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SELECTION OF *TRICHOGRAMMA PRETIOSUM* LINEAGES FOR CONTROL OF *GRAPHOLITA MOLESTA* IN PEACH

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ABSTRACT

Grapholita molesta (Lepidoptera: Tortricidae) is one of the main pests of peach trees in Brazil, causing fruit losses of 3-5%. Among possible biological control agents, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) has been found in peach orchards. Our objectives were to study the rearing of *T. pretiosum* in eggs of *G. molesta* and *Anagasta kuehniella* (Lepidoptera: Pyralidae), and select lineages of this parasitoid that have the potential to control *G. molesta*. Selection of best lineages was made from 5 populations of *T. pretiosum* collected from organically-cultivated peach orchards. The study was done under controlled temperature (25 ± 2°C), relative humidity (70 ± 10%) and 14:10 h (light:dark) photoperiod conditions. *Grapholita molesta* eggs were found to be adequate hosts for the development of *T. pretiosum*, and the parameters for number of parasitized eggs, percent parasitized eggs, and sex ratio were similar to those for *A. kuehniella* eggs. The highest rate of parasitism of *G. molesta* eggs occurred in eggs with up to 48 h of embryonic development. Among the lineages of *T. pretiosum* that were collected, HO8, PO8, PEL, and L3M showed the best biological performance and are therefore indicated for semi-field and field studies for biological control of oriental fruit moth.

Key Words: biological control, temperate climate fruit trees, peach, egg parasitism

RESUMO

Grapholita molesta (Lepidoptera: Tortricidae) é uma das principais pragas do pessegueiro no Brasil, causando perdas de 3-5% da produção. Dentre os agentes de controle biológico *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) tem sido encontrado nos pomares de pessegueiros. O objetivo deste trabalho foi estudar a criação de *T. pretiosum* em ovos de *G. molesta* e *Anagasta kuehniella* (Lepidoptera: Pyralidae) e selecionar as linhagens de *T. pretiosum* com potencial de controle de *G. molesta*. A seleção de linhagens foi realizada com cinco populações de *T. pretiosum* coletadas em pomares de pessegueiro cultivados sob o sistema orgânico de produção. O estudo foi realizado em condições controladas de temperatura (25 ± 2°C), umidade relativa (70 ± 10%) e fotofase (14h). Ovos de *G. molesta* são hospedeiros adequados ao desenvolvimento de *T. pretiosum* uma vez que, nas variáveis estudadas número de ovos parasitados, porcentagem de parasitismo e razão sexual, os valores foram equivalentes aos criados em ovos de *A. kuehniella*. O maior parasitismo de ovos de *G. molesta* ocorreu com posturas de até 48h de desenvolvimento embrionário. Das linhagens de *T. pretiosum* coletadas, H08, PO8, PEL e L3M apresentaram melhor desempenho biológico, sendo, portanto, indicadas para estudos de semi-campo e campo para o controle biológico da mariposa-oriental.

Translation provided by the authors.

Rio Grande do Sul state is the greatest producer of peaches in Brazil, accounting for 50% of the country's production (FNP CONSULTORIA & COMÉRCIO, 2008). It is an economically important crop in this state; however, pest insects, especially the oriental peach moth, *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae), have been among the principal limiting factors for the productivity of peaches (Salles 1998; Botton et al. 2003).

Caterpillars of *G. molesta* attack fruit and new sprouts that have not yet been lignified (Rosenthal et al. 1994; Salles 1998). Fruit loss due to caterpillars can reach 3-5% (Botton et al. 2003). The galleries that the caterpillars construct in branches and on fruit make them difficult to kill; traditionally, sequential applications of broad spectrum insecticides, mainly organophosphates, have been used for control (Kovaleski & Ribeiro 2002; Arioli et al. 2004).

Control of *G. molesta* by peach growers, who apply excessive amounts of insecticide, has negatively affected beneficial entomofauna; consequently, additional insecticide applications become necessary to control secondary pests (Salles 1998; Botton et al. 2003). Abusive use of insecticides also pollutes the environment, animals and humans, and leaves residues in fruit (Salles 1998).

