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Parasitism capacity of *Trichogramma pretiosum* on eggs of *Trichoplusia ni* at different temperatures

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ABSTRACT. *Trichogramma* spp. are egg parasitoids of various pest species of Lepidoptera including *Trichoplusia ni*, an important pest of plants in the genus *Brassica*. Of the climatic conditions that can impair *Trichogramma* spp. parasitism capacity, the temperature is critical. Thus, the objective of this research was to evaluate the parasitism capacity of *Trichogramma pretiosum* on eggs of *T. ni* at 18, 21, 24, 27, 30, and 33ºC; 70±10% RH; and 12/12 hours photophase (L/D). Fresh eggs of the host moth were offered to *T. pretiosum* daily. The parasitism rate varied between 8 and 11.4 eggs/female at the temperatures evaluated for the first 24 hours. The highest number of parasitized eggs per female occurred at 24ºC (53.0 parasitized eggs/female). The period of parasitism and the mean longevity of females were inversely related to the temperature. Temperature heavily influences the parasitism rate of *T. pretiosum* on eggs of *T. ni*, and the best overall performance of the parasitoid occurs from 24 to 27ºC.

Keywords: insecta, biological control, horticultural plants.

Capacidade de parasitismo de *Trichogramma pretiosum* em ovos de *Trichoplusia ni* em diferentes temperaturas

RESUMO. *Trichogramma* spp. são parasitoide de ovos de várias espécies pragas de Lepidoptera incluindo *Trichoplusia ni*, uma importante praga de plantas do gênero *Brassica*. Das condições climáticas que podem influenciar a capacidade de parasitismo de *Trichogramma* spp., a temperatura é uma das principais. Portanto, o objetivo deste trabalho foi avaliar a capacidade de parasitismo de *Trichogramma pretiosum* em ovos de *T. ni* nas temperaturas de 18, 21, 24, 27, 30 e 33ºC; 70±10% UR; e 14/12h fotofase. Ovos frescos de *T. ni* foram oferecidos diariamente para *T. pretiosum*. A taxa de parasitismo nas primeiras 24h variou de 8 a 11,4 ovos/fêmea do parasitoide entre as temperaturas avaliadas. O maior número de ovos parasitados por fêmea ocorreu a 24ºC (53,0 ovos parasitados/fêmea). O tempo de parasitismo e a longevidade média dos parasitoides adultos foram inversamente relacionados à temperatura. Temperatura influência enormemente no parasitismo de *T. pretiosum* em ovos de *T. ni*, e os melhores resultados do parasitoide foram obtidos de 24 a 27ºC.

Palavras-chave: insecta, controle biológico, horticultura.

Introduction

The cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), is a pest with a wide range of hosts including plants of the families Brassicaceae, Solanaceae, and Curcubitaceae, such as cotton, soybean, and different weeds (GRECCO et al., 2010; MILANEZ et al., 2009). The ability of *T. ni* to feed simultaneously on a large variety of host species is crucial to its success. Furthermore, *T. ni* causes large amounts of crop damage, especially among leafy green vegetables (LANDOLT, 1993), which can impair commercialization due to the physical damage caused by the feeding caterpillars.

Trichoplusia ni is most commonly controlled by the use of chemicals. However, the abusive use of insecticides with high levels of biological activity and persistence has caused high economic and environmental costs (BRITO et al., 2004; GRECCO et al., 2010). An alternative measure to mitigate these costs might be biological control. Biological control agents, such as egg parasitoids, constitute an important tool in the development of Integrated Pest Management (IPM) programs, aiming to reduce the use of insecticides and to manage insect resistance to insecticides in a more sustainable manner (PRATISSOLI et al., 2008). In this regard, Godin and Boivin (1998) emphasized that the utilization of egg parasitoids on brassicas is capable of promoting the regulation of the population of pest insects to a level below the economic threshold, which underscores the importance of these biological control agents.

