

- Buena especificidad o preferencia por el huésped

- Buena capacidad de reproducción comparada con la del huésped

- Buena capacidad de adaptación a diferentes condiciones del medio, es decir a diferentes situaciones climáticas, a diferentes nichos ecológicos, a las generaciones del huésped, a los plaguicidas, etc.

Pero la elección de dichas características en el caso de un entomófago, no solo dependerán de los requisitos anteriores sino del método utilizado en su aplicación.

En el control biológico se utilizan tres métodos o estrategias:

- Introducción de organismos exóticos, es decir de organismos útiles no existentes en la zona o país. A este método también se le conoce como Control Biológico Clásico.

- Incremento de enemigos naturales artificialmente mediante sueltas.

- Conservación o mejora de la acción de los enemigos naturales ya existentes.

La introducción y aclimatación de especies exóticas para el control de plagas que carecen de enemigos naturales comenzó hace muchos años, aunque las primeras tentativas serias debemos situarlas a partir del ultimo siglo. Los éxitos alcanzados por algunos casos, (Rodolia (Novius) cardinalis e Icerya purchasi. Prospaltella berlesei y Pseudaulacaspis pentagona), sirvieron para que esta técnica fuera ampliamente utilizada posteriormente.(Segun P. de Bach se han introducido mas de 225 insectos útiles en diferentes países del mundo, de los que un 30% han tenido éxito).

Normalmente en este método no se establecen controles de calidad periódicos ya que generalmente no se realizan crías masivas, aunque en todo caso resulta de gran interés constatar que sus características responden a las exigencias por las que fue seleccionado y observar las siguientes reglas:

- Procurar que exista una gran variabilidad genética en la población de partida.

- Criar los entomófagos antes de su suelta en unidades de cuarentena, para evitar la introducción de alguno de sus hiperparásitos y disponer de un numero mayor de insectos a liberar.

- Elegir el mejor momento para la suelta (máximo de estados del huésped sensibles a su acción, óptimas condiciones climáticas, etc.).

- Diversificar los lugares de suelta y liberar un buen numero de individuos.

- Procurarles zonas refugios para evitar contactos con pesticidas, o liberarlos en zonas donde existan huéspedes alternativos y diversas plantas que puedan proporcionarles otro tipo de alimentos (azúcares).

La segunda técnica consiste en mejorar el control biológico de alguna plaga aumentando las poblaciones de sus enemigos naturales, mediante la cría en masa y posterior liberación.

Existen diferentes estrategias para proceder a su liberación. Veamos algunas de ellas:

- Método estacional. Se liberan los entomófagos para mejorar su acción en momentos climáticos adversos.

- Método inoculativo. Cuando la liberación se realiza con el fin de que su acción se prolongue durante varias generaciones a lo largo de un ciclo vegetativo.

- Método inundativo. Cuando la liberación persigue efectos inmediatos o de choque sin que se complete en muchas ocasiones una generación. Se trata pues de una intervención biológica que pretende conseguir los mismos efectos que un tratamiento químico.

En cualquier caso se debe criar masivamente el entomófago en condiciones controladas y el riesgo de deterioro genético es muy grande, por ello el establecimiento de controles de calidad periódicos es de gran importancia.

La elección de las características o rasgos que la definan si se utiliza esta estrategia, será función no solo de sus atributos sino del método que se quiere utilizar. Así por ejemplo el método inundativo es menos exigente en alguno de los atributos de un buen enemigo natural y quizás en este caso tengan menor importancia los rasgos que definen su reproducción y mas los que definen su adaptabilidad y colonización.

2) Estandarización de las técnicas a utilizar para medir sus parámetros.

Una vez cubierta la etapa anterior debemos establecer los parámetros que queremos medir y fijar las condiciones en que se realizaran las pruebas, es decir la temperatura, la humedad, el numero de repeticiones, la edad, estados y numero de insectos a utilizar etc.

Para ello se debe tener en cuenta lo siguiente:

- Las características bioecológicas del entomófago.

- Los métodos utilizados deben ser fáciles de aplicar y poder ser repetidos en cualquier tiempo y lugar.

- Los resultados deben obtenerse en el mínimo tiempo posible.

- Deben facilitar la comparación de la población elegida como modelo con cualquier otra.

Posteriormente debemos decidir si las pruebas se realizarán en laboratorio, en campo o semicampo. En este sentido indicaremos que muchos de los rasgos que definen la calidad son difíciles de medir en condiciones de campo o semicampo, mientras que otros como la dispersión, supervivencia etc. pueden tener interés en condiciones naturales, aunque siempre tendremos en cuenta que en estos casos los resultados pueden estar sometidos a muchas variables difícilmente identificables.

3) Elección de la población de referencia y fijación de sus parámetros.

En el caso que nos ocupa es norma generalizada tomar la población natural como modelo, aunque en ocasiones la elección de una población criada en cautividad es también admisible.

Una vez elegida debemos establecer sus parámetros mediante la aplicación de las pruebas establecidas en la etapa anterior.

4) Aplicación del control de calidad o monitorización.

Una vez cubiertas las etapas anteriores debemos aplicar la pruebas establecidas a las poblaciones del entomófago criadas en cautividad, para que luego de haberlas comparado mediante métodos estadísticos apropiados con la población de referencia, descubrir si hay cambios importantes en su comportamiento y tomar las medidas oportunas.

POSIBILIDAD DE APLICACION EN EL CASO DE *Cryptolaemus montrouzieri* Muls.

Este depredador hace muchos años que se utiliza en nuestro país para el control de *Planococcus citri* Risso y debido a su falta de aclimatación, hoy existen varios insectarios pertenecientes a organismos oficiales o a asociaciones de agricultores, dedicados a su cría y posterior liberación utilizando el método inoculativo. Por ello hemos creído oportuno abordar el problema del control de su calidad.

A continuación y como ejemplo de la aplicación de las reglas establecidas anteriormente, indicaremos la metodología utilizada en este caso.

1) Elección de los rasgos o características

Teniendo en cuenta el método utilizado se han elegido para definir su calidad, los siguientes componentes mayores y rasgos individuales:

A) Actividad sexual

A.1) Velocidad de acoplamiento
A.2) Capacidad de transferencia del esperma

B) Reproducción

B.1) Proporción sexual
B.2) Capacidad de puesta
B.3) Fertilidad de los huevos

C) Adaptabilidad

C.1) Capacidad de búsqueda del huésped
C.2) Resistencia a bajas temperaturas
C.3) Resistencia a altas temperaturas

2) Estandarización de las técnicas

En la actualidad prácticamente todas las pruebas están estandarizadas.

A.1) Velocidad de acoplamiento

Antes de determinar las condiciones en que se desarrollaría la prueba, se comprobaron cuales eran los momentos en que el insecto presentaba máxima actividad sexual,es decir los días transcurridos desde su emergencia y la hora desde el comienzo de la iluminación en que esta actividad era manifiesta. Gracias a estas pruebas se llego a la conclusión de que la determinación de este parámetro debe realizarse al 5º día de su emergencia y a las 5 horas de haber comenzado la iluminación.

La prueba se realiza pues a 25 ºC, 75% de humedad relativa, 4 repeticiones de 25 parejas que han permanecido separadas hasta que transcurran 5 días de su emergencia y 5 horas desde el inicio de la iluminación; momento en que se inicia la prueba.

El parámetro a medir es el numero de parejas formadas cada 10 minutos durante una hora.

A.2) Capacidad de transferencia del esperma.

De cada repetición de la prueba anterior se toman 10 hembras que se han acoplado, observando en sus órganos genitales la presencia o ausencia de esperma en el interior de su espermateca.

El parámetro a medir es el numero de hembras con su espermateca llena.

B.1)Proporción sexual

Se utilizan cuatro repeticiones de 100 adultos tomados al azar en la mitad del periodo de producción de la unidad de cría, ya que hay diferencias segun el periodo de producción en que se tomen.

El parámetro a medir es el numero de machos y hembras de cada repetición.

B.2) Capacidad de puesta.

Se toman tres repeticiones de 10 parejas formadas en la prueba A.1, valorándose el numero de huevos por pareja y día durante 2 semanas. Dichas parejas se encuentran sometidas a 25 ºC, 75% de humedad y 16 horas luz 8 horas oscuridad.

El parámetro a medir es el numero de huevos por pareja y día.

B.3) Fertilidad de los huevos

Se analizan tres repeticiones de 100 huevos tomados proporcionalmente a la puesta realizada por cada pareja de la prueba anterior y al final de la segunda semana de puesta. Para evitar el canivalismo de las larvas recién nacidas, estos se colocan individualmente dentro de cápsulas de gelatina transparente y se mantienen hasta su nascencia en iguales condiciones a las citadas en la prueba B.2.

El parámetro a medir en cada repetición es el numero de larvas nacidas.

C.1) Capacidad de búsqueda del huésped.

No es fácil medir este parámetro y menos aun si se trata como en este caso de un depredador ya que estos como ocurre con cualquier otro insecto, pueden ser estimulados física o químicamente (estímulos de color, olor, forma o incluso sonido) para localizar su presa, pero está demostrado que el mejor parámetro para medir la capacidad de búsqueda de un depredador es calcular la superficie rastreada, a mayor superficie rastreada mayor capacidad de búsqueda, lo cierto es que resulta muy difícil calcularla. Por ello en principio se utilizaron unos olfatómetros en los cuales los adultos del entomófago se situaban en un extremo y en el otro se situaba <u>P. citri</u>, contando aquellos insectos que durante un cierto tiempo eran atraídos por el alimento , al hacer pasar una leve corriente de aire en dirección a ellos. Estas pruebas se compararon con otras en que no se colocaba alimento en el extremo opuesto, demostrando lo que en principio ya suponíamos es decir que no existían diferencias entre los que tenían alimento y los que si tenían.

Aunque la técnica debemos perfeccionarla, en la actualidad utilizamos cuatro repeticiones de 50 adultos tomados en mitad del periodo de producción de la unidad de cría, que se sitúan en el centro de un circulo con 4 tubos de ensayo opuestos dos a dos, un par con alimento y el otro sin, contando el numero de adultos que durante un determinado tiempo llegan a los tubos que contienen dicho alimento.

C.2) Resistencia a las bajas temperaturas

Luego de varias pruebas que nos permitieron fijar la temperatura de trabajo, la prueba ha quedado establecida del siguiente modo:

Temperatura de trabajo, - 3 ºC

Insectos a utilizar, 3 repeticiones de 20 adultos por periodo de medida mas un testigo que se mantiene a 25 ºC y 75% de humedad relativa. (total 210 adultos)

Parámetro a medir insectos muertos cada dos horas durante 12 horas.

Hay que resaltar que esta prueba podría ser útil para seleccionar razas o líneas resistentes al frío.

C.3) Resistencia a altas temperaturas

Temperatura de trabajo, 35 ºC

Insectos a utilizar; 3 repeticiones de 20 adultos por periodo de medida mas un testigo que se mantiene a iguales condiciones que el de la prueba C.2.

Parámetro a medir, insectos muertos cada 24 horas durante cuatro días.

Los testigos en cada caso se utilizan para corregir la mortalidad si fuera necesario.

3) Elección de la población modelo y determinación de sus parámetros.

Como población de referencia se eligió una raza de C. montrouzieri que se encuentra aclimatada en ciertas zonas cálidas de la provincia de Castellón, y que criada en cautividad se ha constatado su efectividad en condiciones normales de utilización.

Los parámetros de dicha población, fueron determinados cuando se produjo la octava generación. A continuación se exponen los datos obtenidos en cada prueba.

A.1) Velocidad de apareamiento

Parejas a	Numero de repetición 1	2	3	4
10 min.	8	3	2	2
20 min.	2	3	2	3
30 min.	1	6	3	3
40 min.	2	1	1	4
50 min.	3	1	1	4
60 min.	0	1	1	2
Total	16	17	13	22

A.2) Capacidad de transferencia del esperma.

Repetición	1	2	3	4
% esprmat. llenas	86%	82%	80%	82%

B.1) Proporción sexual

Repetición	1	2	3	4
% de hembras	57%	52%	54%	55%

B.2) Capacidad de puesta

Datos referidos a la prueba. (Numero de huevos por pareja al final de la segunda semana)

Pareja	Repetición 1	2	3
1	127	44	178
2	223	309	347
3	228	266	263
4	264	320	210
5	282	301	245
6	380	361	165
7	364	320	259
8	229	284	272
9	270	214	217
10	275	190	197

Datos de 30 parejas referidos al total de su vida.

Media de huevos por hembra y día..... 10
Media total de huevos por hembra..... 1.153
Máximo de huevos por hembra.......... 2.079
Mínimo de huevos por hembra.......... 37

El mínimo anterior pertenece a una hembra enferma, que presentaba deformaciones importantes en su órgano genital externo.

B.3) Fertilidad de los huevos

Repetición	1	2	3	4	5	6
% de huevos eclosionados	74%	70%	75%	71%	73%	74%

C.1) Capacidad de búsqueda del huésped

Parámetros a determinar

C.2) Resistencia a bajas temperaturas

Para comparar estadísticamente esta población con las restantes, se han medido tres tipos de parámetros distintos T6 numero de adultos muertos a las 6 horas, T12 numero de adultos muertos a las 12 horas y A1 área comprendida entre la curva resultante y el eje X. Este ultimo parámetro refleja mejor las diferencias que los dos primeros. Los datos siguientes corresponden a 6 repeticiones de la prueba.

T6	T12	A1
6	6	46
3	9	43
5	6	48
4	9	41
1	8	40
2	7	41

C.3) Resistencia a altas temperaturas

Repetición	Numero de insectos muertos a			
	1 día	2 días	3 días	4 días
1	7	9	19	20
2	10	14	19	20
3	9	19	20	20

Aunque la prueba de supervivencia no se incluye en el esquema presentado anteriormente fundamentalmente por su larga duración; a continuación daremos los datos obtenidos para esta población:

Supervivencia expresada en numero de dias alimentándose de su presa.

	Vida media	Máximo	Mínimo
Hembras	120,7	205	13
Machos	194,1	227	135

Supervivencia expresada en numero de días alimentándose con agua azucarada

	Vida media	Máximo	Mínimo
Hembras	48,74	56	20
Machos	54,60	97	17

4) Monitorización o aplicación del control de calidad.

Una vez cubiertas todas las etapas anteriores, estamos preparados para aplicar las pruebas establecidas a una muestra de las poblaciones del entomófago criadas en los insectarios de nuestro país, para que luego de haberlas comparado estadísticamente (siempre que podemos utilizamos métodos paramétricos y si no tests no paramétricos) con la población de referencia, si existen cambios en su comportamiento tomar las medidas oportunas.

BIBLIOGRAFIA

- Boller E.F. and Chambers D.L., 1.977. Quality aspects of mass-reared insects. From: Biological control by augmentation of natural enemies. Edit. R.L. Ridgway and S.B. Vinson (Plenun Publishing Corporation), 219-235.

- Boller E.F., Katsoyannos B.I., Remund U., Chambers D.L., 1981. Measuring, monitoring and improving the quality of mass-reared Mediterranean fruit flies, Ceratitis capitata Wied. 1. The RAPID quality control system for early warning. Z. ang. Ent. 92, 67-83.

- Boller E.F. and Calkins C.O., 1.984. Measuring, monitoring and improving the quality of mass-reared Mediterranean fruit flies, Ceratitis capitata (Wied.). 3. Improvement of quality by selection. Z. ang. Ent. 98, 1-15.

- Chambers D.L.,Calkins C.O.,Boller E.F., Ito Y. and Cunningham R.T., 1.983. Measuring, monitoring and improving the quality of mass-reared Mediterranean fruit flies, Ceratitis capitata (Wied.). 2. Field tests for confirming and extending laboratory results. Z. ang. Ent. 95, 285-303.

- Garcia A. (sin publicar). Puesta a punto del control de calidad sobre Cryptolaemus montrouzieri Muls.(Tesina)

- Ripollés J.L., 1.986. La lutte biologique: utilisation d'entomophages dans les agrumes en Espagne. Parasitis 86. Geneve

LIMITS AND PERSPECTIVES OF BIOLOGICAL AND INTEGRATED CONTROL METHODS AGAINST PEST OF PROTECTED EGGPLANT CROPS IN ITALY

V. Vacante
Istituto di Entomologia Agraria, Università-Catania, Italy

Summary

The author deals with the problem of egg-plant defense in Italian protected environments. The most widespread diseases and pests, the present possibilities of control and the economic, toxicological and ecological results are discussed. Experimental results of integrated control of harmful Arthropods are briefly showed; limits and perspectives of the new strategy are also discussed.

1. INTRODUCTION

Among vegetable crops growing under tunnel or unheated greenhouse in Italy, egg-plant is of great importance above all in Southern regions which present the most suitable thermical conditions for the crop. Within protected crops, the solanaceous species, as capacity of output, fills the third position, after tomato and pepper. The surface covered with greenhouses and tunnels growing egg-plant amounts to more than 1.260 hectars (statistics ISTAT, 1984), 1.092 of which in Sicily equal to 86% of the global surface. National production is 624.000 ql. In comparison to the two above-mentioned vegetables, egg-plant has a longer cycle of cultivation; infact whereas tomato and pepper could have 2 cycles per year, with a lenght varying from 5 to 7 months each, egg-plant grows for 10-11 months, with a transplantation on September and a harvest from November to June-July, broken by a short standstill (15-20 days). The remarkable lenght of the cultivation cycle and the changeability of the thermic and hygrometric conditions , characterizing the unheated greenhouses, allow the rising of diseases and arthropods pests.

1.1 DISEASES

Plant parasites can cause different damages, with the results of a reduced production even to the death of the plant. Already in the seed-bed, if some particular hygrometric conditions happen and there is excess of water in the soil or peat, the seedlings are frequently liable to mortality in consequence of root or crown soft rots, caused by various fungal species (Rhizoctonia solani Kuhn., Pythium spp., Phytophthora spp.) After transplanting, the plants are liable to "crown-rot" caused by different soil fungi (Phoma lycopersici Cooke, Rhizoctonia solani Kuhn., Sclerotium rolfsii Sacc., Sclerotinia sclerotiorum (Lib.) Massee). In overworked soils, which ospited for a long period just the same crops or alternate solanaceous species, some root fungal diseases like Pyrenochaeta lycopersici Schneider et Gerlach (causal agent of coorky rot), Rhizoctonia solani Kuhn.,Fusarium solani App. et Wr. can occur. The most dangerous disease , occurring expecially outdoors, is caused by the fungus Verticillium dahliae Kleb., which can kill the plant in a varying lapse of time whereas not very often in the greenhouse you find this kind of damage, because of the good preventive action of annual treatments with methyl bromide. Oidium is another common disease caused by the fungus Leveillula taurica (Lèv.) Arn.; leaves are only concerned and just sporadically you need specific treatments. Really more harmful and not stood by the growers is "grey mould" caused by the fungus Botrytis cinerea Pers.; it damages leaves, stems and fruits, causing soft rots on which the mould could develop. Among the protected crops of Ragusa province this last disease is responsible for the bigger lasses of production.

Among bacteric species damaging egg-plant crops, _Pseudomonas solanacearum_ E.F. Smith is of great importance.

Among specific virosis, it must be reminded the presence of"egg-plant mottle dwarf virus"; egg-plant is also exposed to other viral-infections, like AMV and CMV.

1.2 PESTS

Among pests the root-knot nematodes of genre _Meloidogyne_ arouse a particular interest.

In Southern Italy the epigeous and phytophagous species noxious to egg-plant are relatively few. The infestation of the two-spotted spider mite (_Tetranychus urticae_ Koch) are harmful in a particular manner; this species, with the greenhouse whitefly (_Trialeurodes vaporariorum_(Westw.)) and leafminers (_Liriomyza_ spp.) are the key-problems to control the crop in Ragusa area. The attacks of noctuid _Lepidoptera_ (_Spodoptera littoralis_ (Boisd.), _Autographa gamma_ L. and _Chrysodeixis chalcites_ Esper.) are noxious in the same way. Aphids (_Myzus persicae_ (Sulz.), _Macrosiphum euphorbiae_ Thomas) point out frequent problems too. Less common are infestations of thrips (_Thrips tabaci_(Lind.)) and tarsonemid mites (_Polyphagotarsonemus latus_(Banks)), so that it's no necessary a constant control, but in a sporadic case. Absent from protected crops of Southern Italy is the beetle _Leptinotarsa decemlineata_ (Say), extremely noxious to egg-plant in vegetable environment of the Po Valley (Ferro, 1985; Celli _et al._, 1987; Benuzzi and Nicoli, 1988).

The above mentioned phytophagi arrive inside the greenhouse by different ways. The plants coming from the nursery and the different plant supporting materials may be vectors of motile forms of pests. Sometimes these stages come from the uti lity bordering areas where it's easy to find spontaneous flora, and from the neighbouring vegetable fields; little pests can be passively driven by the wind, the irrigation-water or the worker himself. Moreover during the break of cultivation, pests establish and are reproduced on outdoor flora. Also inside greenhouses, between one crop and the other,adult or joung motile stages of pests may remain both in the soil and on supporting structures.

1.3 PRESENT CONDITION OF PHYTOSANITARY DEFENCE

Italian horticulturists are fully aware of phytophatologic and entomological problems. At the present, it's a common notion that the employment of chemicals is the only strategy positively applicable for controlling crops. The undesired effects originating from the indiscriminate and inexperienced use of chemicals are usually omitted. The growers, the cooperative societies, the thecnicians, the commercial and professional organizations accept stoically the results of traditional chemical control as a rule. In a common outlook you can also find worker and buyer's sanitary risks, the environmental pollution, the rarefaction of beneficial organisms and the natural selection of resistant strains towards the most common chemicals.

To obviate these two last drawbacks people in Ragusa protected area are increasing the number of the treatments and the dosage of active ingredients. This cause the establishment of a no-way spiral where a chemical treatment is a real presupposition for the following one. Therefore in Ragusa protected arèa during a whole cycle of cultivation, 15 and over chemical treatments with different active substances are used. It's not unusual to notice treatments on foliage with phorate or other dangerous chemicals during harvesting. It must be recorded that in other Italian regions there is a grave employment of pyrethroids,the use of which can increase the presence of phytophagous mites above misure and the consequent use of acaricides.

2. ALTERNATIVE POSSIBILITIES OF CONTROL

The solution of the problem surely resides in the use and diffusion of integrated control. During these last years many Italian authors (Celli *et al.*, l.c.;Benuzzi and Nicoli,l.c.; Vacante *et al.*, 1988; Vacante *et al.*, in press.) entered upon specific researches in order to descover adequate solutions of the problem. At the first, the attention of the experts has been centralized, with some good results, in controlling the widespread species of epigeous phytophagi (*T. urticae*, *Liriomyza* spp., *T. vaporariorum*, noctuids, aphids, Chrisomelid beetles); but at the moment we don't know good solutions, alternative for controlling fungi or soil nematods.

2.1 RESULTS

2.1.1 *Tetranychus urticae*

The control of this phytophagous mite can be concretely guaranteed by a rational use of the phytoseid mite *Phytoseiulus persimilis* Athias-Henriot. Working in unheated greenhouses of Ragusa coast, Vacante *et al.*, l.c. showed that, at the beginning of the infestation,programmed releases of 6 phytoseids/sq.m. could assure a good success. When the attacks are very serious and distant from the harvesting, a release of the predatory mite must be effected after a selective acaricide treatment and the number of phytoseids/sq.m. can be increased if indispensable.

2.1.2 Liriomyza spp.

The employment of Diglyphus isaea (Walk.) against leafminers has constantly given a good outcome. A release of 200 eulophid adults, effected at the appearance of the early mines on the leaves, can guarantee a constant and profitable control of pests as a rule (Vacante et al., l.c.).

2.1.3 Trialeurodes vaporariorum

This pest representsa key-problem for the health of protected crops and particularly egg-plant. Its biotic potential is conditioned by the host plant. Egg-plant is a vegetable species very sensitive to the pest; on this crop a female of the aleurodid insect can lay as far as 450 eggs at 17°C degrees (Di Pietro, 1977) and layings of over 600 eggs were observed on some varieties (Malausa et al., 1983). Di Pietro showed that its fecondity per day is constant and it's not influenced by temperatures between 17 and 27°C degrees. This demonstrates that T. vaporariorum is a pest with a biotic potential well adapted to the temperature essential for egg-plant cycle.

The best natural antagonist of T. vaporariorum is the aphelinid Encarsia formosa Gahan. Onillon (1985) states that the fecondity of the entomophagous is excellent between 17° and 22°C; it decreases between 27° and 32°C progressively. At this last temperature it's equal at one third. Comparing the above mentioned data with the climatic course of unheated greenhouses of Southern Italy, you can observe that pest-parasitoid interaction is qualified by the unforeseenable climatic condition, which in our environment is commonly characterized, at the end of summer and late spring, from the highest daily temperatures above 30-35°C degrees. During winter time it's also easy to register temperatures below 10°C degrees, above all at the night. These facts have to suggest us to better study the question before proposing alternative solutions difficult to be put into practise and of insecure economic return. Repeated experiences carried out in representative surfaces of Ragusa, in different period of the year and with different modalities, have fomented further doubtful points. Inondative releases with E. formosa both after transplanting and at the end of winter, with various numbers of the parasitoids per square metre of crop from 16 up to 60 in 4-6 stages, have been carried out. Working with the same initial levels of infestation, results never depended on the number of parasitoid introduced; more than once trials carried out by employing a high number of E. formosa puparia assured a negative outcome.

2.1.4 Noctuids, Aphids, Chrisomelid Beetles

The damage caused by noctuids has a real influence in Southern Italy. Sometimes the attacks of Spodoptera littoralis (Boisd.) compromise the production outcome both after transplanting and during the production period. The employment of different strains of Bacillus thuringiensis (Berl.) at the dose suggested by the producing firms, didn't guarantee a good control of the population. Likewise ineffective was the use of the parasite nematods Heterorhabditis.

The effectiveness of natural population of Aphidius matricariae Hal. mass-reared at the insectarium of the Institute of Entomology (Catania) and released in some greenhouse of Southern Sicily, has positively experimented against Myzus persicae (Sulz.); but at the present infestations of Macrosiphum euphorbiae Thom. aren't validly controlled with biological antagonists. Benuzzi and Nicoli, l.c. report that in the

Pô Valley had good success by employing pirimicarb just after transplanting and introducing joung stages of Chrysoperla carnea Sthep. 1 or 2 times when the leaves met. On the contrary, the use of the above-said predator didn't gurantee the same results during spring in Sicily.

Leptinotarsa decemlineata (Say) is a pest very noxious to egg-plant in the Po Valley; in Sicily this insect is present but it isn't dangerous in protected crops. An encouraging biological method of control of this beetle is the employment of the eulophid Edovum puttleri Grissel (Benuzzi and Nicoli, l.c.). At the present we haven't definite methods of control yet.

2.1.5 Minor pests

During the utilization of integrated programmes, many secondary entomological problems often arise; this because of the widespread employment of wide action-spectrum chemicals that have formerly reined them in. So, during the experimentation carried out in Sicily frequent attacks of Thrips tabaci (Lind.), Phthorimaea operculella (Zell.) and Polyphagotarsonemus latus (Banks) have been noticed. Against the first pest, an inondative release of 2-16 motile stages per plant of Amblyseius cucumeris Oud. from the beginning of the infestation didn't assure good results. The presence of P. operculella doesn't alarm the growers and it has been accetable till now. P. latus is controlled at the right moment with the use of sulphur.

3. LIMITS OF THE STRATEGY

In the most important vegetable areas covered with egg-plant crops, the infestations of T. vaporariorum, S. littoralis and L. decemlineata are the principal impediments for the setting out of a realistic programme of integrated control.

As concern the first pest, experimental results showed that in Southern Italy the use of E. formosa is not generally decisive. This observation sets deep delaies for the settlement of real alternative strategies and obliges the experts to better study host-pest interaction expecially referring to the environmental and ecological conditions, in particular the thermical and hygrometric state. The situation of Po Valley greenhouses seems different, because of the spring cultivation period and the better thermic-hygrometric conditions for the success of the parasitoid. Infestations of S. littoralis and other noctuids don't meet adequate solutions now. After transplanting and distant from flowering the use of different active ingredients (methomyl and others) currently solves the problem, but it isn't the optimum solution. At the time of harvesting a localized distribution of poisoned bran is the only way really practicable, able to not alter the agro-ecosystem balance.

At the present we know that in Middle and Northern Italy attacks of L. decemlineata are controlled with widely-effective chemicals, incompatible with integrated control. The making of a strategy based on the use of E. puttleri could obviate the above-said disadvantage and pave the way to the new strategy. It must be added some impediments of socio-cultural, logistic and organizational nature.

The first aspect is sufficiently well-known; it's characteristic of a wide team of growers, both in the North and the South of Italy, not well-disposed to new alternative solutions the results of which, entrusted to biotic factors in some measure, don't seem changeable alike chemical treatment-phytophagous species relation. It follows that, missing an adequate demonstration and clear regulations for the grower, he feels free to choose the own strategy and decides for the so-discussed but still effective traditional methods.

The second and the third aspects are connected with the absence of a global plan, observant of needs and territorial characteristics, and based on a good system of thecnic assistance and the prompt availability of beneficial organisms.

The acarophagous and/or entomophagous species employed till now were bought from foreign bio-factories. This has involved serious problems connected with the quality of auxiliary species. More than once the quantity declared doesn't agree with that payed and moreover the functional response appears rather modest. A typical case is that of E. formosa puparia, coming from all over Europe: in our environment they arrive for the most part already emerged (sometimes above 50%); the remaining adults either die in handling or they are surely not effective beneficial organism. In the same way, the "quality" of P. persimilis populations is proved not sufficient. Experiences repeated into unheated greenhouses of Southern Italy allowed us to conclude that nothing or not much is to be expected from the bio-factories of Middle and Northern Europe.

4. FUTURE PERSPECTIVES

The perspectives for the practical application of integrated control in protected egg-plant crops are connected with the acquisition of further knowledges of some key-phytophagi (T. vaporariorum, S. littoralis and L. decemlineata), their natural antagonists and the production of necessary ausiliary species in loco.

The three aspects are interdependent; they could get done if there will be a political support able to help the finalized research plans, the thecnical assistance and the production of useful organisms. This question is a theme of ample discussion in Italy and in this context some regions are opportunely devoting themselves to the realization of the plan. From this point of view it's desirable that the example was adopted from the other Italian regions. That not just for local patriotism but in the respect of the fact that every strategy has to be matured and arranged in every different environment. Logistic situations connected with the distance between Italian regions suggest the above-said considerations.

REFERENCES

ANNUARIO DI STATISTICA AGRARIA, ISTAT 1984

BENUZZI, M. & NICOLI, G. - 1988. Lotta biologica e integrata nelle colture protette (strategie e tecniche disponibili).-Ed. Centrale Ortofrutticola Cesena, Regione Emilia-Romagna, ENEA Dip. TECAB, 168 pp.

CELLI, G., NICOLI, G. & BENUZZI, M. -1987. Biological control in protected crops in Northern Italy's Po Valley.-Bulletin SROP/OILB, 1987/X/2: 37-39.

DI PIETRO, J.P. - 1977. Contribution à l'étude d'une méthodologie de lutte biologique contre l'aleurode des serres T. vaporariorum (Homopt., Aleurodidae).-Thèse Fac. Sciences Toulouse.

FERRO, D.N. - 1985. Pest status and control strategy of the Colorado Potato Beetle.-Research Bullettin, Massachusett Agricultural Experiments Station, n° 704: 1-8.

MALAUSA, J.C., SICARD, J.C. & DAUNAY, M.C. - 1983. Potential biotique de Trialeurodes vaporariorum West. sur différentes variétés d'aubergine.-Bull. SROP/OILB, 1983 IV:6-9.

ONILLON, J.C.-1985. La lutte biologique contre les ravageurs des parties aériennes sous serre. L'emploi d'ennemis naturels dans la protection des cultures. Versailles, 10 Janvier 1986.-Ed. INRA, 1986 (Les Colloques de l'INRA, n° 34).

VACANTE, V., CALABRETTA, C. & NUCIFORA, A.-1988. Considerations sur les possibilites de lutte biologique et integrèe contre la mouche mineuse, Liriomyza trifolii(Burg.), dans la colture protegèe de gerbera en Italie.-XVIII International Congress of Entomology Vancouver, July 3 September 1988: 330.

