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Biological control of *Sitotroga cerealella* in stored corn using *Trichogramma pretiosum* and *Bracon hebetor*

M. S. R. Inoue, D. E. Nava, J. R. P. Parra

This research was developed aiming to evaluate whether it is possible to control the Angoumois grain moth, *Sitotroga cerealella* in stored corn using the egg parasitoid *Trichogramma pretiosum*, alone or in association with the *Bracon hebetor* larval parasitoid. The results showed that the parasitoid *T. pretiosum* had great control potential against *S. cerealella* in stored corn, both in bulk and on the ear. The parasitoids, released in the mass of corn grains, parasitized *S. cerealella* eggs down to a 40 cm depth. Parasitism by *T. pretiosum* decreased, on average, 1.92% per each centimeter of depth. For ear corn, *T. pretiosum* releases were effective to control *S. cerealella*, providing a 60.7% reduction in adult moth population and 63.1% in damage percentage to ears stored in screened warehouses. The release of *T. pretiosum* in association with *B. hebetor* did not result in *S. cerealella* control advantages, since there were no significant differences between treatments. Despite a numerical tendency for higher efficiency with the association of both parasitoids, with an 8.69% increase in *S. cerealella* adult population reduction and a 1.72% increase in ear damage reduction, as compared with the *T. pretiosum* treatment alone.

M. S. R. Inoue, J. R. P. Parra. Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13418-90, Piracicaba, São Paulo, Brazil
D. E. Nava. Empresa Brasileira de Pesquisa Agropecuária, Clima Temperado, 96001-970, Pelotas, Rio Grande do Sul, Brazil. E-mail: nava@cpact.embrapa.br

Key words: Stored grain, egg parasitoid, larval parasitoid.

INTRODUCTION

Grain storage and conservation is assuming an ever-increasing importance, due to a growing worldwide demand for food. The main concerns during storage refer to losses incurred, among which attack by insects appears as an important factor (Dell'Orto Trivelli, 1984). The Food and Agriculture Organization (FAO) estimates that losses caused by the attack of insects to stored grain are around 10% of the world's yield. In Brazil, such losses amount each approximately 9.8 million tons per year (Conab 2001 cited by Lorini 2001).

Corn coming from the field is often already infested by moths and weevils, as a result of the great flight capacity shown by these insects and the existence of possible infestation spots near the field (Puzzi, 1977). Schulz and Laborius (1986) also ascribe the need for the adoption of control measures right after harvest.

The association that exists between several insect pests and stored corn and its byproducts results in quantitative and qualitative losses, such as weight loss, energy loss in the food, both for human and animal consumption, loss of seed germination, in addition to undesirable fragments that are left in the mass of grains (Puzzi, 1977; Demianyk and Sinhá, 1987; Santos and Fortes, 1990; Almeida and Murta, 1995; Lorini, 2001). In addition to the direct damage to the grain, the insects play an important role in the development and distribution of fungi, thus helping to further depreciate the stored product (LORINI, 2001).

At present, stored grain pests are chemically controlled. This includes fumigations and dustings or sprays with phosphorus and pyrethroid insecticides. Chemical control, although normally effective, may cause serious problems, such as the presence of toxic product residues, reduction in seed germination, or may lead to insect resistance to insecticides, particularly when storage is done for long periods of time, with the repeated use of the same control measure (PUZZI, 1977; SCHULZ AND LABORIUS, 1986; LORINI AND GALLEY, 2000). Technical knowledge and special precautions are required at application time, since factors such as temperature and moisture in the grain mass and the existence of spaces between the kernels that allow the product to spread out are decisive for application success (SANDVOL AND HOMAN, 1986).

When such combination of factors is taken into consideration, the need for the adoption of alternative control methods such as biological control becomes evident, within an Integrated Pest Management scheme (STEIDLE *et al.* 2001; DARWISH *et al.* 2003). Therefore, the objective of this research was to evaluate the effectiveness of *Sitotroga cerealella* (Olivier) control with the *Trichogramma pretiosum* Riley egg parasitoid when released alone or in association with the *Bracon hebetor* Say larval parasitoid, in corn stored either in bulk or on the ear.