Among egg parasitoids, the genus *Trichogramma* is notable because of its wide geographical distribution and high parasitism capacity on eggs of different species, primarily those in the order Lepidoptera (PRATISSOLI et al., 2002). The success of *Trichogramma* spp. releases, however, depends on knowledge of the ecological characteristics of the parasitoid and on its interaction with the targeted host. Therefore, before using *Trichogramma* species to control insect pests in commercial releases, laboratory studies investigating the parasitism capacity of the selected *Trichogramma* species are needed (PARRA et al., 2002). Thus, Milanez et al. (2009) selected the strain 'Tspd' of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) from several different species and strains of *Trichogramma* as the most suitable to parasitize eggs of *T. ni*. Consequently, tests to evaluate the parasitoid in relation to environmental factors should be conducted because the potential that is observed under optimal conditions may be impaired under adverse conditions.

Among abiotic factors, temperature is the most influential, altering life cycle duration, parasitism rate, sex ratio, and longevity of the parasitoids (HOFFMANN; HEWA-KAPUGE, 2000; MOLINA et al., 2005). Therefore, the present work aims to evaluate the parasitism capacity of *T. pretiosum* on eggs of *T. ni* at different temperatures in the laboratory, with the objective of eventually using these data to manage *T. ni* in the field.

Material and methods

The experiment was carried out at the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário (Nudemafi) located at the Agrarian Sciences Center of the Federal University of Espírito Santo in Alegre, Espírito Santo, Brazil.

Trichoplusia ni rearing and maintenance

Trichoplusia ni pupae were sexed and placed into plastic pots containing moistened filter paper at the bottom. After emergence, the adults were transferred to 60 x 50 x 50 cm wooden framed cages containing a leaf of cabbage for oviposition. The adults were fed a 10% honey solution, which was placed inside 20 mL flasks containing cotton pads that were in contact with the honey solution. Food was renewed every 48 hours. The cabbage leaf was replaced daily, and those containing the host eggs were transferred to lidded plastic containers. After eclosion, the caterpillars were transferred to 8.5 x 2.5 cm glass tubes containing the artificial diet proposed by Greene et al. (1976) until the pupal phase.

Trichogramma pretiosum rearing and maintenance

Parasitoid rearing and multiplication was performed on eggs of the factitious host, *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae), according to the methodology described by Parra et al. (2002). Eggs of *A. kuehniella* were glued onto 8.0 x 2.5 cm Bristol board cards with the aid of 30% (w/v) gum arabic and subsequently exposed to ultraviolet light for 45 min. for sterilization. Next, the cards were transferred into 8.5 x 2.5 cm glass tubes containing honey droplets, into which parasitoid females were introduced in sequence. The rearing procedure was performed inside climatic chambers set at 25±1°C, 70±10% RH, and 14/10 hours photophase (L/D).

Trichogramma pretiosum parasitism capacity on eggs of *T. ni* at different temperatures

Based on the results obtained by Milanez et al. (2009), the 'Tspd' strain of *T. pretiosum* was selected for this experiment. Fifteen newly emerged *T. pretiosum* females were separated into 8.5 x 2.5 cm glass tubes containing a droplet of honey for food. New small Bristol board cards containing 20 *T. ni* eggs (< 24 hours old) were introduced daily into the tubes to allow parasitism by *T. pretiosum*. The tubes were maintained in climatic chambers set at 70±10% RH, 14/10h photophase (L/D), and temperatures of 18°±1°C, 21°±1°C, 24°±1°C, 27°±1°C, 30°±1°C, and 33±1°C. The cards containing the parasitized eggs were removed from the tubes daily and transferred to 23.0 x 4.0 cm plastic bags, which were then sealed and maintained at the same climatic conditions until offspring emergence.

The characteristics evaluated were as follows: daily parasitism, cumulative parasitism, lifetime number of parasitized eggs per female, time span of parasitism, parental adult female longevity, sex ratio, number of parasitoid individuals per egg, and parasitism viability (% parasitoid emergence). A completely randomized experimental design with six treatments (different temperatures) and 15 replications was used.