VACANTE, V., TROPEA GARZIA, G. & CALABRO', M.- On the biological control of the two-spotted spider mite (Tetranychus urticae Koch) on egg-plant in unheated greenhouse.- Meeting "Integrated Pest Management in Protected Vegetable Crops", Cabrils, 27-29 may 1987, in press.

BUPROFEZIN: A POWERFUL HELP TO INTEGRATED CONTROL IN GREENHOUSE VEGETABLES AND ORNAMENTALS

M. Van de Veire
Faculty of Agricultural Sciences, State University-Gent, Belgium

V. Vacante
Istituto di Entomologia Agraria, Università-Catania, Italy

Abstract

In greenhouse and laboratory experiments in Belgium and Sicily the IGR buprofezin was tested for whitefly control and side effects on the parasites, Encarsia formosa, Dacnusa sibirica and Diglyphus isaea. The product displayed excellent larvicidal activity (during moult L_1 to L_2 and L_2 to L_3), and also showed ovicidal and vapour activity on the larvae.

Buprofezin efficiently controlled white flies in greenhouse tomatoes with 2 treatments (1 g a.i./10 l) at 8 days intervals without adversely affecting the parasites E. formosa, D. sibirica and D. isaea. Buprofezin-residues on the fruits were very small (less than 0.05 ppm, 8 days after treatment).

INTRODUCTION

Alternative control of pests in greenhouses has progressed very much in recent years all over Europe. Most attention was paid to biological control of whiteflies, leafminers, spider mites, thrips and aphids, with parasites and predators (Van Lenteren, 1987). Recently, attention has also been paid to entomopathogenic fungi (Helyer, 1987), bacteria and nuclear polyhedrosis viruses (Smits, 1987) for their use in IPM. Physical control with yellow sticky traps and thripstick was studied and has proven to be successful for whitefly- and thrips control (Van de Veire, 1984; J. Woets, 1985; Yudin, 1987). Specific insecticides (insect growth regulators) which may be used in IPM, have been developed. Buprofezin is one of these new insecticides which effectively controls the whiteflies Trialeurodes vaporariorum and Bemisia tabaci (Yashui, these new insecticides which effectively controls the whiteflies Trialeurodes vaporiorum and Bemisia tabaci (Yashui, 1986; Martin, 1987; Ishaaya, 1988) and may become very important through its specificity for IPM programs in greenhouses. The compound was discovered by Nihon Nohyaku Co (Yasui, 1985) and showed activities on planthoppers, leafhoppers and scale insects. Buprofezin is reported to be harmless to Phytoseiulus persimilis and Encarsia formosa in lab experiments (Anonymous, 1985). This paper reports on the effectiveness of buprofezin for whitefly control and its effect on the parasites Encarsia formosa, Dacnusa sibirica and Diglyphus isaea.

MATERIALS AND METHODS

1. Whitefly control on greenhouse tomatoes in Belgium

Three thousand tomato plants (cv. Baroso) were planted in a commercial greenhouse (surface: 1,500 M^2) on July 15th 1987. They were planted in 8 rows per subroof (or bay). These were infested by Liriomyza trifolii which had survived from the spring crop (leafmines and pit marks). Whitefly infestation was originally low (1 adult/100 plants) but became extremely high after some weeds (1-3 adults/plant 4 weeks after planting, due to continuous migration of insects from badly infested neighbouring cucumber plants at the sides of the greenhouse.

Adult whiteflies, leafminers and the parasite Dacnusa sibirica were monitored by suspending one yellow sticky trap (1.5 m x 0.1 m) vertically among the plants 30 cm above soil level. The trap was replaced weekly and counts of the afore-mentioned insects were carried out.

Encarsia formosa parasites were released three times at 14 day intervals by the grower (black scales on cucumber leaves) during the growing period. Two consecutive buprofezin treatments (100 mg a.i./liter) (Applaud 25% W.P.) on August 17th and August 25th 1987 were carried out. Further treatments were: dichlofluanide against Botrytis (3 x) and cyhexatin against the two-spotted spider mite. Aphids and caterpillars did not develop to damage levels and were not controlled chemically.

At regular times, leaflets were randomly sampled on which whitefly larvae were checked for mortality; assessment of the percent parasitism by E. formosa were also made. Leaflets containing leafminer-larvae were also randomly sampled, and the percentage of D. sibirica as well as the D. isaea parasitism was determined.

Tomato plants (n = 30), randomly sampled, were checked at different dates on number of leafminers, black and white scales and aphids.

2. Whitefly control on protected Gerbera crops in Sicily

Gerbera plants grown in plastic houses in southern Sicily were treated with buprofezin (Applaud 25 SC) in different concentrations (0.375 g a.i. /10 l; 0.75 g a.i./10 l; 1.125 g a.i./10 l and 2.25 g a.i./10 l). Each dose was applied to 200 plants, which were sprayed to runoff, by using a motor knapsack sprayer (pressure: 15 atm). Three experiments were carried out. In the first experiment, untreated plants were isolated from treated ones with plastic foil; in the 2nd experiment, the control plants grew alongside the treated ones without foil separation and in the 3rd experiment, the control plants were at a considerable distance from the treated ones, but still in the same greenhouse.

Sampling of leaves was done randomly (40 leaves per treatment). Out of each of these leaves a piece of 12.56 cm^2 was taken at random on which mortality of eggs and larvae was checked with a stereomicroscope.

The effect of buprofezin on *E. formosa* and *D. isaea* was also studied. *E. formosa* black scales and *D. isaea*-adults were dipped for 1 min in aquous buprofezin-spray solutions (0.375 g a.i./10 l; 0.75 g a.i./10 l). *D. isaea* and *E. formosa* adults were also confined to boxes which had previously been treated with a buprofezin solution (0.75 g a.i./10 l).

RESULTS AND DISCUSSION

1. Whitefly control on greenhouse tomatoes in Belgium

a. Insect monitoring

The number of weekly captured adult leafminers and *D. sibirica* parasites is given in Table 1.

The yellow sticky trap was rather long; this is necessary because small plates or cards trap different ratios of *L. trifolii*/*D. sibirica* when hung near the soil (30 cm high) or above the plants (2 m high). Plates which are positioned close to the soil, trapped significantly more *D. sibirica* than *L. trifolii*, while the opposite is found when the plates hung 1.5 m-1.8 m high (Van de Veire, unpublished).

Table 1 shows that high numbers of *D. sibirica* wasps emerged or were present in the greenhouse soon after planting the tomatoes (from 22.7.87 to 6.8.87). The grower, relying on these tables decided not to use chemical treatment, hoping natural control of the leafminer would be effective. Leaflets, containing leafminer larvae were collected on August 11th for determination of the *D. sibirica*-parasitization. Counts of emerged adult parasites on August 24th revealed 66% of *D. sibirica* parasites.

This figure coincides nicely with 69% of D. sibirica wasps which were trapped on August 25th. This vould mean that L. trifolii and D. sibirica are equally attracted to the sticky traps.

Table 1. Weekly trapped number of leafminers (L. trifolii) and parasitic wasps (D. sibirica) on a yellow trap

Date	No.of L. trifolii	No.of D. sibirica	%D. sibirica
22.07.87	81	250	75
30.07.87	185	294	61
06.08.87	204	418	67
11.08.87	7	13	65
18.08.87	22	30	58
26.08.87	17	38	69
01.09.87	5	47	90
08.09.87	0	15	100
14.09.87	1	7	87
28.09.87	0	2	100
06.10.87	0	1	100
13.10.87	0	0	-
20.10.87	0	0	-

b. Whitefly control, Encarsia formosa-parasitization

Egg fertilities and larval mortalities are summarized in Table 2. Samples were taken at different times and at different plant heights (leaves numbered from 1 -below- to 28 -top-).

Table 2. Ovicidal and larvicidal activity of buprofezin at different times and different plant heights; side effect on E. formosa

Date	Position of leaves	Egg fertility %	Larval mortality %	Parasitism %
14.9.87	leaf 1->10	-	-	80
14.9.87	leaf 11->14	56	100	0
29.9.87	leaf 17->18	77	100	0
6.10.87	leaf 25->28	96	50	60

Buprofezin was extremely active as larvicide. It interfered with the larval moult from L1 o L2 resulting in complete mortality on the lower and the middle leaves; these leaves had been sprayed twice. Mortality of larvae on the top leaves was only 50%, probably because these leaves had developed after the treatment had been carried out. The 50% mortality of the larvae may be due to residual vapour activity of buprofezin (from spray deposits on low and middle leaves). In an earlier experiment larval mortality on untreated tomato plants, adjacent (less than 1 m) to treated ones, was as high as in the treatments. Larvicidal activity is rather slow, so that long-term observations are necessary to detect the effects.

Ovicidal activity was clearly established on the lower leaves, probably by direct contact of buprofezin to the eggs, or through females having been in contact with buprofezin deposits; females which are in contact with buprofezin for some time may lay unviable eggs. Reports on the latter have been published (Ishaaya, 1988). During the experiment, great numbers of adult E. formosa were found on leaves containing dead L2-larvae. The question arises whether these wasps could feed and survive on these hosts.

At the end of the experiment, parasitized (black scales) were found at the top of the plants. Probably, only a few surviving whiteflies laid viable eggs which fully developed through absence of buprofezin vapours. Surviving E. formosa were probably able to parasitize their hosts. On October 2nd 1987, counts were made of the numbers of adult whiteflies and E. formosa on the plants. Thirty plants were randomly sampled. A mean of 6 adult flies and 7 wasps per plant was found, indicating the compatibility of buprofezin with biological control.

c. Liriomyza trifolii population dynamics; Diglyphus isaea-parasitism

The mean number of leafmines per leaf (n = 30 plants), counted on September 10th, is given in Fig. 1. Leaves were numbered from 1 to 23, beginning at the bottom of the plant. L. trifolii infested the oldest leaves (leaf 1 to 10) seriously but younger leaves were not damaged anymore. This was due to the combined biological control with D. sibirica (which had survived from spring tomato culture in the same house) and natural parasitization by the ectoparasite D. isaea. At different times, leaf samples at different plant heights were taken, and parasitism by D. isaea was checked. Data about the latter are given in Table 3.

The ectoparasitic wasp D. isaea was found at nearly every plant height. Empty leafmines were found as well, but as a fraction of the total; moreover, empty mines could be parasitized by D. sibirica. An indication for the latter is the fact that some D. sibirica were trapped on the yellow trap in September! The aforementioned data show the compatibility of D. isaea with buprofezin-treatments.

Table 3. D. isaea-parasitism at different times on several plant heights

Date	Position of leaves	Nº of counted leaf mines	Nº of empty mines	Nº of *D. isaea* escape-circles	Nº of dead *L. trifolii* larvae	Nº of *D. isaea* larvae	Nº of live *L. trifolii* larvae	Nº of endo-parasites *D. sibirica*
10.9.87	leaf nº4	61	18	30	1	0	0	0
10.9.87	leaf nº8	58	26	9	10	6	3	4
10.9.87	leaf nº10-13	37	14	0	5	2	7	9
22.9.87	leaf nº15-20	71	27	2	22	17	3	0
29.9.87	leaf nº15-20	34	15	3	9	7	0	0

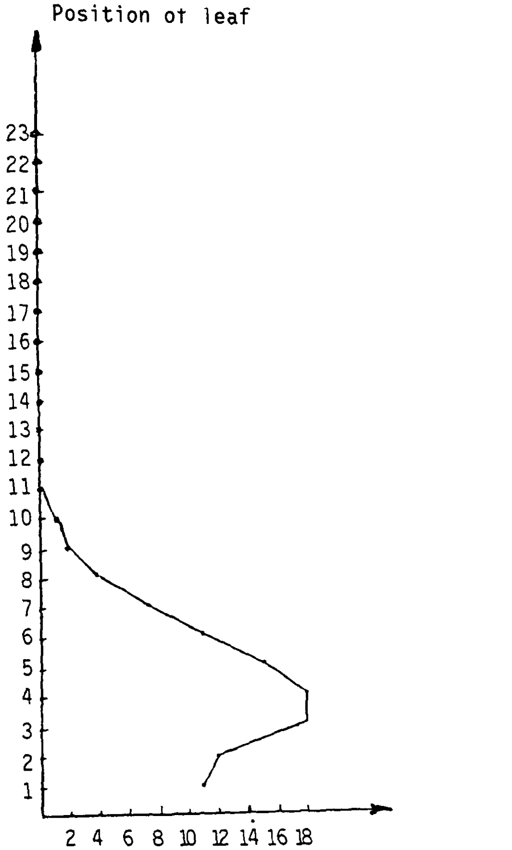


Fig. 1. Mean number of leafmines per leaf (n=30)

d. Aphid parasitism

Normally aphids are always present in greenhouse tomatoes in which biological control (E. formosa, D. sibirica) is carried out. Plant inspection on 26 plants on October 2nd, showed that few aphids were present: only on 5 plants out of 26 checked, aphids were found, while mummies were found on 6 others. It is clear that spontaneous aphid parasitism (probably by the parasite Praon volucre) could occur despite buprofezin-treatments.

2. Whitefly control on protected Gerbera crops in Sicily

a. Ovicidal effect

Egg hatch was checked 30 days post-treatment. Buprofezin sprays of 0.375 g a.i./10 l caused 73%, of 0.75 g a.i./10 l 86% mortality (control: 1% mortality). Egg mortality is probably caused indirectly, through the females, since it is well-known that females which came in contact with buprofezin for a considerable time, may lay unhatchable eggs.

b. Larvicidal effect

Mortality of the larvae was checked 20 days post-treatment (Table 4). In all experiments buprofezin was highly toxic to L_1 -->L_2 and L2 -->L3 moulting larvae, whereas larval mortality in the control of the 2nd experiment was considerably higher than in the other ones, probably due to vapour activity of the compound.

Table 4. Larvicidal activity of buprofezin at different spray-concentrations

Treatment dosage (a.i./10 l)	Experiment I Mortality at L2->L3 moult	Experiment II Mortality at L1->L2 mouly	Experiment III Mortality at L1->L2 and L2->L3 moult
0.375 g	83.3 (10.7)*	-	99.5 (0.17)
0.75 g	94.8 (1.0)	100	99.7 (0.32)
1.50 g	-	100	-
2.25 g	-	100	-
control (untreated)	11.2 (109.4)	29.6	14.44 (14.3)

* between parenthesis: number of adults per Gerbera leaf

c. Effect on E. formosa and D. isaea-parasites

Dipping black scales in buprofezin spray solutions shortened longevity only slightly with the concentration of 0.75 g a.i./10 liter. Exposure to dry spray deposits did not have any effect on adult E. formosa (Fig. 2).

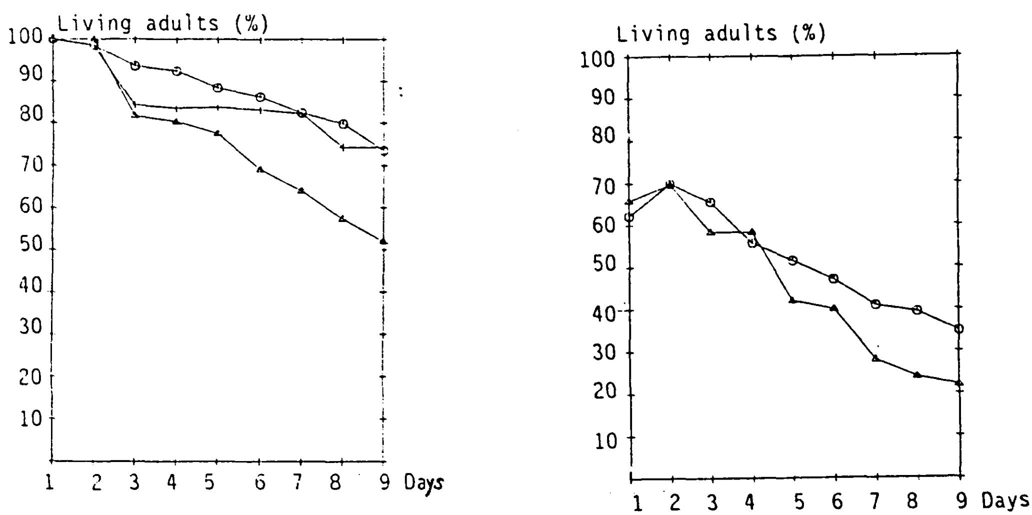


Fig. 2. Effect of buprofezin on the longevity of E. formosa.
Left: dipping of black scales. Right: exposure of adults to spray deposit.
(o: control; Δ: buprofezin 0.75 g a.i./10 l; +: buprofezin 0.375 g a.i./10 l)

Dipping adult D. isaea in a buprofezin-solution of 0.75 g a.i./10 l, and exposure of adults to dry spray deposits (same concentration) had no adverse effect on the ectoparasite (Fig. 3).

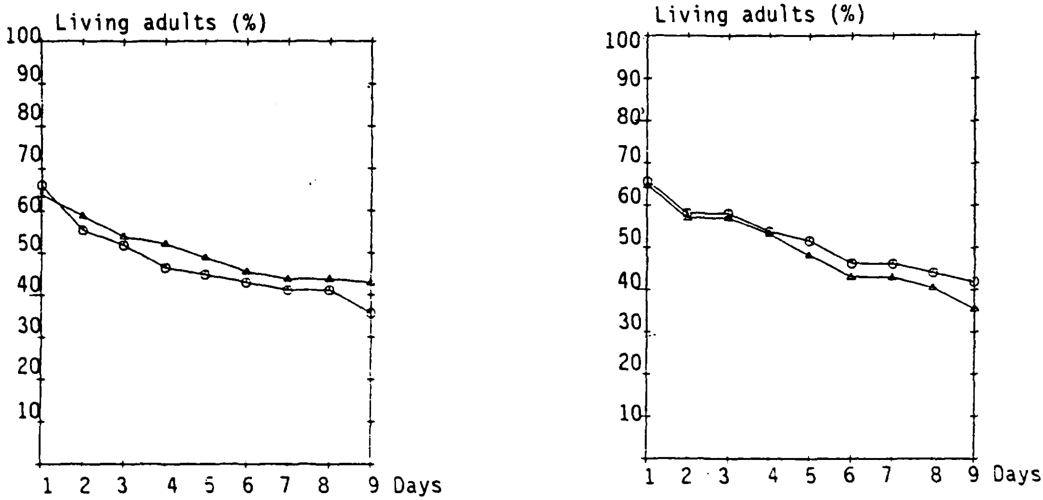


Fig. 3. Effect of buprofezin on the longevity of D. isaea.
Left: dipping of adult D. isaea. Right: exposure of adults to spray deposits.
(Δ: buprofezin 0.75 g a.i./10 l; o: control)

This is of great importance for IPM, since leafminers are nowadays the major pests in Gerbera protected crops.

CONCLUSIONS

Similar results with buprofezin for whitefly control were observed in two countries with a different temperature and humidity regime. The compound efficiently killed whitefly larvae (stage L_1 to L3), exerted ovicidal activity and did not negatively affect the parasites E. formosa, D. sibirica and D. isaea. Vapour activity was established as well.

Two consecutive buprofezin-treatments (0.75 g a.i./10 l) (8 days interval) on tomatoes caused a dramatic decrease in population density, while one treatment (0.75 g a.i./10 l) on Gerbera considerably reduced the number of adult whiteflies.

It looks like it that buprofezin will play a key role in IPM in greenhouses for whitefly control. Up till now, no pesticides were available (except calcium cyanide, legally applied in the Netherlands) to sustain or correct insufficient biological control. Therefore, tomato-growers often decided again to use chemical control, providing a control with less risk than the biological control, but in this case frequent applications (up to 20 per season) are required.

In Sicily, reinfestations from outdoors hamper biological control badly, as well as low winter temperatures; buprofezin will act here as an ideal correcting agent for insufficient E. formosa-activity.

We are convinced that IPM in greenhouses may be stimulated greatly by the use of buprofezin, through a better and much more reliable biological control, which is a key argument for growers, at the same time giving a chance to other parasites and/or predators, which normally are eradicated with broadspectrum pesticides (aphid-, caterpillar-, leafminer parasites and mite predators.

ACKNOWLEDGEMENTS

The authors wish to thank Director R. Nierinck, ICI Belgium, for his generous gift of Applaud and Dr. Calus (IWONL-Bureau for Biometrics) for drawing the figures. Research supported by the IWONL, Center for Integrated Control, Director: Prof. Dr. C. Pelerents.

REFERENCES

Anonymous (1985). Technical Bulletin Buprofezin

Helyer, N.L. & Wardlow, L.R. (1987). Aphid control of chrysanthemum using frequent, low dose applications of Verticillium lecanii
Bull. SROP, X/2, 62-65

Ishaaya, I., Mendelson, Z. & Melamed-Madjar, V. (1988). Effect of buprofezin on embryogenesis and progeny formation of sweetpotato whitefly (Homoptera: Aleyrodidae)
J. Econ. Entomol. 81, 781-784

Martin, N. & Workman P. (1986). Buprofezin: a selective pesticide for greenhouse whitefly control
Proceedings of the 39th N.Z. Weed and Pest Control Conference, 234-236

Smits, P.H. (1987). Nuclear polyhedrosis virus as biological control agent of Spodoptera exigua
Proefschrift ter verkrijging van de graad van doctor in de wetenschappen

Uchida, M. (1987). Inhibition of prostaglandin biosynthesis and oviposition by an insect growth regulator, buprofezin, in Nilaparvata lugens Stal
Pesticide biochemistry and physiology, 27, 71-75

Van De Veire, M. (1984). Yellow sticky plates as an alternative tool for the control of the greenhouse whitefly and leafminers in greenhouse tomatoes
Bull. SROP VII/I, 51-54

Van Lenteren, J.C. (1987). World situation of biological control in greenhouses and factors limiting use of biological control
Bull. SROP, X/2, 78-81

Woets, J. (1985). Integrated programmes for specific crops
Biological Pest Control. Editors: N.W. Hussey & N. Scopes

Yasui, M., Fukada, M. & Maekawa, S. (1985). Effect of buprofezin on different developmental stages of the greenhouse whitefly, Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae)
Appl. Ent. Zool. 20 (3): 340-347

Yasui, M., Fukada, M. & Maekawa, S. (1986). Effects of buprofezin on reproduction of the greenhouse whitefly, Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae)
Appl. Ent. Zool. in press

Yudin, L.S., Mitchell, W.C. & Cho, J.J. (1987). Color preference of thrips (Thysanoptera: Thripidae) with reference to Aphids (Homoptera: Aphididae) and leafminers in Hawaiian lettuce Farms
J. Econ. Entomol. 80: 51-55

THE PROBLEM OF SUPERPARASITISM IN THE PRODUCTION OF NATURAL ENEMIES FOR INUNDATIVE BIOLOGICAL CONTROL: A GENETICAL APPROACH

E. Wajnberg & J. Pizzol
Station de Zoologie et de Lutte Biologique, INRA-Antibes, France

SUMMARY

The release of parasitoids for large-scale inundative biological control programs requires a well-defined method to produce large numbers of insects without reducing their biological potential. In the case of the production of Trichogramma species (Hym.; Chalc.) with the eggs of a factitious, host Ephestia kuehniella, one of the most important problems is the existence of superparasitism, because this phenomenon can lead to a decrease both in the number of individuals produced and in the vigor of the females released (i.e. fecundity, longevity, etc...).

In order to quantify and to avoid as much as possible this phenomenon, experiments were done to see if there is genetic variability of the biological traits involved, in a mass-reared population of Trichogramma maidis PINTUREAU & VOEGELE.

Based on histological staining, which allows us to count the number of Trichogramma eggs deposited inside each host, we compared different wasp isofemale lines (i.e. families), each of which was submitted to different conditions (i.e., parasite/host ratios).

Results show that superparasitism seems to be genetically determined. This appears true both for the average number of eggs deposited in each host, and in the distribution of wasp eggs among hosts.

In terms of applications, these results show that selection programs to reduce the intensity of superparasitism may be feasible. However, they also show that a released wasp population is not genetically fixed, but can change according to environmental pressures. Thus, the concept of "Biological Quality" is often used in too restrictive a sense : the ability of released populations to respond to environmental variations should more often be taken into account.

1 INTRODUCTION

The concept of "Biological quality control" is, in fact, related to the necessity to define accurate methods to produce Insects under laboratory conditions. This is particularly true when we consider large-scale inundative biological control programs, for which large number of Insects must be produced in relatively short times without reducing their biological potential (i.e. efficiency to control pests under field conditions).

Producing such large numbers of Insects is not very easy. In the case of the production of Trichogramma species (Hym.; Chalc.) with the eggs of a factitious host Ephestia kuehniella

(Lep.; Pyralidae), one of the most important problems to solve is the existence of superparasitism. This phenomenon often leads both to a decrease in the fitness of the surviving parasitoids (i.e. fecundity, longevity, searching rate, etc.) (Chacko, 1969; Waage, 1986) and to the emergence of a population with a male-biased sex-ratio (Waage, 1986, 1988).

In order to limit this phenomenon as much as possible, it is of prime importance to understand and to quantify (1) what is its actual limiting effect on the mass-production of wasp populations, and (2) how it is implicated in the demographic and evolutionary characteristics of mass-reared populations.

We decided therefore to see whether or not the biological traits involved in superparasitism are genetically determined in a mass-reared population of Trichogramma maidis PINTUREAU & VOEGELE. If this is the case, it could give us very interesting information on the way parasitoids could respond to mass-production under laboratory conditions, and it would also show that selection programs to reduce the intensity of this phenomenon are feasible.

2 EXPERIMENTAL DESIGN

The method used here was the isofemale lines method (Parsons, 1980) which allows one to see if the measured traits are family characteristics or not, and thus wether or not they are under genetic control.

Ten mated T.maidis females, taken from the laboratory mass-rearing were used to found ten isofemale lines. After two generations of multiplication, emerging females (less than 24 hours old) from each line were offered Ephestia kuehniella eggs using different female/host ratios. These ratios were allowed to take four different values : 1/10, 1/4, 1/2 or 1/1 ; and this was done in two different ways: (1) the number of females was kept constant and equal to 10, and the number of offered hosts was allowed to vary : 100, 40, 20 or 10 eggs, or (2) a fixed number of hosts (40) and different numbers of females (4, 10, 20 or 40).

For each isofemale line and for each female/host ratio, two replicates were done. The whole experimental procedure is summarized Fig. 1.

After 24 hours of parasitization, females were removed and hosts were histologically stained (Toluidin blue) in order to count the number of Trichogramma eggs deposited inside each host.

For the different traits observed, the isofemale lines were compared with analysis of variance procedures, after pooling the two replicates done in each case.

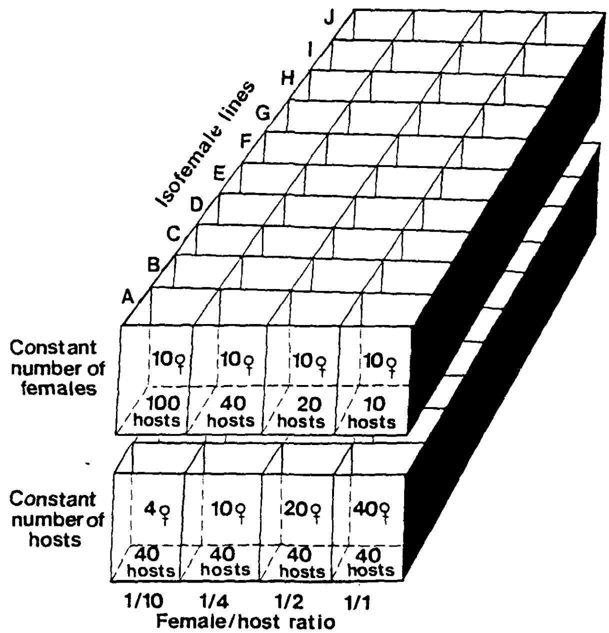


Figure 1 : Diagrammatic representation of the experimental design. For each isofemale line, four female/host ratios were studied. This was done either with a fixed number of wasp females and a varying number of hosts, or with a fixed number of hosts and an increasing number of females. In each case, two replicate were done.

3 RESULTS

Fig. 2 gives all the distributions of wasp eggs per host for all of the female/host ratios and for the ten isofemale lines studied. We can see that there is substantial variations in such distributions when we consider different female/host ratios. Is there also significant differences between the ten families studied?

If we quantify superparasitism intensity by the average number of parasite eggs deposited inside each host, as initially proposed by Fiske (1910), it becomes possible to statistically compare this intensity between the different isofemale lines for all experimental conditions. Results, given in Tab. 1, show that there is a strong female/host ratio effect on this parameter. This fact is an already well-known phenomenon (see for example Rogers, 1975 and Waage, 1986, 1988) : The more the parasite/host ratio increases, the more the average number of eggs deposited in each host rises correspondingly.

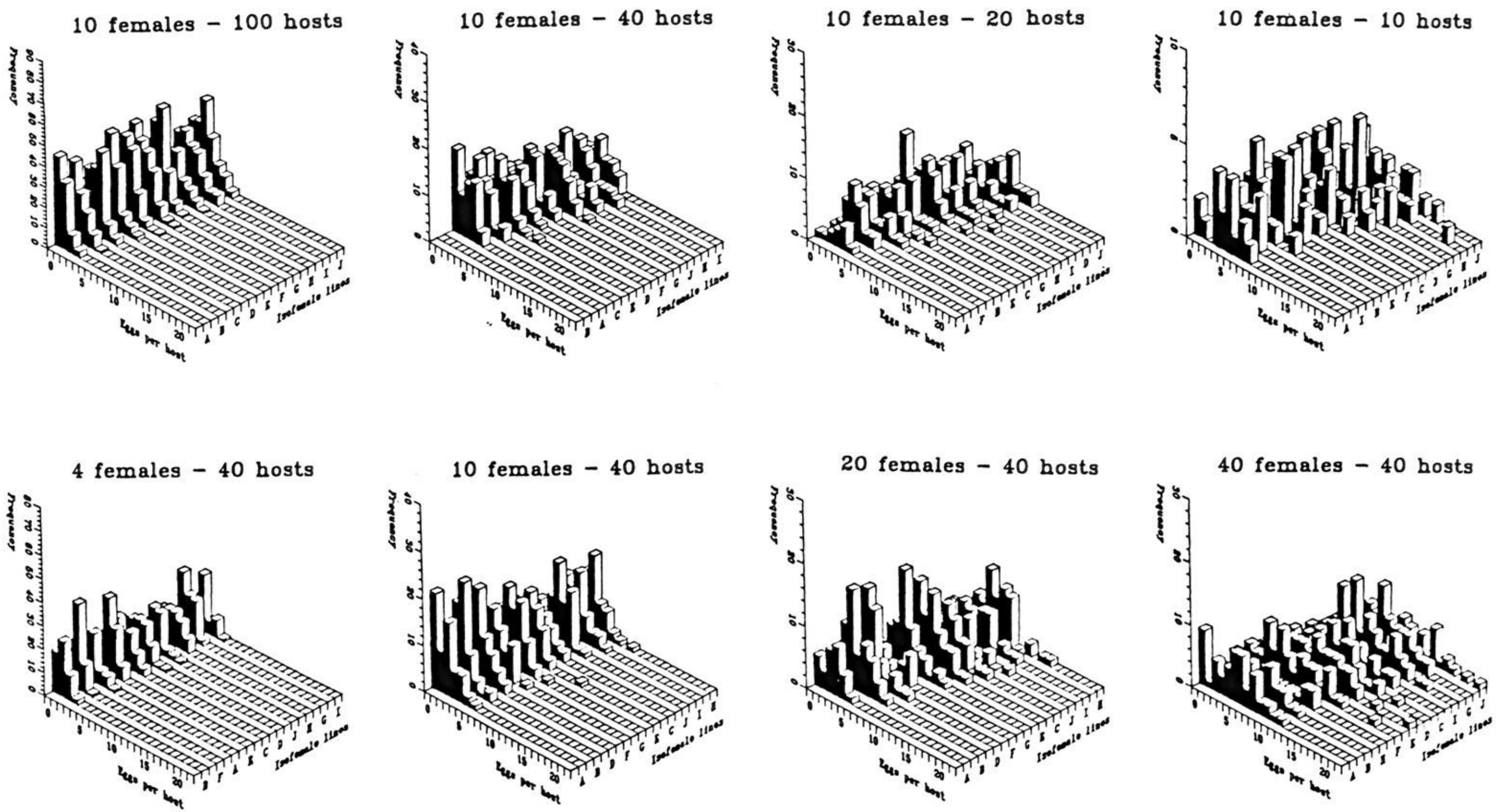


Figure 2 : Distributions of wasps eggs per host for all of the female/host ratios and for the ten isofemale lines studied. In each graph, lines are ordered according to increasing mean value of wasp eggs per host.