In an effort to find ways to avoid abusive overuse of pesticides to control *G. molesta*, research has been directed towards finding alternative control methods, along with application of insecticides that are less toxic to this pest's natural enemies (Pinheiro et al. 2008). Use of a sex pheromone lure is one option, but the cost is high (Arioli et al. 2006). Other studies have searched for biological control agents, such as *Macrocentrus ancylivorus* Rohwer 1921 (Hymenoptera: Braconidae) as a parasitoid of caterpillars, and the egg parasitoid, *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) (Afonso 2001). *Macrocentrus ancylivorus* is widely distributed throughout the major peach-growing regions in the world, and it has been successfully used to control *G. molesta* (Haeussler 1932). In Brazil, it is one of the most abundant parasitoids (Nava, D. E., personal communication). *Trichogramma pretiosum* is an important natural enemy of pest caterpillars; it is found in various agroecosystems, distributed almost worldwide (Pinto 1997). It is easy to culture in the laboratory (Pratissoli & Parra 2000) and the costs are not too high for Brazilian agricultural conditions (Parra & Zucchi 2004). Because it is an egg parasitoid, *T. pretiosum* interrupts the biological cycle of *G. molesta* before the caterpillars hatch, which is highly desirable, and it is recommended for use in the field.

Our objectives were to study the rearing of *T. pretiosum* in eggs of *G. molesta* and *Anagasta kuehniella* (Zeller 1879) (Lepidoptera: Pyralidae), and to select lineages of this parasitoid that have the potential to control *G. molesta*.

Material and Methods

The experiments were conducted at the Insect Biology and Biological Control Laboratory of the Eliseu Maciel Agronomy College, of the Federal University of Pelotas, Capão do Leão, Rio Grande do Sul, Brazil. The experiments were done in a climate chamber maintained at 25 ± 2°C, 70 ± 10% relative humidity and 14:10 (light: dark) photoperiod.

A stock culture of *G. molesta* adults was maintained at 25 ± 2°C, 70 ± 10% relative humidity and 16:8 (light: dark) photoperiod in cylindrical PVC cages PVC (20 × 20 cm), lined internally with smooth plastic for an egg-laying surface and closed at the top with tulle gauze. The adults were fed on 15% honey solution and 0.3 g of nipagin (methyl parahydroxybenzoate) in 200 mL of distilled water. The plastic that lined the cages had eggs laid by the moths; these plastic sheets were removed daily and transferred to a plastic recipient (30 × 20 × 5 cm) containing artificial diet for larval development, based on the methodology proposed by Arioli (2007). Near pupation time, cotton cloth was placed over the container, to serve as a substrate for pupation. The pupae were removed from this cloth and they were maintained in Petri dishes (9 × 15 cm) until the adults emerged and were subsequently transferred to the rearing cages. The alternative host *A. kuehniella* was reared with the methodology described by Parra et al. (1989).

Development of *T. pretiosum* on Eggs of *G. molesta* and *A. kuehniella*

Initially we reared 2 groups of *T. pretiosum* on the 2 different hosts (*A. kuehniella* and *G. molesta*) for 3 successive generations to avoid possible pre-imaginal conditioning. The parasitoids reared on *G. molesta* eggs were tested on *A. kuehniella* and those reared on *A. kuehniella* eggs were tested on *G. molesta*. We tested the lineage of *T. pretiosum* that we named the PEL colony, from a laboratory colony originally started from specimens collected in peach orchards in Pelotas, 31°40'47"S, 52°26'24"W. Each of 20 female parasitoids up to 24 h old was presented with 30 eggs (0-24 h old) of *G. molesta* or *A. kuehniella* on cards. The cards were removed and new ones added every 24 h until the female parasitoid died. The parasitoids were kept individually in glass tubes (12 × 75 mm), closed with PVC film and fed with a drop of honey. We recorded the number of parasitized eggs, emergence of adult parasitoids, and the sex ratio of the adult parasitoids. We reared the parasitoids for 5 successive generations and repeated the entire rearing experiment 3 times for a total of 15 rearings (Table 1).