MATERIAL AND METHODS

Parasitism of *T. pretiosum* on *S. cerealella* eggs at different depths in the mass of corn grain

Five 100-liter capacity plastic barrels with lids were used. Each barrel received 45 kg hybrid corn, previously maintained in a freezer for one week to prevent contaminations by other insects and mites. Each barrel was infested with cards containing *S. cerealella* eggs on the surface and at 10, 20, 30, and 40 cm depths in the mass of corn grain. Five 1 cm^2 cardstock cards were distributed per depth, each containing approximately 600 *S. cerealella* eggs killed by UV light to prevent corn contamination with the moth, since the same corn was used in the five replicates of the experiment. The cards were placed inside a 6˘2 cm plastic box for protection, with a screened lid to allow the parasitoids to pass through and to prevent the eggs attached to the card from being damaged by the weight of the grain mass. The *S. cerealella* rearing technique on wheat grain was based on BLEICHER *et al.* (1987).

The parasitoids, selected among three lines from the *Trichogramma* spp. collection of the Insect Biology laboratory of the Departamento de Entomologia, Fitopatologia e Zoologia Agrícola at ESALQ/USP, were multiplied on eggs of the alternate host *A. kuehniella*, based on PARRA (1997), for subsequent releases. Each barrel received the release of a different number of *T. pretiosum*, obtained by varying the number of *T. pretiosum*: number of *S. cerealella* eggs, at ratios of 1:1, 3:1, 5:1, 8:1, and 10:1, in a randomized complete block experimental design, totaling five blocks. Five days after release, the cards were removed from the barrels, identified, and kept in an incubator adjusted at 25 ± 2°C for five days, after which the number of dark eggs (denoting parasitism) was counted.

The mean number of parasitized eggs in the five cards placed at each depth was calculated. The results were then submitted to analysis of variance, and the means were compared by the Tukey test ($P \geq 0.05$) at the 5% probability level. The experimental design consisted of 25 treatments (five parasitoid-to-egg ratios at five depths) and five replicates.

Effect of *T. pretiosum* alone and in association with *B. hebetor* for the control of *S. cerealella* in corn ears stored in a warehouse

Three "warehouses" (1x1x1m) were made of wood, with nylon screen walls covered with asbestos cement shingles. The warehouses received the following treatments: 1) infestation with *S. cerealella* eggs only; 2) infestation and release of *T. pretiosum*; 3) infestation, release of *T. pretiosum* and release of *B. hebetor*.

We placed 1,000 unhusked hybrid corn ears in each warehouse. In order to obtain a 50% infestation in the grain, the following procedures were adopted: a calculated corn grain average of 400 kernels was used, and *S. cerealella* egg viability average of 75% was estimated. Consequently, because 1g of *S. cerealella* eggs corresponds 50 thousand eggs, 5.34g eggs (≅ 267 thousand eggs) of the moth were "inoculated" in each warehouse, distributed on the surface of the ear mass.

The *T. pretiosum* release calculations were made based on the rate of 1 *T. pretiosum* to 5 *S. cerealella* eggs. We therefore released 67 thousand *T. pretiosum* females in each warehouse (treatments 2 and 3), considering an 80% *T. pretiosum* viability. Cards containing eggs parasitized by ready-to-emerge *T. pretiosum* were placed on the surface of the ear mass.

In warehouse number 3, besides the release of the egg parasitoid, we also tested the effectiveness of a subsequent release of the ectoparasitoid *B. hebetor* larval. Temperature in the warehouse was measured daily and showed an average value of 25°C in the initial days. Based on INOUE AND PARRA (1998) the moth's total cycle duration was approximately 50 days, whereas the egg stage duration was around seven days. Under these conditions, CÔNSOLI AND AMARAL FILHO (1989) determined the duration of the pupal stage at this temperature to be 12 days; therefore, we were able to estimate the larval stage to last from day seven to day 38. Thus, it was possible to synchronize the *B. hebetor* release with the occurrence of the last larval instar, which is when the highest parasitism takes place (SERRA, 1992). Release was therefore made 25 days after infestation with *S. cerealella* eggs.