Source of variation	d.f.	Variances	F
Female/host ratio	7	50.124	66.74 (**)
Isofemale line	9	6.249	8.32 (**)
Error	62	0.751	
Total	78	5.816	

Table 1 : Analysis of variance of the average number of parasite eggs deposited in each host. (** : $p<0.01$).

Additionally, we can see that there is also a strong isofemale lines effect ($p<0.01$). This result shows that superparasitism intensity is a family characteristic and seems to be therefore genetically determined in the <u>Trichogramma</u> population studied. This seems to be confirmed by the fact that the sorting out of lines in Fig. 1, on the average number of parasite eggs per host, is statistically the same for all of the eight female/host ratio conditions (Friedman test : $chi^2 = 49.9$, 9 d.f., $p<0.01$).

Instead of considering average values of wasp eggs deposited inside each host as done above, we will try now to see if there are also variations between the ten families studied for global characteristics of distributions shown on Fig. 1.

One of the parameters frequently used in such a case is the mean/variance ratio which indicates here, at constant average values, if the parasite eggs are randomly distributed among hosts or not. Values close to one indicate that the average is approximately equal to variance, which is characteristic of a random (Poisson) distribution. Values larger than one show that we are dealing with more regular distributions, and, on the other hand, a mean/variance ratio less than one indicates that ovipositing wasp females lay their eggs in an aggregative manner. Tab. 2 shows results of analysis of variance done on that parameter in order to compare the ten isofemale lines. We can see, here again, that there is a strong parasite/host ratio effect. This fact seems to show that wasp females are able to change the way they distribute their eggs among hosts in response to the experimental conditions encountered.

More interesting is that there is also a significant isofemale lines effect ($p<0.05$), which suggests that distribution of wasp eggs among hosts is a family feature : Some lines aggregate their eggs more intensively than others. Thus, this trait seems also to be genetically determined.

Source of variation	d.f.	Variances	F
Female/host ratio	7	0.5332	9.63 (**)
Isofemale line	9	0.1249	2.26 (*)
Error	62	0.0554	
Total	78	0.1063	

Table 2 : Analysis of variance of the mean/variance ratio of the distributions shown in fig. 1. (* : $p<0.05$; ** : $p<0.01$).

Finally, Fig. 3 shows that there is no correlation between the two traits studied (average number of eggs per host, and mean/variance ratio) among families. This suggests that these traits seem not to be controlled by the same genetic mechanism.

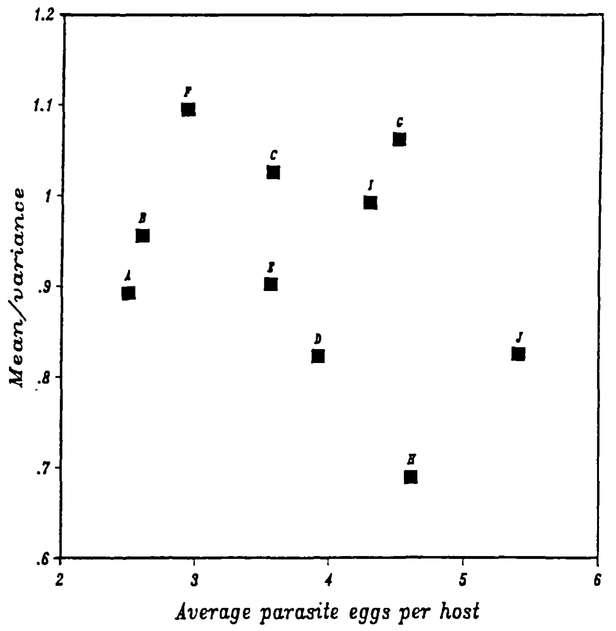


Figure 3 : Relationship between the two traits calculated on each family : average number of wasp eggs per host (superparasitism intensity) and mean/variance ratio. Points represent Trichogramma isofemale lines which are labeled as in fig. 1. Data shown are mean values of the eight female/host ratio conditions. Spearman rank correlation = -0.055 (NS).

4 DISCUSSION

Results presented here show that there are substantial variations in superparasitism characteristics in the mass-reared *Trichogramma maidis* population studied. Using the isofemale lines method, we have shown that these variations appear to be, at least in part, under genetic control. This is true both for the average number of wasp eggs deposited in each host, and for the distribution of parasite eggs among available hosts. Furthermore, no correlation could be found between these two traits, suggesting that genetical mechanisms involved are not the same in both cases.

If we restrict this discussion to an application point of view, with respect to "biological quality control" programs, such results lead to several conclusions. They show, firstly, that selection programs to reduce the intensity of this phenomenon may be feasible, at least for the population here studied. Indeed, if the results shown in Tab. 1 are confirmed, it should be possible to reduce the intensity of superparasitism (i.e. average number of parasite eggs per host) in a few breeding generations of selection.

Secondly, they lead, in our opinion, to a more interesting conclusion : that a released wasp population is not genetically fixed, and is thus able to change according to variations in environmental conditions (i.e. variations in climatic characteristics, in the density and in the size of available hosts, etc.).

Such genetic variation may lead to some additional difficulties when we want to estimate and control the "quality" of a released wasp. The use of this term "quality", in its rigorous meaning, presupposes to have a thorough knowledge of the mass-production characteristics, and chiefly of the field conditions encountered by the released population, and this is unfortunately almost never the case.

The concept of "biological quality control" seems often to be used in too restrictive a sense : In fact, the ability of released populations to respond to environmental variations should more often be taken into account.

oOo

REFERENCES

CHACKO, M.J. - 1969. The phenomenon of superparasitism in Trichogramma evanescens minutum Riley. I. Beiträge zur Entomologie. 19: 617-635.

FISKE, W.F. - 1910. Superparasitism : an important factor in the natural control of insects. J. Econ. Ent. 3: 88-97.

PARSONS, P.A. - 1980. Isofemale strains and evolutionary strategies in natural populations. in: HECHT, M., STEERE, W. & WALLACE, B. (eds.), Evolutionary biology. - Plenum. 13: 175-217.

ROGERS, D. - 1975. A model for avoidance of superparasitism by solitary insect parasitoids. J. Anim. Ecol. 44: 623-638.

WAAGE, J.K. - 1986. Family planing in parasitoids: adaptative patterns of progeny and sex allocation. in: WAAGE, J.K. & GREATHEAD, D. (eds.), Insect parasitoids. - Academic press. 63-95.

WAAGE, J.K. - 1988. Understanding progeny and sex allocation in egg parasitoids. in: VOEGELE, J. (ed.), Trichogramma and other egg parasites. - 2nd International Symposium. Guangzhou. China. November 1986. 283-294.

EMPLEO DE ELECTROFORESIS EN LA IDENTIFICACION DE BIOTIPOS DE PARASITOS DE AFIDOS Y SU IMPORTANCIA EN PROGRAMAS DE CONTROL BIOLOGICO CLASICO

D. González, M. Moratorio
Departamento de Entomología, Universidad de California-Riverside, USA

E. N. Botto
Laboratorio de Control Biológico, Instituto de Patología Vegetal, INTA-Buenos Aires, Argentina

Resumen

La electroforesis como técnica analítica aplicada a estudios biológicos, ha contribuido enormemente al desarrollo de la sistemática y la genetica de poblaciones.

El presente trabajo analiza el valor de esta técnica en relación con su empleo en investigaciones sobre control biológico clásico (CBC) de plagas agrícolas y discute su uso en aspectos tales como: identificación de biotipos de enemigos naturales, control de calidad genética en crias masivas y evaluación del establecimiento de enemigos naturales con especial referencia a parásitos de áfidos.

1 INTRODUCION

El control biológico de la cochinilla algodonosa *Iceria purchasi* (MASK.) por el coccinélido *Rodolia cardinalis* (MULS.) en cítricos de California en 1888, señala el primer éxito de un programa de control biológico clásico (CBC) (DOUTT, 1964). Desde entonces, diferentes resultados se han ido alternando en el empleo del CBC (GREATHEAD, 1985).

Los factores que determinan el éxito o el fracaso del CBC, no siempre conocidos o convenientemente estudiados, por lo general están relacionados con alguna de las cuatro etapas operativas del método: 1-Introducción de enemigos naturales, 2-Cría masiva de éstos; 3-Colonización de los enemigos naturales introducidos; y 4-Evaluación de los mismos.

El CBC, ha experimentado un importante desarrollo en aspectos teóricos y prácticos; no obstante ésto, la implementación de cada una de sus etapas continúa planteando las mismas preguntas básicas.

En relación con la introducción, la correcta identificación y el mantenimiento de la máxima variabilidad genética de los organismos a introducir son dos aspectos fundamentales en el proceso de selección de los enemigos naturales.

La correcta identificación sistemática es un punto clave, no sólo por los riesgos que implica una mala identificación, sino porque la variabilidad genética que se trata de proteger depende de la posibilidad de detectar diferencias entre las problaciones de enemigos naturales a introducir. Estas diferencias, que no siempre son evidenciadas morfologicamente, pueden ser ecológicas, fisiológicas o de comportamiento y afectan notablemente el potencial biológico de los enemigos naturales.

El mantenimiento de la variabilidad genética de los enemigos naturales a introducir, plantea interrogantes tales como: 1- ¿cuál es el número mínimo de enemigos naturales a colectar? (tamaño de la futura colonia fundadora), y 2- ¿cuántas regiones deben ser examinadas durante la colecta de individuos? (mantenimiento de la diversidad genética), etc.

En la etapa de cría masiva, la producción óptima de enemigos naturales es el objetivo mas importante. Esto plantea un problema práctico difícil de resolver, ya que aumentar la cantidad y mejorar la calidad (= atributos biológicos) no siempre son aspectos compatibles. El mantenimiento de la calidad biológica de los enemigos naturales introducidos depende en gran medida del tamaño de la colonia colectada. Si dicha colonia es pequeña se corre el riesgo de un "cuello de botella genético" y sus posibles consecuencias sobre los atributos biológicos de los enemigos naturales.

La endocría, otro problema que ocurre frecuentemente en los procesos de cría masiva, conduce tambien a la disminución de la variabilidad genética (pérdida de heterozigosis) tal como se observó en *Aphidius ervi* HALIDAY (Hymenoptera: Aphidiidae), UNRUH Y COL. (1983).

La pérdida de variabilidad genética puede afectar seriamente a los atributos biológicos de los enemigos naturales introducidos, jugando consecuentemente un papel de importancia en el éxito de la etapa de colonización.

En la etapa de colonización el principal objetivo es establecer, en forma permanente, los enemigos naturales introducidos. Los problemas de tipo operativo que se plantean aquí son, entre otros: ¿cuál es el número mínimo de enemigos naturales a liberar en cada región?, ¿en qué momento?, ¿bajo qué condiciones?, etc.

La evaluación de enemigos naturales es la última etapa en el desarrollo de un proyecto de CBC. Esta etapa abarca dos tipos de evaluaciones: biológica y económica. En la evaluación biológica se analizan los aspectos que han influido en el éxito o fracaso del establecimiento de los enemigos naturales, mientras que en la evaluación económica interesa saber cuál de las especies introducidas y establecidas es la mas efectiva desde el punto de vista económico.

Los aspectos mencionados para cada una de las etapas del CBC, si bien representan problemáticas distintas, estan interrelacionados por tener una necesidad común: la correcta identificación de los enemigos naturales y el conocimiento de sus principales atributos biológicos. Estos dos aspectos son críticos en CBC.

2 IDENTIFICACION DE BIOTIPOS POR MEDIO DE ELECTROFORESIS

Poblaciones de enemigos naturales morfologicamente idénticas y sin aislamiento reproductivo entre ellas, pueden diferir en propiedades biológicas fundamentales tales como: requisitos térmicos, fecundidad, proporción de sexos, comportamiento de búsqueda, especifidad de huéspedes, etc. En algunos casos dichas poblaciones se han denominado biotipos (GONZÁLEZ Y COL., 1979; DIEHL Y BUSH, 1984; CALTAGIRONE, 1985).

La importancia de la discriminación y evaluación de biotipos en el control biológico de plagas ha sido considerada por varios autores: NEMEC Y STARY, 1983a,b; DIEHL Y BUSH, 1984; CALTAGIRONE, 1985; UNRUH Y COL., 1986; BOTTO Y COL., 1988; GONZÁLEZ, 1988.

La identificación y discriminación de biotipos de enemigos naturales es una tarea difícil que requiere el empleo de mas de un método de análisis (morfológico, biológico, molecular).

Entre las técnicas que emplean análisis a nivel molecular, las usadas mas frecuentemente son la electroforesis de enzimas y los análisis de ADN (Secuencia; hibridación; etc.) (BERLOCHER, 1984; UNRUH, en prensa).

La electroforesis de enzimas (proteinas) como técnica analítica aplicada a la química y a la biología se ha popularizado enormemente en los últimos 30 años (RICHARDSON, 1986). Basada en la capacidad migratoria de las enzimas al ser sometidas a un campo eléctrico y a su posible visualización (zimogramas) por medio de técnicas de coloración histoquímicas, la electroforesis provee importante información genética de los organismos estudiados tanto a nivel individual como poblacional. Basicamente dicha información es cuantitativa (frecuencias genéticas), contándose actualmente con programas de computadora como el BIOSYS (SWOOFFORD Y SELANDER, 1981) que permite el empleo de técnicas para el análisis estadístico de los datos y para su correcta interpretación biológica. AVISE (1974), BERLOCHER (1979, 1984), RICHARDSON Y COL. (1986), proporcionan información importante sobre el uso y alcance de la electroforesis y sus diferentes métodos en estudios de sistemática y de genética de poblaciones de organismos. Comparada con otros métodos moleculares, ésta técnica es una de las mas empleadas en la actualidad por su sencillez y bajo costo operativo.

3 ESTUDIOS ELECTROFORETICOS DE ENEMIGOS NATURALES UTILIZADOS EN EL CONTROL BIOLOGICO DE PLAGAS

No obstante los antecedentes mencionados, el empleo de la electroforesis en estudios de enemigos naturales es relativamente escaso (UNRUH Y COL., 1986) y su aplicación en programas de control biológico es reducido. A continuación se presentan algunos ejemplos del uso de este método en relación con distintos aspectos del CBC.

3.1 Sistemática

Entre los primeros trabajos en los que se utilizaron técnicas electroforéticas en estudios sistemáticos de enemigos naturales se destacan los efectuados sobre *Trichogramma* en Francia (VOEGELE Y COL., 1976; JARDAK Y COL., 1979; PINTUREAU Y COL., 1980; PINTUREAU Y BABAULT, 1980, 1981, 1982; BERGE Y COL., 1983). Estos estudios posibilitaron el esclarecimiento de la posición sistemática de varias especies de *Trichogramma* y la evaluación de diferentes razas (biotipos) del parásito empleadas en el control biológico de *Cydia pomonella* (L.). PUNGERL (1986) empleó la electroforesis como técnica complementaria en estudios sobre la variabilidad de caracteres morfométricos utilizados frecuentemente en la taxonomía de parásitos de áfidos de importancia económica.

3.2 Genética y biologia

KAWOOYA (1983) utilizó el método electroforético para el estudio genético de poblaciones de *Muscidifurax* spp. y *Spalangia endis* WALKER, himenópteros parásitos de dípteros de importancia veterinaria. Mediante la electroforesis de isoenzimas, UNRUH Y COL. (1983) evaluaron la pérdida de variabilidad genética en poblaciones de *A. ervi* criadas en laboratorio para su empleo en el control biológico de áfidos de la alfalfa. De modo semejante, HUNG Y COL. (1988) evaluaron la variabilidad genética de razas telitocas y arrenotócas del parásito *Mesochorus nigripes* RATZEBURG (Hymenoptera: Ichneumonidae).

Las técnicas electroforéticas han sido empleadas también con éxito en la detección de enemigos naturales endoparásitos de varios insectos plagas.

En áfidos: WOOL Y COL. (1978); TOMIUK Y COL. (1979); CASTAÑERA Y COL. (1983); en lepidópteros: MENKEN (1982); en moscas blancas (Aleurodidae): WOOL Y COL. (1984).

3.3 Colonización:

La electroforesis de isoenzimas ha sido empleada con éxito en la evaluación del establecimiento de enemigos naturales de plagas y malezas. Mediante el análisis electroforético de esterasas, WHALON Y COL. (1982) comprobaron el establecimiento de dos razas de ácaros predadores (Phytoseiidae) resistentes a piretroides. En relación con el control biológico de malezas, la electroforesis tambien ha brindado aportes importantes. El análisis electroforético de alloenzimas permitió a GOEDEN Y KOK (1986) separar distintos biotipos de *Rhynocyllus conicus* FROELICH (Coleoptera: Curculionidae), un enemigo natural empleado en el control biológico de cardos (Asteraceae) en California, USA. Similarmente, UNRUH Y GOEDEN (1987) determinaron cuál de los biotipos de *R. conicus* introducidos en California se estableció posteriormente a su liberación.

4 BIOTIPOS DE PARASITOS DE AFIDOS, ELECTROFORESIS Y CONTROL BIOLOGICO

La determinación de biotipos de enemigos naturales empleados en el CBC de plagas, se ha visto facilitada por el uso de técnicas electroforéticas. Tal es la situación observada por ejemplo, en parásitos de áfidos del género *Aphidius* NEES (Hymenoptera: Aphidiidae), entre cuyas especies figuran importantes enemigos naturales de varios áfidos de importancia económica.

El estudio electroforético de diferentes sistemas enzimáticos en seis especies y 17 poblaciones de *Aphidius*, permitió a UNRUH Y COLABORADORES (en prensa) esclarecer el controvertido "status" taxonómico de algunas especies (ej. *A. pisivorus* - *A. ervi*); corroborar la existencia de numerosos biotipos entre las diferentes poblaciones de *A. ervi* y constatar cuales de dichos biotipos se establecieron en algunas de las diferentes áreas agroecológicas de California, USA, donde *A. ervi* es el principal enemigo natural de los áfidos plaga de la alfalfa, *Acyrtosiphon pisum* HARRIS y *A. kondoi* SHINJI. Entre las poblaciones de *A. ervi* analizadas, aquellas de orígen asiático, presentaron la mayor variabilidad genética. Esto sugiere que dichas regiones (Asia central y Japón), serían las mas indicadas para efectuar búsquedas de nuevos biotipos (o especies) que eventualmente podrían contribuir a mejorar el control biológico de estos áfidos.

Mediante el análisis electroforético de esterasas de *A. ervi* y *Diaeretiella rapae* McINTOSH, obtenidos de distintos áfidos huéspedes, NEMEC Y STARY (1983a,b; 1984) hallaron que una alta variabilidad genética en las poblaciones de parásitos indicaría la preferencia de huéspedes, siendo ésto un aspecto importante a tener en cuenta en el análisis de la estructura de poblaciones en diferentes agroecosistemas.

5 CONCLUSIONES

De lo expuesto se desprende que la electroforesis es una técnica potencialmente útil para el CBC. No sólo por su aplicación directa en la identificación de biotipos de enemigos naturales, un aspecto importante dentro del CBC, sino porque es una herramienta que puede ser empleada en todas las etapas del CBC. Como ocurre con la mayoría de las técnicas usadas en estudios biológicos, la electroforesis no es la panacea, pero su aplicación, complementada con otros métodos, es importante para la correcta implementación tanto del CBC como de otras alternativas biológicas empleadas en el control de plagas.

Agradecimientos:

Se agradecen los comentarios y sugerencias vertidos sobre el texto al Dr. Ricardo Biurrum (Instituto Técnico del Cereal, Navarra, España), y al Dr. Ing. Agr. Manuel Alvarado (El Califa de Sevilla, España).

6 REFERENCES BIBLIOGRAFIA

AVISE, J.C. - 1974. Systematic value of electrophoretic data. - Syst. Zool. 23: 465-481.

BERGE, J.B., DALMASSO, A., PINTUREAU, B., BABAULT, M., BONGIOVANNI, M. & BRIDE, J.B. - 1982. Variabilite genetique intraspecifique de *Trichogramma maidis* (Hym.: Trichogrammatidae), 15-21. - In: Les Trichogrammes, Colloq. INRA (France), No. 9.

BERLOCHER, S.H. - 1979. Biochemical approaches to strain, race, and species determination. - In: HOY, M. & McKELVEY, J. (eds.), Genetics in relation to insect management, - The Rockefeller Foundation, NY, 179 pp.

BERLOCHER, S.H. - 1984. Insect molecular systematics. - Ann. Rev. Entomol. 29: 403-433.

BOTTO, E., GONZÁLEZ, D. & BELLOWS, TOMÁS. - 1988. Effect of temperature on some biological parameters of two populations of *Aphidius ervi* HALIDAY (Hymenoptera: Aphidiidae). - In: BRILL, E.J. (ed.), Advances in parasitic Hymenoptera research, - Leiden/New York, 550 pp. (in press).

CALTAGIRONE, LEOPOLDO. - 1985. Identifying and discriminating among biotypes of parasites and predators, 189-200. - In: HOY, M. & HERZOG, D. (eds.), Biological control in agricultural IPM systems, - Academic Press, San Diego, 589 pp.

CASTAÑERA, P., LOXDALE, H.D. & NOWAK, K. - 1983. Electrophoretic study of enzymes from cereal aphid populations. II. Use of electrophoresis for identifying aphidiid parasitoids (Hymenoptera) of *Sitobion avenae* (F.) (Hemiptera: Aphididae). - Bull. Ent. Res. 73: 659-665.

DIEHL, S. & BUSH, G. - 1984. An evolutionary and applied perspective of insect biotypes. - Ann. Rev. Entomol. 29: 471-504.

DOUTT, R.L. - 1964. Biological characteristics of entomophagous adults, 145-167. - In: DeBACH, P. (ed.), Biological control of insect pests and weeds, - Chapman and Hall, London.

GOEDEN, R.D. & KOK, L.T. - 1986. Comments on proposed "new" approach for selecting agents for the biological control of weeds. - Can. Ent. 118: 51-58.

GONZÁLEZ, D., GORDH, G., THOMPSON, S. & ADLER, J. - 1979. Biotype discrimination and its importance to biological control, 129-136. - In: HOY, M. & McKELVEY, J. (eds.), Genetics in relation to insect management, - The Rockefeller Foundation, NY, 179 pp.

GONZÁLEZ, D. - 1988. Biotypes in biological control - Examples with populations of *Aphidius ervi*, *Trichogramma pretiosum* and *Anagrus epos*. - In: BRILL, E.J. (ed.), Advances in parasitic Hymenoptera research, - Leiden/New York, 550 pp. (in press).

GREATHEAD, D.J. - 1986. Parasitoids in classical biological control, 289-318. - In: WAAGE, J. & GREATHEAD, D.J. (eds.), Insect parasitoids, - Academic Press.

HUNG, A.C.F., DAY, W.H. & HEDLUND, R.C. - 1988. Genetic variability in arrhenotohoud and telythokous forms of *Mesochorus nigripes* (Hym.: Ichneumonidae). - Entomophaga 33(1): 7-15.

JARDAK, T.B., PINTUREAU, B. & VOEGLE, J. - 1979. Mise en evidence d'une nouvelle espece de *Trichogramma* (Hym.: Trichogrammatidae). Phenomene d'intersexualite; etude enzymatique. - Ann. Soc. Entomol. Fr. (N.S.) 15: 635-642.

KAWOOYA, J.K. - 1983. Electrophoretic discrimination of species of *Muscidifurax* (Hymenoptera: Pteromalidae) complex. - Ph.D. Dissertation, Univ. of Illinois (unpublished).

MENKEN, S.B.J. - 1982. Enzymatic characaterization of nine endoparasite species of small ermine moths (Ypomeutidae). - Experientia 38: 1461-1462.

NEMEC, V. & STARY, P. - 1983a. Genetic polymorphism in *Aphidius ervi* HAL. (Hym.: Aphidiidae), an aphid parasitoid on *Microlophium carnosum* (BCKT.) - Z. ang. Ent. 95: 345-350.

NEMEC, V. & STARY, P. - 1983b. Elpho-morph differentiation in *Aphidius ervi* HAL. biotype on *Microlophium carnosum* (BCKT.) related to parasitization on *Acyrthosiphon pisum* (HARRIS) (Hymenoptera: Aphidiidae). - Z. ang. Ent. 95: 524-530.

NEMEC, V. & STARY, P. - 1984. Population diversity of *Diaeretiella rapae* (M'INT.) (Hym.: Aphidiidae), an aphid parasite in agroecosystems. - Z. ang. Ent. 97(3): 223-233.

PINTUREAU, B. & BABAULT, M. - 1980. Comparison des esterases chez 19 souches de *Trichogramma* (Hym.: Trichogrammatidae) appartenant au groupe d'especes *evanescens*. - Arch. Zool. exp. gen. 121(4): 249-260.

PINTUREAU, B. & BABAULT, M. - 1981. Characterization enzymatique de *Trichogramma evanescens* et de *T. maidis* (Hym.: Trichogrammatidae): etude des hybrides. - Entomophaga 26: 11-22.

PUNGERL, N.B. - 1986. Morphometric and electrophoretic study of *Aphidius* species (Hymenoptera: Aphidiidae) reared from a variety of aphid hosts. - Syst. Entomol. 11: 327-354.

RICHARDSON, B.J., BAVERSTOCK, P.R. & ADAMS, M. - 1986. Allozyme electrophoresis. A handbook for animal systematics and population studies. - Academic Press, Australia, 410 pp.

SWOOFFORD, D.L. & SELANDER, R.B. - 1981. BIOSYS-1: A FORTRAN program for the conprehensive genetic analysis of data in population genetics and systematics. - J. Hered. 72: 281-283.

TOMIUK, J., WOHRMANN, K. & EGGER-SCHUMACHER, H.A. - 1979. Enzyme patterns as a characteristic for the identification of aphids. - J. Appl. Ent. 88: 440-446.

UNRUH, T.R., WHITE, W., GONZÁLEZ, D., GORDH, G. & LUCK, R.F. - 1983. Heterozygosity and effective size in laboratory populations of *Aphidius ervi* (Hym.: Aphidiidae). - Entomophaga 28(3): 245-258.

UNRUH, T.R., WHITE, W., GONZÁLEZ, D. & LUCK, R.F. - 1986. Electrophoretic studies of parasitic Hymenoptera and implications in biological control. - Misc. Publ. Ent. Soc. Am. 61: 150-163.

UNRUH, T.R., WHITE, W., GONZÁLEZ, D. & WOOLLEY, B. - (en prensa). Genetic relationships among seventeen *Aphidius* populations including six species. - Ann. Ent. Soc. Am.

UNRUH, T.R. - (en prensa). Molecular methods for classical biological control. - FISHER, T. *et al.* (ed.), Principles and application of biological control, - Univ. Calif. Press, Berkeley.

VOEGELE, J. & BERGE, J.B. - 1976. Les Trichogrammes (Insectes, Hymenop., Chalcidens, Trichogrammatidae), caracteristiques isoesterasiques de deux especies: *Trichogramma evanescens* WESTW. et *T. achaeae* NAGARAJA *et* NAGARKATTI. - C. R. Acad. Sci. (Paris) 283: 1501-1503.

WHALON, M.E., CROFT, B.A. & MOWRY, T.M. - 1982. Introduction and survival of susceptible pyrethroid resistant strains of *Amblyseius fallacis* (Acari: Phytoseiidae) in a Michigan apple orchard. - Env. Ent. 11: 1096-1099.

WOOL, D., GERLING, D. & COHEN, I. - 1984. Electrophoretic detection of two endoparasite species, *Encarsia lutea* and *Eretmocerus mundus* in the whitefly, *Bemisia tabaci* (GENN.) (Hom.:Aleurodidae). - Z. ang. Ent. 98: 276-279.

WOOL, D., VAN EMDEN, H.F. & BUNTING, S.W. - 1978. Electrophoretic detection of the internal parasite, *Aphidius matricariae* in *Myzus persicae*. - Ann. Appl. Biol. 90: 21-26.

MASS-PRODUCTION AND QUALITY OF INSECT-PARASITIC RHABDITID NEMATODES

P. N. Richardson

AFRC, Institute of Horticultural Research-Littlehampton, Great Britain

Summary

Insect-parasitic rhabditid nematodes have emerged as highly effective and desirable biological control agents for a wide range of pests. Major advances are being made in their *in vitro* mass-production both in solid and liquid substrates. It is essential that the nematodes that are being commercialised are subject to stringent quality control checks. Monitoring for quality is a continuous performance that begins prior to the production process with the evaluation of raw diet components and bacterial symbionts, continues with assessments of life-cycle criteria and ends with rigorous examination of the quality and efficacy of the final product.

1 INTRODUCTION

The use of nematodes as biological control agents of insect pests has been extensively studied and comprehensively reviewed (POINAR, 1975; 1979; NICKLE & WELCH, 1984; POINAR, 1986). Mermithid and rhabditid nematodes are two groups that have received most attention. Both kill their hosts and both include species that have reached the status of commercial products in recent years. *Romanomermis culicivorax*, a mermithid parasite of mosquitoes made a brief market appearance as 'Skeeter Doom' from 1975 to 1978 (PETERSEN, 1984). Interest in it dwindled, however, and, largely due to environmental limitations, host specificity, and inherent problems associated with handling, storage, shipping and application procedures, the company ceased production.

Rhabditid nematodes possess many features that, together, make them highly adaptable biocontrol agents (RICHARDSON, 1987). Even though they are easily mass-produced, and several commercial products have been developed most have disappointingly disappeared within a very short time-scale.

The need for quality biocontrol products utilising rhabditid nematodes is greater now than it has ever been; only stringent and well-considered programmes of quality control will ensure that this is achievable.

This paper considers the various developmental stages necessary in the mass-production of rhabditid nematodes and examines the quality control procedures that can be employed to ensure that these parasites provide customer satisfaction.

2 THE RHABDITID INFECTIVE LARVA

In steinernematid and heterorhabditid nematodes, infection of an insect host can only be achieved by the infective stage of the life-cycle. Nematodes can penetrate passively (e.g. with the insects' food), or actively, through natural body openings (e.g. the spiracles) or, in the case of Heterorhabditis spp., by boring through the cuticle (Fig. 1).

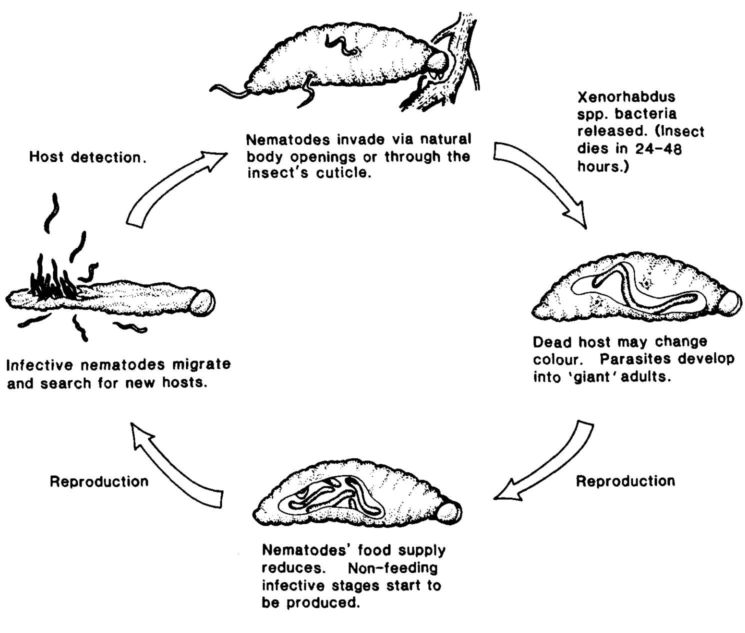


Fig. 1 Rhabditid life cycle

The infective larva is the third larval stage and is comparable to the dauer larva of free-living nematodes. It differs from the latter not only in its proclivity for insect hosts but also in its relationship with specific insect-pathogenic bacteria of the genus Xenorhabdus. Species of Steinernema co-exist symbiotically with X. nematophilus and Heterorhabditis species have a similar association with X. luminescens. Bacteria are held within a pharyngeal vesicle and are released into the insects' haemolymph resulting in death of the host. The infective larva (Fig. 2) is the only phase of the life-cycle that is capable of transmitting the bacteria and it also exhibits physiological and behavioural adaptations that suit it for survival in the environment and detection of fresh hosts. Production of infective larvae is the primary objective of all mass-culture schemes for rhabditid nematodes; only infective larvae can be successfully applied to horticultural and agricultural cropping systems.

