Temperature Effects on the Development and Reproduction of Three Trichogramma (Hymenoptera: Trichogrammatidae) Species Reared on Trichoplusia ni (Lepidoptera: Noctuidae) Eggs

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ABSTRACT. The cabbage looper, Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae) is a generalist species and an important pest of Brassicaceae worldwide. Egg parasitoids are a feasible alternative for the control of this species. We evaluated the suitability of T. ni eggs as hosts for three Trichogramma Westwood (Hymenoptera: Trichogrammatidae) species and their tolerance to survive and develop within a range of temperatures between 15 and 30 °C under laboratory conditions. The species evaluated were Trichogramma pretiosum Riley, Trichogramma atopovirilia Oatman and Platner, and Trichogramma acacioi Brun, Moraes and Soares. Parasitism rate was affected by temperature, parasitoid species, and by the interaction between these two factors. Parasitoids developed and reproduced in the range of temperatures evaluated, but Trichog. acacioi failed to parasitize T. ni eggs at 30 °C. The highest parasitism rates of Trichog. atopovirilia and Trichog. pretiosum occurred at 20 and 25 °C and Trichog. acacioi at 25 °C, with parasitism rate above 70% in the three species. Parasitoid emergence was not affected by temperature or species. The estimated thermal constant and lower temperature threshold were 134.6 DD and 10.6 °C for Trichog. pretiosum and 130.1 DD and 11.2 °C for Trichog. atopovirilia. The results demonstrated that Trichog. pretiosum and Trichog. atopovirilia are the most suitable species for the control of T. ni, as they can remain active throughout the year in subtropical regions.

Key Words: biological control, cabbage looper, crucifer, egg parasitoid, thermal requirement

The cabbage looper Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae) is a generalist pest that feeds on more than 160 species of plants comprising 36 families (Sutherland and Green 1984). This species is an important pest of cucumber [Cucumis sativus (L.)], pepper [Capsicum annum (L.)], tomato [Solanum lycopersicum Miller] (Sutherland and Green 1984), and one of the most important pests of brassicaceous crops, causing direct damage and significant yield losses (Maxwell et al. 2006). The excessive dependence on chemical insecticides affects natural enemies and promotes the selection of resistant strains of pests (Akhtar et al. 2007, 2010), in addition to causing environmental damage. Thus, biological control can be an alternative to the use of insecticides for controlling of T. ni (Milanez et al. 2009, Altoé et al. 2012).

The development of mass rearing and release techniques of parasitoids has promoted the use of Trichogramma Westwood (Hymenoptera: Trichogrammatidae) species (Jalali and Singh 1992, Jalali et al. 2007, Firake and Khan 2014). Inoculative biological control Westwood in eggs of T. ni (Marston and Ertle 1973, Ashley et al. 1974, Parker and Pinnel 1974, Hohmann et al. 1988). However, these species do not occur in Brazil.

Other less studied species of Trichogramma may also have potential to control T. ni. Trichogramma atopovirilia Oatman and Platner and Trichogramma acacioi Brun, Moraes and Soares, as well as Trichog. pretiosum, are naturally found in Southern Brazil and were collected parasitizing eggs of the velvetbean caterpillar Anticarsia gemmatalis Hübner in soybean fields, during summer, in Southeastern Paraná State (Avanci et al. 2005, Foerster and Foerster 2009). Milanez et al. (2009) compared the development and reproduction of six species of Trichogramma, including Trichog. acacioi and Trichog. atopovirilia in T. ni eggs, at 25 °C. However, subtropical mean temperatures in Southern Brazil range from 13 to 15 °C in winter (with periods with temperatures near 0 °C), to 22 °C in summer (sometimes exceeding the range of 25 °C) (Foerster and Foerster 2009), exposing Trichogramma species to a wider range of temperature than in tropical areas. Therefore, naturally occurring species in subtropical region tend to be more successful as biological control agents in this temperature regime, since they are adapted to a wider range of temperature. Under such conditions, the biological characteristics of parasitoids and their interactions with their host will determine the suitability of the species to effectively control the target host (Bourchier and Smith 1996).