The parasitoid *B. hebetor* was reared on *A. kuehniella* caterpillars, maintained at 25°C, according to SERRA's methodology (1992). The number of females of the braconid to be released was also calculated based on that work, that was on parasitism on *A. kuehniella* caterpillars and found that each *B. hebetor* female can parasitize 40 caterpillars on average. Because the weight of one *S. cerealella* caterpillar was calculated to be 35.81% in relation to the weight of one *A. kuehniella* caterpillar, we obtained by calculation that one female of the parasitoid could parasitize around 2.79 times more *S. cerealella* caterpillars, i.e., 112 caterpillars, which resulted in the release of 1,800 females of the parasitoid in warehouse number 3, on the surface of the mass of ears, where we estimated the presence of 200 thousand caterpillars.

Ten ears were separated on the surface and at each depth of the warehouses (10, 20, 30, and 40 cm), grouped for identification by means of a plastic string. The ears were removed 10 days after the last parasitoid release (*B. hebetor*). These ears were maintained at 25°C inside PVC® tubes closed with voile fabric, allowing biological control effectiveness to be subsequently evaluated.

In order to evaluate effectiveness, we ran an analysis of variance and compared the means by the Tukey test ($P \geq 0.05$) for the number of emerged adults and the percentage (%) of damaged grain (evaluated by the presence of adult exiting holes and by the presence of insects inside the kernel). These variables were obtained for the various treatments and depths. The percentage reductions of the treatments in relation to the control was also calculated for these variables. A linear correlation analysis was carried out to determine whether an association existed between the number of emerged adults and percentage of damaged grain.

RESULTS AND DISCUSSIONS

Parasitism of *T. pretiosum* on *S. cerealella* at different depths in the mass of corn grain

Total parasitism by *T. pretiosum* on *S. cerealella* eggs was low for the conditions

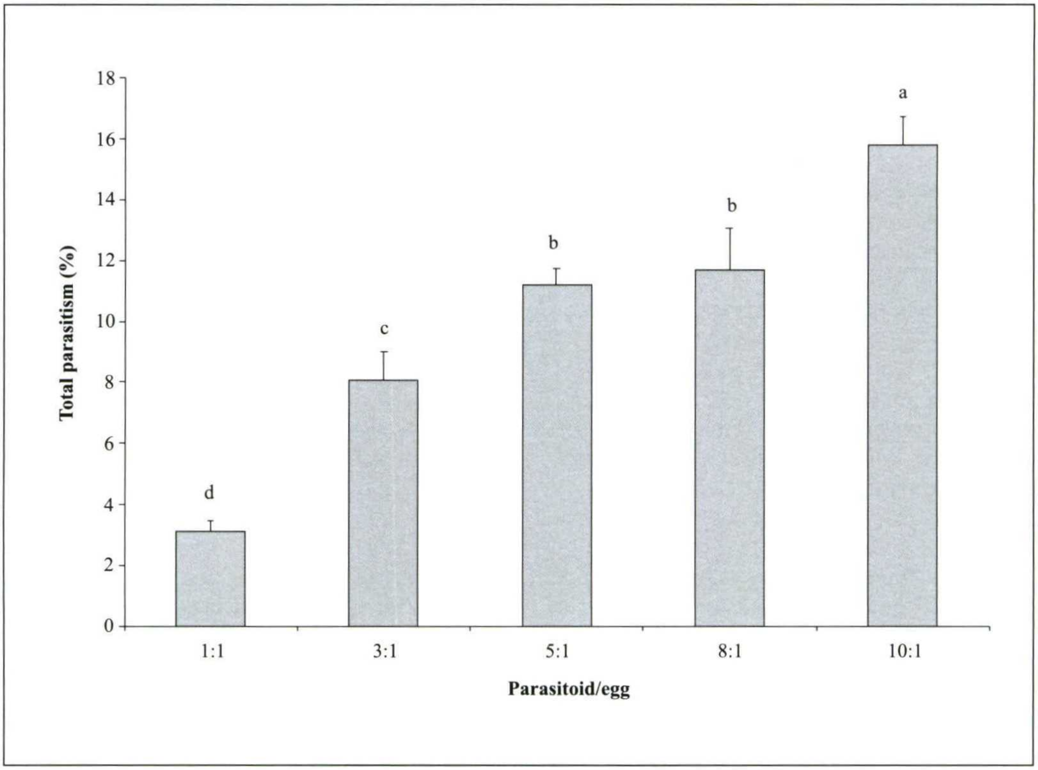

Figure 1. Total parasitism (% over egg total) of different numbers of *Trichogramma pretiosum* released on *Sitrotroga cerealella* eggs located at a depth of up to 40 cm in the mass of grains. Means followed by the same letter do not differ by Tukey test (P≥0.05).