Fig. 2 Steinernema bibionis infective larva

3 MASS-PRODUCTION, QUALITY AND QUALITY CONTROL

Present-day commercial pressures are intense and it is easy to understand how strict mass-production schedules can result in manufacturers of nematode biocontrol products placing emphasis on low-cost rearing rather than high quality products. The goal of a mass-culture programme was defined by FINNEY & FISHER (1964) as 'the production with minimum man hours and space of the maximum numbers of fertile females in as short a time and as inexpensively as possible'. By substituting 'infective larvae' for 'fertile females' the above definition could come uncomfortably close to the attitude of some companies on the brink of investing in nematode production technology.

Quality in nematode production is of the essence. If we consider 'quality' to be all those features and characteristics of a product that bear on its ability to satisfy a given need then 'quality control' is the regulation process for those activities which measure actual product performance, compare that performance with established standards and act on the difference. As far as good manufacturing process is concerned, quality control encompasses not only the sampling, specification and testing of the product. Equally important are the organisation, documentation and release procedures that ensure that the necessary tests are carried out. In addition to the functional aspect, a quality product is one that can be used safely.

4 TOWARDS A NEMATODE MASS-PRODUCTION CAPABILITY

Any biological control project involving the release of massive numbers of nematodes contains two major elements: (i) the production of the nematodes, (ii) the subsequent performance of the nematodes for accomplishing the objective of release. These factors can be visualised as a series of stages and events at each of which quality control procedures that contribute to high quality assurance can be invoked.

4.1 Production of the nematodes

Mass-production of *Steinernema* and *Heterorhabditis* species can only occur through a gradual step-by-step procedure starting with the isolation and identification of a source colony. From source colonies, by scale-up, research colonies large enough to provide material for infectivity tests, or small-scale field trials can be acquired. When the most effective nematode isolate for a particular pest target has been identified, research colonies give rise to mass-cultures and ultimately to commercial products.

4.1.1 The source colony

Infective larvae of many species of *Steinernema* and *Heterorhabditis* occur naturally in a wide range of soil types throughout the world. Source colonies arise from soil samples in which insect larvae, most commonly those of the highly-susceptible and easily-reared wax-moth (*Galleria* mellonella) are exposed. Dead larvae are removed from the soil samples and, if parasitised, may yield thousands of infectives that can be used to infest further G. mellonella larvae. Even at this early stage, the effects of the size and of the origin of the source colony on the ultimate genetic, and the potential behavioural, profiles of nematode production stocks should not be underestimated. When new source colonies are being multiplied for identification and scale-up a large representative sample of the new nematode population should be set aside, preferably in refrigerated conditions, as a standard against which the future performance of mass-produced stocks can be compared.

4.1.2 The research colony

Artificial diets and in vitro culture techniques are well-established for rhabditid nematodes (BEDDING, 1981; WOUTS, 1981) and are used by research workers to provide large numbers of infectives for laboratory trials. Bedding's method, using 500 ml flasks containing a substrate consisting of polyurethane sponge foam and a diet rich in protein and fat is very successful, giving yields as high as 100 million infectives per flask for Steinernema spp.

Though inexpensive and ideal for research purposes, the 'flask method' is not suitable for the purposes of large-scale commercialisation of rhabditids. It is the technique, however, that will be used in the present paper to illustrate the scope of quality control during the production process.

4.1.3 The mass-culture

Mass-culture of rhabditid nematodes has been achieved in two ways, the first of which is a modification of the flask method. BEDDING (1984) introduced a system of large autoclavable bags holding up to 5 kg of a similar diet to that used in his 1981 technique. Yields as high as 2000 million S. bibionis infectives per bag have been produced using this method. Even with this level of success, bags are highly labour-intensive, particularly during the extraction, settling and cleaning periods of harvesting. The future seems to lie with the second method - liquid fermentation - a commercially-sensitive area in which much progress is being made by several companies throughout the world at the present time. A company in California, Biosys, has for example, recently announced plans to build fermentation tanks containing 40,000 litres of medium and capable of producing many billions of nematodes (MILLER, 1987).

The technology to allow companies to mass-produce rhabditid nematodes is now available and several commercial concerns have already demonstrated their ability to provide economically-viable nematode products for a range of pest control purposes. The success of these products is dependent upon the quality of the control achieved with them. This is itself greatly influenced by the extent of quality control monitoring before, during and after the production process.

5 MONITORING FOR QUALITY PRIOR TO PRODUCTION

Any *in vitro* production scheme for rhabditid nematodes consists essentially of three components: the sterile diet, the monoxenic bacterial inoculum (*Xenorhabdus* spp.) and the contaminant-free nematode inoculum.

5.1 Diet quality

BEDDING (1986) listed a variety of factors that could complicate the simple flask culture technique and adversely affect the final yield of nematodes. The factors included the density of the polyurethane sponge foam, its crumb size, the type of medium, the ratio of the medium to foam, the quantity and depth of the medium per flask, the water content of the diet and autoclaving time

5.2 Quality of *Xenorhabdus* spp.

Xenorhabdus spp. produce two forms of colony on agar media (AKHURST, 1980). The primary form, that isolated from the intestine of the infective stage nematode, may be unstable in *in vitro* conditions, producing the secondary form. The primary form produces antimicrobial compounds, more-effectively provides nutrients for the nematodes and, in *Galleria*, results in as many as seven times more infectives being produced (AKHURST, 1980). It is essential that cultures of steinernematid and heterorhabditid nematodes should always be monoxenic and be started with pure primary forms of their naturally-occurring strains of Xenorhabdus. Aseptic technique should be observed throughout.

5.3 Quality of nematode inoculum

Bacteria and fungi commonly contaminate cold-stored nematode cultures and must be eliminated before mass-cultures are initiated. Care should be taken to select healthy stocks of nematodes and to avoid those infected with nematophagous fungi such as Arthrobotrys oligospora (POINAR & JANSSON, 1986) or with microsporidia (POINAR, 1988). Washing in a surface sterilant such as merthiolate (AKHURST, 1980) is a useful technique for the cleaning of nematodes.

6 MONITORING FOR QUALITY DURING PRODUCTION

Nematode cultures, whether produced in solid or liquid substrates, may take 2-4 weeks to mature. Monitoring of the culture's progress is important and should be done at regular intervals. Samples of sponge foam removed aseptically from flasks, for example, and examined microscopically will reveal valuable information on a range of aspects of the nematodes' development and also allow tests to be made to determine whether or not the Xenorhabdus bacteria have started to revert to the secondary form. Observations of life-cycle duration, growth rate, fecundity, sex ratio, size and yield of adults all serve to confirm, when compared with past experience, that a satisfactory production-run is underway. The final density and yield of infective larvae is the most crucial factor and a point at which important decisions are made. High yields of infectives may be taken as an indication that the current stock is still viable and capable of supplying inocula for further mass-cultures. Low, or disappointing, yields that cannot be explained by any obvious problem during production may indicate that continuous artificial culturing is having a detrimental effect and that fresh stock should be brought into the production schedule. Visual assessments of infective quality can also be made. High quality infectives are active individuals with extensive quantities of stored food reserves (Fig. 3); freshly-harvested nematodes should not appear lethargic, thin and starved.

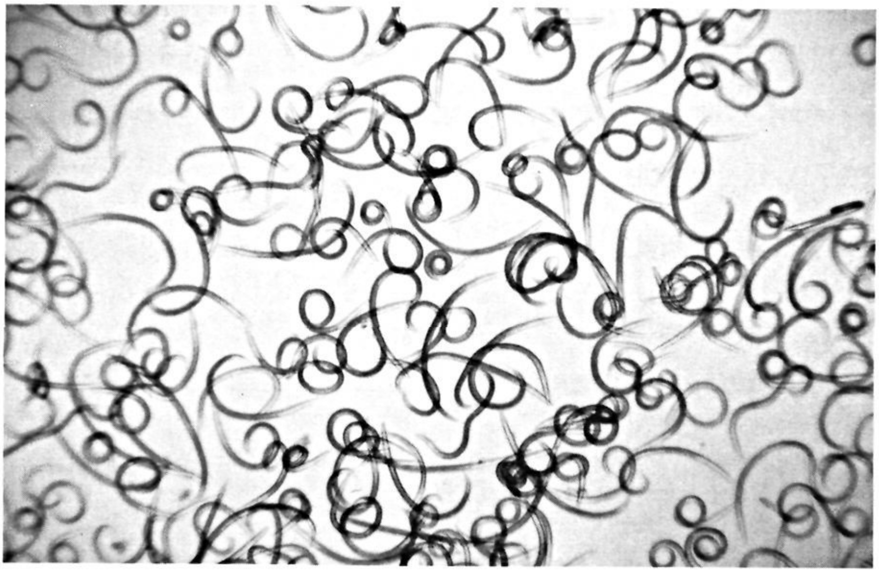


Fig. 3 Freshly harvested mass-produced infective larvae

7 MONITORING PRODUCT QUALITY

Continuous mass-rearing of rhabditid nematodes may result in inbreeding, selection, and other processes leading to genetic decay, loss of fitness and the development of laboratory ecotypes that are totally unsuitable for field release. Apparent loss of fitness during production may be due to unnatural maintenance conditions leading to physiological acclimatisation. It has been suggested that, in such circumstances, fitness might be restored through back-crossing laboratory stocks of nematodes with wild isolates, or by completely replacing laboratory cultures with fresh field-collected material at regular intervals (MACKAUER, 1976). Factors such as dispersal, survival, and host recognition are aspects of the behaviour and performance of a mass-produced stock that may be altered by the constant conditions of temperature and pH, and the high nutrient levels inherent in *in vitro* culture methods. It is important that attributes relating to the overall pathogenicity of rhabditids should not become impaired. The most widely-used and generally-accepted technique for monitoring nematode product quality over time, before shipping, after shipping, or between productions, is to conduct bioassays. All aspects of bioassay technique were reviewed by BURGES & THOMSON (1971).

7.1 Bioassay

Bioassay techniques for rhabditid nematodes have most frequently been developed for the purpose of selecting the most effective species, or isolate, of parasite for a particular purpose. As a result, many variations on the theme exist and there is no standard method that can be recommended for use in quality control testing.

Neither is there a standard bioassay organism. Galleria mellonella larvae are easy to produce but considered by some to be too susceptible to nematodes to give a true measure of pathogenicity. Target hosts such as the black vine weevil (Otiorhynchus sulcatus) would appear to be the ideal insects for bioassay but the majority cannot easily be cultured on artificial diets and are expensive to rear on their natural host plants. Fly larvae such as Calliphora erythrocephala, mass-produced for the angling market, are generally available but difficult to parasitise with Steinernema and Heterorhabditis species. KAYA (1985) called for the universal use of final-instar larvae of Musca domestica and suggested that 'International Housefly Units', or some other convenient international unit of comparison, might result. Even when suitable bioassay systems have been developed, problems, particularly regarding wide variability in results, are sometimes encountered (ROVESTI, GAVIOLI & DESEO, 1986).

Whatever method is devised, it is important that companies providing nematodes for the biocontrol market have conducted rigorous programmes of tests on their products before releasing them. It is essential that LD_{50} and LD_{90} values are calculated using probit analysis for each batch of nematodes produced.

8 CONCLUSIONS

Insect-parasitic nematodes will provide an extra dimension to pest control strategies and may prove particularly useful in those intensively-grown high-value crops associated with the European glasshouse and mushroom industries (RICHARDSON, 1987).

Existing around the roots of plants, parasitising and killing damaging insect larvae, nematodes provide a highly-desirable alternative to chemical insecticides and are readily integrated into pest management programmes. But Steinernema and Heterorhabditis species have a poor commercial history to live down. Products labelled 'Seek', 'Spear', 'Exit' and 'Bionem' are amongst those nematode preparations no longer produced even though growers, and other consumers, were willing to try this new approach to pest control. It is of the utmost importance for the future credibility of these adaptable and effective parasites that economic, reliable and safe products become established, maintain a long-term market presence and ensure customer satisfaction. Companies paying particular attention to high standards of quality control will have the best chance of achieving that goal.

BIBLIOGRAPHY

AKHURST, R.J. - 1980. Morphological and functional dimorphism in Xenorhabdus bacteria symbiotically associated with the insect pathogenic nematodes Neoaplectana and Heterorhabditis. J. Gen. Microbiol., 121: 303-309.

BEDDING, R.A. - 1981. Low cost, in vitro mass-production of Neoaplectana and Heterorhabditis species (Nematoda) for field control of insect pests. Nematologica, 27: 109-114.

BEDDING, R.A. - 1984. Large scale production, storage and transport of the insect-parasitic nematodes Neoaplectana spp. and Heterorhabditis spp. Ann. Appl. Biol., 104: 117-120.

BEDDING, R.A. - 1986. Mass rearing, storage and transport of entomopathogenic nematodes, pp 308-311. In: Fundamental and applied aspects of invertebrate pathology (R.A. SAMSON, J.M. VLAK & D. PETERS eds). ICIP Wageningen, The Netherlands, 711 pages.

BURGES, H.D. & THOMSON, E.M. - 1971. Standardization and assay of microbial insecticides, pp 591-622. In: Microbial control of insects and mites (H.D. BURGES & N.W. HUSSEY eds). Academic Press, London, 861 pages.

FINNEY, G.L. & FISHER, T.W. - 1964. Culture of entomophagous insects and their hosts, pp 328-355. In: Biological control of insect pests and weeds (P. DeBACH ed). Chapman & Hall, London, 844 pages.

KAYA, H.K. - 1985. Entomogenous nematodes for insect control in IPM systems, pp 283-302. In: Biological control in Agricultural IPM Systems (M.A. HOY & D.C. HERZOG eds). Academic Press, London, 589 pages.

MACKAUER, M. - 1976. Genetic problems in the production of biological control agents. Ann. Rev. Ent., 21: 369-385.

MILLER, J.P. - 1987. Bug-eating nematodes hold promise as natural pesticide. The Wall Street Journal, August 28.

NICKLE, W.R. & WELCH, H.E. - 1984. History, development and importance of insect nematology, pp 627-653. In: Plant and insect nematodes (W.R. NICKLE ed). M. Dekker, New York, 925 pages.

PETERSEN, J.J. - 1984. Nematode parasites of mosquitoes, pp 798-820. In: Plant and insect nematodes (W.R. NICKLE ed). M. Dekker, New York, 925 pages.

POINAR, G.O. - 1975. Entomogenous nematodes - a manual and host list of insect - nematode associations. E.J. Brill, Leiden, 317 pages.

POINAR, G.O. - 1979. Nematodes for biological control of insects. CRC Press, Florida, 277 pages.

POINAR, G.O. - 1986. Entomophagous nematodes, pp 95-122. In: Biological plant and health protection (J.M. FRANZ ed). Gustav Fischer Verlag, New York.

POINAR, G.O. - 1988. A microsporidian parasite of Neoaplectana glaseri (Steinernematidae : Rhabditida). Revue Nematol., 11: 359-361.

POINAR, G.O. & JANSSON, H-B. - 1986. Infection of Neoaplectana and Heterorhabditis (Rhabditida : Nematoda) with the predatory fungi, Monacrosporium ellipsosporum and Arthrobotrys oligospora (Moniliales : Deuteromycetes). Revue Nematol., 9: 241-244.

RICHARDSON, P.N. - 1987. The use of insect-parasitic nematodes in integrated pest control programmes on protected crops, pp 163-174. In: Integrated and biological control in protected crops (R. CAVALLORO ed). A.A. Balkema, Rotterdam, 251 pages.

ROVESTI, L., GAVIOLI, F. & DESEO, K.V. - 1986. Bioassay problems with insect-parasitic nematodes in Cossus cossus L., p 315. In: Fundamental and applied aspects of invertebrate pathology (R.A. SAMSON, J.M. VLAK & D. PETERS eds). ICIP Wageningen, The Netherlands, 711 pages.

WOUTS, W.M. - 1981. Mass production of the entomogenous nematode Heterorhabditis heliothidis (Nematoda : Heterorhabditidae) on artificial media. J. Nematol., 13: 467-469.

Report on the Session 6: QUALITY ASSESSMENT OF BIOLOGICAL CONTROL AGENTS

J. P. Aeschlimann
CSIRO, Biological Control Unit-Montpellier, France

Premier du genre à se tenir en Europe dans le domaine de la lutte biologique contre les plantes et les animaux nuisibles, le symposium a mis en évidence les points essentiels suivants:

1. Des contrôles de qualité sont déjà régulièrement effectués par la plupart des agences et firmes impliquées dans la lutte biologique.

2. Ces contrôles de qualité portent sur toutes les catégories d'agents naturels utilisés jusqu'ici (microorganismes, nématodes, parasitoides, prédateurs). En revanche, ces contrôles ne sont pas toujours pratiqués de manière à fournir des résultats comparables. Il est donc urgent que les chercheurs travaillant dans ce domaine adoptent une terminologie commune, des critères et des techniques similaires. Par rapport aux entomologistes qui s'attachent à mesurer la qualité des phytophages produits en masse sur milieu artificiel, les spécialistes de l'utilisation d'ennemis naturels contre des espèces nuisibles sont confrontés à un problème autrement plus complexe, et qui n'est pas résolu. Il s'agit en effet de tenir compte ici du fait que l'efficacité des ennemis naturels varie également en fonction de la qualité des espèces-hôtes.

3. A l'avenir, il est recommandé d'identifier une série de biotypes de tout organisme susceptible d'être utilisé en lutte biologique et d'en comparer les performances afin de sélectionner celui qui s'avèrera le plus adéquat pour chaque type de situation.

4. Il existe un important décalage entre les spécifications des producteurs d'organismes utiles et les observations des praticiens utilisateurs de ces mêmes organismes. Un gros effort doit être entrepris afin de rapprocher ces points de vue et d'améliorer la fiabilité du matériel commercialisé.

SESSION 7

STORAGE OF FOOD

Chairman: E. Celma Calamita

FOOD STORAGE AND STORAGE PROTECTION IN ANCIENT EGYPT

H. Z. Levinson & A. R. Levinson
Max-Planck-Institut für Verhaltensphysiologie-Seewiesen, F. R. Germany

Abstract

The invention of food stores as a safeguard measure against poor harvests in ancient Egypt, the insect species infesting dried foodstuffs in bulk storage as well as the early attempts of pest control are described and briefly compared with contemporary storage protection.

1 Design and construction of ancient grain stores

Poor harvests and severe famine, caused by lacking inundations of the Nile, probably motivated the ancient Egyptians to establish large scale storage of food and seed reserves approximately five millennia ago [LEVINSON and LEVINSON 1985, 1988]. Large reed baskets [diameter 0.3 - 1.8 m, height 0.3 - 0.6 m] or clay jars [height ca. 1 m] immersed to the soil were the archetypes of granaries which had been used by the neolithic inhabitants of the Delta region of lower Egypt. The granaries of the first dynasty [ca.2920 - 2770 B.C.] were cylindrical mud silos with a chamfered roof opening and two square apertures at different height levels.

The more capacious store chambers [capacity up to 40.000 grain sacks] employed during the Middle kingdom [ca.2040 - 1785 B.C.] and New kingdom [ca.1554 - 1080 B.C.] were predominantly of cylindrical shape with a vaulted roof [Fig. 1] and less commonly of rectangular shape with a flat roof [Fig.2].They were usually built of Nile mud, sand and straw and had a roof opening for the introduction of dried seeds or fruits as well as a sliding door for withdrawing the former, above or near the base [Fig. 3]. The physical advantages of sun-baked mud [bricks or other shape] include adequate insulation against

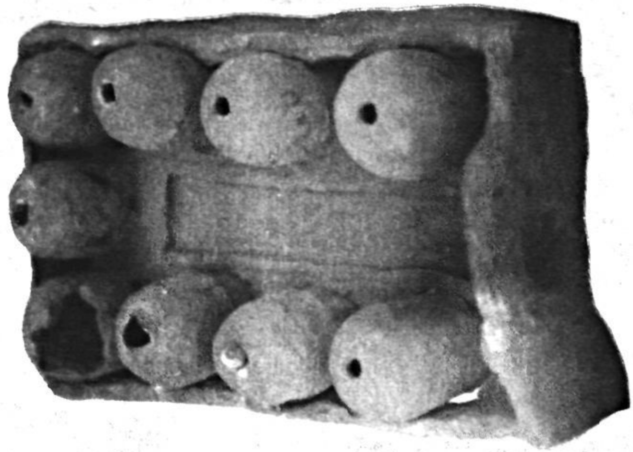


Fig. 1. Mud-shaped model [∿ 33 x 20 x 12 cm] of a granary originating from the Old kingdom [∿ 2635 - 2154 B.C.]. This model was found in the Necropolis G e b e l e i n and consists of nine dome-shaped silos arranged on a platform along three walls of a rectangular courtyard. The silos are filled from an apical aperture which can be closed by means of a conical plug [evident on one of the apertures], while grain may be removed through a sliding shutter above the base [not seen in the photograph]. Voluminous granaries of this design were employed throughout the Middle and New kingdom [Museo Egizio, Turin].

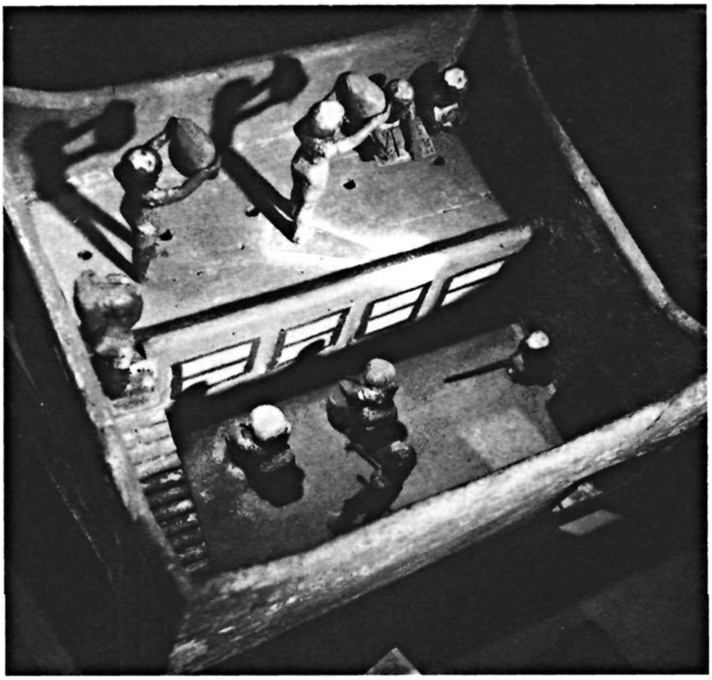


Fig. 2. Wooden model of a granary with flat roof found in Saqqara, originating from the Middle Kingdom [~ 2040 - 1785 B.C.]. This model [~ 50 x 46 x 25 cm] consists of five adjacent storage chambers surrounded by a high wall with an entrance. One can see a worker carrying a sack of grain to the flat roof, two workers pouring grain from sacks into the openings of two storage chambers as well as a scribe and his assistant recording the amount of grain stored. Two workers filling grain in sacks and two supervisors holding sticks are evident in the courtyard [Egyptian Museum, Cairo].

heat, cold, humidity, inflammability and sound as well as air permeability and render it thus particularly suitable as a building material for warm and dry climates. The above mentioned types of stores became known to us due to some existing ruins as well as several models and drawings which were discovered in tombs of various pharaonic dynasties.

2 Pest species of foodstuffs stored in ancient Egypt

When maintainance of dried foodstuffs in large stores had become a common practice throughout the ancient Orient, about one dozen of cereal and fruit-feeding insect species as well as a small number of carnivorous insect species migrated to these ample accumulations of food sources. Predominant among them were the hump beetle [Gibbium psylloides Czempinsky], the tobacco beetle [Lasioderma serricorne Fabricius], the drugstore beetle [Stegobium paniceum Linné], the saw-toothed grain beetle [Oryzaephilus surinamensis Linné], the lesser grain borer [Rhizoperta dominica Fabricius], the rustred flour beetle [Tribolium castaneum Herbst], the granary weevil [Sitophilus granarius Linné], storage moths [Ephestia spp.], a meal moth [Plodia spp.] as well as the carnivorous leather beetle [Dermestes frischii Kugelann], the ham beetle [Necrobia rufipes de Geer] and the parasitic wasp species Bracon hebetor [Say.]. The occurrence of the above pest species in foodstuffs stored in ancient Egypt is documented by the presence of these species in edible offerings [e.g. emmer, barley, flour, bread and meat] which had been placed in ample amounts in the tombs of pharaos and officials at the time of their burial [Fig. 4], in order to support the Ka [vitality] of the deceased.

3 Early attempts of pest control

During the Middle and New kingdom a considerable number of previously harmless insect and mite species left their scanty and scattered habitats and eventually became voracious pests

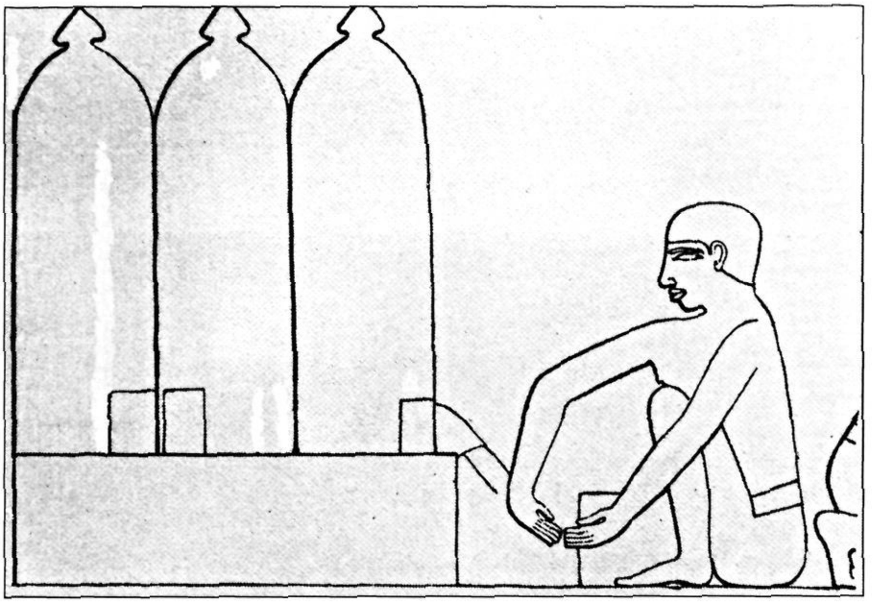


Fig. 3. Outline of a wall painting of three dome-shaped silos originating from the fifth dynasty.
It is evident that each silo is provided with a sliding shutter near the base. A seated worker is transferring grain from the first silo to a sack by means of an adapter inserted to the open shutter [Tomb no. 60 of the civil servant T i in Saqqara].

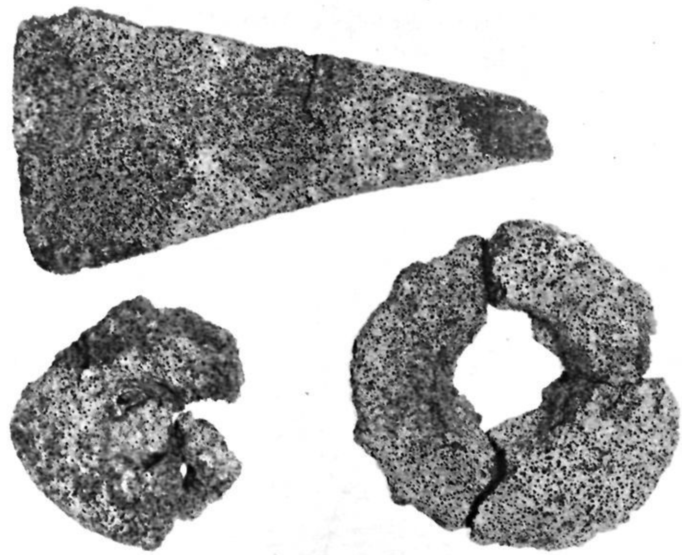


Fig. 4. Remains of three breads, being baked ~ 3400 years ago, which were excavated from the tomb of the architect K h a [18th Dynasty] in De'ir el Medina.
Bread was baked during the New kingdom [~ 1554 - 1080 B.C.] in more than fifteen shapes [approximately 2 - 3 times larger than our rolls], indicating the popularity of this kind of food. The depicted remains probably originate from a triangular, round and ring-shaped bread. The numerous perforations evident in those remains display the past infestation of the dessicated breads by two anobiid species, probably Stegobium paniceum and Lasioderma serricorne [Museo Egizio, Turin].

of stored foodstuffs. Being aware of the great damage caused by these pest species, the ancient Egyptians believed in divine protection of their crops and livestock and thought that Horus [main tutelary god] and Renenutet [goddess of agriculture] will decimate populations of harmful arthropods and rodents and thus save the harvests. The first traceable measures of pest control were described in the so-called Papyrus Ebers [a hieratic scroll written ca. 1550 B.C.] which is regarded as a compilation of therapeutic medicine practised during the Old and Middle kingdom. Among the suggestions made in this Papyrus are thorough washing by aqueous natron [sodium carbonate], application of repellents, viz. fat of certain birds and cats as well as dung ash of some animals, to the walls and seeds of store chambers. It is conceivable that the repellent effect of rancid fats was due to the release of short-chain fatty acids, while the respective influence of ashes depended on the irritant and abrasive action of mineral salts. Fumigation by burning incense [various spices, resins and gums], used as a religious ceremonial in temples and tombs, was also employed for pest control in houses and foodstores during the New kingdom. Moreover the pest-averting property of sulphur applied as dust or fumigant [by burning it to sulphur dioxide] became known to Mediterranean peoples during the above-mentioned period and provided a useful tool for the protection of stored foodstuffs.

4 Epilogue

It is worth recalling that the number of species infesting stored foodstuffs has certainly increased from the period of the New kingdom until present time [KLAUSNITZER 1988], while the contemporary methods of storage protection still resemble those practised in ancient Egypt. Modern store chambers and silos have basically the same design as the stores of ancient Egypt, whereas both variety and efficiency of storage protectants have increased since antiquity. Hence, the salient problems arising from bulk storage of dried foodstuffs [BENZ 1987] are rather similar to those encountered in pharaonic times and their solution remains as a challenge for inventive minds.

References

BENZ G. [1987]
Integrated pest management in material protection, storage and food industry.
Integrated pest management: quo vadis ?
Parasitis <u>86</u> [ed. V. Delucchi], pgs. 31 - 69.

KLAUSNITZER B. [1988]
Verstädterung von Tieren.
A. Ziemsen Verlag, Wittenberg Lutherstadt, 315 pgs.

LEVINSON H.Z., LEVINSON A.R. [1985]
Storage and insect species of stored grain and tombs in ancient Egypt.
Zeitschr. angew. Entomol. <u>100</u>, 321 - 339.

LEVINSON H.Z., LEVINSON A.R. [1988]
Hungersnot und Nahrungsspeicherung im alten Ägypten.
Spektrum der Wissenschaft <u>11</u>, 1988, 40 - 47.