Statistical analysis

The data were subjected to ANOVA, and the means were compared by Tukey's test at 5% probability ($p \leq 0.05$).

Results and discussion

The rate of parasitism during the first 24 hours varied between 8 and 11.4 eggs per *T. pretiosum* female between 18 and 33ºC (Figure 1). After the first day, the parasitism rate decreased at all temperatures, with the highest figures observed in the first three days of parasitism activity. These results demonstrate that the parasitism rate is not constant at all temperatures and may depend on the intrinsic characteristics of the species and/or strain of the parasitoid and host. The rate of parasitism at different temperatures is a biological characteristic specific to each parasitoid strain or species reared on each host (PRATISSOLI; PARRA, 2000, 2001; PRATISSOLI et al., 2004). Similar results were reported by Pratissoli et al. (2004) and Bueno et al. (2010), who recorded that the rate of *T. pretiosum* parasitism varied with temperature on *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) and *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) eggs. Other authors have reported high parasitism during the first 24 hours when studying different species or strains of *Trichogramma* and host species (INOUE; PARRA, 1998). This behavior might be associated with the lower longevity of the parasitoids when reared under higher temperatures compared to lower temperatures (INOUE; PARRA, 1998). Under higher temperatures the metabolic expenses are higher so it is advantageous for the parasitoid to conduct the parasitism in the first hours (GERLING, 1972). Having the majority of parasitism concentrated on the first day is a positive feature for mass releases in the field as this might guarantee quick pest control and allow growers to apply herbicides or fungicides shortly after the parasitoid release if necessary. Therefore, when choosing a release strategy it is important to consider whether parasitism is concentrated in the first days of life or evenly distributed throughout adulthood and to consider that this might vary due to differences in temperature (REZNIK; VAGHINA, 2006), hosts (REZNIK et al., 2001), or parasitoid species/strain (PIZZOL et al., 2010). These factors can influence the success of biological control programs using egg parasitoids of the genus *Trichogramma* (SMITH, 1996).

Moreover, in *T. pretiosum* these characteristics should also be considered when choosing the most suitable parasitoid species or strain to be used in the field. It is better if the parasitoid reaches 80% of its lifetime parasitism soon after release. A longer amount of time between release and parasitism increases the chances of being influenced by biotic and/or abiotic factors that may impair parasitism. In this context, the higher rate of parasitism in the first 24 hours observed in this study is a positive characteristic of the parasitoid strain because they are less vulnerable to the side effects of any pesticide spraying that might take place after the field release. After releasing parasitoids into the field, it is not unusual to use a fungicide or herbicide that might impair parasitism. Egg parasitoids are typically tiny little wasps that may be more susceptible to chemicals used in agriculture, including herbicides and fungicides, than their hosts (CARMO et al., 2010). Furthermore, parasitoids differ from herbivorous insects by their inability to synthesize lipids as adults, and this makes them more vulnerable to temperature increases than most pest species (DENIS et al., 2011). Similar results have been observed by several authors using different species of parasitoids and hosts. Pratissoli et al. (2004) studied T. pretiosum parasitism on eggs of *P. xylostella* and verified a higher rate of parasitism on the first day, with the number of parasitized eggs decreasing with time. The decrease in the performance of the females may be directly related to the age of the insects (PASTORI et al., 2007; SÁ, PARRA, 1994; ZAGO et al., 2007). The highest parasitism rate of *T. exiguum* Pinto and Platner, 1978 on eggs of *P. xylostella* occurred at 25ºC (PEREIRA et al., 2007), and for *T. pratissolii* Querino and Zucchi, on eggs of *Corcyra chephalonica* Stainton, 1865 (Lepidoptera: Pyralidae) and *A. kuehniella*, the highest parasitism rates observed were between 24 and 30ºC (ZAGO et al., 2007).