Effect of Age of *G. molesta* Eggs on Parasitism by *T. pretiosum*

Grapholita molesta eggs that were 24, 48, 72, and 96 h old were offered to *T. pretiosum* to determine the age of eggs that allowed the most parasitism. One-hundred eggs of each age were offered to 20 female parasitoids (0-24 h old). This was repeated 5 times. The wasps were confined in

TABLE 1. NUMBER OF EGGS PARASITIZED, PERCENT EMERGENCE, AND SEX RATIO OF *TRICHOGRAMMA PRETIOSUM* LINE, PEL, REARED ON EGGS OF *GRAPHOLITA MOLESTA* AND *ANAGASTA KUEHNIELLA*, DURING 5 SUCCESSIVE GENERATIONS.

	Number of eggs parasitized		
Generation	*A. kuehniella*	*G. molesta*	(*F*; *P*; *df*)
1	7.47 ± 1.11 bA	8.06 ± 0.90 bA	0.28; 0.64; 1
2	14.65 ± 1.61 aA	13.37 ± 1.05 abA	0.35; 0.56; 1
3	11.29 ± 1.35 abA	15.13 ± 1.27 aA	4.71; 0.56; 1
4	9.18 ± 1.68 abA	14.28 ± 1.60 aA	3.75; 0.08; 1
5	14.61 ± 0.89 aA	13.33 ± 1.82 abA	0.29; 0.59; 1
(*F*; *P*; *df*)	6.16; <0.001; 4	3.99; <0.001; 4	
	Emergence* (%)		
	A. kuehniella	*G. molesta*	(*F*; *P*; *df*)
1	88.52 ± 3.55 aA	79.77 ± 4.97 bA	8.99; 0.007; 1
2	92.93 ± 2.40 aA	81.70 ± 5.97 abA	1.89; 0.18; 1
3	91.77 ± 2.21 aA	95.09 ± 1.61 aA	6.59; 0.02; 1
4	97.98 ± 2.02 aA	85.11 ± 3.37 abB	4.17; 0.06; 1
5	98.37 ± 5.46 aA	81.47 ± 5,41 abB	3.54; 0,06; 1
(*F*; *P*; *df*)	2.61; 0.04; 4	8.42; 0.012; 4	12.82; 0.02; 1
	Sex ratio		
	A. kuehniella	*G. molesta*	(*F*; *P*; *df*)
1	0.59 ± 0.05 aA	0.61 ± 0.05 aA	0.21; 0.67; 1
2	0.53 ± 0.05 aA	0.61 ± 0.09 aA	1.05; 0.35; 1
3	0.33 ± 0.15 aA	0.72 ± 0.03 aA	7.27; 0.11; 1
4	0.42 ± 0.07 aA	0.59 ± 0.08 aA	3.88; 0.30; 1
5	0.46 ± 0.07 aA	0.71 ± 0.04 aA	4.54; 0.17; 1
(*F*; *P*; *df*)	0.90; 0.48; 4	1.43; 0.26; 4	

Means followed by the same letter, lower case in the column and capitalized in the row, do not differ significantly, based on the Tukey test ($P = 0.05$). * Data transformed by arcsine √(x/100). Degrees of freedom = *df*. Parasitism period: 24 h, Temperature: 25 ± 2ºC, RH: 70 ± 10%, and 14:10 h (light: dark) photoperiod.

glass tubes (8 × 2.5 cm) and fed with honey. The *G. molesta* eggs were offered for 24 h, after that time the females were removed from the tubes, and the eggs were transferred to a climatic chamber to evaluate the percentage parasitism, evidenced by darkening of the eggs.

Selection of *T. pretiosum* Lineages

We tested 3 lineages of *T. pretiosum* (T08, P08 and H08) collected from the municipality of Bento Gonçalves, RS, Brazil (29º09'64" S, 51º90'12" W, 690 m de altitude), in Nov 2007 from peach orchards and a vegetable garden, both maintained under an organic production system, and 2 lineages (PEL and L3M) from the Biology and Biological Control laboratory of UFPEL that originated from specimens collected from peach orchards in Pelotas (31°40'47"S, 52°26'24"W). Recently-emerged females of each line were placed individually in glass tubes (12.0 × 75.0 mm) and were fed with a drop of honey placed on the tube wall. Cards with 30 eggs (0-24 h old) were offered to each female. The parasitism was allowed for 24 h, and after this time the cards with egg were removed from the glass tubes and placed inside the other tube together with a moistened filter paper and transferred to a climatic chamber.

