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Effects of crude plant extracts and mineral oil on reproductive performance of the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae)

M. J. CARIAC, A. A. FERRERO, T. STADLER

The effects of crude plant extracts of *Gossypium hirsutum* L. (Malvaceae), *Glycine max* (L.) Merr. (Leguminosae) and *Diplotaxis tennuifolia* (L.) De Candolle (Cruciferae) on the fecundity and fertility of *Cydia pomonella* L. (Lepidoptera: Tortricidae), a key-pest of apple orchards were evaluated in the laboratory and compared in efficacy with a mineral spray oil (Dapsa-DRV). All products tested caused a drop in fecundity of treated females. The order of effectiveness in reducing fecundity was: *D. tennuifolia* > *G. hirsutum* > DAPSA-DRV > *G. max*, yielding 29%, 41%, 45%, and 65% of the fecundity, respectively, relative to the control. A delay of the oviposition was observed in females treated with *D. tennuifolia.* All oils tested, with the exception of the extract of *G. max*, interfere with the normal development of the eggs and the hatching process of this species. Our study highlights the importance that plant extracts could have in the framework of an integrated pest management program for the control of the codling moth, providing an alternative strategy to the current management practices used in southern Argentina.

M. J. CARIAC, A. A. FERRERO: Laboratorio de Zoología de Invertebrados II. Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur. San Juan 670 (8000) Bahía Blanca, Argentina.
T. STADLER: Laboratorio de Parasitología y Ecotoxicología. Consejo Nacional de Investigaciones Científicas y Técnicas-CONICET- LIBIQUIMA. Facultad de Ingeniería. Universidad Nacional del Comahue. Buenos Aires 1400 (Q8300BCX) Neuquén, Argentina.

Keywords: *Cydia pomonella*; plant extracts; sublethal effects; oviposition; fertility.

INTRODUCTION

The codling moth, *Cydia pomonella*, is an important pest of pome fruits throughout the world and the key pest in apple orchards in the Alto Valle, the main apple producing area in Argentina, causing losses of US$15-30 million a year. The intensive insecticide treatments against *C. pomonella* give rise to secondary pest problems. Moreover, chemical control looses effectiveness from year to year because of the high levels of insecticide resistance and cross resistance. In addition to control costs, yield reduction, and ecological disruption, environmental contamination is also a consequence of dealing with this pest. Public concern about the use of toxic chemicals in fruit production, and increasing resistance of codling moth and other orchard pests against available insecticides demand the development of new pest control strategies (WITZGALL *et al.*, 1999). The interest in biological pest management and the use of plant extracts and mineral oils against the codling moth have recently increased. Plant-derived insecticides have been important tools for codling moth control before modern synthetic insecticides replaced them. In recent years, there has been a considerable effort to find phytochemicals with insecticide activity (RIEDL *et al.*, 1998). Thanks to modern chemistry, the structures of many of these biologically active agents are now known, and systematic studies on the natural products that protect plants from pests have become a recognized area of research within the fields of chemistry and pest control (HEDIN & HOLLINGWORTH, 1997).

Crude extracts obtained from plants are usually composed of crude oils, essential oils and miscellaneous compounds. These plant extracts as well as mineral oils have several benefits: they are selective to natural enemies and other non-target species, suppress pests such as mites and pear psylla, are safe to the applicator, and residues do not represent a food safety concern. The most outstanding property of spray-oils is that they address multiple targets in the insect body (STADLER *et al.*, 1996). Because of this, oils do not elicit resistance responses in insects as chemical insecticides do.

Mineral oils act primarily as ovicides by depressing the respiratory rate when applied directly to codling moth eggs (RIEDL *et al.*, 1995). The length of respiratory depression and the dose of oil in contact with the egg are critical for causing mortality (SMITH & PEARCE, 1948). Several plant essential oils seem promising as arrestants and repellents against larval stages of codling moth (LANDOLT *et al.*, 1999). Studies carried on neonates of *C. pomonella* fed on a diet treated with crude extracts of *Schinus molle* L. (Anacardiaceae) showed that this plant extract exhibited an antifeedant and insect growth regulator activitiy and yielded adult malformations (CHIRINO *et al.*, 2001). Although numerous publications report on pesticide-induced repellency or reproductive stimulation in insects, few studies have addressed the reproductive response of *C. pomonella* to pesticides. Therefore there is enough evidence to draw attention to the potential impact of sublethal concentrations of pesticides on fecundity and fertility of the codling moth. A number of non-host plants extracts have shown to be ovipositional deterrents against some agricultural pests (CHEN *et al.*, 1996a). Seeking for new alternatives and tools for a sustainable integrated pest management strategy in apple orchards, the aim of this work was to assess the effect of crude seed extracts of *G. hirsutum*, *G. max*, *D. tennuifolia* on the reproductive performance of *C. pomonella* and compare their efficacy with a commercial mineral spray-oil, Dapsa-DRV.