MASS TRAPPING OF TOBACCO BEETLES (*LASIODERMA SERRICORNE* F.) BY MEANS OF PHEROMONE TRAPS

C. Th Buchelos
Laboratory of Economic Entomology, «Benaki» Phytopathological Institute-Kiphis

A. R. Levinson
Max-Planck-Institute für Verhaltensphysiologie-Seewiesen, F. R. Germany

Mass trapping of male tobacco beetles [Lasioderma serricorne F., Anobiidae] was carried out for three successive years in tobacco stores [size: 18 x 12 x 4 m] which were not treated by an insecticide. The tobacco stores were located in Piraeus [southern Greece] and had a relatively warm climate [annual average temperature: 18.0 °C]. Adhesive stripes, singly furnished with 10 mg of anhydroserricornin [2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran] in a polyethylene dispenser, served as pheromone traps. Each tobacco store was provided with 2 pheromone traps and 2 control [unbaited] traps. Anhydroserricornin is a presumed storage form of the pheromone, from which the actual sex attractant for male tobacco beetles [4S,6S,7S-serricornin] is continuously released into the atmosphere.

Monthly catches of tobacco beetles on pheromone traps during a three year period revealed continuous presence of a fluctuating population of adult L. serricorne from April through December with peak densities during August. A total of 37.905 tobacco beetles were trapped throughout the three year's period extending from January 1983 to December 1985.

Approximately 2.7 times more L. serricorne were caught on pheromone traps [73.3%] than on control traps [26.7%]. The annual catches of L. serricorne on four pheromone traps in two tobacco stores revealed a population decrease of 34.3 % in 1984 as compared to 1983 and an additional decrease of 66.3 % in 1985 as compared to 1983. The tobacco beetle population was thus suppressed to o n e t h i r d of its initial density due to permanent presence of four pheromone traps only [Figure 1].

It follows that adhesive traps furnished with 10 mg of anhydroserricornin provide a useful tool for monitoring and mass-trapping populations of Lasioderma serricorne and can thus be employed for the causation of i n s e c t i s t a s i s in tobacco and food stores.

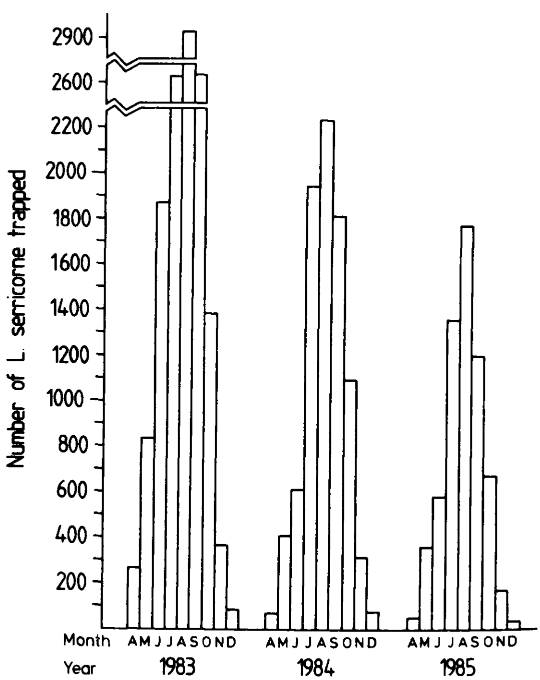


Fig. 1. Monthly captures of male tobacco beetles on 4 pheromone traps (10 mg anhydroserricornin per trap) in two untreated tobacco stores (Piraeus region) during 3 successive years.

RURAL AND URBAN STORAGE OF GRAIN IN GREECE

C. Th. Buchelos
Laboratory of Economic Entomology, «Benaki» Phytopathological Institute-Kiphissia, Greece

Summary

A brief description of the annual production of cereals, the procedures concerning harvest, long-term storage and protection of stored grain will be presented. Greece is usually self-sufficient in its grain productivity, it often exports cereals and imports them only occasionally, when harvests were unfavourable. The cereal crops are chiefly wheat and maize, while rice and barley are grown to a lower extent.

The external structures, size and storing caracity of silos, stores and warehouses as well as the kind of cereals stored in rural and urban regions are described. The predominant type of grain stores of village farms is the horizontal made of stones or bricks and concrete, while the principal type of harbors and towns is the vertical one with huge metal or concrete constructions.

The most important arthropod and vertebrate pests infesting cereals include the following insect, mite, bird and rodent species:

Insects: *Oryzaephilus surinamensis*, *Physopertha dominica*, *Sitophilus granarius*, *S. oryzae*, *Cryptolestes* spp. *Cadra cautella*, *Anagasta kühniella*, *Plodia interpunctella*, *Sitotroga cerealella*

Mites : *Acarus siro*, *Glycyphagus destructor*, *Caloglyphus krameri*

Birds : *Passer domesticus* (Sparrows)

Rodents: *Mus* sp., *Rattus-rattus*

The damage caused by those pest species is correlated with the type of stores and cereals involved. The most common preventive and curative pest control measures practiced in villages and towns are usually centralized on the effort to maintain a high standard of hygiene in the stores by cleaning or and treating the structure of premises with an approved contact insecticide but fumigation with phosphine is the most effective and main application during storage and before export.

1 INTRODUCTION

Greece is usually self-sufficient in its grain productivity, it often exports cereals and imports them only occasionally, when harvests were unfavourable. The cereal crops are chiefly wheat and maize, while rice and barley are grown to a lower extent. The annual amount of grain stored throughout Greece is about 2.000.000 tons of which about 1.000.000 tons are hard as well as soft wheat, about 850.000 tons are maize and about 150.000 tons are rice, barley and rye (Fig. 1). After harvesting, which in most

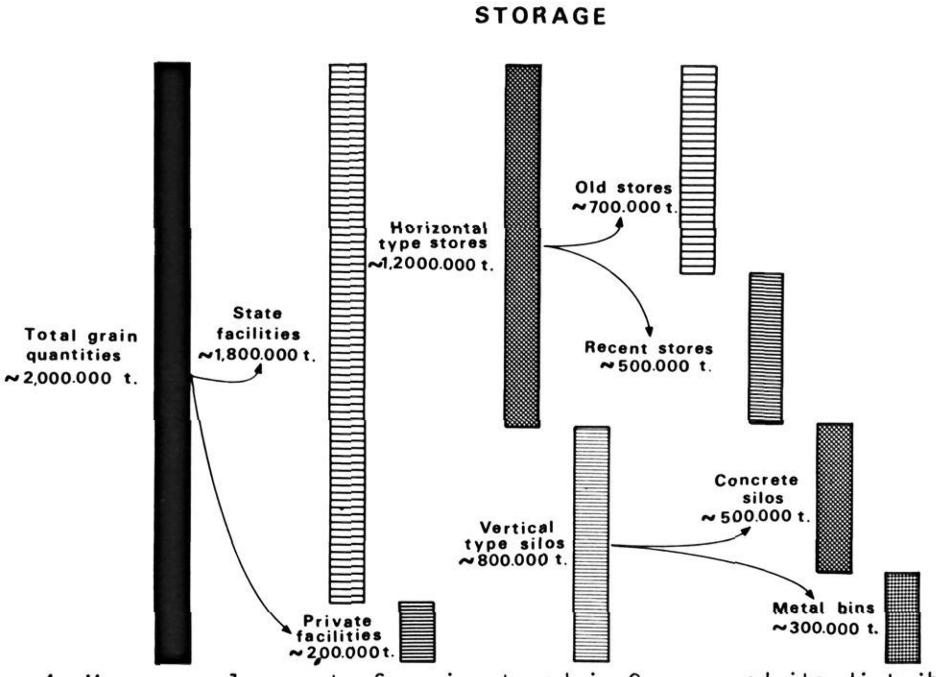


Fig. 1: Mean annual amount of grain stored in Greece and its distribution to different types of storage

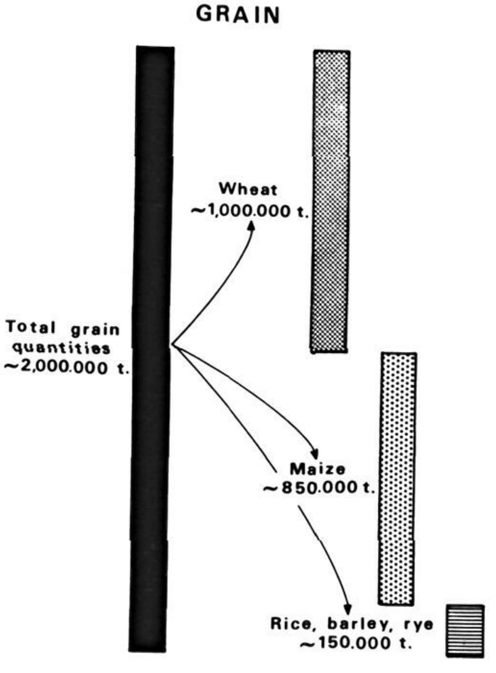


Fig. 2: Capacity of concrete and metal silos

greek regions takes place in July, the grain is sold by the farmers mainly to the State Products Handling Cooperative Administration (K.Y.D.E.P.) or to a lesser extent to private milling companies. Usually most of the grain is sold to the above agencies within three months following the harvest, whereafter it is stored. It is worth mentioning that K.Y.D.E.P. is the state intervention organization, being responsible for storage of grain, adjusting its cost, intervention purchases, improvement of production by means of financial assistance to the farmers as well as distribution of selected seeds for planting. Grain purchased by K.Y.D.E.P. may subsequently be sold to millers or be coloured by a red dye and then resold to farmers as animal fodder.

In this paper I have attempted to briefly review the structure of the prevalent grain stores, techniques of storing, major pest species and some of the methods used for controlling them. The distribution of the pest species occuring in various stores is based on extensive population surveys carried out during the past five years.

2 TYPES OF STORES

2.1. Horizontal type

Many rural stores which were built some time ago, are made of either hard stone (common greek limestone) or to a lesser extent of sun-baked mudbricks, are found abundantly in most villages throughout Greece. Their walls are rather thick (40-60 cm) and their pointed roof (inclination 22%) is covered by clay tiles. The above building materials provide a fairly good insulation against excessive temperature, rainfall and humidity. Those stores are always of rectangular shape and largely differ in dimension according to the quantities of grain to be stored (Fig. 3).

More recently constructed stores of horizontal type have double walls, about 40 cm thick, of ovenbaked bricks and concrete while their slightly pointed roof (inclination 14%) is covered by corrugated plates (usually 1,40 x 0,60 m) made of a mixture of asbestos and cement. Those stores are constructed to contain either 1000 or 2000 tons of cereals. The first, mainly in semimountainous regions, consist of two chambers (external dimension 14 x 14,46 x 5,5 m each) and the later, mainly in regions of the plains, consist of two pairs of the above chambers (external dimension 14 x 57,85 x 5,5 m). The ceiling is from concrete planted with blocks of inflated polysterine (felizol) blocks (0,50 x 0,50 x 0,30 m) for heat insulation. The

Fig. 3: Old rural store of stone and mudbricks

Fig. 4: Cooperative store

floor is elevated to approximately 0,50 m above ground surface, in order to facilitate loading and unloading from tracks; this procedure ensures that the stored grain is not directly exposed to the ground humidity. Those stores usually have 2 large sheet-iron doors (4,20 x 4,95 m) of which one is infront and the other in the rear side (Fig. 4). Inside the store an assembly line is employed for convenient translocation of the sacked grain. Horizontal stores are provided with a variable number (4-8) of windows (usually 1,50 x 3,50 m) which are internally covered with a dense wire net.

Such rural stores usually belong to the farmers' cooperative organizations pertaining to a group of villages.

2.1.1. Vertical type

This type of stores consists of large cylindrical silos in upright position which are made of either metal or concrete, provided with a roof opening for the input and an opening near the base for the withdrawal of grain.

(a) Metal bins (Fig. 5) are made of successive zones of corrugated zinc plated (galvanized in hot) steel sheets. The minimal thickness of those sheets is 2 mm in the lower zones of the bin and 1 mm near its conic roof. The diameter of the bin is 12,5 ± 0,5 m and its useful hight 10,00 ± 0,50 m to achieve capacity of 1000 tons. The bottom is flat provided with aeration ducts and the floor is from 0,80 to 1,00 m above ground surface.

(b) The bins of concrete (Fig. 6) have either circular or octagonal cut, diameter about 9,00 or 12,00 m and about 36,00 m hight. The thickness of the walls is 20 cm. Their bottom is either conical permitting natural flow of the grain towards the horizontal assembly line underneath or flat provided with sweeping screws and aeration ducts; the floor is again from 0,80 to 1,00 m above ground surface.

In both, metal and concrete type of vertical stores, the aeration system is not automatic but it is turned on whenever the grain temperature, depending on the humidity, rises above 30°C.

3 TECHNIQUE OF STORAGE

Most of the grain is stored in the harbour regions of big cities in central and nothern Greece, i.e. the main areas of grain fields. Approximately 1.200.000 tons of grain i.e. 60% of the total amount of stored grain, is stored in the horizontal stores of rural areas. About 700.000 tons of the

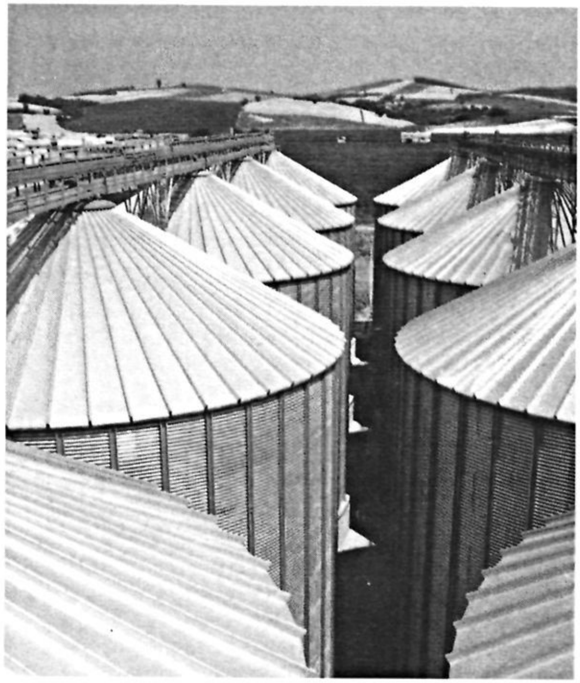


Fig. 5: Metal bins

Fig. 6: Concrete silos

above quantity are kept in the old farmers' stores, while about 500.000 tons of the above quantity are maintained in the newer type of grain stores. The remaining amount of grain (~800.000 tons) is purchased by the State Administration, private milling companies and cereal food factories and stored in the large vertical silos. It is estimated that private facilities cover about 20% of the whole needs in storage capacity. Millers and factory owners may hire state owned silos to store grain, while the time of storage is then up to two years. Time of storage is usually up to 3 months in rural (farmers and Cooperatives) stores and up to 5 or 6 months in State silos; in exceptional cases such as Tsernobil accident,the time in the later stores can be even 3 years.

Main way of storing cereals is "in bulk" while storage in sacs is reserved for selected seed only; the capacity of a standard jute sac is about 50 kgs of seed. When grain is purchased by the State Administration (K.Y.D. E.P.), it is collected from farmers in one-use (not returnable) jute sacs. In spite of their relative cheapness, returnable sacs, used in previous times have the serious disadvantage that thigmotactic insects may adhere to the hairy surface of the sacs and reinfest healthy product.

The capacity of all concrete silos in Greece amounts to about 500.000 tons and that of the metal silos about 300.000 tons (Fig. 2).

4 MAJOR PEST SPECIES

Table I lists the more important arthropod and vertebrate pest species infesting grain during storage, in accordance to their abundance which also roughly reflects the economic importance of the pests. Although the distribution of each pest in the various types of stores can not yet be given, it is worth mentioning that in addition to those pests, there are occasional infestations by other species, e.g. *Trogoderma inclusum, Tenebrioides mauritanicus, Palorus subdepressus, P. ratzeburgii.*

The presence and abundance of the of the listed insect species was chequed during the last five - year period, in regular intervals by the following methods : a) for the beetles (coleopterous species), several samples consisting of 1 kilogram of infested grain were taken from different parts of the stores then carried to the laboratory where the insects found were identified and counted. b) for the moths (lepidoptera species) brown paper stripes (size : 75 x 4 cm) adhesive on both sides, were hung up in vertical position 1 m above the sacs' or the bulk level mainly in

Table I. Approximate abundance of major grain pests in Greece

Insect species :	Abundance 1)
Coleoptera	
Oryzaephilus surinamensis	18
Rhyzopertha dominica	17
Oryzaephilus mercator	10
Sitophilus granarius	9
Sitophilus oryzae	7
Cryptolestes ferrugineus	6
" *turcicus*	3
" *pusilus*	1
Lepidoptera	
Cadra cautella	12
Sitotroga cerealella	11
Anagasta kühniella	4
Plodia interpunctella	2
Mite species :	
Acarus siro complex	relatively high
Glycyphagus destructor	
Caloglyphus krameri	moderate in humid grain
Birds :	
Passer domesticus	
Rodents :	unknown 2)
Mus musculus	
Rattus - rattus	

1) The figures for the insects species are expressed in percentages.
2) Surveys have not been carried out yet.

the corners of the stores. Adhesive stripes were replaced by new ones every 15-20 days while the moths were attracted to configuration of the stripes, were trapped, brought to the laboratory and counted after their separation and determination.

5 CONTROL MEASURES

In order to minimize infestations by insect pests, grain is usually stored for the shortest possible period of time (3 - 24 months). The walls of empty stores are always thoroughly cleaned, treated with lime solution and subsequently sprayed with either Actellic, Malathion, Lindane or K-Othrine, as a preventive control measure. Unless any of the above species is present in more than 4 individuals per kilogram sample, no control measure is permormed. If larger numbers of any of the major pest species (Table I) are detected, fumigation of the grain is carried out; extra fumigation could also be decided in case of presence of any quarantine pest species such as *Trogoderma granarium*. Fumigation is usually performed by alluminium phosphide which is liberated from an adequate formulation (tablets, bags or blankets) resulting in a concentration of 5 to 10 g of phosphine per ton of grain, depending on the obtained air-tightness of each store. As most of stores are not equiped with an incorporated fumigation system,treatments are performed by means of dispensers releasing pellets or tablets of the fumigant into the bulk of grain. Such treatments are usually carried out in September and, if necessary, also in May. In a number of horizontal stores either Actellic or Malathion is admixed to the grain in bulk.

The moisture content of bulk stored grain is regularly examined and maintained between 11% and 13% which would exclude infestation by mite species, e.g. *Acarus siro*.

Measures taken against insects, control also mites. Against birds and rodents, no measures are taken except in cases of heavy infestations.

PHEROMONE TRAPPING AND MATING DISRUPTION LEADING TO INSECTISTASIS

P. Trematerra
Istituto di Entomologia Agraria, Università-Milano, Italy

SUMMARY

A relatively new approach to pest management involves the causation of "insectistasis" (stasis = stagnation) by means of naturally occurring or artificially produced agents which specifically decimate undesirable insect populations. Insectistasis can be caused by retarding or interrupting insect development and reproduction or by reducing the number of insects in a pest population. Among the insectistatics, both sex and aggregation pheromones have been intensely investigated from a practical viewpoint. In the storage environment insect monitoring and mass-trapping as well as mating disruption (by lowdosed pheromones), disorientation (by overdosed pheromones) and attracticide methods (by pheromones and contact insecticide) can be utilized for pest manipulation. The main target species belong to the orders of Lepidoptera (Gelechidae, Pyralidae) and Coleoptera (Anobiidae, Bostrichiidae, Cucujidae, Curculionidae, Dermestidae, Tenebrionidae). The prospects for the use of pheromones in the pest management of stores and mills are discussed. Insectistasis could lead to a drastic reduction of chemical treatments, limiting the probability of the development of insecticide-resistant strains. It moreover produces economic and qualitative advantages, protecting goods from residual products noxious to the consumer and improving te image of the firm.

1. INTRODUCTION

Storage pest manipulation can be divided into curative and preventive measures; prominent among the curative measures are the use of gaseous insecticides (application of methyl bromide and emission of hydrogen phosphide from precursors), contact insecticides (dichlorvos, malathion or pyrethroids) as well as the accumulation of carbon dioxide (CO2) under airtight storage conditions. Insect collision in entoleters, application of specific pathogens, induction of sterility by chemosterilants or irradiation, are curative measures either already partly in use or under development.

Preservation of products at low temperatures, protection of stored foodstuffs by means of insect-resistant packing materials, sex pheromones, hormone-mimetics agents, repellents, antifeedants and feeding stimulants are preventive measures which can be usefully employed.

2. CONCEPT OF INSECTISTASIS

A relatively new approach to pest management involves the causation of insectistasis, a state wherein the population density of harmful insect species is diminished to the extent of allowing the storage of foodstuffs without significant damage (Levinson and Levinson, 1985).

Insectistasis can be induced by employing two main tactics, these are: the retardation or interruption of insect development and reproduction as well as the reduction of the number of insect in a pest population (Levinson and Levinson, 1982; Levinson, 1983). The first tactic involves specifically thef use of nutrient antagonists, juvenoids and ecdysoids while the second tactic includes the employment of food attractants, repellents, sex and aggregation pheromones.

Insectistasis can be readly achieved by continual supervision of an environment by these kinds of attractant traps in combination with a limited number of curative measures being timed according to the extent of trap catches (Levinson, 1983).

The use of conventional insecticide is necessary only when the insect population has incurred significant economic damage. It is then, as a rule, reduced to the level of insectistasis, but will gradually increase again to its previous level. Insecticidal measures should be timed in accordance with trap catches as long as the population density is beyond the limits of insectistasis.

Sometimes mass trapping alone is sufficient to suppress the population density to the level of insectistasis. Potential preventive measures using pheromones requiring further development include mating disruption and attracticide methods.

3. SURVEY OF PHEROMONE USES IN STORED-PRODUCT PESTS CONTROL

There has been considerable progress in the use of pheromones for the monitoring and control of stored-product insects, since the pheromone of Attagenus unicolor (= megatoma) (black carpet beetle) was identified (Silverstein et al., 1967). Pheromones from nearly all of the species of major importance have been identified (Burkholder, 1982 and 1984; Suss, 1984; Burkholder and Ma, 1985).

Two general types of communication and reproductive strategies characterize stored-product insects: adults which are short-lived (>1 month) and require no feeding for reproduction to take place, such as the moths, some dermestids, bruchids and anobiid beetles which rely on sex pheromones (usually produced by the female) for communication. Adults which are long-lived (<1 month) and must feed in order to reproduce, e.g. grain weevils and the flour and grain beetles use male-produced aggregation pheromones for long distance communication. Both males and females respond to these pheromones.

Pheromone usage has developed in four main ways:

- monitoring insect population with pheromone baited traps;
- mass-trapping, where traps are used to reduce population levels;
- mating disruption, whereby the pheromone prevents communication between the sexes and hence subsequent mating;
- attracticide method, combining the attractant effect of the pheromone with the killing effect of the insecticide.

3.1. Monitoring

Pheromone traps can be used to detect both the presence and the density of pests, with the aim of obtaining a more precise control so that insecticides are used only when necessary.

Pheromone traps are generally effective when pest numbers are very low and so they can be used qualitatively to provide an early warning of pest incidence. They can also be useful to define areas of pest infestation, particularly where the overall distribution and life cycle are poorly understood.

Lepidoptera - The use of synthetic sex pheromones for pest management of moths in stored-product environments was first reported by Haines (1976) and Reichmuth et al. (1976). Haines' studies with Cadra cautella were conducted in maize and wheat stores in Kenya and confirmed the attractiveness of (Z,E)-9,12-tetradecadyenil acetate (or TDA). Reichmuth et al. reported the employment of TDA traps for early detection of infestations by Plodia interpunctella and Ephestia elutella in granaries and chocolate factories. The pheromone TDO, is produced by P.interpunctella , as a second component, according to Vick et al.(1981). Ten mg of 60:40 ratio of TDO:TDA was more effective in trapping P.interpunctella than 0.1 mg TDA, demonstrating the synergizing role of TDO in the activity of attraction of TDA. A mixture of TDA+TA in doses of 1+0.5 μg (10 ng released daily) showed activity which was greater than when TDA alone in doses of 1 μg was used (Trematerra, 1986).

In cacao warehouses, de Abreu and Williams (1981) showed that greater numbers of Cadra cautella were captured in light traps when sex pheromones were added as bait. Monitoring of C.cautella by means of pheromone traps in lower Egypt has been reported by Metwally and Hrdy (1987). According to the number of captured males, the most effective type was the"Stuttgart"trap (fig. 1) consisting of two flowerpots, followed by the cylindrical trap Etokap (both primed with adhesive); a balloon water trap was less effective.

The incidence of P.interpunctella and C.cautella in food distribution warehouses was observed as determined by sex pheromone baited traps by Vick et al. (1986). Significantly more moths were captured in some warehouses than in others; bird seed and chicken feed were associated with the increased moth captures.

Levinson and Levinson (1979) utilized adhesive stripes with a polyethylene capsule containing 50 ug of TDA in granaries and mills in West Germany and Greece (fig. 2). The monthly catch of male Ephestia kuehniella resulted in the identification of peak activity periods in February and July. The periods of greatest activity for C.cautella were June and August. The males of P.interpunctella occurred primarily from July through November in a flour mill in Piraeus.

In similar moth trapping studies in a chocolate factory, curves based on weekly moth captures reflected the start, peak and termination of the flight period as well as the fluctuations in moth catches following control attempts (Hoppe and Levinson, 1979). These curves has been employed for selecting the suitable time for control measures as well as for checking the results of such treatments.

Pheromone application methods with TDA were also reported by Bommer and Reichmuth (1980) suggesting a dosage for the optimum catch of E.kuehniella on adhesive traps. The seasonal occurrence of the warehouse moth E.elutella in West Berlin granaries as well as the correspondingly timed application of control measures were reported by Reichmuth et al. (1980).

A qualitative and quantitative survey of storage moth species (Pyralidae and Gelechiidae) occurring in a flour mill of southern Greece was carried out for two years by Levinson and Buchelos (1981).

From Czechoslovakia, Šifner et al. (1983) reported pheromone traps of different designs, suitable for monitoring stored-product phycitids and the optimum dose of TDA via a rubber dispenser.

The results of research carried out in Italy and in France in food preparation industries and warehouses are reported by Suss and Trematerra (1986) and by Fleurat-Lessard (1986) in which the major sex pheromone component of C.cautella , E.kuehniella and P.interpunctella was used.

Sitotroga cerealella could be readily trapped by (Z,E)-7,11-hexadecadien-1-yl acetate (HDA), this insect was generally predominant in grain stores and less frequent in flour stores. Kanaujia and Sidhu (1980) reported factors affecting the responsiveness of males to female sex pheromone in Angoumois moth.

Distribution of S.cerealella , P.interpunctella and C.cautella in rice fields and rice stores in Texas, as indicted by pheromone-baited adhesive traps have been reported by Cogburn and Vick (1981). Monitoring for the Angoumois grains moth in corn has been reported by Stokel and Sureau (1981); this paper contains data on the optimum dose of pheromone for sex trapping applications. Satisfactory results for S.cerealella are obtained using pheromone amounts between 300 and 900 μg/dispenser.

Coleoptera - Males of the five dermestid species were trapped successfully in grain stores of Marrakech with layered gunny bag traps containing 10 µg of the Z isomer of the T.granarium pheromone (Levinson and Levinson, 1979). Various Trogoderma species were monitored and successfully trapped with pheromone baited cardboard traps (fig. 3) in two large warehouses in which no infestations were previously known using the main pheromone component, viz. Z-trogodermal (Smith et al., 1983).

The natural ratio of the Rhyzopertha dominica pheromone is 1:2 for dominicalure 1 and dominicalure 2. In tests with adhesive traps conducted in Texas, the individual pheromone components or mixtures of these were equally attractive, but the attractancy increased with the dosage and trap catches of adults were inversely proportional to the height of the traps.

Laboratory and field studies have been conducted to study the responses of another bostrichid, Prostephanus truncatus , to the same pheromones (Cogburn et al., 1984). Field trials were conducted in Tanzania to monitor R.dominica and P.truncatus in farm stores. Dominicalure 2 was the most effective pheromone in trapping P.truncatus (Hodges et al., 1983).

Sitophilus oryzae , S.zeamais and S.granarius , have male-produced aggregation pheromones. Corrugated cardboard traps were used to test the attractive properties of combinations of synthetic pheromones and various food odors (cracked wheat, whole wheat, wheat germ oil, or water) to S.zeamais (Walgenbach et al., 1987). A pheromone produced by the first two species has been identified and named sitophilure. Moreover trace amounts of "sitophilate" have been found in the granary weevil and both males and females respond to the pheromone (Phillips et al., 1987).

Trapping experiments with Lasioderma serricorne were conducted in a Japanese cigarette manufacturing plant with flat adhesive traps containing serricornin and the food attractant, methyl phenylacetate (Wanabe et al., 1982). The trap was effective in catching beetles and in determining seasonal emergence patterns. Management of the beetles was aided, because infestation sites were located using the traps.

Male T.castaneum produce a pheromone which attracts both males and females, but the response of females appears to be stronger than that of males (Faustini et al., 1981). The male produced aggregation pheromone of T.castaneum and T.confusum was used with corrugated paper pitfall. Significantly more beetles were trapped in the pheromone baited traps as compared to controls (Barak et al., 1984). Larvae of T.castaneum were attracted by both contact and vapour of the synthetic adult aggregation pheromone at concentrations of 1.5 and 10 µg/g of medium (Mondal and Pont, 1984).

Field evaluation of a pheromone to detect adults of Cryptolestes ferrugineus using a mixture of the synthetic ferrulactone I and ferrulactone II were tested in grain stored in Canada by Loschiavo et al. (1986). Population fluctuations of adults of C.ferrugineus in wheat stored in a steel granary in Manitoba (Canada) were monitored by Loschiavo and Smith (1986) using insect detection traps 35 cm below the surface of the grain during autumn 1983 and spring, summer and autumn of 1984. Lindgren et al. (1985) reported a newly designed trap (fig. 4), which confines beetles responding to attractive odours within an enclosed chamber, was also very effective in recapturing released adults of C.ferrugineus when baited with a 13:16 mixture of ferrulactone I and (+) ferrulactone II released at 1.25 μg/24 h, as well as in recapturing released adults of T.castaneum, in response to the aggregation pheromone, 4,8-dimethyldecanal, released at 0.08 μg/h. Moreover, the capture of each species in traps baited with both pheromones of the species was not different than in traps baited with one pheromone alone. Thus the pheromones of both species can be used together in the same trap in a semiochemical-based, pest monitoring system.

Aggregation pheromones were isolated from C.pusillus and C.turcicus (Millar et al., 1985a and 1985b).

The silvanids O.surinamensis and O.mercator utilize male-produced macrolide aggregation pheromones, as a result of laboratory bioassays as reported by Pierce et al. (1984).

In the Bruchidae, Hope et al. (1967) first demonstrated the existence of a male produced sex pheromone in the bean weevil, Acanthoscelides obtectus. Preliminary evidence for a female sex attractant in Callosobruchus chinensis which attracts males was reported by Honda and Yamamoto (1976) and in C.maculatus by Rup and Sharma (1978) and Qi and Burkholder (1982).

3.2. Mass-trapping

A powerful, highly specific insect attractant should catch a sufficiently large number of the target insect species to reduce population increase to economically acceptable levels.

Early attempts of mass-trapping were conducted by the pheromone blends of many target insect species; designs of traps have been developed, generally on an empirical basis. Various release mechanisms have been developed to dispense the pheromones at a controlled rate. Part of the problem is to quantify the number of traps necessary per unit area to achieve control.

Pheromone trapping with TDA in a granary slightly infested with E.elutella permitted a postponement of fumigation (Reichmuth et al., 1978). The key to control appears to be effective mass trapping when low insect populations prevail. This situation often occurs in well maintained and modern food processing and storage facilities. In instances where few insects are known to be present, the use of pheromone traps to either eliminate the insects or to locate infested commodities is feasible.

The continual employment of TDA-traps in tobacco stores (in the harbour region of Piraeus, Greece) appears to be adequate for the detection and surveillance of populations of adult E.elutella as well as for the proper timing and checking the efficacy of control measures against this species. It is likely that the employment of additional TDA-traps in tobacco stores would reduce the warehouse moth population (due to mass trapping) to the level of insectistasis and thus limit the number of control measures to a minimum (Buchelos and Levinson, 1985).