The index of 80% cumulative parasitism, which represents an estimate of the efficiency of the parasitoid in the field (due to abiotic factors, this index will never reach 100%), also varied with the temperature (Figure 1). The observed variation was approximately four days. At the lowest temperatures (18 and 21ºC), the time required was six and eight days, respectively; at the median temperatures (24 and 27ºC), it was six and five days, respectively; and at the highest temperatures (30 and 33ºC), it was five and three days, respectively. The variation observed may be associated with the characteristics of the parasitoid species and/or strain, as well as with the choice of the host studied because the parasitoids used in this experiment were reared on eggs of *A. kuehniella*.

Pereira et al. (2007) reported similar results to those found here. Studying different species of parasitoids under distinct thermal conditions, those authors attributed the variation of the parasitism of *Trichogramma* sp. to temperature differences. Pastori et al. (2007), studying the performance of *T. pretiosum* on eggs of *Bonagota salubricola* Meyrick (Lepidoptera: Tortricidae), verified that the strain collected on eggs of *B. salubricola*, and even those reared on eggs of *Sitotroga cerealella* (Oliv., 1819) (Lepidoptera: Gelechiidae), did not show reduction of potential, being influenced solely by the thermal regime to which they had been submitted. Another possibility is that the intrinsic characteristics of the host species might influence the parasitoid performance, as verified by Pratissoli et al. (2004)

and Zago et al. (2007) in studies performed with *T. pretiosum* and *T. pratissolii*, respectively, on eggs of different hosts. Hoffmann et al. (2001), however, reported that the development of *T. ostriniae* collected on *Ostrinia nubilalis* (Lepidoptera: Crambidae), on different hosts and during several generations of the parasitoid did not affect their performance on the original host. These differences in results emphasize the importance of studying these biological characteristics for each parasitoid strain and target host.