of the experiment, ranging from 3.3 to 15.8% in the releases of different numbers of the parasitoid (Fig. 1). We also verified that the increased numbers of released *T. pretiosum* did not result in increased parasitism on the surface, starting at a proportion of three parasitoids for each egg of the moth (Fig. 2), which could be due to the occurrence of superparasitism, as recorded by SCHÖLLER *et al.* (1996). These authors, testing releases of different numbers of the parasitoid, verified that superparasitism occurred at smaller depths when great releases were made. These authors drew attention to the fact that the parasitism evaluation method that uses counts of numbers of black eggs may result in errors, since superparasitized eggs do not show parasitoid development and do not become black.

The parasitoids released on the mass of corn grains were able to parasitize *S. cerealella* eggs located as far down as 40 cm in depth (Table 1, Fig. 2), in agreement with SCHÖLLER *et al.* (1996), who detected penetration of *T. evanescens* females to a 55 cm depth in wheat. Parasitism decreased as the eggs were located at greater depths.

Higher numbers of released parasitoids resulted in greater parasitism. Studies conducted by BROWER (1990) confirmed that the release of a higher number of *T. pretiosum* results in greater parasitism. Although this parasitoid is normally attracted to light, it remains in the mass of grains, even in open environments, searching for eggs, attracted

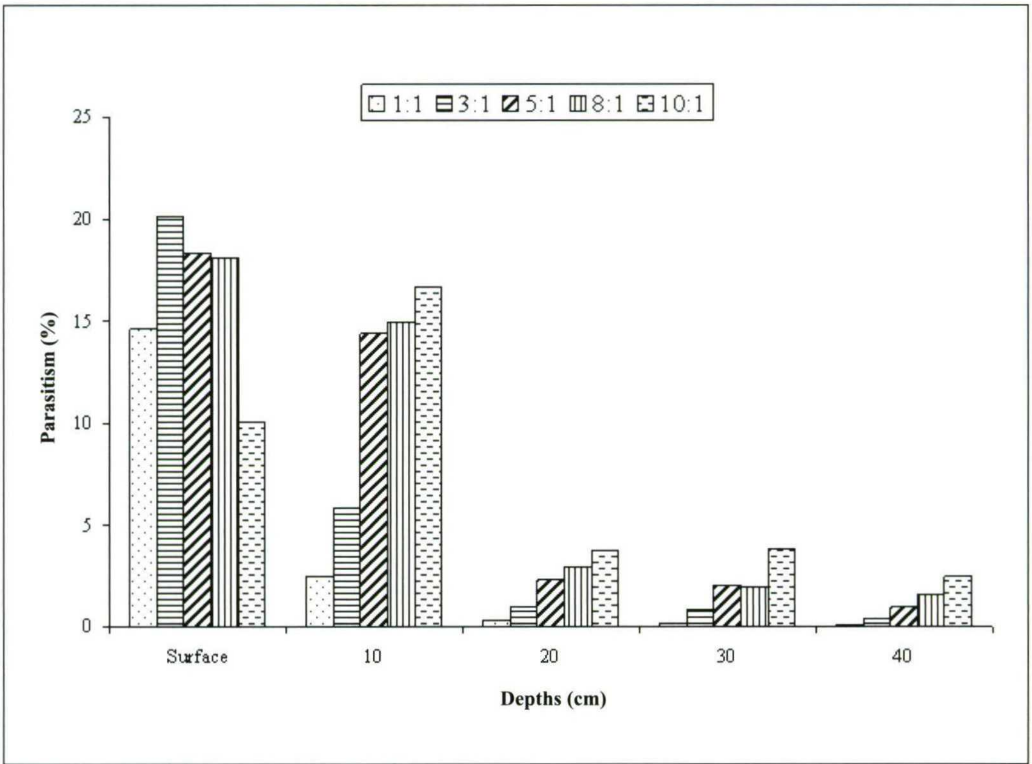

Figure 2. Percentage of *Sitrotroga cerealella* eggs distributed on the surface and at 4 depths, parasitized by different numbers of *Trichogramma pretiosum* released in the mass of corn grains. Temperature: 25±2°C; RH: 60±10%; photophase: 14 hours.

by chemical substances produced by the host (kairomones). At greater parasitoid populations (referred to by the author as "high releases"), a 76% parasitism on *Cadra cautella* eggs (Walker) was observed on the surface, with a mean reduction of 2.5% for each centimeter of depth.