The biological variables evaluated were number of parasitized eggs, percent parasitism and emergence, longevity of the males and females, and sex ratio. The sex ratio was calculated by the formula: sex ratio = ♀/(♀+♂). To determine longevity, 20 pairs were placed in individual glass tubes and were fed with a drop of honey. Daily observations were made from emergence until the parasitoids died.

Data Analysis

The experimental design was completely randomized. The data were submitted to analysis of

variance and the means compared with the Tukey test, with an alpha level of 5%.

Results and Discussion

Development of *T. pretiosum* in *G. molesta* and *A.* kuehniella Eggs

The host species did not significantly affect parasitism by the PEL *T. pretiosum* line (Table 1). However, the number of parasitized eggs per female varied significantly among the generations in both host species. The number of eggs parasitized during the first (7.47) and the fifth (14.61) generations of *T. pretiosum* reared on eggs of *A. kuehniella* differed significantly, and between the first generation (8.06) and the third and fourth generations (15.13 and 14.28, respectively), when *T. pretiosum* was reared on *G. molesta* eggs, indicating adaptation occurring during successive generations.

The percent emergence of *T. pretiosum* reared for successive generations on eggs of *A. kuehniella* did not differ significantly, but the parasitoids reared on eggs of *G. molesta* showed significantly superior performance by the third generation (95.09%) (Table 1). When we analyzed emergence of *T. pretiosum* from the different hosts, by generation, we observed a significant difference between the fourth and fifth generations (Table 1). Although there were significant differences in the emergence of parasitoids between the fourth and fifth generations, the percent emergence was above 80% for all generations (Table 1), demonstrating that it is viable to rear this parasitoid on *G. molesta* eggs.

The sex ratio of the third, fourth, and fifth generation parasitoids reared on *A. kuehniella* was lower than 0.50, indicating a larger number of males than females, but on eggs of *G. molesta* more females than males emerged in each of the 5 generations.

Effect of Age of *G. molesta* Eggs on Parasitism by *T. pretiosum*

The percent parasitism by *T. pretiosum* on *G. molesta* eggs of different ages did not differ significantly between 12 (70.8%) and 48 h (63.4%), but the percentage was significantly lower for 72 and 96 h eggs (30.4 and 10.6%, respectively (Fig. 1). A reduction in the number of eggs parasitized with increasing egg age was also demonstrated by Nava & Parra (2006), who did not observed a significant difference in the percent parasitism in eggs of *Stenoma catenifer* Walsinghan (Lepidoptera: Elachistidae) up to 60 h old, although it did fall significantly after 84 h of egg development. According to Vinson (1997), this could be a consequence of changes in egg composition, as the nutrient reserves become converted into tissues

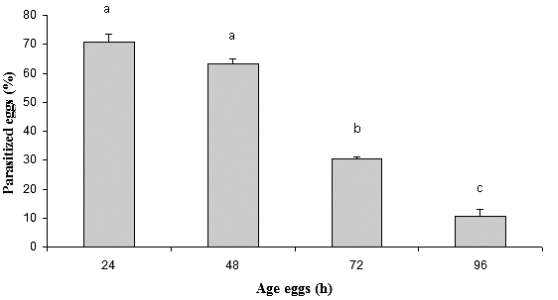

Fig. 1. Effect of age of eggs of *Grapholita molesta* on the percentage parasitism by *Trichogramma pretiosum*. Temperature: 25 ± 2ºC, RH: 70 ± 10%, and 14:10 h (L:D) photoperiod. Numbers in columns followed by the same letter are not significantly different by the Tukey test ($P = 0.05$).

that are more complex chemically. Similarly, older eggs of *Helicoverpa zea* (Bod.) resulted in longer egg to adult development time and smaller *T. pretiosum* parasitoids (Sá & Parra 1994). Consequently, both for laboratory work and for field studies, we recommend using *G. molesta* eggs up to 48 h old, or successive liberations so that eggs of different ages will be found in the field.

