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Biological characteristics and thermal requirements of a Brazilian strain of the parasitoid *Trichogramma pretiosum* reared on eggs of *Pseudoplusia includens* and *Anticarsia gemmatalis*

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ABSTRACT

A new strain of the parasitoid *Trichogramma pretiosum*, was collected in Rio Verde County, State of Goiás, Central Brazil, and designated as *T. pretiosum* RV. This strain was then found to be the most effective one among several different strains of *T. pretiosum* tested in a parasitoid selection assay. Therefore, its biological characteristics and thermal requirements were studied, aiming at allowing its multiplication under controlled environmental conditions in the laboratory. The parasitoid was reared on eggs of *Pseudoplusia includens* and *Anticarsia gemmatalis* at different constant temperatures within an 18–32 °C temperature range. The number of annual generations of the parasitoid was also estimated at those temperatures. Results have shown that *T. pretiosum* RV developmental time, from egg to adult, was influenced by all temperatures tested within the range, varying from 6.8 to 20.3 days and 6.0 to 17.0 days on eggs of *P. includens* and *A. gemmatalis*, respectively. The emergence of *T. pretiosum* RV from eggs of *A. gemmatalis* was higher than 94% at all temperatures tested. When this variable was evaluated on eggs of *P. includens*, however, the figures were higher than that within the 18–30 °C range (more than 98%), and were also statistically higher than the emergence observed at 32 °C (90.2%). The sex ratio of the parasitoids emerged from eggs of *A. gemmatalis* decreased from 0.55 to 0.29 at 18–32 °C, respectively. However, for those emerged from eggs of *P. includens*, the sex ratio was similar (0.73, 0.72 and 0.71) at 20, 28 and 32 °C, respectively. The lower temperature threshold (*Tb*) and thermal constant (*K*) were 10.65 °C and 151.25 degree-days when the parasitoid was reared on eggs of *P. includens*; and 11.64 °C and 127.60 degree-days when reared on eggs of *A. gemmatalis*. The number of generations per month increased from 1.45 to 4.23 and from 1.49 to 4.79 when the parasitoid was reared on eggs of *P. includens* and *A. gemmatalis*, respectively, following the increases in the temperature.

1. Introduction

Soybean (*Glycine max* L. Merril), is a worldwide commercially important field crop, and from Argentina to the Southeast United States the velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae)] is one of the most important defoliator insects occurring on that crop (Panizzi and Corrêa-Ferreira, 1997; Hoffmann-Campo et al., 2003; Embrapa, 2006). Another insect, the soybean lopper [*Pseudoplusia includens* (Walker, 1857) (Lepidoptera: Noctuidae)] used to be considered of secondary importance and kept under control mainly by the natural occurrence of entomophatogenic fungi and parasitoids (Bueno et al., 2007). However, during recent years, there has been an increase in the occurrence of *P. includens* on soybean fields, as a consequence of the abusive use of non-selective chemicals and consequential reduction of these two biocontrol agents, among other natural enemies, which usually prevent *P. includens* outbreaks (Bueno et al., 2007; Van Lenteren and Bueno, 2003).

Regarding chemical control, it is important to consider that controlling *P. includens* is much more difficult than controlling *A. gemmatalis*. While *P. includens* remains mainly on the bottom and middle leaves, *A. gemmatalis* prefers the top leaves, therefore being easily reached by the insecticides sprayed on the crop. These factors altogether might be playing an important role, leading *P. includens* to become a key soybean pest (Sosa-Gomez and Oliveira, 2007). Thus, *P. includens* outbreaks have occurred in several Brazilian states (Mato Grosso, Goiás, São Paulo and Paraná for example), usually together with *A. gemmatalis* populations (Bueno et al., 2007; Sosa-Gomez and Oliveira, 2007). Therefore, nowadays *P. includens* has frequently caused damages to the soybean crop in

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several different regions, not only in Brazil but in other countries, from South and North America, as well (Mascarenhas and Boethel, 2000).

Among other different control techniques, a procedure that has shown efficient results in controlling pest outbreaks, mainly from the order Lepidoptera, is the use of egg parasitoids from the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) (Parra et al., 1987). Increasingly, the practice of biocontrol employing egg parasitoids has been used in Latin America to fight pests which damage crops such as cotton, sorghum, soybean, and sugar cane (Van Lenteren and Bueno, 2003). Some of the advantages in using *Trichogramma* spp. in biological control programs are: these parasitoids are easily reared on alternative hosts and are highly aggressive in parasitizing eggs of different pest species as well (Botelho, 1997; Haji et al., 1998; Parra, 1997; Smith, 1996; Wajnberg and Hassan, 1994). Studies on *Trichogramma* spp. have been carried out in more than 50 countries and commercial releases occur in approximately 32 millions hectares every year (Smith, 1996). Despite its great importance, the success of a *Trichogramma* release depends basically on the knowledge of the biological characteristics of the parasitoid species or strain used, and on its interaction with a specific host, since these characteristics will determine if the parasitoid is efficient or not in controlling the targeted pest species (Bourchier and Smith, 1996). Therefore, previous laboratory studies are required to better evaluate the parasitoid biology, parasitism capacity and viability, and biological cycle of the parasitoid, among other biological features that might differ among species or strains, and may also vary according to host and temperature (Hassan, 1997; Scholler and Hassan, 2001). It is also important to determine its thermal requirements as to allow predicting the ideal temperature for parasitoid development. This is especially important in planning parasitoid mass rearing in laboratory, since through the knowledge of the thermal requirements it is possible to predict the day of the parasitoid eclosion, thus allowing fulfillment of field experiments needs (Haddad et al., 1999).