In the present research, the low total parasitism obtained could be due to the artificial moth egg distribution conditions in the mass of grains, which was uniform for all depths. Studies can be conducted to determine the manner by which *S. cerealella* populations are distributed as depth becomes greater, in an attempt to obtain more realistic data.

The parasitism reductions in the various depths were variable depending on the released rate (Table 1), with a 1.9% mean reduction for each centimeter of depth. SCHÖLLER *et al.* (1996) also verified that the dispersal of *Trichogramma* females in the mass of wheat grains in the vertical direction is not uniform, decreasing as depth becomes greater.

For stored-grain moths, BROWER (1988) observed that control success depends on the number of *T. pretiosum* released and on the frequency of releases. Its use can be viable due to their specificity (allowing the joint use with other control agents) and reduced size (causing small grain contamination, as well as facilitating their movement in the mass of grain).

Even though parasitism was high in relation to the surface in releases where higher parasitoid numbers were used, it also

Table 1. **Parasitism (% in relation to the surface) of different numbers of *Trichogramma pretiosum* released in the mass of hybrid corn grains, on *Sitrotroga cerealella* eggs distributed at 4 depths. Temperature: 25±2°C; RH: 60±10%; photophase: 14 hours.**

Treatments[a]	Depth (cm)[b]			
	10	20	30	40
1:1	20.92±2.34 cA	8.08± 0.74 cB	5.73±0.69 cB	1.63±0.24 dC
3:1	38.91±3.70 bA	26.95±1.93 bA	15.89±1.37 bB	12.81±1.22 cB
5:1	76.64±6.21 aA	57.77±4.18 aA	49.30±4.66 aA	20.66±2.08 bcB
8:1	81.24±5.50 aA	63.20±3.40 aAB	43.67±4.03 aBC	27.68±0.85 abC
10:1	84.42±6.44 aA	76.86±8.11 aAB	76.38±8.45 aAB	53.66±6.64 aB

[a]No. *Trichogramma pretiosum*: no. eggs
[b]Means followed by the same upper case letter in the row and lower case letter in the column are not different by Tukey test (P≥0.05). Data transformed to log (x + 1).

decreased significantly as depth increased. Based on this, it can be concluded that a more effective control can be obtained with the use of this parasitoid as a preventive agent, in the very beginning of the storage period or right after fumigation, when the attack by the pest is still at the surface, before a large population becomes established, as proposed by BROWER (1982, 1983b, 1984).

On the other hand, LEWIS AND REDLINGER (1969) showed that a reduction in parasitism occurs according to host-egg age: greater parasitism is obtained in the first 60 hours of incubation. REZNIK AND UMAROVA (1990) suggested that parasitism can be even inhibited in the presence of old eggs, at a more advanced stage of larval development of the pest, reiterating the necessity for continuous storage-environment monitoring, allowing *T. pretiosum* to be used as soon as the first adults of the moth are detected, when a minimum amount of moth kairomones are already present to stimulate parasitism (VINSON 1976; REZNIK AND UMAROVA 1991).

Effect of *T. pretiosum* alone and in association with *B. hebetor* for the control of *S. cerealella* in corn ears stored in a warehouse

The number of emerged adult moths in treatments involving parasitoids was significantly smaller when compared to the control (Fig. 3). No interaction occurred between depths and treatments, and the parasitoids always had a similar behavior at the various depths (Table 2). There were no differences between treatments with or without the addition of *B. hebetor*, although numerically there was a tendency of reduction in the

Table 2. **Number of *Sitrotroga cerealella* adults emerged from corn ears at 5 depths, after infestation with *Sitrotroga cerealella* eggs followed by releases of *Trichogramma pretiosum* or *Trichogramma pretiosum* in association with *Bracon hebetor*. Temperature: 25±2°C; RH: 60±10%; photophase: 14 hours.**

Treatments	Depth (cm)[a]					
	surface	10	20	30	40	Mean
Control	57.8±22.91	57.8±12.42	59.4±16.28	56.6±18.41	74.6±14.23	61.2 a
T. pretiosum	19.6± 4.47	18.6± 8.56	20.4± 4.88	13.8± 8.03	47.8±13.52	24.0 b
T. pretiosum + *B. hebetor*	11.8± 6.88	25.4±10.07	22.6± 9.43	11.0± 2.95	22.8± 5.16	18.7 b
Mean	29.7 A	33.9 A	34.1 A	27.1 A	48.40 A	

[a]Means followed by the same upper case letter in the column or lower case letter in the row are not different by Tukey test (P≥0.05). Data transformed to $\sqrt{x} - 0.5$.