Brassicaceous crops are grown year-round in subtropical areas of Brazil and elsewhere, exposing both pests and their natural enemies to a wide range of temperatures. For two species, Trichog. atopovirilia and Trichog. acacioi, there are no data available on the effect of temperatures as low as 15 °C on the development and reproduction of these species on eggs of T. ni. Trichog. pretiosum is a widely studied species on many hosts; however, the host egg, as well as its nutritional characteristics are known to alter the thermal requirements and biological characteristics of the parasitoid (Bueno et al. 2009). For this reason, Trichog.
**Materials and Methods**

**Origin and Rearing Procedures of T. ni and Trichogramma Species.** A colony of *T. ni* was established in 2008 with larvae and pupae collected from commercial crops of broccoli and cauliflower located in Colombo (25° 17′ 31′′ S, 49° 13′ 26′′ W), Southeastern Paraná State, Southern Brazil. Approximately 30 newly hatched caterpillars were kept per Petri dish (18 by 1 cm) containing a disk of absorbent paper and leaves of broccoli. This initial number of caterpillars in the dishes was reduced as they increased in size. Pupae were maintained in plastic containers (12 by 5 cm) until adult emergence. Adults were fed a 10% honey solution soaked in a cotton ball in a Petri dish. This concentration has been shown to increase fertility and longevity for adults (Binder 1996, Tisdale and Sappington 2001, Marchioro and Foerster 2012) and is commonly used as food for noctuid adults. Adults were reared in glass cages (45 by 33 by 35 cm) at ambient temperature (18–22°C), with the inner sides covered with acrylic plates and openings for ventilation and maintenance. An excised leaf of broccoli or cauliflower was provided to stimulate egg laying and as a substrate for oviposition. The eggs laid on the leaves were removed with a fine brush, and those laid on the acrylic plates were washed and then collected using a strainer with fine mesh. All eggs were transferred to Petri dishes (15 by 1 cm) lined with paper towel and placed in a chamber at 20 ± 1°C and RH 70 ± 10%.

The colonies of *Trichog. pretiosum*, *Trichog. atopovirilia*, and *Trichog. acacioi* were established in the laboratory with parasitized eggs of *A. gemmatalis* collected from soybean crops in Southeastern Paraná State, Southern Brazil. The species were reared on eggs of the wheat armyworm *Pseudaletia sequax* Franclemont for 15 generations. Subsequently, the parasitoids were maintained for three generations in *T. ni* eggs before of the experiments. The rearing methods followed Foerster and Foerster (2009).

**Temperature Effects on Parasitoids.** The experiments were conducted in climatic chambers at constant temperatures of 15, 20, 25, and 30 ± 1°C, photoperiod of 12:12 (L:D) h, and 70 ± 10% RH. Paper cartons (0.5 by 1.5 cm) containing 20 eggs of *T. ni* less than 24 h old were exposed to ultraviolet light for 30 min for sterilization. Newly emerged female parasitoids were kept in the presence of males to mate for 24 h. After this period, females were maintained individually in 0.5 by 6 cm glass tubes sealed with a piece of cotton and presented with 20 eggs of *T. ni* for 24 h, at each temperature evaluated. After this period, females were removed and the eggs placed inside the tubes and maintained in the climatic chambers. The eggs were monitored daily to check the development of the parasitoids, indicated by the blackening of the eggs (Knutson 1998). After the majority of parasitoids emerged, the remaining eggs were kept for three additional days to ensure that any delayed emergence was recorded. Twenty replicates were performed for each temperature. All emerged adults were fed daily with honey and their longevity and sex ratio were recorded.

**Thermal Requirements.** The lower temperature threshold (\(T_0\)) and thermal constant (\(K\)) were estimated for *Trichog. pretiosum* and *Trichog. atopovirilia*. The development rate (1/development days) was regressed against temperature using the linear regression equation (\(Y = a + bx\)). The lower temperature threshold was calculated with the x-intercept method (\(T_0 = -a/b\)), and the thermal constant by the reciprocal of the slopes of the estimated regression lines (\(K = 1/b\)) (Campbell et al. 1974). The linear regression requires a minimum of four points to accurately estimate the parameters (Haddad et al. 1999), thus the thermal requirements of *Trichog. acacioi* were not estimated, because this species did not parasitize at 30°C. Additionally, survival in different temperature regimes was compared using survival curves constructed according to the Kaplan–Meier function (Kaplan and Meier 1958).

**Statistical Analysis.** The normality of the data and the homogeneity of the variance were assessed with the Shapiro–Wilk’s test and Levene’s test, respectively. The data on parasitism, emergence, sex ratio, and longevity were not normally distributed and were transformed to log \((x + 1)\). The transformed data were compared using a factorial analysis of variance (ANOVA) with parasitoid species and temperature as sources of variation (3 by 4). When significant, the means were compared with the Tukey’s test (\(P < 0.05\)). All statistical procedures were performed using the software Statistica v.7 (Statsoft 2004).
Table 1. Mean (±SE) percentage of parasitism and developmental time of *Trichog. pretiosum*, *Trichog. atopovirilia*, and *Trichog. acacioi* reared on eggs of *T. ni* at different temperatures