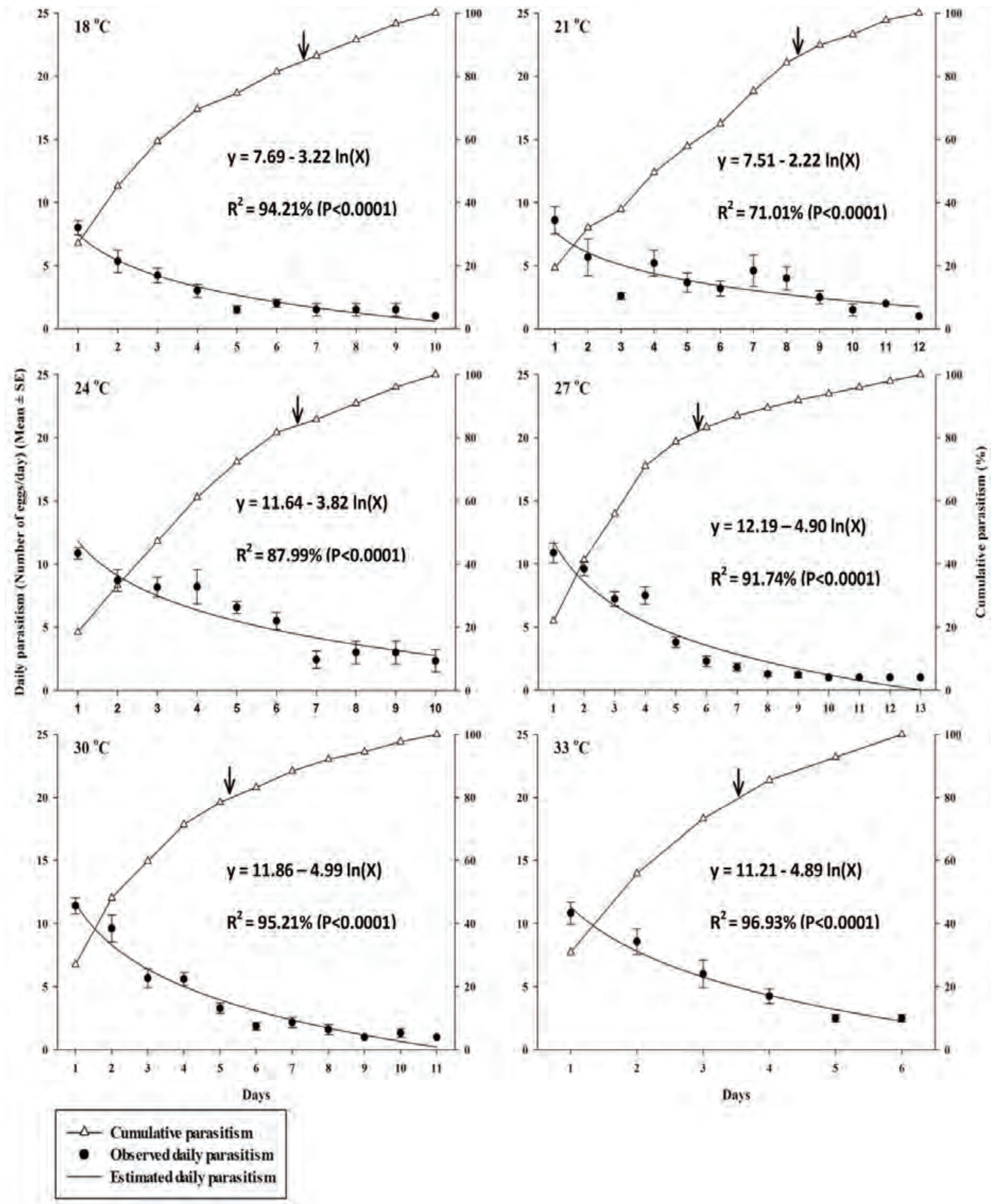

Figure 1. Daily and accumulated parasitism rates and parasitism time span of the egg parasitoid *Trichogramma pretiosum* on eggs of the cabbage looper *Trichoplusia ni* at different temperatures (18°±1°C, 21°±1°C, 24°±1°C, 27°±1°C, 30°±1°C, and 33±1°C); 70±10% RH; and 14/10 hours photophase (L/D) (↓ 80% parasitism).

The lifetime number of parasitized eggs varied with temperature (Table 1). However, there were no statistically significant differences at 18, 21, 30, and 33ºC. The highest number of parasitized eggs occurred at 24ºC (53.0 parasitized eggs/female) and the lowest number was observed at 33ºC (23.2 parasitized eggs/female). The figures obtained in this study were higher than those reported for different species of *Trichogramma* and different hosts at the same temperatures, as observed by Pratissoli et al. (2004), Pereira et al. (2007), Zago et al. (2007) and Pratissoli et al. (2008), demonstrating that *T. pretiosum* displays a real potential for use in biological control programs of the caterpillar *T. ni*. Contrary to the results presented in this study, Pastori et al. (2007) found that *T. pretiosum*, strain bonagota, is better adapted to 18ºC, a fact that is linked to adaptation of the parasitoid strains to specific climatic conditions, according to the authors. This illustrates the importance of this type of study for each parasitoid strain to successfully develop a biological control program.

The period of parasitism varied between six and 13 days at the temperatures studied, with the smallest value observed at 33ºC (Figure 1). This might be directly correlated with the intrinsic characteristics of the *Trichogramma* strain studied, which displays differentiated responses as a function of thermal adaptability. Reinforcing such results, Zago et al. (2007), studying *T. pratissolii*, verified an inverse correlation of the parasitism period with the temperature, observing figures between two and 10 days from 18 to 33ºC. Pratissoli et al. (2009), in studies with *T. acacioi* on eggs of *A. kuehniella* and *S. cerealella*, found that between 20 and 35ºC, there was variation in the period of parasitism between five and 16 days. Pereira et al. (2007) verified an inferior variation on the parasitism period in the same temperature range (nine to 13 days).