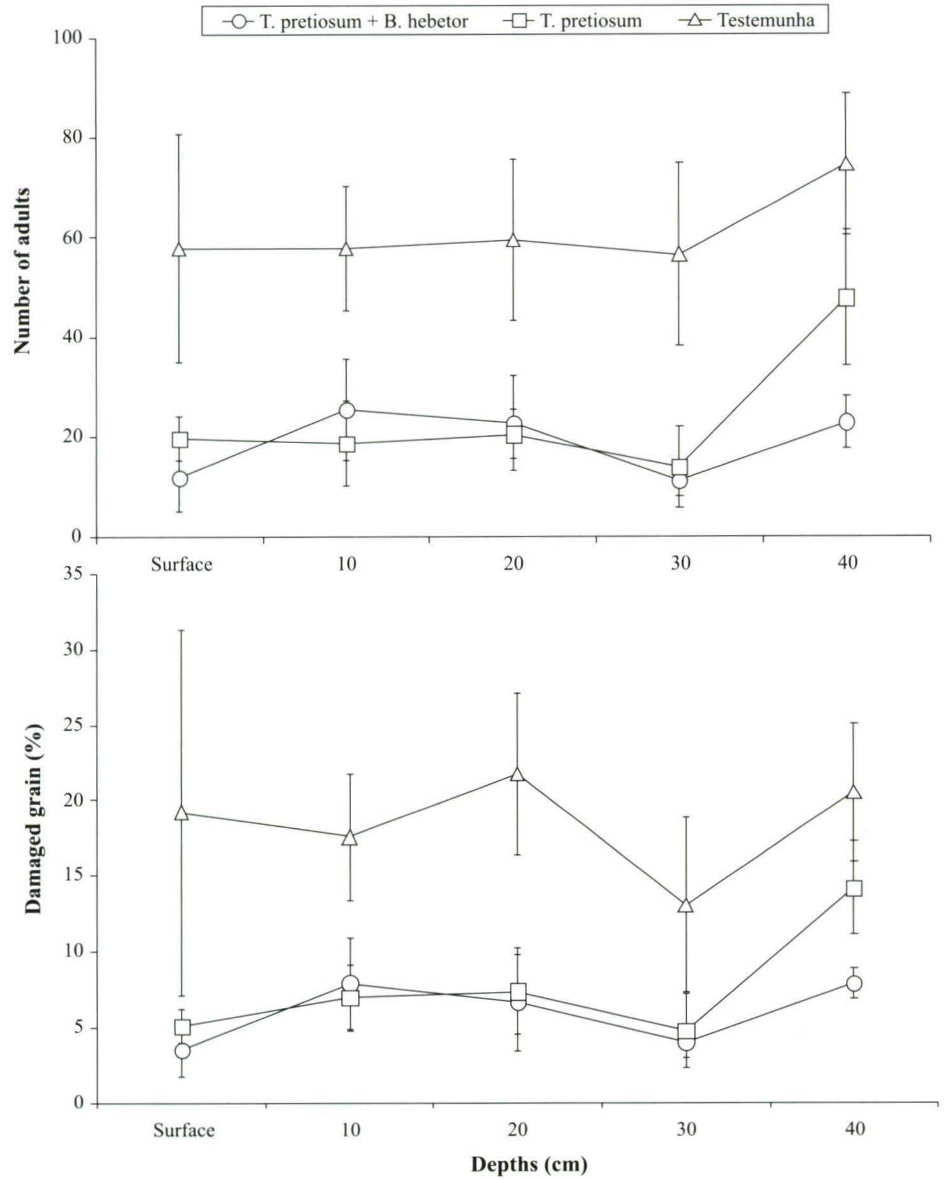

Figure 3. Variation in *Sitrotroga cerealella* population and damage produced, on the surface and at 4 depths in the mass of corn ears, stored in a warehouse after being submitted to infestation and release of *Trichogramma pretiosum* alone, or release of *Trichogramma pretiosum* in association with *Bracon hebetor*. Temperature: 25±2°C; RH: 60±10%; photophase: 14 hours.