Šifner et al. (1983) reported that a 2-year mass trapping trial performed in a chocolate factory (in Czechoslovakia) demonstrated the applicability of the pheromone method for the maintenance of an acceptably low population density of C.cautella suppressed initially by insectidides.

Süss and Trematerra (1986) and Fleurat-Lessard (1986) showed that it was possible to effectively control infestation of E.kuehniella and P.interpunctella in infested environments using the technique of mass-trapping. The experiments reported by Trematerra and Battaini (1987) indicate that E.kuehniella infestation in flour mills can be controlled by a mass-trapping program of funnel traps (fig. 5) baited with 2 mg of TDA (daily release of 13 µg); one placed for every 260-280 cubic meters.The continued presence of the traps in the environment stabilize the infestation to levels as low as those observed during months unfavourable to development with a drastic reduction of chemical treatments with consequent economic and qualitative advantage.

The areal density of Rhyzopertha dominica , flying in a warehouse was recorded using lindgren funnel traps baited with dominicalure (Leos-Martinez et al., 1986). This crepuscolar species had a small peak of flight activity around sunrise and a very large one at sunset. At night flight was minimal under normal conditions but increased when warehouse lights were on.

3.3. Mating disruption

Mating disruption is assumed to be achieved by permeating the area under treatment with a synthetic pheromone so as to reduce mate finding or aggregation, the result being mating suppression. The mechanisms involved to achieve this effect could consist of one or a combination of any of the following:

- the constant exposure of the insect to a relatively high level of pheromone leads to the adaptation of the antennal receptors and habituation of the central nervous system. Under such circumstances, the responding insects would be unable to respond to any normal level of the stimulus;
- a sufficiently high background level of the applied pheromone masks the natural pheromone plume and therefore trail following is not possible;
- the synthetic pheromone is applied in a relatively large number of discrete sources so that insects flying within the treatment area can be diverted from the naturally occurring plumes.

Knowledge as to which disruption mechanisms are operating is important, since otherwise the design of appropriate formulations would remain guesswork. In fact, the situation may differ for various insect species.

Mating disruption trials have been conducted for species of Cadra and Ephestia in warehouse situations by permeation with TDA. The isolated environment within such large stores certainly provides a situation where

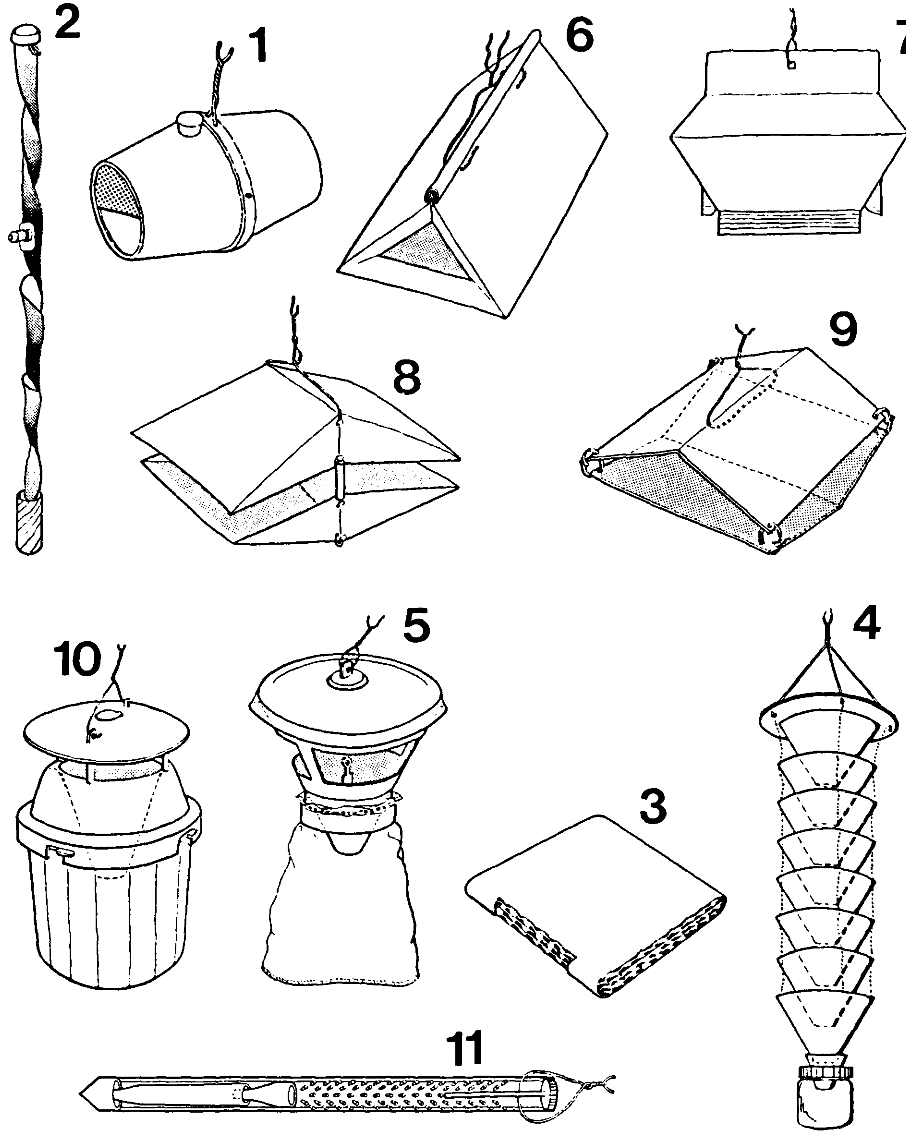


Figure 1-11 - Various types of pheromone traps.
Stuttgart trap (1); stripe trap (2); cardboard trap (3); Lindgren funnel trap (4); funnel trap "Mastrap" (5); delta trap (6); tent trap (7); wing trap (8 and 9); funnel trap "Moth trap" (10); grain-probe trap (11).

immigration is virtually excluded. The relatively high population densities that occur within such enclosures do not, however, suggest this approach to be promising (Barrer, 1976; Haines and Read, 1977).

The reduction in mating of C.cautella caused by permeation of the atmosphere with synthetic sex pheromone components was investigated in the laboratory and in cocoa stores. Decreased numbers of copulating pairs were observed in the stores treated with pheromone and F1 emergence was reduced by 91 to over 99% when densities of moths of 0.1-0.3 moths/m2 surface area were exposed to pheromone concentration of 4-10 µg/m3 (Hodges et al., 1984).

Moreover, results of mating disruption of C.cautella in warehouse of a confectionery industry containing nuts were reported by Suss and Trematerra (1986).

Regarding E.kuehniella a considerable reduction of the infestation in flour mills was reached over a two year period by distributing 0.2 mg of TDA per 1 cubic meter (Trematerra and Capizzi, 1987).

Regarding the control of S.cerealella ,some information has been reported by Stockel and Sureau (1981).

Pheromone biology of the tobacco beetle, Lasioderma serricorne , with notes on the pheromone antagonism between 4S, 6S, 7S - and 4S, 6S, 7R - sericornin were reported by Levinson and Levinson (1987).

3.4. Attracticide method

In these trials one side of particular laminar dispensers can be treated with an insecticide in the attempt to combine the attractant effect of the pheromone with the killing effect of the insecticide (fig. 12). The first attempt to kill males with the treated dispensers has been employed with encouraging success on E.kuehniella by Capizzi et al. (1986). They used a dispenser made of a layer of felted polyetylene fibrils coated with a heat-sealed aluminium-polyethylene film (of square shape with 20 mm sides and 0.46 mm thickeness) so that the pheromone is released from the edges only.

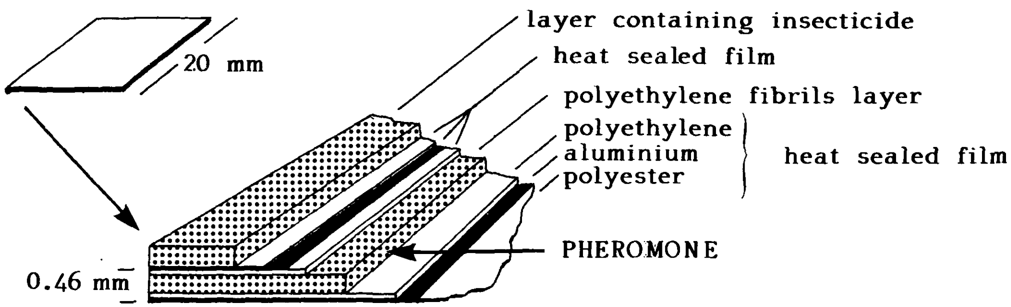


Figure 12 - Diagram of laminar dispenser.

Trematerra and Capizzi (1987) controlling E.kuehniella in flour mills by the attractioo method eliminated over 90% of the males, stabilizing residual infestation to tolerable levels.

Surely this can be ascribed to the males which touch the attraction source again and again. According to the indications of Trematerra and Battaini (1987) the dispensers, baited with 2 mg of TDA and treated with a pyrethroid, were placed on the walls and machineries, from 1.80-2.00 m from the floor (one dispenser for every 220-280 cubic meters).

4. TRAPS AND TRAPPING PROCEDURE

Before mating, most male or female insects respond to their pheromone in combination with supplementary key stimuli. This sequence of sensory stimuli can be utilized in the design of attractant traps to be employed in the manipulation of storage pests. New traps have been developed and are being employed in monitoring and management systems that utilize pheromones. Trapping procedures vary depending on whether the objective is monitoring or control.

4.1. Trap design

Trap design is vital in developing trapping systems. In this regard, e.g. the preferential flight of the Plodia interpunctella and Cadra cautella toward figures varying in shape, size and position as well as the influence of combined optical and olfactory stimuli were investigated by Levinson and Levinson (1985). It would be difficult to review comprehensively the numerous types that have been tested. Instead, we will cover some of the major types and consider some of their attributes and deficiencies.

In the control of Lepidoptera the most common types of traps in use employ a sticky surface to retain the attracted insect.

Four of the most common sticky traps are the stripe trap (fig. 2) the delta trap (fig. 6), the tent trap (fig. 7), and the wing trap (fig. 8 and 9). If the sticky surface on these traps is fresh, these kinds of traps retain most insects that come in contact with their sticky surface. As the sticky surface ages, it becomes covered with debris or with dust, or it is filled with the target insects, its ability to retain new arrivals is reduced or even eliminated. Then the trap must be replaced by a new one.

Thus, the suitability of sticky traps is dependent upon the population levels of the target species and the necessity of trapping a high proportion of the attracted insects.

In survey and detection programs, the foremost concern is sensitivity of the system, i.e., detecting the presence of the target species. Similarly, when trap catch is used to time phenological events such as the onset of adult emergence, trap saturation may be of no concern. But in other trapping programs, saturation can pose serious problems. When a trap catch is used as an indicator of population density, the limited capacity will make it difficult to develop predictable relationships between trap catch and density. This can be overcome by frequent replacement of the sticky surface.

In mass trapping applications, superior trap efficiency necessary for management would appear to eliminate sticky traps, except at very low pest densities. In the control of phycitid and gelechiid moths a variety of nonsticky traps with large capacity and unvarying efficiency offer alternative approaches. Insects can be trapped and kept alive with lures in funnel traps (fig. 5 and 10). Their capacity exceeds several thousand insects, in contrast to sticky traps which become saturated, according to the species, with about 150-400 males.

However when a funnel trap contains an insecticide to kill the insects, some attention should be paid to the possibility of the insecticide repelling the insects before they enter the trap.

Crawling insects, generally Coleoptera, both larvae and adults, can be successfully trapped in corrugated paper traps (Levinson et al., 1981). Insects retreat into and hide in the corrugations just as they do in the cracks of floors or walls. This behavioural response was the key influence in the development of this type of trap. Originally, this trap was treated with an insecticide to kill the insects. In response to concerns about pesticide use in food plants, the trap has been modified using adhesive on the floor of the trap.

A particular trap used in grain bins is the plastic grain probe (Burkholder, 1984) (fig. 11). Insects fall or crawl into the trap and drop through a plastic funnel into the lower part of the trap. The trap may be used near the surface of the grain bin or it may be pushed several meters into the grain with the aid of a metal sleeve and a standard seedburo T-handle and threaded extensions.

4.2. Trap placement

The confined and relatively small area of warehouses, factories, flour-mills, silos is more likely to have a homogeneous density of insects in flight than a large open area. Traps placed at several points in the warehouse would be expected to catch a representative number of the insects available in the building and also could indicate locations of high population densities or hot spots in the warehouse.

The precise position of a trap within a habitat can have a substantial effect on the levels of trap catch. Placement of traps in warehouses is dependent in part on the size of the warehouse and on available supporting posts or other places where there is little or no traffic.

In general, the traps should be placed away from open doors o windows to avoid attracting insects into a facility from outside. Traps may be placed outside of a warehouse to catch migrant insects and therefore may intercept insects before they have a chance to move inside.

Moth traps are usually more effective if placed near ceiling levels (about 2.20-2.50 meters to the floor). Traps for crawling insects such as Coleoptera can be placed near the corner, under machinery or in proximity to goods on hand.

5 . CONCLUSIONS

As the above-mentioned review shows, considerable progress has been made in recent years in monitoring and controlling stored-product insects with pheromones.

In England, pheromone traps were used in ca. 60% of government flour stores to monitor pyralid moths, especially the E.kuehniella , during 1981. The trapping system was successful in improving integrated control measures undertaken in the stores using less pesticide than in previous years.

A pheromone monitoring program for Trogoderma spp. is currently used by the Plant Protection and Quarantine Programs, Animal and Plant Health Insection Service (APHIS), USDA, at major United States port facilities and in the interior of California.

In West Germany, Italy and France, pheromone traps have been valuable for monitoring and controlling infestations of pyralid moths in warehouses, grain stores , flour mills, and chocolate factories.

Different tolerance thresholds should be established for the various groups of insects depending on their economic impact and where they are found. For example, a limited number of insects can be tolerated at times in a storehouse containing raw materials, but in food-processing plants and storehouses containing finished products the threshold must necessarily be zero.

An important role should be assigned to all the prevention techniques which effectively keep infested goods out of food-processing plants, mills, storehouses, etc.

In the case of complex infestation, where more than one species of moths or beetles are involved, treatments which use only pheromones become complex and aren't effective, whereas integrated treatments which use different technical means, not excluding chemicals, have better results. Insectistasis can be readly achieved by continual supervision of environments by pheromone traps in combination with a limited number of curative measures appropriately timed.

The use of pheromones, therefore, is one of several modern techniques that shows promise in controlling stored-product insects. The utilization of pheromones could lead to a drastic reduction of chemical treatments with consequent economic and qualitative advantages, protecting goods from residual products noxious to consumers and improving the image of the firm.

6. LITERATURE CITED

BARRER P.M., 1976 - The influence of delayed mating on the reproduction of Ephestia cautella (Walker) (Lepidoptera, Phycitidae). J.stored Prod.Res., 12: 165-169.

BOMMER H., REICHMUTH Ch., 1980 - Pheromone der vorratsschadlichen motten (Phycitinae, speziell Mehlmotte Ephesta kuehniella Zeller) in der biologischen Schadlingsbekampfung. Mitt.Biol.Bundesanst. Land Forstwirtsch. Berlin-Dahlem, 198: 102-103.

BUCHELOS C. TH., LEVINSON A.R., 1985 - Population dynamics of Ephestia elutella (Hubner) in tobacco stores with and without insecticidal treatments: a survey by pheromone and unbaited traps. Z.ang.Ent., 100: 68-78.

BURKHOLDER W.E., 1982 - Reproductive biology and communication among grain storage and warehouse beetles. J.Ga.Entomol.Soc., 17 (4): 1-10.

BURKHOLDER W.E., 1984 - The use of pheromones and food attractants for monitoring and trapping stored-product insects. In: Insect Control for the Food Industry, ed. Baur F.J., Am.Assoc.Cereal.Chem.: 69-86.

BURKHOLDER W.E., MA M., 1985 - Pheromones for monitoring and control of stored-product insects. Ann.Rev.Entomol., 30: 257-272.

CAPIZZI A., ARSURA E., SPINELLI P., 1986 - I feromoni: esperienze applicative con un nuovo tipo di erogatore. Atti Giornate Fitopatol., 1986, 1: 177-186.

COGBURN R.R., VICK K.W., 1981 - Distribution of Agoumois grain moth, almond moth, and Indian meal moth in rice fields and rice storages in Texas as indicated by pheromone-baited adhesive traps. Environ.Entomol., 10: 1003-1007.

COGBURN R.R., BURKHOLDER W.E., WILLIAMS H.J., 1984 - Field tests with the aggregation pheromone of the lesser grain borer (Coleoptera: Bostrichidae). Environ.Entomol., 13: 162-166.

De ABREU J.M., WILLIAMS R.N., 1981 - Effectiveness of fluorescent lamps and synthetic sex pheromone on suction-trap catches of Ephestia cautella . Rev.Theobroma, 11: 245-248.

FAUSTINI D.L., BURKHOLDER W.E., LAUB R.J., 1981 - Sexually dimorphic setiferous sex patch in the male red flour beetle Tribolium castaneum (Herbst.) (Coleoptera: Tenebrionidae): site of aggregation pheromone production. J.Chem.Ecol., 7 (2): 465-480.

FLEURAT-LESSARD F.C, 1986 - Utilisation d'un attractif de synthése pour la surveillance et le piégeage des pyrales Phycitinae dans les locaux de stockage et de conditionnement de denrées alimentaires végétales. Agronomie, 6 (6): 567-573.

HAINES C.P., 1976 - The use of synthetic sex pheromones for pest management in stored-product situations. Pestic. Sci., 7: 647-649.

HAINES C.P., Read J.S., 1977 - The effect of synthetic female sex pheromones on fertilization in warehouse population of Ephestia cautella (Walker) (Lepidoptera, Phycitidae). Rap. L 45, Tropical Product Institute, London: 10 pp.

HODGES R.J., BENTON F.P., HALL D.R., DOS SANTOS SERODIO, 1984 - Control of Ephestia cautella (Walker) (Lepidoptera, Phycitidae) by synthetic sex pheromones in the laboratory and store. J.stored Prod.Res., 20 (4): 191-197.

HODGES R.J., HALL D.R., GOLOB P., MEIK J., 1983 - Responses of Prostephanus truncatus to components of the aggregation pheromone of Rhyzopertha dominica in the laboratory and field. Entomol.Exp.Appl., 34: 266-272.

HONDA H., YAMAMOTO I., 1976 - Evidence for and chemical nature of a sex pheromone present in azuki bean weevil, Callosobruchus chinensis L. Proc. Symposium of Insect Pheromones and their Applications, Negaoka and Tokyo: 164 pp.

HOPE J.A., HORLER D.F., ROWLANDS D.G., 1967 - A possible pheromone of the bruchid Acanthoscelides obtectus (Say). J.stored Prod.Res., 3: 387.

HOPPE T., LEVINSON H.Z., 1979 - Detection of infestations and population surveillance of lepidopteran storage pests (Phyticinae) in a chocolate factory by use of pheromone baited traps. Anzeiger fur Schadlingskunde, Pflanzenschutz, Umweltschutz, 52: 177-183.

KANAUJIA K.R., SIDHU H.S., 1980 - Factors affecting the responsiveness of males to female sex pheromone in Angoumois grain moth, Sitotroga cerealella (Olivier). Z.ang.Ent., 89: 387-390.

LEOS-MARTINEZ J., GRANOWSKY T.A., WILLIAMS H.J., VINSON S.B., BURKHOLDER W.E., 1986 - Estimation of aerial density of the Lesser grain borer (Coleoptera: Bostrichidae) in a warehouse using dominicalure traps. J.Econ.Entomol., 79: 1134-1138.

LEVINSON H.Z., 1983 - The scientific background of pest manipulation involving insectistasis. Annls Inst.Phytopath.Benaki (N.S.), 14: 69-79.

LEVINSON H.Z., 1983 - Integrated manipulation of storage pests involving insectistasis. Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte Entomologie, 4: 101-103.

LEVINSON H.Z., BUCHELOS C.Th., 1981 - Surveillance of storage moth species (Pyralidae, Gelechiidae) in a flour mill by adhesive traps with notes on the pheromone-mediated flight behaviour of male moths. Z.angew.Ent., 92: 233-251.

LEVINSON H.Z., LEVINSON A.R., 1979 - Trapping of storage insects by sex and food attractants as a tool of integrated control. In: Chemical Ecology: Odour Communication in Animal (ed. Ritter F.J.). Elsevier, Biomedical Press, North Holland: 327-341.

LEVINSON H.Z., LEVINSON A.R., 1982 - Attractifs, répulsifs et phéromones en tant qu'insectistatiques dans le milieu de stockage. Les cahiers de la recherche agronomique, Al Awamia, 39: 189-216.

LEVINSON H.Z., LEVINSON A.R., 1985 - Use of pheromone traps for the proper timing of fumigation in the storage environment. Bull. OEPP, 15: 43-50.

LEVINSON H.Z., LEVINSON A.R., 1987 - Pheromone biology of the tobacco beetle (Lasioderma serricorne L., Anobiidae) with notes on the pheromone antagonism between 4S, 6S, 7S - and 4S, 6S, 7R -

serricornin. J.Appl.Ent., 103: 217-230.

LEVINSON H.Z., LEVINSON A.R., BURKHOLDER W.E., SILVERSTEIN R.M., CASSIDY R.F., 1981 - A trap for khapra beetles and a method of controlling the beetles. U.K. Patent nb. 1584856, London.

LINDGREN B.S., BORDEN J.H., PIERCE H.D., PIERCE A.M., OEHLSCHLAGER A.C., WONG J.W., 1985 - A potential method for simultaneous, semiochemical-based monitoring of Cryptolestes ferrugineus and Tribolium castaneum (Coleoptera: Cucujidae and Tenebrionidae). J.stored Prod.Res., 21: 83-87.

LOSCHIAVO S.R., WONG J., WHITE N.D.G., PIERCE H.D., BORDEN J.H., OEHLSCHLAGER A.C., 1986 - Field evaluation of a pheromone to detect adult rusty grain beetles, Cryptolestes ferrugineus (Coleoptera: Cucujidae) in stored grain. Can.Ent., 118: 1-8.

LOSCHIAVO S.R., SMITH L.B., 1986 - Population fluctuations of the rusty grain beetle, Cryptolestes ferrugineus (Coleoptera: Cucujidae), monitored with insect traps in wheat stored in a steel granary. Can.Ent., 118: 641-647.

METWALLY M.M., HRDY I., 1987 - Monitoring of Ephestia cautella (Lepidoptera, Pyralidae) by means of pheromone traps in lower Egypt. Acta Entomol.Bohemoslov., 84: 408-413.

MILLAR J.G., PIERCE H.D., PIERCE A.M., OEHLSCHLAGE A.C., BORDEN J.H., BARAK A.V., 1985a - Aggregation pheromones of the flat grain beetle, Cryptolestes pusillus (Coleoptera: Cucujidae). J.Chem.Ecol., 11 (8): 1053 - 1070.

MILLAR J.G., PIERCE H.D., PIERCE A.M., OEHLSCHLAGER A.C., BORDEN J.H., 1985b - Aggregation pheromones of the grain beetle, Cryptolestes turcicus (Coleoptera: Cucujidae). J.Chem.Ecol., 11 (8): 1071-1081.

MONDAL K.A.M.S.H., PORT G.R., 1984 - Response of Tribolium castaneum larvae to synthetic aggregation pheromone. Entomol.Exp.Appl., 36 (1): 43-46.

PHILLIPS J.K., MILLER S.P.F., ANDERSEN J.F., FALES H.M., BURKHOLDER W.E., 1987 - The chemical identification of the granary weevil aggregation pheromone. Tetrahedron Letters, 28 (49): 6145-6146.

PIERCE H.D., PIERCE A.M., MILLAR J.G., WONG J.W., VERGIN V.G., OEHLSCHLAGER A.C., BORDEN J.H., 1984 - Methodology for isolation and analysis of aggregation pheromones in the genera Cryptolestes and Oryzaephilus (Coleoptera: Cucujidae). Proc. Third Int. Working Conf. stored-product Ent., Manhathan: 121-137.

QI T.Y., BURKHOLDER W.E., 1982 - Sex pheromone biology and behavior of the cowpea weevil Callosobruchus maculatus (Coleoptera: Bruchidae). J.Chem. Ecol., 8 (2): 527-534.

REICHMUTH Ch., WOHLGEMUTH R., LEVINSON A.R., LEVINSON H.Z., 1976 - Untersuchungen uber den Einsatz von pheromonbekoderten Klebefallen zur Bekampfung von Motten in Vorratsschutz. Z.ang.Ent., 82: 95-102.

REICHMUTH Ch., SCHMIDT H.U., LEVINSON A.R., LEVINSON H.Z., 1978 - Die Faengigkeit pheromonbekoederter Klebefallen fur Speichermotten (Ephestia elutella Hbn.) in unterschiedlich dicht befallenen Getreidelagern. Z.ang.Ent., 86: 205-212.

REICHMUTH Ch., SCHMIDT H.U., LEVINSON A.R., LEVINSON H.Z., 1980 - Das jahreszeitliche Auftreten von Speichermotten (Ephestia elutella HbN.) in Berlin Getreideschuttbodenlargern sowie der zeitentsprechende Einsatz von Bekampfungsmbnahmen. Z.ang.Ent., 89: 104-111.

RUP P.J., SHARMA S.P., 1978 - Behavioural response of males and females of Callosobruchus maculatus (F.) to the sex pheromones. Indian J.Ecol., 1: 72-76.

ŠIFNER F., ZDAREK J., HRDY I., HALVODA L., 1983 - Pheromone traps for the pest management of phycitid moths. Crop.Prot., 2: 463-472.

SILVERSTEIN R.M., RODIN J.O., BURKHOLDER W.E., GORMAN J.E., 1967 - Sex attractant of the black carpet beetle. Science, 157: 85-87.

SMITH L.W., BURKHOLDER W.E., PHILLIPS J.K., 1983 - Detection and control of insects in stored food: the effect of the pheromone-baited traps and their placement on the number of Trogoderma species captured. Natick Technical Report TR 83/008. US Army Natick Res.Dev.Lab., Natick, Mass., 13 pp.

STOCKEL J., SUREAU F., 1981 - Monitoring for the Angoumois grain moth in corn. In: Management of insects pest with semiochemicals, Mitchell E.R., Plenum Press, New York: 63-67.

SÜSS L., 1984 - Il monitoraggio. In:Gli artropodi infestanti le derrate, problemi e prospettive per la difesa, Domenichini G.. CNR, Piano finalizzato: IPRA, mon. 1: 107-138.

SÜSS L., TREMATERRA P., 1986 - Control of some Phycitidae infesting stored-products with synthetic sex pheromone in Italy. Proc.4th Intern.Working Conf. stored-product prot., Tel-Aviv: 606-611.

TREMATERRA P., 1986 - Valutazione dell'attività di richiamo di due componenti il feromone sessuale femminile di Plodia interpunctella Hubner. Atti Giornate Fitopatol., 1986, 1: 187-194.

TREMATERRA P., BATTAINI F., 1987 - Control of Ephestia kuehniella Zeller by mass-trapping. J.appl.Ent., 104: 336-340.

TREMATERRA P., CAPIZZI A., 1987 - Esperienze di controllo delle infestazioni di Ephestia kuehniella Zeller nei mulini mediante feromoni. Atti IV Simposio sulla difesa antiparassitaria delle industrie alimentari e sulla protezione degli alimenti (in press).

VICK K.W., COFFELT J.A., MANKIN R.W., SODERSTROM E.L., 1981 - Recent developments in the use of pheromones to monitor Plodia interpunctella and Ephestia cautella . In: Management of Insect Pests with Semiochemicals, Mitchell E.R., Plenum Press, New York, 3: 19-28.

VICK K.W., KOEHLER P.G., NEAL J.J., 1986 - Incidence of stored-product Phycitinae Moths in food distribution warehouses as determined by sex pheromone-baited traps. J.Econ.Entomol., 79: 936-939.

WALGENBACH C.A., BURKHOLDER W.E., CURTIS M.J., KHANZ Z.A., 1987 - Laboratory trapping studies with Sitophilus zeamais (Coleoptera: Curculionidae). J.Econ.Entomol., 80: 763-767.

WANABE T., KATO J., ARAI K., KOHNO M., CHUMAN T., 1982 - Integrated control of cigarette beetle (Lasioderma serricorne F.) with a synthetix sex phe romone and food attractant in the cigarette manifacturing factory. Sci. Pap. Cent. Res. Inst. Jpn. Tobacco Salt Publ. Corp., 124: 23-32.

THE SAFETY OF PESTICIDE RESIDUES IN FOOD TOWARDS THE CONSUMER
THE EXAMPLE OF DELTAMETHRIN

G. De Wilde
Roussel-Uclaf-Paris, France

Summary

The need to use pesticides in modern agriculture in order to increase yields and quality of agricultural products is generating a legal question in many countries : How safe are these pesticide residues in food towards the consumer eating these "contaminated" foods ?
To answer this question for a well defined population one has to know the average food consumption habits (statistics about average portion of different food consumed), the ADI (Acceptable Daily Intake) and the MRL's (Maximum Residue Limits) for this pesticide fixed by the governmental authorities of this population.
Knowing these factors a first approach is the calculation of the TMDI (Theoretical Maximum Daily Intake) which is the sum of all the MRL's multiplied by the average food consumption for each commodity.
If TMDI < ADI x B.W (B.W.= Bodyweight) safety for the population is guaranteed and no further examinations have to be performed.
Whenever TMDI > ADI x B.W., a second more realistic approach of pesticide residue intake through diet can be done by calculating the EMDI (Estimated Maximum Daily Intake) which takes also into account the effects of the preparation, processing and cooking of the food and related MRL's.
If still EMDI > ADI x B.W., a third still more realistic approach can be made by calculating EDI (Estimated Daily Intake) taking into account the actual residue levels "in the plate" (not always MRL's), the market share of the pesticide for the different crops, the ratio local production/import for the crop and the disappearance of the pesticide during storage.
The EDI is mostly one tenth or one hundredth of the TMDI and results in figures far below the ADI so that in any case the safety of the average consumer eating food contaminated by pesticide residues is totally guaranteed.
We will illustrate this approach by an example of TMDI, EMDI and EDI calculation with deltamethrin on a typical french diet.

1- INTRODUCTION

The need to use pesticides in the production of crops in modern agriculture in order to increase yields and quality became a must during the last fifteen to twenty years. Modern agriculture had to produce more (increase of population on earth) at a lower price, using less manpower (costs) and more automatic and mechanical means.

The use of pesticides contributed for a large part to succeed this challenge because the use of herbicides increased the yields by 20 - 30 % and makes it possible that field crops as peas, beans, cereals, oil seeds etc... can be harvested by high performant agricultural machinery. The use of fungicides and insecticides boosted yields and quality of the agriculture products (visual aspects).

Observing the intensive use of pesticides in modern agricultural practices, the consumer is wondering nowadays if these products do not leave residues in crops and if these residues are totally safe for human health.

In order to answer these questions, pesticide industry has to make enormous investment costs before putting on the market a new and safe product. These pre-marketing costs concern the constitution of a complete chemical, toxicological and environmental file including residue studies in treated crops. The complete file is reviewed by official toxicological experts of the countries were the pesticide will be put on the market and by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). These national or international experts estimate the Acceptable Daily Intake which is based on a complete review of the available biochemical, metabolic, pharmacological, toxicological data from a wide range of experimental animal studies and/or available relevant human data.
The No-Observed-Adverse-Effect-Level (NOAEL) for the most sensitive toxicological parameter which is normally for the most sensitive species of experimented animal, is used as the basis for estimating the ADI which is :

$$\frac{\text{NOAEL}}{\text{safety factor (min. 100x)}}.$$

As this ADI is established in mg per kg of bodyweight we have to multiply ADI by the mean bodyweight (B.W.) to find the max quantity of pesticide that a person can ingerate safely per day for a whole lifetime.