| Parameter          | Temperature (°C) | Trichog. pretiosum | Trichog. atopovirilia | Trichog. acacioi |
|--------------------|------------------|--------------------|----------------------|------------------|
| Parasitism (%)a   | 15               | 19.1 ± 2.8 bB      | 46.3 ± 7.6 cA        | 25.5 ± 4.2 bB    |
|                    | 20               | 70.5 ± 7.6 aA      | 74.0 ± 8.2 aB        | 42.1 ± 6.9 bB    |
|                    | 25               | 72.8 ± 6.1 aB      | 90.3 ± 1.2 aA        | 74.1 ± 7.2 bA    |
|                    | 30               | 59.1 ± 6.2 aA      | 60.0 ± 8.1 bA        | 32.5 ± 6.2 bA    |
| Developmental timea | 15              | 28.8 ± 0.1 aB      | 32.5 ± 0.2 aA        | 28.6 ± 0.1 aB    |
|                    | 20               | 15.6 ± 0.1 bB      | 15.9 ± 0.1 bAB       | 16.0 ± 0.0 bA    |
|                    | 25               | 9.0 ± 0.0 cB       | 9.0 ± 0.0 cB         | 10.0 ± 0.0 cA    |
|                    | 30               | 7.0 ± 0.0 dA       | 7.0 ± 0.0 dA         | —b               |

aMeans followed by the same lowercase letter (columns) and means followed by same uppercase letter (rows) do not differ significantly by Tukey test (*P* < 0.05).

bParameter not assessed due to absence of parasitism.

Table 2. Mean (±SE) emergence percentage, sex ratio, and longevity (days) of females and males of *Trichog. pretiosum*, *Trichog. atopovirilia*, and *Trichog. acacioi* reared on eggs of *T. ni* at different temperatures

| Parameter          | Temperature (°C) | Trichog. pretiosum | Trichog. atopovirilia | Trichog. acacioi |
|--------------------|------------------|--------------------|----------------------|------------------|
| Emergence (%)a     | 15               | 84.4 ± 6.6 AA      | 96.5 ± 1.7 AA        | 95.0 ± 3.1 AA    |
|                    | 20               | 83.1 ± 8.5 AA      | 95.9 ± 3.6 AA        | 85.8 ± 5.7 AA    |
|                    | 25               | 99.5 ± 0.4 AA      | 98.7 ± 1.3 AA        | 98.5 ± 1.1 AA    |
|                    | 30               | 79.5 ± 7.5 AA      | 82.1 ± 8.3 AA        | —b               |
| Sex ratioa         | 15               | 0.66 ± 0.07 aA     | 0.85 ± 0.02 aA       | 0.60 ± 0.06 aA   |
|                    | 20               | 0.59 ± 0.09 aA     | 0.77 ± 0.07 aA       | 0.54 ± 0.06 aA   |
|                    | 25               | 0.75 ± 0.05 aA     | 0.81 ± 0.03 aA       | 0.73 ± 0.07 aA   |
|                    | 30               | 0.68 ± 0.06 aA     | 0.82 ± 0.03 aA       | —b               |
| Longevity (γa)     | 15               | 19.7 ± 1.2 AA      | 22.2 ± 0.6 AA        | 16.3 ± 0.6 AB    |
|                    | 20               | 9.5 ± 1.4 BA       | 8.2 ± 1.9 cA         | 9.4 ± 1.0 BA     |
|                    | 25               | 12.1 ± 0.9 BA      | 13.6 ± 0.7 BA        | 6.2 ± 0.2 cB     |
|                    | 30               | 2.3 ± 0.2 CB       | 4.4 ± 0.5 cA         | —b               |
| Longevity (δa)     | 15               | 15.7 ± 1.1 AA      | 16.6 ± 0.9 aA        | 12.1 ± 0.8 AB    |
|                    | 20               | 10.1 ± 1.8 BA      | 7.3 ± 1.7 cA         | 9.3 ± 0.8 BA     |
|                    | 25               | 9.6 ± 0.7 BA       | 11.2 ± 0.7 BA        | 6.5 ± 0.2 cB     |
|                    | 30               | 2.3 ± 0.2 CB       | 4.4 ± 0.5 cA         | —b               |

aMeans followed by the same lowercase letter (columns) and means followed by same uppercase letter (rows) do not differ significantly by Tukey test (*P* < 0.05).

bParameter not assessed due to absence of parasitism.