Selection of Lineages of *T. pretiosum*

Among the 5 lineages (PEL, L3M, T08, P08, and H08) of *T. pretiosum* that we evaluated for control of *G. molesta*, from 14.29 to 16.38 eggs were parasitized, corresponding to 47.61 to 54.58% parasitism in 24 h for lineages T08 and L3M, respectively, but emergence of adult parasitoids was always above 84% and not different among the 5 lineages, (Table 2).

The sex ratio differed significantly among the lineages of *T. pretiosum* (Table 2). Lineages L3M, H08, and PEL had a sex ratio above 0.5 (more females than males). Lineages T08 and P08 had more males than females, when compared to L3M (Table 2). The higher values are close to those found on *S. catenifer* eggs (0.79-0.61) (Nava et al. 2007). Higher ratios were reported for 20 lineages of *T. pretiosum* on *S. frugiperda* eggs (0.71-0.80) (Beserra et al. 2003). Because biological control depends on females for parasitizing, a line with a higher proportion of females would be desirable.

There were significant differences in the longevity of the females among several different lineages of wasps: PEL (5.0 d), L3M (8.75 d) and H08 (8.53 d) (Table 2); lineages T08 (7.33 d) and P08 (7.05 d) did not differ significantly.

According to Molina et al. (2005) the host and the origin of the parasitoid can affect parasitoid longevity. Gomes (1997) reported that longevity is an important characteristic that should be taken into consideration in biological control programs,

TABLE 2. NUMBER OF PARASITIZED EGGS, PERCENTAGE PARASITISM, PERCENT EMERGENCE, SEX RATIO, AND LONGEVITY (D) OF MALES AND FEMALES OF 5 LINEAGES OF *TRICHOGRAMMA PRETIOSUM* REARED ON *GRAPHOLITA MOLESTA* EGGS.

Line	Parasitized eggs (*n*)	Parasitism (%)	Emergence* (%)	Sex ratio	Female longevity (d)	Male longevity (d)
PEL	15.68 ± 1.89 a	52.28 ± 6.29 a	86.28 ± 4.63 a	0.70 ± 0.04 ab	5.0 ± 0.30 b	3.11 ± 0.17 b
L3M	16.38 ± 2.02 a	54.58 ± 6.72 a	86.50 ± 5.66 a	0.77 ± 0.05 a	8.75 ± 0.74 a	4.72 ± 0.22 a
T08	14.29 ± 1.99 a	47.61 ± 6.64 a	84.12 ± 4.50 a	0.31 ± 0.09 c	7.33 ± 0.55 ab	3.25 ± 0.19 b
P08	16.05 ± 1.24 a	53.50 ± 4.14 a	91.25 ± 3.25 a	0.45 ± 0.08 bc	7.05 ± 0.61 ab	4.69 ± 0.22 a
H08	15.32 ± 1.10 a	51.05 ± 3.67 a	89.32 ± 3.06 a	0.63 ± 0.07 ab	8.53 ± 0.69 a	3.88 ± 0.33 ab
F	1.41	1.24	0.44	6.14	5.96	7.84
P	0.23	0.30	0.77	<0.001	<0.001	<0.001
df	4	4	4	4	4	4

Means followed by the same letter in the same column do not differ significantly, based on the Tukey test ($P = 0.05$).
*Data transformed by arcsine √(x/100). Parasitism period: 24 h, temperature 25 ± 2°C, RH: 70 ± 10% and 14 h photophase.

since longer-living parasitoids will probably be more efficient in the field and able to parasitize more pest insect eggs. Male longevity was significantly longer in lineages L3M and P08 (4.72 and 4.69 d, respectively), than in lineages PEL and T08 (3.11 and 3.25 d) (Table 2).

Based on biological parameters observed, the lineage H08 showed the best performance, however the lineages PEL, L3M and P08 also gave good performance, and can be used for biological control of oriental fruit moth.

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