On the other hand, Maximum Residue Levels are estimated from globally generated pesticide residue data, respecting Good Agricultural Practices. Taking into account that statistically the maximum quantity intake of the same crop is 0.4 kg per person and per day, the MRL can theoretically not exceed :

$$\left(\frac{\text{NOAEL}}{\text{Safety Factor}} : 0.4\ \text{kg} \right) \times \text{B.W} \qquad \text{(see Fig.1)}$$

Neither ADI nor MRL are immutable, both are established according to the best judgement of a group of internationally or nationally recognized experts on the data available to them at the time of evaluation but when new data become available, ADI and/or MRL can be revised.

Knowing ADI and MRL fixed by local or international authorities, we need to know exactly in order to answer our question about the safety of pesticide residues in food towards a population, the average daily portions of food consumed by this population.
Prediction of pesticide residue intake through the diet can be done with different degrees of realism.

Comparing estimated pesticide residue intake and maximum quantity of pesticide, a person can safely ingerate enables an assessment of the potential human health risk of these pesticide residues.
The risk assessment starts with the most exagerated intake predictions and proceeds towards more and more realistic ones.

This approach makes it possible to eliminate at an early stage, pesticides for which the intake is clearly unlikely to exceed the maximum quantity considered as safe.

A second more realistic approach using more refined data would make it possible to eliminate other pesticides from further consideration. This type of approach facilitates acceptance of national or Codex MRL which seems to exceed in a first approach the maximum safe limit. And so we can go on by still more and more realistic approaches. We can even be obliged to study the real residue intake for a pesticide by residue determination in the plate in order to demonstrate the complete safety of a compound.

FAO/WHO proposes a four-stage approach for predicting pesticide residue intake (see Fig.2). This approach will be explained hereafter.

2- THEORETICAL APPROACHS - DEFINITION OF TMDI, EMDI AND EDI

2.1) TMDI or Theoretical Maximum Daily Intake or crude estimate TMDI

TMDI is an estimation of dietary intake that is calculated by using the MRL and the average daily portion of each food commodity for which an MRL has been established. It is calculated by multiplying the MRL by the average food consumption for each commodity and then summing the products :

$$TMDI = \sum F_i \times M_i$$

where Σ indicates the sum of the products of $F_i \times M_i$ of all commodities for which an MRL was established

F_i average food consumption for the commodity in the national or international diet in kg food per person per day

M_i the MRL for the commodity in mg/kg

So TMDI is given in mg/person and on the otherhand ADI is expressed in mg/kg body weight, in order to compare the TMDI with the ADI, the ADI must be multiplied by the average body weight (B.W) for a population which is 60 kg for an european population and 50 kg for asian people.

This TMDI is overestimating the real pesticide residue intake because :

1) never 100 % of a crop is treated with the same pesticide,
2) only a small part of a crop will contain residues as high as the MRL,
3) we do not take into account the fate of the residues (storage, transport, preparation and processing)
4) the MRL is fixed for the raw commodity containing inedible portions.

For these reasons, it should not be concluded that if TMDI exceeds the maximum safe quantity, there is some danger for the health and safety of the consumer. At that moment, we have to consider a more realistic approach to estimate the daily intake of pesticide by food.
On the other hand, if already TMDI is lower than the maximum safe quantity, no further consideration of intake of pesticide residue by food is needed to guarantee the safety of the consumer taking into account the enormous overestimation we made by calculating TMDI.

2.2) <u>Estimated maximum daily intake</u>

A second more realistic approach to evaluate pesticide residue intake is to calculate estimated maximum daily intake by taking into account the maximum residue level in the edible portion of each crop, the effects of preparation, processing and cooking of the food.

So $$EMDI = \Sigma\ Fi \times Ri \times Pi \times Ci$$

Fi : average food consumption per head/per day for a crop
Ri : max residue level in the edible portion of the crop
Pi : correction factor for the reduction or the increase in residue on commercial processing (milling f.i)

Ci : the correction factor taking into account the reduction or increase in the residue on preparation or cooking of the food.

This EMDI is still an overestimation of the real pesticide residue intake because it does not take into account that :

- a crop is never treated 100 % by the same pesticide,
- only a few of the treated crops contain residue levels as high as the MRL.

Once again if EMDI is lower than the maximum safe limit, no further consideration of intake of pesticide residue by food is needed to guarantee the safety of the consumer taking into account the overestimation by calculating EMDI.

2.3) Estimated daily intake

If EMDI still exceeds the maximum safe limit, a third still more realistic approach for pesticide intake estimation can be made by calculating EDI (Estimated Daily Intake) taking into account :

- the food consumption data for the population (more detailed than in 2.2)
- known uses of the pesticide and not all registered crops
- real residue levels and not MRL
- percentage of the crop treated with the pesticide (market share)
- ratio home grown/import
- disappearance of the pesticide during storage.

2.4) Residue intake study

If still EDI exceeds the maximum safe limit, the final and fourth approach to guarantee the safety of customers is to undertake a so called measured pesticide residue intake study. This is a study measuring every day during a certain period the real residue level of a pesticide in the plate of the consumer.

3- THE PRACTICAL EXAMPLE OF DELTAMETHRIN

Let us now try to put into practice this theoretical approach for a well known insecticide, produced by Roussel-Uclaf, Deltamethrin. Roussel-Uclaf registered this product allover the world on the most different crops but the maximum diversification of use was until now granted in France.

First of all, a quick physico-chemical review of the product (Fig. 3). Note the very low vapour pressure and the low water solubility.
A summary of the toxicological file (Fig.4) and the estimation by international toxicological experts (JMPR, France, Germany...) of the ADI and MRL (Fig.5).

First of all, let us see on Fig. 6, 7 and 8 what are the Codex Maximum Residue Limits established for deltamethrin and the Maximum Residue Levels we detected by respecting Good Agricultural Practices.
Fig. 9 and 10 explain the fate of Deltamethrin residues (location) in tomatoes and oranges and give an idea of the level of residues in processed food (tomatoepaste and orange pulp).
Fig. 11 indicates the maximum residue level for Deltamethrin in raw crops treated after harvest and the level of residue found in the processed food made from these crops.
As we have most diversification in Deltamethrine applications in France, it is most likely that if TMDI exceeds the maximum safe limit, it is in the country where the product has its most important and intensive uses.

The following Fig.12 summarizes the official decisions concerning ADI and LMR by the French Ministry of Agriculture.
On fig.13, we listed the average individual portion per day (Q) (OCDE source 1982) eaten by the french population in 1982.
Let us now calculate TMDI by multiplying Q x MRL and making the sum of all the intakes.

As you can see, the average food portion in France per head and per day is 1.7 kg and in this portion, deltamethrin can represent maximum 0.41 mg which is 70 % of maximum safe limit. As we already explained this is exaggerated for the reasons mentionned before in this lecture.

So normally as TMDI in this case is inferior to maximum safe limit, we have no more approach to perform and can conclude to a complete safety for the consumer.

However to further illustrate this lecture we made the second approach by calculating EMDI taking into account Maximum Residue Levels in edible portions of the food and reduction factors to prepare and process the food (Fig.13).

So again on the same french diet, we calculate the sum of the estimated maximum intakes and we come to the conclusion that only 29 % of the ADI is consumed which if 2.5 x less than TMDI but remember that EMDI still overestimated for the reasons we repeat on the following Fig.14.

When we take into account only real (= average) residue levels in food, market share of Deltamethrin in France and the ratio home grown/import (abroad less diversification in crops) we can calculate EDI and at that moment, we come only to about 10 % of the EMDI or 0.017 mg Deltamethrin/day what is only about 3 % of the quantity considered as safe (Fig.14).

When we are looking to the different applications of Deltamethrin in France and the important market share of the product in France, we can conclude that if in this case the EDI is only 3 % of the maximum quantity considered as safe, complete safety for the consumer eating food containing Deltamethrin residues is worldwide guaranteed.

Conclusion

Prenant comme exemple la deltaméthrine en France, le calcul de l'IMJT (Ingestion Maximale Journalière Théorique), de l'IMJE (Ingestion Maximale Journalière Estimée) et de l'IJE (Ingestion Journalière Estimée) permet de s'assurer que l'IJE est bien inférieure à la DJA (Dose Journalière Acceptable).
Ceci confirme que les résidus de deltaméthrine dans les aliments n'entraînent aucun risque pour la santé de la population française.

Bien entendu, et selon la même approche, les autres populations sont tout aussi en sécurité suite aux usages autorisés dans le monde avec cet insecticide.

References

WHO/EME/FOS/88.2 Guidelines for predicting dietary intake of pesticide residues - Geneva, World Health Organisation, 1988

FAO Pesticide Residues in Food (several years) - Reports of the Joint Meeting of the FAO Panel of experts on Pesticide residues in food and the Environment and the WHO Expert Group on Pesticde Residues - Roma, Food and Agriculture Organisation of the United Nations.

Codex limits for pesticide residues in food and consumer safety -Discussion paper by the Ad Hoc Working Group on Regulatory Principles, Codex Committee on Pesticide Residues (CS/PRT 86/12, February 1986)

OCDE - Statistiques de la consommation des denrées alimentaires : Situation fin 1982 - Paris 1985

Roussel Uclaf - Deltamethrin Monograph - 1982

NATIONAL OR INTERNATIONAL EXPERTS
IN TOXICOLOGY, CHEMISTRY AND OECOLOGY
ESTABLISH FOR EACH PESTICIDE :

N.O.A.E.L.

$$A.D.I. = \frac{N.O.A.E.L.}{\text{Safety Factor}} \qquad \text{Safety Factor} > 100$$

$$M.R.L.\ \text{for one crop} = \left(\frac{N.O.A.E.L.}{\text{Safety Factor}} : 0.4\ \text{kg} \right) \times B.W.$$

Figure 1 : NOAEL, ADI and MRL

(increasing realistic approach ↑)

Step IV ► Measured Pesticide Residue Intake ► MPRI
Direct study of real residues in the plate

EDI > ADI x BW

Step III ► Estimated Daily Intake ► EDI = known uses
known residues
ration local/import

EMDI > ADI x BW

Step II ► Estimated Maximum Daily Intake ► $EMDI = \Sigma\ Fi \times Ri \times Pi \times Ci$

TMDI > ADI x BW

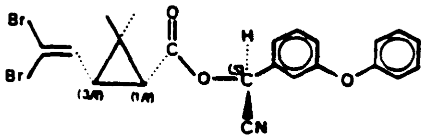
Step I ► Crude estimate Theoretical Maximum Daily Intake ► $TMDI = \Sigma\ Fi \times Mi$

Figure 2 : Different approaches to evaluate the safety of the consumer towards pesticide residues in food.

FIGURE 3-

DELTAMETHRIN

PHYSICO-CHEMICAL CHARACTERISTICS :



$C_{22}H_{19}Br_2NO_3$ = 505.2

CHEMICAL NAME :

(1 R,3R)-3(2,2-DIBROMOVINYL)-2,2-DIMETHYLCYCLOPROPANE CARBOXYLATE OF (S)-ALPHA-CYANO-3-PHENOXYBENZYLE

ASPECT : WHITE CRYSTALLINE POWDER

PURITY : > 98 %

VAPOUR PRESSURE : 3×10^{-10} MMHG AT 25°C

MELTING POINT : 101 - 102°C

ROTATION ANGLE : $[\alpha]_D^{20}$ = + 58° (± 1°) IN 4 % TOLUENE

SOLUBILITY :

LESS 1%	- ISOPROPANOL, GLYCEROL, ETHYLENEGLYCOL, PEG 200
1 - 10 %	- ETHANOL, CYCLOHEXANE, ACETONITRILE
10 - 50 %	- BENZENE, TOLUENE, XYLENE, TRICHLOROETHANE, ETHYLACETATE, DMSO, ACETONE
MORE THAN 50 %	- DMF, CYCLOHEXANONE, DIOXANE ...
IN WATER	< 0,1 PPM AT 20°C

FIGURE 4 -

LACK OF ADVERSE TOXIC EFFECTS

- Studies conducted or sponsored by Roussel-Uclaf
- Studies conducted by independant organisms or by Research Centers (IARC in Lyon at the request of WHO)

NOT MUTAGENIC : more than 10 tests (France, Netherlands, Italy, IARC, Bulgaria, etc...)
both in vitro or in vivo ⟶ all negative

NOT TERATOGENIC nor EMBRYOTOXIC : on rats, mice, rabbits (USA, Kavlock)

NO EFFECTS ON REPRODUCTION : 3-generation study on rats (USA)

LACK OF NEUROTOXICITY :
- Special test on hens (UK)
- Special examination on dogs after 2 years feeding study
  no lesion on central or peripheric nervous system

NOT CANCEROGENIC : 2-year studies on rats and mice (USA and IARC)

FIGURE 5 -

LONG TERM TOXICITY STUDIES - N.E.L.

COMBINED STUDIES CHRONIC + CARCINOGENICITY STUDIES / IARC + IRDC

2 YEARS RAT AND MICE

CHRONIC STUDY

2 YEARS DOG

⇓

N.E.L. ESTIMATED BY 1982 FAO / WHO JMPR

AND NATIONAL REGULATORY BODIES

1 MG/KG/BODYWEIGHT

ON DOG (MOST SENSIBLE)

SAFETY COEFFICIENT : 100 (CLEAN COMPOUND IN SPECIAL TOXICOLOGICAL STUDIES)

A D I = 0,01 MG/KG/BW

OR FOR A 60 KG B.W. PERSON : 0.6 MG/DAY

MAX MRL ON CROP = 0,01 MG/KG X 60 KG X $\frac{1000}{400\text{ G.}}$

MAX M R L = 1,5 MG/KG

FIGURE 6

RESIDUE LEVELS ON FIELD CROPS

MRL RESPECTING G.A.P.			CODEX MRL	
GRAINS		STRAW	GRAINS	STRAW
CEREALS :				
. WHEAT	⩽ 0.03	⩽ 0.03	) 1 (INCLUDING POST-HARVEST)	0.5 (C X L)
. BARLEY	⩽ 0.05	⩽ 0.1	)	
. OAT	N.D.	⩽ 0.5	)	
. CORN	< 0.05		)	
. RICE	⩽ 0.02		)	
. SORGHUM	< 0.05		)	
COTON	⩽ 0.02		0.1 (C X L)	
SOYBEAN	N.D.	⩽ 0.4	0.1 (C X L)	0.5
SUNFLOWER	⩽ 0.02		0.1 (C X L)	
RAPE	⩽ 0.05	N.D.	0.1 (C X L)	
SUGARBEET ROOTS	N.D.	< 0.1 (LEAF + TOP)	0.01 (C X L)	
POTATOES	N.D.		0.01 (C X L)	
TEA	⩽ 4		10 (C X L)	
TOBACCO	⩽ 3		-	

ON THESE CROPS LAST SPRAY (ACCORDING G.A.P.) IS NEVER CLOSE TO HARVEST.

FIGURE 7

RESIDUE LEVELS ON VEGETABLES

	MRL RESPECTING GAP	MRL CODEX	SITUATION IN EUROPE	
			MRL	WAITING PERIOD
• ROOT VEGETABLES	< 0.001	⩽ 0.01 (C X L)		
• BULB VEGETABLES	0.05	⩽ 0.1 (C X L)		
• FRUIT VEGETABLES :				
. EDIBLE PEEL (TOMATO)	< 0.05	⩽ 0.2 (8)	0.05 - 0.5	MOST BETWEEN
. NON EDIBLE PEEL (MELON)		⩽ 0.01 (C X L)	0.05 - 0.5	0 - 3 DAYS SOMETIMES 7 DAYS
• LEGUME VEGETABLES :				
. WHOLE	< 0.05	) ⩽ 0.1 (C X L)	0.05 - 0.5	
. BEANS	< 0.001	)		
• LEAF VEGETABLES :				
. LETTUCE	⩽ 0.1	) ⩽ 0.5 (8)	0.05 - 0.5	
. SPINACH	⩽ 0.5	)		
• CABBAGE	< 0.2	⩽ 0.2 (5)	0.2 - 0.5	

FIGURE 8

RESIDUE LEVELS ON FRUIT AND GRAPES IN PPM

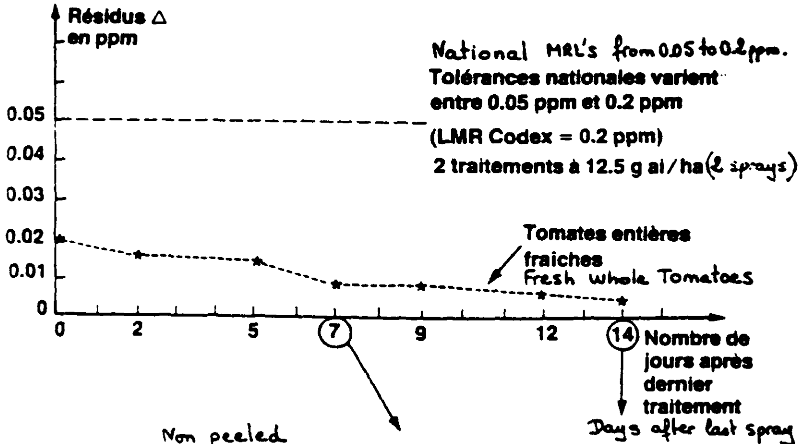
MAXIMUM RESIDUE LEVELS RESPECTING G.A.P.			CODEX MRL's	SITUATION IN EUROPE	
				MRL	WAITING PERIOD
FRUIT TREES	APPLES	≤ 0.07	0.1 (C X L)	0.1 - 0.5	0 - 7 DAYS
	PEARS	≤ 0.03			
	APRICOTS	≤ 0.03	0.05 (C X L)	0.05 - 0.5	0 - 7 DAYS SOMETIMES 14 D.
	CHERRIES	≤ 0.05			
	PEACHES	≤ 0.05			
	PLUMS	≤ 0.05			
VINE (GRAPES)		≤ 0.05	0.05 (C X L)	0.05 - 0.5	0 - 3 DAYS OR SEVERAL WEEKS LINKED WITH LAST SPRAY ON BERRYMOTHS

FIGURE 9

- INCIDENCE OF INDUSTRIAL PROCESSING ON Δ RESIDUES FOR CANNED TOMATOES / FRANCE -

INCIDENCE DE TRANSFORMATION INDUSTRIELLE SUR TOMATES DE CONSERVERIE

(Institut Technique Puyricard-France)

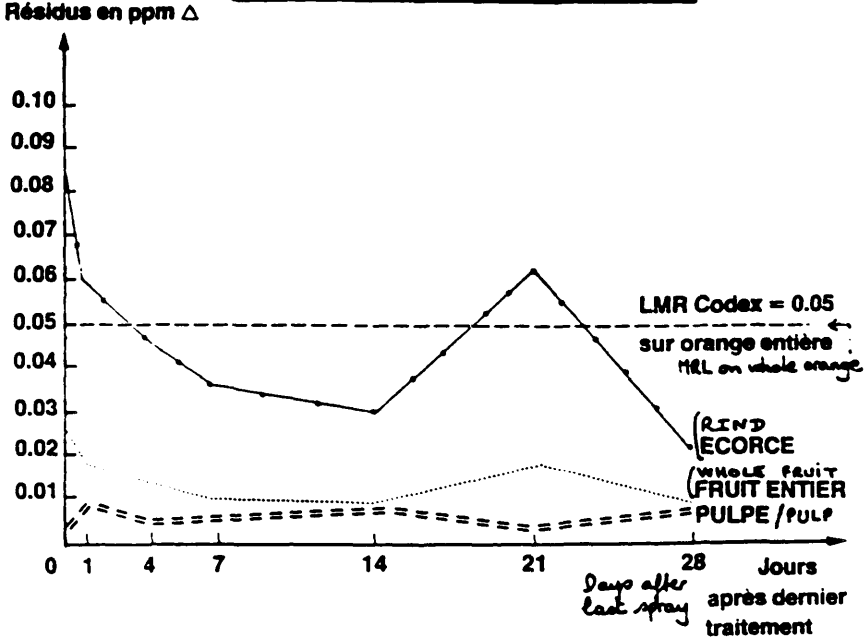


		7	14
FRUITS FRAIS / FRESH TOMATOES	- NON PELEES (Non peeled)	0.008 ppm	0.004 ppm
	- id + RINCAGE eau froide (do + Rincing (cold water))	0.008	0.003
	- PULPE / Pulp	<0.002	<0.002
PRODUITS / PROCESSED PRODUCTS	Concentré à 14 % (Concentrate (14%))	0.003	0.002
	Marc/pelage à chaud (Pomace/hot proc.)	0.049	0.043
	Eau de rinçage (Rincing water.)	0.0019	0.0010

FIGURE 10

EXEMPLE DE LOCALISATION DES RESIDUS DANS L'ECORCE D'ORANGES (essai MAROC - 1978)

EXAMPLE OF LOCALIZATION OF RESIDUES IN ORANGE RIND (MOROCCO/1978)



Dose massive : 50 ml DECIS CE 25 g/l par hl pour 6600 l/ha) = 82,5 g ma/ha

Huge dosage (experimental) corresponding to 82.5 g ai/ha.

FIGURE 11

MAXIMUM LEVEL OF RESIDUES OF DELTAMETHRIN

AFTER 6-MONGH STORAGE

COUNTRIES WITH TEMPERATE CLIMATE : MAXIMUM RATE OF USE = 0.5 PPM

ON RAW OR INTERMEDIATE PRODUCT (MG/KG)		ON THE PRODUCT AS EATEN (MG/KG)	
WHEAT WHITE FLOUR	< 0.04	WHITE BREAD	< 0.02
HUSKED RICE	< 0.01	COOKED RICE	N.D.
BARLEY MALT	< 0.01	BEER	N.D.
SOYBEAN OILCAKE	< 0.02	COOKED MEAT	N.D.
PEAS / CHICK PEAS	< 0.4	COOKED PEAS	< 0.05
LENTILS	< 0.3	COOKED LENTILS	< 0.05
BEANS	< 0.3	COOKED BEANS	< 0.05
COFFEE BEANS, UNROASTED	< 0.3	COFFEE BEVERAGE	N.D.
COCOA BEANS, UNROASTED	< 0.15	CHOCOLATE	< 0.01

FIGURE 12: **For France : Deltamethrin decisions from the authorities were :**

ADI = 0.01 mg/kg

- **Max. Daily Intake for a person of 60 kg : 0.6 mg/kg**

LMR were fixed on :

- ► **fruits and vegetables** 0.2 mg/kg (except salad 0.5 mg/kg)
- ► **cereals** 1 mg/kg
- ► **white flour** 0.3 mg/kg
- ► **dried beans** 1 mg/kg
- ► **wheat bran** 3 mg/kg
- ► **green coffee beans** 2 mg/kg
- ► **eggs** (0.01 mg/kg) (*)
- ► **meat** (0.015 mg/kg) (*)
- ► **offals** estimated 0.2 mg/kg
- ► dairy products (0.06 mg/kg) (*)
- ► butter (0.5 mg/kg) (*)
- ► vegetable oil (0.2 mg/kg) (*)
- ► potatoes (0.01 mg/Kg) (*)

(*) propositions

FIGURE 13: **% of ADI consumed taking into account possible applications of DELTAMETHRIN.**

	Quantity in kg/day	MRL in mg/kg	TMDI in mg/day	Reduction factors	Quantity in kg/day	MRL in mg/kg	EMDI in mg/day
cereals	0.2	1	0.2	as bread noodles bran	0.13 0.05 0.02	0.02 0.01 1.00	0.002 0 0.02
potatoes, starch products	0.215	0.2	0.043	peeling, cooking	0.215	0.01	0.002
dried beans	0.003	1	0.003	cooking	0.003	0.05	0
vegetables + tomatoes	0.34	0.2	0.068	as such	0.34	0.2	0.068
fruits + berries	0.15	0.2	0.03	as such	0.150	0.2	0.03
citrus	0.055	0.2	0.011	peeling	0.055	0.05	0.003
meat (beaf, veal, porc, mutton)	0.205	0.02	0.004	cooking	0.205	0.02	0.004
meat (poultry)	0.045	0.015	0.001	cooking	0.045	0.01	0
offals (liver, kidney)	0.03	0.2	0.006	cooking	0.03	0.05	0
eggs	0.042	0.01	0	cooking	0.042	0.01	0
dairy products	0.393	0.06	0.024	as such	0.393	0.06	0.024
butter	0.022	0.5	0.011	as such	0.022	0.5	0.011
vegetable oil	0.045	0.2	0.009	cooking	0.045	0.2	0.009
	Σ= 1.70 kg		Σ= 0.41 = 70 % ADI x Bw				Σ= 0.173 = 29 % ADI x Bw

Source France (consumption in kg/day/person) OCDE 1982

FIGURE 14 :

REAL DAILY INTAKE

Overestimation with EMDI

* because supposing all cereals sprayed with Deltamethrin (local + import)
* all these food cannot be eaten by the same person in one day
* all the food contains the max. of Deltamethrin

Looking to EDI and so taking into account:

* average residue levels in the food
* market share
* ratio home grown/import

only 10 % of EMDI what means 0.017 mg Deltamethrin/day

or ≈3 % of ADI x b.w.

TRATAMIENTOS DE POS-COSECHA DE FRUTOS DE PEPITA

J. García De Otazo López
Servicio de Protección de los Vegetales-Lérida, Spain

RESUMEN

Se estudia la problemática planteada durante la conservación frigorífica de peras y manzanas por las enfermedades y alteraciones fisiológicas.
En lo referente a las alteraciones fisiológicas, el escaldado (Scald) es la fisiopatía contra la que se puede luchar preventivamente mediante el tratamiento con antioxidantes y también con las nuevas técnicas de Atmósfera controlada con reducción de etileno. Contra el Bitter-pit sólo el Cloruro de cal da resultado, aunque parcialmente satisfactorio.
En la prevención de las enfermedades de conservación se debe empezar por una buena desinfección de cámaras y envases, seguido por los tratamientos de post-cosecha.
Los fungicidas autorizados en post-cosecha son un número reducido, lo que viene agravado por la aparición de cepas resistentes de las enfermedades más importantes y por el hecho de las diferentes tolerancias en cuanto a residuos en diferentes paises.

1. SITUACION ACTUAL DE POST-COSECHA DE FRUTOS DE PEPITA

La provincia de Lérida es el área más importante de España en cuanto a la producción de pera y manzana, ya que en ella se concentra el 33% de la producción total española de manzanas y el 39% de la producción total de pera.
La distribución varietal por especies es la siguiente,
En manzanas: Golden Delicious 42'1%; Starking Delicious 36%; Belleza de Roma 9'3%; Varias 12'6%. La variedad más importante es la Golden Delicious, que ha ido aumentando con los años y no creemos que, a corto plazo, se invierta la tendencia.
En peras: Limonera o Dr. Jules Guyot 36'9%; Blanquilla 32'8%; Ercolini 4'9%; Otras 25'4%. En el grupo de otras se incluyen variedades como Buena Luisa, Passa Crassana, Williams, For de Invierno, Devoe, Max Red Barlett, etc.
Las producciones de peras y manzanas de mesa en los tres últimos años y las previsiones de cosecha para 1988 (en miles de Tm.) son las siguientes,

	1985	1986	1987	1988 (prev. de Julio)
Peras				
Lérida	239	111	221	217
Aragón	120	97	100	-
España	594	361	519	-
Manzanas				
Lérida	389	278	319	321
Aragón	129	151	140	-
España	988	828	971	-

(Fuentes: DARP y MAPA)

2. CAPACIDAD ACTUAL DE CONSERVACION

Actualmente existen en la provincia de Lérida 299 instalaciones frigoríficas censadas, con una capacidad total de - - - - 1.780.446 m^3, que se distribuyen de la siguiente manera,

Atmósfera normal	809.717 m3	45'48%
Atmósfera controlada	970.729 m3	54'52%
Total ...	1.780.446 m3	100 %

Se ve que la atmósfera controlada es mayoritaria en su participación en el total del frío, aunque en conjunto creemos que las dos orientaciones están equilibradas.

3. PRINCIPALES PATOGENOS CAUSANTES DE PODREDUMBRES DURANTE LA CONSERVACION FRIGORIFICA

Durante la conservación frigorífica de peras y manzanas se - producen mermas atribuibles a diferentes causas que podemos - agrupar en tres apartados,

a) Pérdida de peso por deshidratación:
Esta pérdida varía entre un 2 y un 8%.

b) Pérdida por diferentes enfermedades:
En fruta tratada oscila entre un 0'25 y un 3%.

c) Pérdida debida a fisiopatías:
En conjunto, estas pérdidas suponen entre un 2 y un 3% globalmente.

Dejando aparte las pérdidas de peso por deshidratación que se deben evitar al máximo con una correcta manipulación de la - fruta y de las condiciones de conservación, vamos a entrar en el segundo apartado.

PALAZON & COLS (1984) establecieron una lista de agentes patógenos encontrados entre 1971 y 1984 en cámaras frigoríficas - del Valle medio del Ebro. La lista es la siguiente:

Tab. 1.- Inventario e importancia de especies criptogámicas causantes de podredumbres sobre la fruta de pepita de cámara en el Valle medio del Ebro. PALAZON & RODRIGUEZ (1977-84)

ESPECIE	MANZANO	PERAL
1. Alternaria chartarum Preuss.	+	+
2. Alternaria tenuis Nees.	+ +	+ +
3. Botrytis cinerea Pers.	+	+
4. Cladosporium herbarum Ell. y Arth.	+	+ +
5. Coniothyrium sp.		+
6. Fusarium lateritium Ness.		+
7. Fusarium roseum var. avenaceum (Sacc.) Sn.y H		+
8. Fusiclaudium dentriticum Fuck.	+ +	
9. Fusiclaudium virecens (Lib.) Fuck.		+
10. Gloeosporium album Osterw.	+ +	
11. Gloeosporium perennans* Zell y Childs.		+
12. Monilia fructigena Pers.	+	+
13. Monilia laxa Enrenb.	+	+
14. Penicillium expansum Link.	+ + +	+ + +
15. Peyrenollaea glomerata (Corda) Goid.		+
16. Phoma sp. *	+	+
17. Phytophthora cactorum Schröet.	+	
18. Rhizopus nigricans Ehrh.	+	+
19. Sphaeropsis malorum Peck.		+
20. Stemphylium botryosum Wallr.	+	+
21. Trichothecium roseum Link.	+	

(*) = nueva cita ++ = importancia media o variable
+++ = muy importante + = poco importante

La relación podría ser alargada hasta la cifra de 41 especies, pero muchos de los patógenos encontrados lo son con caracter - prácticamente anecdótico.

Las principales especies patógenas se pueden agrupar por su importancia en cuanto a las pérdidas por podredumbre que provocan; así, 14 especies agrupan en conjunto el 99% de las pérdidas, y de éstas hay una, el P. expansum que supone por si sola el 70-80% de las pérdidas. El P. expansum es pues un patógeno - constante en el tiempo y causante de daños graves.

El segundo grupo en importancia está constituido por patógenos generalizados pero de aparición irregular; aquí, se incluirán los siguientes: Alternaria tenuis, Rhizopus nigricans, Gloeosporium album, Monilia sp. . Este grupo de patógenos es responsable en conjunto del 10-15% de las pérdidas por podredumbres, y es de destacar el incremento que está tomando últimamente el Rhizopus .

En el tercer y último grupo se incluyen los patógenos de apari ción esporádica que, en determinadas circunstancias, pueden provocar daños graves. En conjunto este grupo es el responsable del 3-5% de las pérdidas. Aquí, se incluirían las siguientes especies: Phytophthora cactorum, Phytophthora syringae, Trichothecium roseum, Stemphylium botryosum, Fusiclaudium pirinum, Spilocaea pomi .

4. LAS ALTERACIONES FISIOLOGICAS O FISIOPATIAS DURANTE LA CONSERVACION FRIGORIFICA

Las fisiopatías o alteraciones fisiológicas de caracter no parasitario que aparecen durante la conservación frigorífica de pera y manzana son muy variadas, y, en la mayoría de los casos, su aparición e importancia está directamente relacionada con la interacción de diversos factores de campo (abonado, riegos, estado de madurez, etc.) y de cámara, por lo que en muchos casos es extraordinariamente difícil establecer la causística que ha provocado su aparición.

Las principales alteraciones fisiológicas que se pueden observar en manzana son las siguientes,

Tab. 2.- Principales fisiopatías en manzana.