*Trichog. atopovirilia* and temperature, as indicated by the coefficient of determination of 0.99 for both species (Fig. 2). The values of *T*₀ were 10.6 (± 0.09) and 11.2 (± 0.08) °C for *Trichog. pretiosum* and *Trichog. atopovirilia*, respectively. The estimated thermal constants were 137.6 (± 8.55) DD for *Trichog. pretiosum* and 130.1 (± 7.72) DD for *Trichog. atopovirilia* (Fig. 2). The thermal requirements of *Trichog. acacioi* were not estimated, as this species did not parasitize eggs of *T. ni* at 30°C.

**Discussion**

Temperature affects immature development, adult emergence, reproduction, and longevity of insects, since they are constantly exposed to temperature fluctuations in their natural habitat and may show differences in tolerance to extreme temperatures and in their acclimation responses (Lessard and Boivin 2013, Firake and Khan 2014). The number of eggs parasitized by a female of *Trichogramma* is a determining factor in the choice of a species or strain to be used in a biological control program (Kalyebi et al. 2005). This study recorded that the three investigated species parasitized at 15°C, besides, parasitism rates above 70% were recorded at temperatures of 20 and 25°C for *Trichog. atopovirilia* and *Trichog. pretiosum*, and at 25°C for *Trichog. acacioi*. Although, in some cases differences were not statistically significant, *Trichog. atopovirilia* tended to have higher rates of parasitism throughout the range of temperatures evaluated. Milanez et al. (2009) evaluated the suitability of *T. ni* eggs as hosts for four *Trichogramma* species at 25°C. The eggs of *T. ni* were accepted by all species, but the percentage of parasitism was lower than that reported in our study. This might have occurred because the parasitoids used in their experiment were previously reared on the small eggs of *Anagasta kuehniella* (Zeller, 1879). Previous studies concluded that larger eggs with higher nutritional content generate larger females, with higher parasitism capacity and longevity (Kazmer and Luck 1995, Doyon and Boivin 2005). Therefore, the more vigorous parasitoids obtained from large eggs of *P. sexula*, and posteriorly *T. ni*, used in our experiment, might explain the better performance of the *Trichogramma* species.

According to Pak and van Lenteren (1988), species and strains with good performance in the laboratory are more likely to have a better performance in the field. In this study, with the exception of *Trichog. acacioi*, which did not parasitize at 30°C, the emergence of the three parasitoid species was not affected by the temperatures tested. The rates of parasitism and emergence of *Trichog. pretiosum* and *Trichog. atopovirilia* at all examined temperatures indicate that these species have a high potential to control *T. ni* in brassicaceous crops year round. *Trichog. acacioi* proved to be effective in temperatures below 30°C, thus becoming a good option for biological control except during summer months. In brassicaceous crops, because of their continuous cultivation, hosts are also available in the field throughout the year. Even in periods when *T. ni* is not available on the field, parasitoids may use other lepidopteran pests of Brassicae as hosts, such as *Platella xylostella* (L.) and *Spodoptera eridania* (Cramer) (Prati ssoi et al. 2008, Goulart et al. 2011, Goulart et al. 2012). Thus, the thermal tolerance of the tested species in this study, to both winter and summer temperatures
in Southern Brazil, along with a constant availability of hosts in the field make these species suitable candidates as biocontrol agents of *T. ni* in subtropical regions.

The development time of all species increased as temperature decreased, as previously described for other *Trichogramma* species (Haile et al. 2002, Bueno et al. 2009, Altoé et al. 2012, Lessard and Boivin 2013). The nutritional requirements of insects and their body mass have been reported as important factors affecting the thermal constant (K) and base temperature (*T*) (Haddad et al. 1999, Charnov and Gilloby 2003). The base temperature (*T*) of *Trichog. pretiosum* (10.6°C) and *Trichog. atopovirilia* (11.2°C) were below average winter temperatures in Southern Brazil (13–15°C). *Trichogramma* species show different strategies to survive during winter, which varies depending on the host, individual characteristics of the species, and its geographic origin (Boivin 1994). Some species are able to stay active using alternative hosts (Babendreier et al. 2003, Özder and Saglam 2005), while others enter a state of quiescence or diapause under unfavourable environmental conditions (Curl and Burbutis 1977, Garcia et al. 2002, Boivin 1994).
Rundle and Hoffmann 2003). It was not possible to estimate the lower threshold temperature of Trichog. acacioid in this study; however, Foerster et al. (2015) reported that Trichog. acacioid and Trichog. pretiosum are able to remain active during winter in Southern Brazil.

In conclusion, based on parasitism and emergency rates, as well as the estimated base temperature, Trichog. pretiosum and Trichog. atopoeditrilla are the most suitable species for the control of T. ni, since they can remain active throughout the year in subtropical regions.

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