Furthermore, efficiency has been proved to be different among species or strains of *Trichogramma* and regarded to this subject very scarce research has been carried out so far in soybean, despite its importance (Bueno et al., 2009). Among several different strains of *Trichogramma pretiosum*, a strain which had been collected in Rio Verde County, State of Goiás, Central Brazil, designated as *T. pretiosum* RV was much more efficient in controlling *P. includens* than the other strains of *T. pretiosum* tested (Bueno et al., 2009). The higher efficiency detected for the *T. pretiosum* RV strain may probably be attributed to the following fact: since it was collected in a soybean field in which *P. includens* had actually been a major pest for many years, the co-evolution between this pest species and that strain of *Trichogramma* might had allowed an adaptation of the parasitoid to the eggs of this specific host higher than the other *T. pretiosum* strains. This strain (voucher specimen number TP-17) was deposited at the "Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças, NUNEMAFI", Federal University of the State of Espírito Santo, Brazil).

The most important biocontrol agent used on the soybean crop up to the present has been the Nucleopolyhedrosis virus of *A. gemmatalis* (AgMNPV) (Moscardi, 1999). Nevertheless, the use of other biocontrol agents became of crucial importance for biological control programs as well as for the success of the Soybean Integrated Pest Management, since the AgMNPV does not control *P. includens* and other lepidopteran pests besides *A. gemmatalis*. Therefore, this study was carried out aiming at evaluating the biological characteristics, thermal requirements, and number of generations per year of this specific strain of *T. pretiosum* reared on eggs of *P. includens* and *A. gemmatalis*, under several different constant temperatures as to generate information which would allow future mass production of the parasitoid in the laboratory as well as the use of this egg parasitoid in extensive soybean biocontrol programs.

2. Material and methods

The experiments were carried out in the Biology of Insects Laboratory of the Department of Entomology, Plant Pathology and Agricultural Zoology of the Luiz de Queiroz College (ESALQ), University of São Paulo (USP), Piracicaba County, State of São Paulo from January to December of 2006.

2.1. Cultures of the parasitoid and hosts

Cultures of *P. includens* and *A. gemmatalis* were kept in the laboratory under controlled environmental conditions [25 ± 2 °C temperature, 70 ± 10% RH, 14/10 h photoperiod (L/D)]. The caterpillars were reared on the artificial diet proposed by Greene et al. (1976) and Parra (2001). After eclosion, adults were fed with a 10% honey/water solution inside 10 cm Ø × 21.5 cm tall cages with the walls covered with A4 paper, where their eggs were laid. These eggs were then removed and either used for the trials or for maintaining the insect colonies.

Cultures of *T. pretiosum* RV were accomplished according to Parra (1997). Eggs of the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), were glued on cardboard and turned nonviable by exposing them to a ultraviolet light (Stein and Parra, 1987) and then offered to parasitism for 24 h. Newly emerged parasitoids were either used for trials or for maintaining cultures.

2.2. Biological characteristics of T. pretiosum RV reared on eggs of P. includens and A. gemmatalis

The experiments were carried out specifically for each pest species although applying the same standardized methods. All parasitoids used were priorly reared for one generation on eggs of each pest species (*P. includens* or *A. gemmatalis*) to avoid the pre-imago experience due to the rearing on eggs of the alternative host (*A. kuehniella*), which was used for parasitoid culture maintenance.

Twenty *T. pretiosum* RV females (24 h old) were individualized into 12 mm Ø × 75 mm tall glass vials containing 20 eggs (also 24 h old) of one of the hosts (*P. includens* or *A. gemmatalis*), which were then kept under controlled environmental conditions [25 ± 2 °C temperature, 70 ± 10% RH, 14/10 h photoperiod (L:D)]. The parasitism was allowed for 24 h. After that period, the parasitoids were removed, and the vials were then placed into environmental chambers set at different constant temperatures (treatments) of: 18, 20, 22, 25, 28, 30, and 32 °C; 70 ± 10% relative humidity (RH); and 14/10 h light/dark photoperiod (L:D). The following biological parameters were then evaluated: developmental time (egg to adult); percent emergence (viability); number of parasitoids per host egg; longevity; and sex ratio (female/(female + male)).

Trials were carried out in a completely randomized design with seven treatments (temperatures) and 20 replications per treatment. Evaluations were performed on a daily basis in order to precisely determine *T. pretiosum* developmental time (egg to adult). The parasitoid emergence was evaluated by counting the hatched eggs with the aid of a stereomicroscope. Results data were computed for normality and then submitted to ANOVA. If data had normal distribution, means were compared by Tukey test ($P \leqslant 0.05$). Data were also transformed when necessary according to Bartlett's Homogeneity Variance Test for Statistical Analysis (SAS Institute, 2001).