MATERIALS AND METHODS

Insects. Codling moths used in the bioassays were obtained as pupae reared in our lab cultures. The insects were reared according to the POITONT & BLUES (1970) method, modified by GUENNELON *et al.* (1981), at 25 ± 1 °C, 60-70 % of r.h., and L24:D0.

Chemical products. The commercial crude extracts of *G. hirsutum and G. max* were procured from EEA-INTA Chaco (Argentina). The hexanic crude extract of *D. tennuifolia* was obtained from the organic chemistry laboratory at the Universidad Nacional del Sur. The mineral spray oil Dapsa-DRV is a commonly used spray-oil in apple and grape fruit orchards. The product was obtained from Destilerías Argentinas de Petróleo.

Bioassays. Products were applied topically to the ventral abdominal surface of 48-hour-old *C. pomonella* adults by using a Hamilton repeating dispenser PB 600 supplied with a 50μl syringe, calibrated to deliver 1μl droplets. Insects were anesthetized with CO_2 and treated with 1 μl of the test products. Untreated adults were used as control. After the treatment, pairs of male and female moths were transferred to oviposition cages where fecundity and fertility were assessed.

Fecundity assessment. The oviposition cages were assembled as a cylinder of wax paper to provide a substrate on which the eggs were laid. Two PVC rings (5 cm in diameter) provided structural support for each cage. Both ends of the cylinder were covered with a fine mesh to prevent the insects from escaping the cage. The wax paper was replaced every two days until the end of the bioassay, and the number of eggs laid per female was recorded.

Fertility assessment. Batches of 50 eggs obtained from fecundity bioassay cages were transferred and incubated in Petri dishes containing a wet cotton basis covered with filter paper dish to prevent drying. Hatched eggs were recorded during eight days, twice the required time for the normal development of this species.

The bioassay cages and Petri dishes were maintained at 25 ± 1°C, 60-70% of h.r. and L24:D0. The adults were fed a 5% sucrose solution, which was dispensed from a cotton wick.

Analysis of results. Differences in fecundity between treated and untreated insects were assessed by Student t-tests, after transforming the data when necessary. We performed Wilcoxon-Mann-Whitney tests when the data distribution was not normal (ZAR, 1999). The Tm50 (time at which 50% of the total eggs are laid) was also calculated by using a Probit analysis computer program (ROBERTSON & PREILER, 1992). Differences in fertility were determined by ANOVA and treatment means were separated by the Tukey and Kramer procedure (ZAR, 1999). The data were previously arc-sin transformed. Mortality percentages were corrected with ABBOTT'S (1925) formula.

RESULTS

Fecundity

The three crude plant extracts as well as the mineral oil tested show a significant inhibitory effect on the oviposition of *C. pomonella*. In each case, the mean number of eggs laid by treated females was significantly lower than that of controls (Table 1). The effectiveness of the tested extracts and mineral oil in reducing fecundity was: *D. tennuifolia* > *G. hirsutum* > Dapsa-DRV > *G. max*, yielding 29%, 41%; 45%, and 65% of the fecundity, respectively, relative to controls. The *D. tennuifolia* was the only plant extract that performed better than Dapsa-DRV, dropping fecundity 30% below the values achieved (assets performed) with spray-oil treatments.