CASTELLANO	INGLES
Escaldado	(Scald)
Escaldado blando	(Soft Scald)
Manga amarga	(Bitter Pit)
Plara	(Leutecil bloteh pit)
Vitrescencia (Corazón vidrioso)	(Water core)
Congelación	(Freezing injury)
Enfermedad del frío	(Low temperature breakdown)
Textura harinosa	(Mealiness)
Mancha lenticelar	(Red spots)
Corazón marrón	(Brown core)
Corazón rosado	(Core flush)
Golpe de sol	(Sunscald)
Mancha Jonathan	(Jonathan spot)
Carencia de boro (acorchado)	(Cork, Boro deficiency)

Tab. 3.- Principales fisiopatías en pera.

CASTELLANO	INGLES
Escaldado	(Scald)
Congelación	(Freezing injury)
Corazón pardo	(Pithy brown core)
Descomposición interna	(Core breakdown-Internal breakdown)
Oscurecimiento interno de la Passa Crassana	
Daños por amoniaco. Mancha amarga	(Bitter pit)

5. MEDIOS DE LUCHA CONTRA LAS FISIOPATIAS

Las únicas alteraciones fisiológicas que tienen un trtamiento preventivo, tanto en pera como en manzana, son el Escaldado , mediante los tratamientos de post-recolección con antioxidantes y el bitter pit, mediante cloruro cálcico. El resto de las fisiopatías vienen condicionadas por los factores de campo (cultivo y recolección, siendo muy importante el estado de madurez de los frutos) y los de cámara, sobre todo en atmósfera controlada, y a que los niveles de O_2 y CO_2 y temperatura sean los adecuados para la variedad en cuestión.

De los factores de cámara, hoy en día, se tiene claro que es importante la reducción del etileno generado por los propios frutos o de otra procedencia, aunque la baja temperatura y la atmósfera controlada con concentración de CO_2 alta limitan la síntesis de etileno y por tanto de sus efectos.

La acumulación de etileno en las cámaras frigoríficas puede favorecer la aparición de las siguientes alteraciones fisiológicas: escaldado superficial, alteraciones de la senescencia, manchas en la piel, corazón pardo en pera y pulpa harinosa.

En la Tab. 4, se da la capacidad de producción de etileno por parte de diferentes variedades de pera y manzana.

Tab. 4.- Capacidad máxima de producción de etileno por diversos vegetales recolectados (según RYALL et al.,1979; BIALE et al., 1981; ABELES, 1973)

FRUTO	T. ºC.	PRODUCCION ETILENO ml/kg
MANZANA:		
- Golden Delicious	25 º	35
- Cox's Orange	3 º	41
PERA:		
- Passa Crassana	25 º	8
- Anjou	18 º	30
- Williams	20 º	222

Actualmente diversos trabajos experimentales han demostrado que la reducción del etileno de las cámaras de conservación aporta ventajas, tanto de cara a la prevención de las fisiopatías antes citadas, como a la calidad de los frutos.

Así GRAELL et al. (1987) demostró que mediante la instalación

de un sistema de eliminación catalítica de etileno, en una cámara de atmósfera controlada donde se almacenaron manzanas Starking Delicious tratadas en campo con daminocida (Alar); al cabo de 8 meses de conservación, durante los cuales se mantuvo el nivel de etileno por debajo de 5-10 ppm, mostraban una diferencia de penetromía de 0'9 - 1'5 kg. con los frutos conservados en otra cámara de atmósfera controlada en la que no se eliminó el etileno.
Otras experiencias anteriores son las de LIU (1980 con manzanas McIntosh tratadas con daminozida, que mostraban mayor índice de penetromía si se conservaban en una atmósfera con nivel bajo de etileno (1 ppm), frente al testigo conservado en una atmósfera con nivel alto (10-100 ppm), y las realizadas en el East Mallins Research Institute donde se observó que la reducción del etileno aumentaba la penetromía de la Bramley's Seedling y, lo que es más importante, reducía la incidencia del escaldado superficial, incluso en frutas no tratadas con DPA o etoxiquina, lo que desde el punto de vista legal (residuos) tiene un gran interés, ya que la DPA no está autorizada en algunos paises. DOVER (1985) también obtuvo un buen control del escaldado en manzanas sin usar antioxidantes, manteniendo bajos los niveles de etileno en las cámaras frigoríficas.
De todos los sistemas de eliminación del etileno:
Renovación del aire de las cámaras, oxidación con permanganato potásico, oxidación con ozono y radiaciones UV y oxidación catalítica (sistema convencional o sistema "swing-therm"), es éste último el más interesante para la conservación de fruta de pepita, habiendo dado lugar al desarrollo de la técnica de Atmósfera controlada con Reducción de Etileno, que además contempla el tratamiento en campo con productos inhibidores de la biosíntesis del etileno como es la daminozida y la recolección del fruto en un estado de preclimaterio.

5.1. El Escaldado

Del escaldado se pueden distinguir hasta cuatro tipos, tres se producen durante la conservación frigorífica y son: Escaldado común (Scald), Escaldado blando (Soft Scald) y Escaldado de senescencia.

El cuarto tipo de escaldado es del tipo blando y se produce en el campo, provocado por factores como son el exceso de vigor y nitrógeno entre otros.

Las causas que provocan el Escaldado común durante la conservación frigorífica son básicamente la emisión por parte del fruto de sustancias volátiles que se acumulan en la piel, hasta alcanzar niveles tóxicos. Así, la oxidación del α-farnaseno, se cree que produce sustancias tóxicas que causan la muerte de las células epidérmicas. El Escaldado de senescencia se provoca por la misma causa, pero en este caso se asocia con frutos que han rebasado un límite de conservación.

El Escaldado blando es provocado por temperaturas próximas al punto de congelación de la variedad en cuestión y se ve favorecido por un exceso de madurez de los frutos, entre otros factores.

Tratamientos del Escaldado

La prevención del escaldado se hace con antioxidante, mediante los tratamientos de post-recolección con baño o ducha (drencher).

Actualmente en España están autorizados: la difenilamina (DPA) y la etoxiquina.

La DPA tiene como ventajas, frente a la etoxiquina, una eficacia ligeramente mayor en el control del escaldado, una mayor facilidad de uso en razón a su tipo de formulación líquida, frente a la formulación pastosa de la etoxiquina. El hecho de tener un ligero efecto fungicida y el tener en general LMRS permitidos superiores a los de la etoxiquina, como puede verse en la Tab. 5.

Tab. 5.- Niveles máximos de residuos autorizados (ppm)

PAIS	D P A	ETOXIQUINA
Bélgica	3	No permitida
Dinamarca	5	--
Irlanda	pendiente	3
Finlandia	5	--
Francia	3	3
Alemania	3	No permitida
Holanda	3	No permitida
Italia	3	3 (?) 1'5
Gran Bretaña	3	3
EE.UU.	5	3

Canadá	5	3
España	5	3
Codex	5	3

Como inconvenientes la DPA tiene la posible fitotoxicidad sobre la variedad Golden D., en la que puede causar necrosis lenticelares, así como el bloqueo del color verde en variedades amarillas (en la Golden Delicious, por ejemplo).
Otro problema que presenta la DPA es su toxicidad para la fauna acuicola, con una LD-50 de 10 ppb, por lo que hay que extremar las precauciones con los vertidos para no provocar problemas ecológicos.
Las dosis recomendadas para cada uno de los productos se recogen en la Tab. nº 6.

Tab. 6.- Dosis recomendadas de DPA y etoxiquina en peras y manzanas.

VARIEDAD	DPA (ppm)	ETOXIQUINA (ppm)
Manzanas :		
Golden Delicious	500 - 600	900 - 1200
Granny Smith	2000	2500
Belleza de Roma	2000	1000 - 1900
Stayman	2000	1600 - 1900
Red Delicious y otras rojas	2000	2500
Starking D.	2000	2500
Reineta	700	1500 - 1800
Verde Doncella	900	1500 - 1800
Idared	1500	-
Mutsu	2000	-
Peras :		
Blanquilla	1200 - 1400	1900 - 2500
Packam's Triumph	1100	-
Resto de peras	900 - 1200	200 - 2500

5.2. La mancha amarga o bitter-pit

La lucha más eficaz contra la mancha amarga o bitter-pit es la que se basa en medidas culturales, agronómicas y de manejo de la fruta. El tratamiento de post-cosecha es la última oportunidad para tratar la fruta a fin de prevenir la aparición del biterr-pit, para ello se recomienda el cloruro cálcico a la dosis de 1'5 al 2%. En general, no hay problema de mezcla de este producto con los autorizados en post-cosecha, si no se sobrepasa la dosis del 2%. Este tipo de tratamientos sólo tienen resultados parcialmente satisfactorios, si bien por sus efectos colaterales sobre la evolución y senescencia del fruto y sus bajos costes se recomienda.

6. MEDIOS DE LUCHA CONTRA LAS ENFERMEDADES

La fruta que va a ser conservada en cámara frigorífica puede sufrir contaminación por hongos en el campo o en la misma central frutícola. Contra la contaminación de campo, los tratamientos de precosecha suelen ser de gran eficacia, recomendándose una o dos aplicaciones, la última respetando el plazo de seguridad del fungicida empleado.

Las contaminaciones que se producen en la central provienen del inoculo que se ha conservado en los envases (palots), paredes, suelo y en el mismo aire. Para evitar la contaminación, en lo posible, y que al mismo tiempo los tratamientos de postcosecha tengan la máxima eficacia, se recomienda en primer lugar la desinfección previa de cámaras y embalajes.

6.1. Desinfección de cámaras y embalajes

Los tratamientos de desinfección de cámaras y embalajes mediante diversos productos, disminuyen significativamente la cantidad de inoculo presente. Este hecho, demostrado y corroborado por diversos autores, hace que hoy día se recomiende de una manera generalizada esta desinfección previa al inicio de cada campaña frutera.

La desinfección de envases (palots y cajas) que ya hayan sido usados en la anterior campaña se puede llevar a cabo con pistola o ducha o por inmersión (baño).

Los productos que se recomiendan son los siguientes: Hipoclorito sódico del 3 al 5%. Ortofenilfenato sódico a la dosis del 4 al 5'5% (8000 ppm).

Para la desinfección de cámaras se puede emplear lechada de cal viva, a la cual se añade un 5% de hipoclorito sódico. Este producto resulta interesante en cámaras que por su revestimiento permitan este tipo de aplicación. Además tiene interés para el tratamiento de los locales anexos a las cámaras frigoríficas.

Fungicidas gaseosos. Los más aconsejables son:

Formol.- La manera más práctica de emplearlo es en depósitos metálicos u hornillos donde se hace evaporar el producto. La dosis recomendada es de unos 20 cc. de producto comercial del 40% por m^3. Una vez aplicado el producto, las cámaras tienen que permanecer cerradas unas 48 horas, transcurridas las cuales se ventilarán, dado que el producto es lacrimógeno y fitotóxico.

Mezcla de Fenol y Formol.- Se aplica mediante microdifusor electrotérmico. Este tipo de productos tiene la ventaja de poder ventilar las cámaras a las 8-12 horas y entrar a trabajar.

Driol (Para-dicloro-dihidroxisalialamida).- Se aplica a la dosis de 0'6 g/m^3, debiendo parar un mínimo de 4 horas antes de abrir las cámaras. Este producto actua por contacto sobre la espora de los hongos.

El formol y la mezcla de fenoles y formol se pueden emplear para la desinfección simultánea de cámaras y embalajes vacios, pero la dosis a emplear será entre un 50 y un 100% superior, ya que habrá una mayor superficie a desinfectar.

Para la desinfección de la maquinaria (clasificadoras) se pueden emplear los detergentes alcalinos, sosa o hipoclorito sódico, pero lo más práctico es usar los amonios cuaternarios que no plantean problemas de corrosión, como los derivados del cloro y los detergentes alcalinos.

6.2. Tratamientos de post-cosecha contra las enfermedades de conservación

Los tratamientos con fungicidas se aconseja realizarlos:

a) Cuando la futa se conserve un periodo de tiempo superior a los 4-5 meses.
b) En las variedades de pera y manzana destinadas a larga conservación y sensibles al escaldado, ya que el baño o ducha es una fuente de contaminación.

Actualmente en España están autorizados expresamente los siguientes productos para los tratamientos de post-recolección: Imazalil; Imazalil + Iprodine; Ortofenilfenol; Tiabendazol; Tiabendazol + Folpet (este producto está autorizado en plan experimental para la campaña de 1988, sólo en la provincia de Lérida).

Tab. 7.- Eficacia teórica de los productos recomendados contra las enfermedades de conservación

PRODUCTOS	Alternaria tenuis	Botrytis cinerea	Gloeosporium sp	Monilia sp	Penicillium expansum	Rhizopus nigricans
Benomilo	---	+++ (SNR)	+++	++	+++ (SNR)	---
Captan	+	+	+	+	+	+
Carbendazim	---	+++ (SNR)	+++	++	+++ (SNR)	---
Diclofuanida	+	++	++	+	++	---
Dicloran	---	++		++	---	++
Folpet	---	++	+	+	+	+
Imazalil (PC)	++	+	+	++	+++	---
Imazalil + Iprodione (PC)	++	+++	+	++	+++	---
Metil-tiofanato	---	+++ (SNR)	+++	++	+++ (SNR)	---
Ortofenilfenol (PC)	++	++	---	---	++	++
Tiabendazol (PC)	---	+++ (SNR)	+++	++	+++ (SNR)	---
Tiabendazol + Folpet (PC)	---	+++	+++	++	+++	+
Ziram	+	+	---	+	+	+

+++ = Eficacia buena
++ = Eficacia medianamente buena
\+ = Poca eficacia

--- = Sin eficacia
(SNR)= Cepas no resistentes
(PC) = Productos que se pueden utilizar en post-cosecha

Vemos pues que el primer problema que se plantea es la existencia de una gama de productos muy pequeña para aplicar en los tratamientos de post-cosecha que junto con la aparición de cepas no resistentes sobre todo de Penicillium agrava el problema.

Los LMRs admitidos en diferentes paises se dan en la siguiente Tab. nº 8.

Tab. 8.- LMRs admitidos en diferentes paises

	TBZ	IMZ	IPRODIONE	BENOMILO	FOLPET+CAPTAN (*)
Alemania	3	-	-	2	3
Bélgica	2	5	5	2	3
Dinamarca	10	2	-	-	3
España	3	-	-	5	3
FAO/OMS	10	5	10	5	15+15
Finlandia	3	2	10	0'2	-
Francia	6	-	10	6	3
Gran Bretaña	-	-	10	-	3
Holanda	6	5	-	3	3
Italia	3	-	5	1	3

(*) Directiva del Consejo de las Comunidades Europeas de 16-05-88

Se entiende por Residuo de un plaguicida, los restos del producto, de sus impurezas y de sus productos de metabolización o degradación, puestos en el producto vegetal. Ahora bien, - hay que reseñar que en ninguna legislación al definir los residuos, se hace referencia al origen potencial del residuo, - dado que analíticamente no puede determinarse ese origen, ni tiene valor alguno para los consumidores. Lo único importante en la cuestión de residuos es sí hay o no, y en este último - caso si se supera el LMR establecido.
A este respecto en 1982-83, se llevó a cabo por BARELLES,F. y GARCIA DE OTAZO, J. (1983) un estudio de los residuos de los fungicidas Captan, Folpet y Captafol, aplicados en post- recolección de manzanas, con la finalidad de ver si los residuos de dichos productos eran inferiores a los señalados por el Codex Alimentarius (FAO/OMS) y la legislación de los paises de la CEE. En dicho estudio se llegó a la conclusión de que los residuos del Captan, Folpet y Captafol estaban por debajo de los permitidos en aquel momento (15 ppm para el Captan, 10 para el Folpet y 5 ppm para el Captafol). En las conclusiones de dicho estudio se abogaba que para los tratamientos de post cosecha se autorizaran expresamente estos productos, señalándoles un P.S. de acuerdo con sus curvas de degradación, con la finalidad de poder hacer frente legalmente a la problemática planteada por las podredumbres durante la conservación fri

gorífica.

En 1985, en un ensayo de pre-registro oficial de la asociación de Tiabendazol al 17% + Folpet al 40%, se vió que los residuos de Folpet a los 7 días del tratamiento eran inferiores a los 3 ppm. Al reducirse recientemente el LMR de Captan + Folpet a 3 ppm, se ha realizado un estudio de los posibles residuos, contemplando la posibilidad de tratamientos de Captan de pre-cosecha. Teóricamente, de acuerdo con las curvas de degradación del Captan aplicado en pre-recolección con P.S. de 7 días y la asociación del Tiabendazol + Folpet en post-recolección, los posibles residuos serán inferiores a 3 ppm si se establece un P.S. de 90 días. Este estudio, en este momento, se está llevando a cabo.

Tab. 9.- Evolución de los residuos (ppm) de Folpet y Tiabendazol aplicados en post-recolección.

Conservación	nº días desde el Tratº	Folpet	Tiabendazol
Atm. Normal	7	2'46	0'86
	15	2	1'06
	76	1'83	1'03
Atm. Controlada	231	> 0'5	0'6

Tab. 10.- Evolución de los residuos de Captan aplicado en pre recolección de manzanas, a diferentes dosis y con reiteración de tratamientos.
COSIALLS JR. y GARCIA DE OTAZO J. (1986) (medias de tres repeticiones)

Tratº	1 Tratº	2 Tratº (2º a los 18 días)	2 Tratº (2º a los 7 días)	3 Tratº
125 g. i.a	0'63	1	0'9	1'9
150 g. i.a	1'96	1	0'9	1'6
200 g. i.a	1'86	1'5	4'2	1'2

En definitiva, para poder hacer frente a la aparición de cepas resistentes de los principales hongos patógenos, es necesario disponer de una mayor gama de productos para los tratamientos de post-cosecha y establecer plazos de seguridad concretos para cada producto o asociación de productos, de acuerdo con sus curvas de degradación.

La aparición de cepas resistentes de Penicillium expansum y de Rhizopus nigricans fueron detectadas por PALAZON en la campaña 1977-78.

Actualmente, la única forma legal de luchar contra esas cepas resistentes son los tratamientos de pre-recolección con fungicidas de amplio espectro y cuya eficacia como se ha podido demostrar, es incluso superior a la de los baños fungicidas, PALAZON et al (1984).

Para solventar el problema de las resistencias PALAZON (1984) propone como productos más interesantes frente a las cinco cepas contra las que trató con 72 materias activas, la diclofuanida, imazalil, iprodione, procloraz y el propiconazol, cuyas únicas limitaciones pueden ser las toxicológicas.

Tab. 11.- Concentración mínima inhibidores (CMI) de diferentes fungicidas sobre la germinación de conidios y el desarrollo miceliano de los principales hongos causatnes de podredumbres de la fruta de pepita (ppm), PALAZON et al (1985)

Materia activa	P. expansum		A. tenuis	
	conidios	micelio	conidios	micelio
diclofuanida	100-100	1-10	100-1000	10-100
folpet	> 1000	100	> 1000	100-1000
imazalil	10-100	1-10	10-100	10-100
procloraz	10-100	< 1	10-100	1-10
propiconazol	100-1000	< 1	10-100	10-100

Materia activa	B. cinerea		R. nigricans	
	conidios	micelio	conidios	micelio
diclofuanida	10-100	1-10	> 1000	> 1000
folpet	100-1000	1-10	10	1000
imazalil	10-100	10-100	100-1000	1000
procloraz	10-100	1-10	> 1000	10
propiconazol	10-100	1-10	100-1000	10-100

Otros problemas que presentan los tratamientos de postcosecha son :

a) El comportamiento de los productos en disolución acuosa, - tanto al inicio como durante el tratamiento de la fruta.

b) La compatibilidad de los diversos productos y su posible fitotoxicidad.

Respecto al primer problema, durante la campaña 1987, se llevó a cabo por el Laboratorio Agrario del DARP de la Generalitat - de Cataluña un estudio del comportamiento de diferentes formulados en disolución acuosa, tanto al inicio como después del

tratamiento aplicado sobre frutas. Dicha iniciativa se debió a que los resultados de los análisis de una serie de muestras tomadas de los baños o duchas de diversas centrales frutícolas, con la finalidad de analizar el contenido de dichos caldos, dio resultados sorprendentes, pues los contenidos encontrados de las diferentes i.a. estaban muy por debajo de lo que teóricamente debía haber de acuerdo con las dosis empleadas.
El resultado de dicho ensayo se corresponde con una primera fase inicial orientativa del comportamiento de los Formulados Fitosanitarios en este tipo de aplicación de Post-cosecha, y se estima que es necesario hacer un estudio más detallado, repetitivo y programado respecto de las cinéticas de descomposición de cada producto, con el correspondiente estudio estadístico, así como la consideración de la influencia de otros parámetros GRASSES JM. (1988).
Este tipo de estudio, creemos que es de una gran importancia, entre otras razones, para que los tratamientos de post-cosecha se realicen adecuadamente.
Veamos, a continuación, las conclusiones extraidas en dicho estudio,
Etoxiquina: Inicialmente en contacto con el agua, hay una clara evidencia de disociación de la molécula por una parte, y de otra se piensa que por oxidación, hidrolisis o las dos a la vez, de Degradación. Una hora después de la preparación del baño hay una degradación aproximada del 50%. Durante los once días siguientes aumenta entre un 25 y un 30%, sin adicionar ninguna fruta. En contacto directo con la fruta (manzanas) al cabo de once días la degradación llega al 95%.

Difenilamina: Inicialmente ya aparece disociación o presencia de isomeros que no se acusa o aumenta en disolución acuosa. Tampoco se aprecia ninguna degradación del producto al cabo de los once días de preparado el baño, ni siguiera en presencia de fruta.

Iprodione: Se observa disociación o presencia de isomeros, incluso en un patrón metanólico. En disolución acuosa la disociación no es más acusada. Durante la primera hora, la disolución aparece inalterada y lo mismo sucede durante los once siguientes días. Contrariamente, en contacto con la fruta se degrada un 50% aproximadamente en el mismo número de días.

Folpet: Inicialmente, por razón de mala suspensibilidad del formulado (polvo mojable), no se aprecia contenido de folpet ni de ningún producto de degradación durante la primera hora, después de preparado el baño. No obstante, con el tiempo y probablemente a causa de fenómenos de difusión y/o lenta disolución del producto (hay que tener en cuenta las repetidas agitaciones a que ha estado sometido el baño), resulta que al cabo de los once días no se aprecia ninguna diferencia entre presencia o ausencia de fruta y la degradación es del orden del 10 al 12%.

Ortofenilfenol: Inicialmente, aparece una clara disociación, pero no se degrada durante la primera hora. Durante los once días siguientes e independientemente de si está o no en contacto con la fruta, se degrada entre un 85 y un 90%.

Tiabendazol: Inicialmente, por razones de mala suspensibilidad del formulado (P.M.), casi no se aprecia un 10% del contenido del producto (ingrediente activo) en el baño, transcurrida una hora desde la preparación. Contrariamente, a los once días no se aprecia degradación en el caso de disolución acuosa, pero sí se observa una degradación total del 100% en el baño con fruta.

Imazalil: No aparece ninguna disociacion y la degradación que se observa en el baño con fruta a los 22 días de su preparación oscila entre el 90 y el 95%.

Tab. 12.- % de i.a. en el baño de diferentes productos GRASSES JM. (1988)

Producto	A los 60 min.	A los 11 días	
		con fruta	sin fruta
Etoxiquina	50	5 - 7	25 - 30
DPA	100 con disociación	100 con disoc.	100 con disoc.
Iprodine	100 con disociación	50	100
Folpet	0	88 - 90	88 - 90

Ortofenilfenol	100 con disociación	10 - 15	10 - 15
Tiabendazol	10	0	100
Imazalil	100	-	-

Está previsto continuar con este estudio, ajustando al máximo las condiciones reales de aplicación de los tratamientos de - post-cosecha.

Por último, señalar que en un estudio realizado por MASCAROS J y MIRANDA G (1988) en los laboratorios de Pennwalt, sobre - la influencia del tipo de recipiente (hierro o plástico) y la presencia o no de tierra, en la evolución de la concentración de Imazalil, Iprodione y DPA, llegan a la conclusión de que - en recipientes de hierro y con presencia de tierra, con indiferencia del tipo de recipiente, las concentraciones de los - i.a. disminuyen más rápidamente; en vista de lo cual se recomienda que los recipientes de los baños o duchas se revistan de plástico.

Compatibilidad de los diversos productos:

En los tratamientos de post-cosecha, normalmente, se asocian un anti-escaldado, un fungicida y el Cloruro de cal. En numerosos ensayos realizados por diversos autores, en general esa asociación carece de fitotoxicidad, con excepción de algunas mezclas concretas. Normalmente se recomienda no superar la dosis del 2% para el Cloruro de cal.

CONCLUSIONS

a) Le principal champignon qui occasione des pourritures pendant la conservation frigorifique c'est le Penicillium expansum qui lui même supose le 70-80% du total.

b) L'apparition des cèpes résistents de Penicillium expansum et Rhizopus nigricans aux foungicides autorisés c'est un fait demontré.

c) Entre toutes les physiopaties l'unique qui a un traitement préventif éfficace c'est l'échaudure.

d) On emploie encore des traitements avec Chlorure de chaux pour le contrôle en post-récolte du Bitter Pit, malgré que les résultats soient seulement partiellment satisfactoires, à cause de leurs éffets colateraux - profitables dans l'évolution et la sénescence des fruits. On ne doit - surpasser la dose du 2% pour éviter fhitotoxicités ou incompatibilités.

e) La désinfection des chambres et des récipients est nécessaire réduire -

la quantité d'inocule au début de la campagne et rompre le cicle des champignons.
La désinfection ensemblée des chambres et des récipients est possible en utilisant le Formaldeido qui a une excellente éfficacie.

f) La gamme des fungicides autorisés pour les traitements de post-récolte est trés réduite, ce qui établit des problemes à l'heure de lutter de forme éfficace contre l'apparition de cèpes résistents de Penicillium expansum aux benzimidazoles.

g) En l'établisement d'une stratégie de lutte contre les pourrissements on doit donner la préférence aux produits qui ont plus éfficacie contre le Penicillium, et on doit alterner leur usage pour éviter l'apparition de résistences.

h) C'est trés important le connaissement de la conduite des différentes formulations dans le bain. En principe les études réalisés conduisent à la conclusion de que les formulations liquides ou de tipe "flow" - sont les préférables et que la duration du bouillon de bain ne doit pas passer de 8 heures.

i) C'est urgent l'autorisation des nouvelles matéries actives pour les - traitements de post-récolte, en établissant leur terme de sécurité , d'acord avec leurs courbes de dégradation en ce qui concerne en A.C. comme en A.N.

BIBLIOGRAFIA

ABELES, F.B. - 1973. Ethylene in Plant Biology- New Yowk, London, Acad. Press.

BARELLES, F. & GARCIA DE OTAZO, J. - 1983. Estudio de los residuos de tres fungicidas aplicados en post-recolección de manzanas - IX Jornadas de Productos Fitosanitarios. I.Q. Sarriá, Oct-83. p. 99-110.

BIALE. J.B.& YOUNG, R.E. - 1981. Respiration and ripenining in fruits. Retrospect and prospect. Recent Advances in the Biochemistry of fruits and vegetables - Friend, J. & Rhodes, M.J.C. Adid. Pres London.

BIONDE, G. - 1987. Strategie nella difesa post-raccolta degli ortofrutticoli. Revista Frutticoltura, 9-10, 47-51.

CELMA, E. & YAGUE, A. - 1988. Seguridad para el consumidor. Establecimiento de Límites Máximos de Residuos de Plaguicidas - Phytoma España - (0), 12-16.

COSIALLS, J.R. & GARCIA DE OTAZO, J.- 1986. Estudio de los residuos de Captan aplicado en pre-recolección de manzanas - Frut, vol.III nº 2, p. 82-88.

GRAELL, J. - 1987. Reducción del etileno en cámaras de conservación de fruta. Frut. Profesional, 10, 56-61.

GRASSES, J.M. - 1988. Informe: Baños de tratamientos de post-cosecha. Resumen de actividades nº 1, p. 41.

HERRERO, A. - 1986. La nueva conservación frigorífica de la fruta -Frut, 1(0), p. 15.

HERRERO, A. - 1988. Alteraciones fúngicas y alteraciones fisiológicas de la fruta en el: Curso de Manipulación y conservación frigorífica de la fruta - Fundación Narcis Monturiol. Universidad Técnica de Verano de Cataluña.

LIU, FW. et al. - 1986. Low-ethylene CA storage of "Mcintosh" apples in a semicommercial sized room. - Hort Science 21(3), 480-484.

MASCAROS, J. & MIRANDA, A. - 1988. Imazalil, Iprodione y Difenilamina. Evolución de la concentración en su uso comercial. Pennwalt Iberica S/A Fruticultura Profesional (46), 71-74.

NARDIN, C; CASERA C.; HAEUSL, H.- 1986. Influenza di atmosfere con bassi tenori di O2 (L.O.) sue riscaldo e l'imbrunimento del cuore e sue mantenimento dell'aciditè di alcune varieta di mele. Frutticoltura, 9-10, 77-80.

PALAZON, J. & RODRIGUEZ, M.C.- 1977. Las podredumbres de la fruta de pepita conservada en cámaras frigorífica. Cuadernos INIA, 5, p.92.

PALAZON, J. - 1981. Eficacia de diferentes productos en la desinfección de cámaras frigoríficas y de embalajes utilizados para la conservacion de fruta de pepita. Hoja Técnica INIA nº 35. Mº Agricultura.

PALAZON, J. et al.- 1984. Estudio de los problemas patológicos de la conservación de peras y manzanas en la provincia de Zaragoza - Institución Fernando el Católico. Publ. nº 990, 147 p.

PALAZON, J. - 1987. Productos utilizables en tratamientos de post-cosecha sobre fruta de pepita - Frut. Profesional, 8, 48-54.

PLAZA, J.L. - 1986. La vida post-recolección de los frutos. Fruticultura 2, 28-35.

PRATELLA, G.C. & BERTOLINI, P. - 1987. Trattamendi post-raccolta delle mele: aspetti fitoiatrici e legali. Frutticoltura, 4, 33-39.

LIST OF AUTHORS

Aeschlimann, J. P.

Bailly, R.
Barahona Ruiz, D. E.
Baumgärtner, J.
Bertazzoni, U.
Blanco, J.
Botto, E. N.
Buchelos, C. Th.

Calvi Parisetti, C.
Cavalloro, R.
Chaisse, E.
Croizier, G.

Dartigues, V.
De Jong, M. D.
Delucchi, V.
De Wilde, G.
Di Cola, G.

Edwards, C. A.

Fages, J.
Fougeroux, A.
Fricot, L.
Fujiki, N.

García, A.
García De Otazo López, J.
González, D.
Gutiérrez, A. P.

Holden, W. T. C.
Holt, D.

Kmieciak, S.
Komjanc, M.

Levinson, A. R.
Levinson, H. Z.
Lorenzoni, C.

Magnien, E.
Mann, B. P.
Marmiroli, N.
Martiniello, P.
Meyer-Roux, M. J.
Mols, P. J. M.
Monzón Fernández-Peña, D. G.
Moratorio, M.

Olivier, A.
Oshima, S.

Peix, D. J.
Perry, J. N.
Pizzol, J.

Quelennec, G.

Rabbinge, R.
Regev, U.
Riba, G.
Richardson, P. N.
Rieder, P.
Ripolles, J. L.
Rossing, W. A. H.
Rudolph, R.

Saarenmaa, H.
Sala, F.
Stanca, A. M.
Stone, N. D.

Terzi, V.
Trematerra, P.

Ueda, O.

Wajnberg, E.
Woiwod, I. P.
Wratten, S. D.

Vacante, V.
Van Der Werf, W.
Van de Veire, M.
Vassarotti, A.

parasitis INTERNATIONAL ASSOCIATION

PROCEEDINGS OF A SCIENTIFIC CONGRESS

Barcelona, Spain, 25-28 October 1988

parasitis 88

Edited by

R. CAVALLORO, President
V. DELUCCHI, Former President

EDITA

MINISTERIO DE AGRICULTURA PESCA Y ALIMENTACION

SECRETARIA GENERAL TECNICA

Depósito Legal: M. 43415-1989
ISBN: 84-7479-783-7
NIPO: 251-89-088-2
Imprime: GRAFOFFSET, S. L.