Table 3. **Population reduction (% over the control) of *Sitrotroga cerealella* obtained in corn ears at 4 depths, after infestation with *Sitrotroga cerealella* eggs followed by release of *Trichogramma pretiosum* or *Trichogramma pretiosum* in association with *Bracon hebetor*. Temperature: 25±2°C; RH: 60±10%; photophase: 14 hours.**

Treatments	Depth (cm)					
	Surface	10	20	30	40	Mean
T. pretiosum	66.09	67.82	65.66	75.61	35.92	60.74
T. pretiosum + *B. hebetor*	79.58	56.05	61.95	80.56	69.43	69.43
Mean	72.84	61.94	63.80	78.09	52.67	

Table 4. **Percentage of grains damaged by the attack of *Sitrotroga cerealella*, obtained in corn ears at 5 depths, after infestation with *Sitrotroga cerealella* eggs, followed by release of *Trichogramma pretiosum* alone or *Trichogramma pretiosum* in association with *Bracon hebetor*. Temperature 25±5°C; RH: 60±10%; photophase: 14 hours.**

Treatments	Depth (cm)[a]					
	Surface	10	20	30	40	Mean
Control	19.2±2.13	17.5±4.22	21.7±5.40	16.1±5.73	20.5±4.54	18.9 a
T. pretiosum	5.1±1.13	6.9±2.19	7.8±2.84	4.82.43	14.2±3.05	7.7 b
T. pretiosum + *B. hebetor*	3.5±1.77	7.9±3.00	6.6±3.17	4.0±1.02	7.8±0.99	5.9 b
Mean	8.9 A	10.8 A	11.9 A	8.3 A	14.2 A	

[a]Means followed by the same upper case letter in the column or lower case letter in the row are not different by Tukey test (P≥0.05). Data transformed to log (x + 0.5).

number of adults emerged in the associated treatment.

These results agree with those by Brower and Press (1990) for *C. cautella*, where no additional effect of *B. hebetor* release after the release of *T. pretiosum* on moth control was detected. However, for *Plodia interpunctella* (Hübner) populations, those authors obtained reductions of 37.3%, 66.1%, and 84.3% in relation to the control with releases of *T. pretiosum*, *B. hebetor*, and *T. pretiosum* in association with *B. hebetor*, respectively.

The control percentage, calculated according to Keever *et a*l. (1986), showed that the degree of reduction in moth population (as compared to the control) was 60.7% at the *T. pretiosum* release site, and a little higher (69.43%) for the *T. pretiosum - B. hebetor* combination (Table 3). The results obtained in the present study are promising, since authors such as McGaughey and Kinsinger (1978) reported reductions only at the second generation of a *S. cerealella* population, achieving a on averege 78% with the use of *Bacillus thuringiensis* Berliner; Storey (1975) recorded a mean reduction of 56.7% in the population of the same moth when submitted to an atmosphere treatment with an O_2 content that was reduced to 1% for 24 hours.

The parasitoid releases reduced the percentage of damaged grain in stored corn ears (Fig. 3). No significant difference was detected between depths, either in the release of *T. pretiosum* alone or in the joint release of *T. pretiosum* and *B. hebetor* (Table 4). Consequently, in order to achieve the same control level herein obtained to occur, the release of *T. pretiosum* alone would have been sufficient.

Press *et al*. (1982) obtained a 97% reduction in the population of *Ephestia cautella* (Walker) using *B. hebetor*. According to Nickle and Hagstrum (1981), the effectiveness of this parasitoid can be higher if food

Table 5. **Reduction in percentage of damaged grain (in relation to the control) caused by *Sitrotroga cerealella*, obtained in corn ears at 4 depths, after infestation with *Sitrotroga cerealella* eggs, followed by release of *Trichogramma pretiosum* in association with *Bracon hebetor*. Temperature 25±5ºC; RH: 60±10%; photophase: 14 hours.**