The oils applied topically affected the egg-laying schedule (Tables 2 to 4). For all

Table 1. **Mean of eggs laid per female after topical treated.**

OIL			CONTROL			Difference
Treatment	Re[a]	M[b] (CL 95%)[c]	Re	M (CL 95%)	P level	in fecundity (%)
Dapsa-DRV	33	**96** (69-122)	42	**210** (185-235)	p=2,1517E-08	55
G. max	30	**138** (101-175)			p=0,0006994	35
G. hirsutum	31	**69** (49-89)	44	**169** (152-186)	p=3,13E-07	59
D. tennuifolia	39	**48** (32-64)	39	**165** (137-193)	p=6,3341E-10	71

[a] Re: number of replicates.
[b] M: mean total number of eggs laid per female.
[c] CL: confidence limits.

treatments the mean number of eggs laid on the 4th and 6th days was significantly higher in controls. As shown in table 3, for *G. hirsutum* there were differences in the oviposition rate on day 2nd, 8th and 10th; for *D. tennuifolia*, there were differences on the 2nd and 8th days (Table 4). However, significant differences in Tm50 were achieved only for

Table 2. **Schedule of egg production by codling moth females after topical treatment with Dapsa-DRV and G. max.**

Treatment (number of pairs)		Days after treatment							
		2 [a]	4 [b]	6 [b]	8	10 [b]	12 [b]	14 [a]	16 [b]
Control	Mean n° eggs/female	**17,36**	**54,60**	**55,07**	**29,27**	**24,62**	**17,25**	**12,86**	**11,68**
'(42)	SE[c]	4,90	7,44	5,51	3,27	2,91	2,01	2,26	2,74
	Cumulative %	7,79	32,31	57,04	70,18	81,24	88,98	94,76	100
Dapsa-DRV	Mean n° eggs/female	**4,24**	**20,27**	**20,36**	**23,65**	**26,04**	**10,20**	**12,69**	**11,11**
'(33)	SE	2,31	6,28	4,83	4,09	5,77	1,79	4,87	5,28
	Cumulative %	3,30	19,07	34,90	53,30	73,55	81,49	91,36	100
	P level	(p=0,25)	(p=8,73E-05)	(p=5,59E-07)	(p=0,1439)	(p=0,2505)	(p=0,0542)	(p>0,25)	(p=0,2128)
G. max	Mean n° eggs/female	**6,27**	**32,53**	**35,63**	**31,37**	**22,09**	**20,17**	**10,00**	**7,86**
'(30)	SE	2,87	8,30	7,12	7,47	3,76	2,11	1,55	2,11
	Cumulative %	3,78	23,38	44,86	63,77	77,08	89,24	95,26	100
	P level	(p>0,25)	(p=0,0056)	(p=0,0028)	(p=0,399)	(p=0,1564)	(p=0,0943)	(p>0,25)	(p=0,3813)

[a]: data were compared by Wilcoxon-Mann-Whitney test. The remaining data were compared with Student test, [b] data transformed to ln(x+1) for analysis. All tests were made at one tail.
[c] SE: Standard error.

Tabla 3. **Schedule of egg production by codling moth females after topical treatment with *G. hirsutum***

Treatment (number of pairs)		Days after treatment							
		2 [a]	4 [a]	6 [a]	8 [b]	10 [b]	12 [b]	14	16 [a]
Control	Mean n° eggs/female	**20,70**	**45,86**	**32,09**	**33,65**	**23,86**	**12,94**	**10,13**	**8,47**
'(44)	SE[c]	4,87	6,82	4,04	4,34	3,15	2,18	1,42	1,42
	Cumulative %	11,03	35,46	52,56	70,48	83,20	90,09	95,49	100
G. hirsutum	Mean n° eggs/female	**1,13**	**8,68**	**14,20**	**11,11**	**11,54**	**12,00**	**9,74**	**6,21**
'(31)	SE	0,49	2,93	4,68	2,17	3,27	2,88	1,46	1,63
	Cumulative %	1,51	13,15	32,18	47,07	62,54	78,62	91,68	100
	P level	(p<0,0005)	(p<0,0005)	(p<0,0005)	(p=2,48E-05)	(p=0,0002)	(p=0,4017)	(p=0,4254)	(p>0,1)

[a]: data were compared by Wilcoxon-Mann-Whitney test. The remaining data were compared with Student test, [b] data transformed to ln(x+1) for analysis. All tests were made at one tail.

[c]: SE: Standard error.

Tabla 4. **Schedule of egg production by codling moth females after topical treatment with *D. tennuifolia***

Treatment (number of pairs)		Days after treatment							
		2 [a]	4 [a]	6 [a]	8 [a]	10 [b]	12 [b]	14	16 [a]
Control	Mean n° eggs/female	**29,36**	**50,74**	**40,50**	**24,51**	**20,42**	**10,90**	**8,36**	**3,00**
'(39)	SE[c]	6,37	6,56	5,62	3,23	3,93	2,12	1,62	1,74
	Cumulative %	15,63	42,65	64,22	77,27	88,15	93,95	98,40	100
D. tennuifolia	Mean n° eggs/female	**3,26**	**7,18**	**9,29**	**8,50**	**11,77**	**11,83**	**7,64**	**7,63**
'(39)	SE	2,38	2,27	2,16	2,13	2,00	3,01	1,83	2,51
	Cumulative %	4,86	15,56	29,40	42,07	59,61	77,24	88,63	100
	P level	(p<0,0005)	(p<0,0005)	(p<0,0005)	(p<0,0005)	(p=0,08465)	(p=0,4932)	(p=0,3862)	(p>0,1)

[a]: data were compared by Wilcoxon-Mann-Whitney test. The remaining data were compared with Student test, [b] data transformed to ln(x+1) for analysis. All tests were made at one tail.

[c] SE: Standard error.

Tabla 5. **Required time for the laid of 50% of total eggs**

	OIL		CONTROL	
Treatment	Tm50a	CL 95%b	Tm50	CL 95%
Dapsa-DRV	7,32	(5,46-8,68)	5,41	(3,91-6,64)
G. max	6,26	(4,79-7,43)		
G. hirsutum	7,88	(5,86-9,28)	5,42	(3,84-6,69)
D. tennuifolia	8,52	(6,22-10)	4,62	(3,31-5,72)

[a] Tm50: required time for the laid of the 50% of total eggs.
[b] CL: confidence limits.

D. tennuifolia, showing an effective delay in oviposition of the treated females (Table 5).

Fertility

All products tested, except the *G. max* extract produced disruption in the development by preventing normal hatching or causing egg mortality. Egg mortality data in the various treatments are presented in Table 6.

DISCUSSION AND CONCLUSION

Our results show that *D. tennuifolia, G. hirsutum* and *G. max* extracts, affected the fecundity and fertility of the codling moth *C.*

Table 6. **Percent fertility of codling moth after treatment**

Treatment	Re[a]	Mortality (%) ± SE	Efficacy[b] (%)
Control	183	**19,98** ± 1,69 a	
Dapsa-DRV	40	**49,9** ± 5,74 b	37
G. max	48	**28,75** ± 3,95 a	11
G. hirsutum	21	**60,29** ± 6,98 b	50
D. tennuifolia	16	**73,25** ± 4,26 **b**	67

[a] Re: number of replicates per treatment; each cage contained 50 eggs.
Means followed by the same letter are not significantly different (Tukey-Kramer procedures, α=5%). Data were transformed to arcosen (x) for analysis.

[b] Efficacy: percent corrected mortality (Abbot 1925)= (% alive in control - % alive in treatment) / % in control * 100.

pomonella. Therefore, natural extracts as well as mineral oils should be considered as potential tools for the control of this widespread pest, as has been previously shown by different authors for other insect pests (CHEN *et al.*, 1996a y b; KOUL *et al.*, 2000; NASSEF, 1999; KUMAR & BHATT, 1999; RENIPRABHA *et al.*, 1999; BAUTISTA *et al.*, 1998; DWIVEDI & GARG, 1997; RAJAPAKSE & SENANAYAKE, 1997; GRANT & LANGEVIN, 1995; GE & WESTON, 1995). Among the products tested in this study, *D. tennuifolia* extract caused the largest reduction in codling moth fecundity. These results highlight the potential benefit of this extract for the control of *C. pomonella*, since this crucifer is highly abundant in Argentina, in the provinces of Buenos Aires and La Pampa, and it has even been declared agricultural plague (AGULLO *et al.*, 1987). Furthermore, this weed is not eaten by cattle and is quite invulnerable to the attack of plant lice and caterpillars. Therefore, *D. tennuifolia* extracts could be obtained easily and at low cost. Studies on extracts from *G. max*, show that it is also effective in reducing the oviposition of other pest species as *Callosobruchus chinensis* (RAJAPAKSE & SENANAYAKE, 1997). In addition to the effect caused by *G. hirsutum* on *C. pomonella* oviposition, other authors have pointed it as an effective carrier (HAYES & SMITH, 1994) and to be responsible for the alteration of cuticle hardness of other insect pest species (STADLER *et al.*, 1996; STADLER *et al*, 2001). Moths treated with mineral oil Dapsa-Drv laid less eggs than controls, but there was no difference in the required time for oviposition of 50% of the total eggs. These findings dissent with previous results who stated that mineral oils of similar physicochemical characteristics had no significant effect on the total number of eggs produced by treated females. In addition, treated moths laid a larger proportion of their total eggs (57%), during the first 4 days, compared with 33% for untreated females (RIEDL *et al.*, 1995).