Treatments	Depth (cm)					
	Surface	10	20	30	40	Mean
T. pretiosum	78.71	67.45	57.63	73.18	38.61	63.12
T. pretiosum + *B. hebetor*	85.39	55.96	67.00	76.43	65.50	70.06
Mean	82.05	61.70	62.31	74.80	52.05	

is supplied to the adults. They even recommended the distribution of parasitized caterpillars on the surface of the grain, as long as it is periodically cleaned to remove insect remains from the storage facility. KEEVER *et al.* (1985, 1986) stated that for control to be successful, releases must be done in the beginning of the storage season, when a small pest population is present, but before the product has been damaged, since this ectoparasitoid will only attack the later instars of the larval stage of lepidopterans, when damage has already been inflicted.

The mean of reduction in grain damaged by *S. cerealella* in the mass of corn ears stored in the warehouse with the release of *T. pretiosum* was 63.1%, and 70.1% for *T. pretiosum* in association with *B. hebetor* (Table 5). These are acceptable values when compared with those obtained by BITRAN *et al.* (1981) with chemical control. Those authors obtained grain damage reductions of 30, 46, 37, 48, and 83% in corn ears using malathion, decamethrin, fumigation, fumigation followed by malathion, and fumigation followed by decamethrin, respectively.

With regard to the linear correlation analysis for number of emerged adults and percentage of damaged grain, a significant correlation (r=0.94) was observed, indicating that a relation existed between the two variables; the positive value indicated that the increase in number of adults implied an increase in percentage of damaged grain, or vice versa.

The 30 cm depth showed numerically divergent results because, although there was no statistical difference in relation to other depths, a little higher control level could be noted, with greater population reduction and lower grain damage. At this depth, the control also showed smaller attack values, which could be due to a deficiency in *S. cerealella* egg distribution uniformity in the various depths. This moth's attack simulation could have been more true to reality if, after infesting with eggs, parasitoid releases were made after the development of one or two generations.

Because in the present research the release of *B. hebetor* was performed based on a theoretical model, at 25 days after infestation with *S. cerealella* eggs, maybe such effectiveness could have been increased if the release was done previously or with a higher number of parasitoids. Researches in this respect are suggested. Nevertheless, there was a numerical tendency of damage reduction, either in relation to the percentage of emerged adult moths (Table 3), or in relation to the percentage of damaged grain (Table 5), which also demonstrates the potential use of this parasitoid in combination with *T. pretiosum*, whose feasibility was clearly demonstrated in this work, as well as the use of other *Trichogramma* species for several crops and pests in Brazil (PARRA AND ZUCCHI 2004).

RESUMEN

INOUE, M. S. R., D. E. NAVA, J. R. P. PARRA. 2008. Control biológico de *Sitotroga cerealella* en maíz almacenado usando *Trichogramma pretiosum* y *Bracon hebetor*. *Bol. San. Veg. Plagas*, **34**: 517-527.

Con el objetivo de evaluar la posibilidad de control de *Sitotroga cerealella* (Olivier) en maíz almacenado con el parásito de huevos *Trichogramma pretiosum* Riley, solo ó asociado al parásito larval *Bracon hebetor* Say, se llevó a cabo el siguiente trabajo. Los resultados mostraron que el parásito *T. pretiosum* presentó un gran potencial para el control de *S. cerealella* en maíz almacenado como granos y como espigas. Los parásitos liberados sobre los granos de maíz parasitaron huevos de *S. cerealella* hasta 40 cm de profundidad. El parasitismo de *T. pretiosum* disminuyó un 1,92% para cada centímetro de profundidad. Para el maíz en espigas, la liberación de *T. pretiosum* fue eficiente en el control de *S. cerealella*, provocando una reducción de 60,7% de la población de adultos de la polilla y de 63,1% en el porcentaje de daños causados a las espigas almacenadas en silos de alambre. La liberación de *T. pretiosum* asociada a *B. hebetor* no mostró ventajas para el control de *S. cerealella*, ya que no fueron observadas diferencias significativas entre tratamientos, a pesar de existir una tendencia numérica de mayor eficiencia para la asociación de los dos parásitos, con un aumento de 8,69% en la reducción de la población de adultos de *S. cerealella* y de 1,72% en la reducción de daños en espigas, en comparación al tratamiento con *T. pretiosum*.

Palabras clave: Granos almacenados, parásito de huevos, parásito de larvas.

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