The decline in egg fertility after the treatment with Dapsa-DRV and *G. hirsutum* could be partially explained by the ovicidal effect of these substances (FERRERO *et al.*, 2001). Many studies have reported that ovicidal nature of the oils is due to the physical nature of oils to block the oxygen supplied from the developing embryo or due to the toxicity of some inherent constituents of oils (RAJAPAKSE & SENANAYAKE, 1997).

On the other hand, several studies have addressed the effect of insecticides like insect growth regulators, insect growth inhibitors, organophosphates and others on the oviposition of *C. pomonella.* Continuous exposure to surfaces treated with tebufenozide or methoxyfenozide significantly reduce the mean number of eggs laid and the percentage of eggs that hatched (SUN & BARRET, 1999). The fecundity of the codling moth was slightly affected but its fertility decreased with the increase in the concentration when it was exposed to dry residues of CGA 184'699 (5% EC) Ciba-Geigy, an insect growth inhibitor and fenoxycarb, an insect growth regulator (CHARMILLOT & PASQUIER, 1992). Azinphosmethyl inhibited oviposition strongly but at low concentrations moths produced more eggs than in the controls (ABIVARDI *et al.*, 1998). Similar results where addressed by using natural plant extracts and spray oils.

At present, codling moth populations have been reported to be resistant to diflubenzuron (CHARMILLOT *et al.*,1999; SAUPHANOR *et al.*, 1997; RIEDL, 1994; MOFFIT *et al.*, 1988; SAUPHANOR *et al.*, 2000a), deltamethrin (SAUPHANOR *et al.*, 2000a) and organophosphates (SAUPHANOR *et al.*, 2000b), some of the most common insecticides used for its control. Consequently, our findings provide evidence that plant extracts or spray-oils could be a useful complementary or alternative tool for the control of this key pest.

Because of the toxicity and synergism to chemical insecticides shown by some hydrocarbons these products could be alternated with chemical pesticides or added as coadjuvants to neurotoxic insecticides or insect growth regulators in the framework of a resistance-management strategy. However, further studies on the nature of the chemical compounds of plant extracts and the potential for phytotoxicity to fruit and foliage need to be carried out for a better understanding of their mode of action and to assess possible restrictions for the use of these products.

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ABSTRACT

CARIAC M. J., A. A. FERRERO, T. STADLER. 2003. Efectos de extractos crudos de vegetales y un aceite mineral en el comportamiento reproductivo del gusano de la manzana, *Cydia pomonella* L. (Lepidoptera: Tortricidae). *Bol. San. Veg. Plagas*, **29**: 471-479.

Se evaluaron en laboratorio los efectos de extractos crudos de *Gossypium hirsutum* L. (Malvaceae), *Glycine max* (L.) Merr. (Leguminosae) y *Diplotaxis tennuifolia* (L.) De Candolle sobre la fecundidad y fertilidad de *Cydia pomonella* L. y se compararon en eficacia con un aceite mineral (Dapsa-DRV). Todos los productos evaluados causaron una caída en la fecundidad de las hembras tratadas. El orden de efectividad en reducir la fecundidad fue: *D. tennuifolia* > *G. hirsutum* > Dapsa-DRV > *G. max*, presentando el 29%, 41%, 45% y 65% de la fecundidad de los controles respectivamente. Se observó un retraso efectivo en la oviposición de las hembras tratadas con *D. tennuifolia.* Todos los aceites, excepto el extracto de *Glicine max*, interfirieron con el desarrollo normal de los huevos y el proceso de eclosión de esta especie. Nuestro estudio enfatiza la importancia que podrían tener los extractos de plantas como parte de los programas de manejo integrado de plagas para el control del gusano de la manzana, proveyendo una estrategia alternativa a las prácticas de manejo actualmente usadas en la Argentina.

Palabras clave: *Cydia pomonella*, aceites minerales, extractos vegetales, efectos subletales, oviposición, fertilidad.

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