The mean longevity of parental adult *T. pretiosum* females reared on eggs of *T. ni* presented statistically significant variation at different temperatures, demonstrating an inverse behavior to increases in temperature (Table 1). The longevity of the female parasitoids were statistically similar at 18, 21, 24, and 27ºC (11.5, 10.9, 10.1, and 9.7 days, respectively), but significantly different at 30 and 33ºC. At 27 and 30ºC, this parameter was not significantly different, displaying values of 9.7 and 7.7 days, respectively. The lowest mean longevity of females occurred at 33ºC (4.9 days) illustrating that the increase in temperature places more stress on the parasitoid than the lower temperatures. Other authors have found similar results from different species of the same parasitoid genus on the same host (PASTORI et al., 2007; PEREIRA et al., 2007; PRATISSOLI et al., 2004; ZAGO et al., 2007). According to Pastori et al. (2007), the reduction of the temperature promotes an increase in parasitoid longevity due to a reduction of metabolic rate.

Temperature is not the only factor responsible for variations of longevity. Other factors, such as photoperiod, relative humidity, interspecific and intraspecific competition (PRATISSOLI; PARRA, 2001), and the presence of the host (CAÑETE; FOERSTER, 2003), can interfere with the biological characteristics of an insect. Nevertheless, in the present study, temperature was the primary factor responsible for the variation of parasitoid longevity.

The sex ratio of *T. pretiosum* descendants reared on eggs of the moth *T. ni* was statistically different at 24 and 33ºC, varying between 0.57 and 0.80 (Table 1). At 18, 21, 27, and 30ºC, the sex ratios were statistically similar. This may have occurred due to the intrinsic characteristics of the parasitoid species and/or strain. However, the results are within the range found by other authors (BUENO et al., 2009; DIAS et al., 2008; PEREIRA et al., 2007; PRATISSOLI et al., 2010), but environmental factors such as temperature and relative humidity, as well as the host used, can influence this parameter. In their study with *T. pretiosum*, Pastori et al. (2007) observed a variation in the sex ratio between 18 and 32ºC, detecting a higher number of females at 32ºC. In contrast, Pratissoli and Parra (2000) studying *T. pretiosum* on eggs of *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) found no variation in sex ratio of the descendants from the eggs of *T. absoluta* in the same thermal range (18 to 32ºC), while the highest sex ratio figures were found between 20 and 32ºC in the descendants reared on eggs of *P. operculella*.

Table 1. Biological characteristics of the egg parasitoid *Trichogramma pretiosum* reared on eggs of the cabbage looper *Trichoplusia ni* at different temperatures (18º±1°C, 21º±1°C, 24º±1°C, 27º±1°C, 30º±1°C, and 33±1°C); 70±10% RH; and 14/10 hours photophase (L/D).

Temperature (°C)	Lifetime number of parasitized eggs/female (± SE)[1]	Parental adult female longevity (days ± SE)[1]	Sex ratio[1]	Number of parasitoids/egg[1]	Parasitism viability (%)[1]
18	26.6±2.18 c	11.5±0.51 a	0.65±0.04 bc	1.9±0.08 ab	100.0±0.00 a
21	29.2±4.24bc	10.9±0.78 a	0.74±0.02 ab	1.9±0.04 ab	100.0±0.00 a
24	53.0±3.11a	10.1±0.61 a	0.57±0.01 c	1,3±0.03 c	100.0±0.00 a
27	40.2±2.82b	9.7±0.56 ab	0.66±0.03 bc	1.7±0.16 ab	95.4±2.39 ab
30	38.4±2.54bc	7.7±0.49 b	0.74±0.02 ab	1.5±0.14 b	88.2±5.16 b
33	23.2±2.29 c	4.9±0.46 c	0.80±0.04 a	2.0±0.12 a	93.5±3.41 ab

[1]Means (Mean±Standard Error) followed by the same letter in the column are not significantly different from each other by Tukey's test at 5% probability.

The number of descendants that emerged per egg significantly differed between 24, 30, and 33ºC (Table 1). Despite the variation, the number of individuals emerged per host egg was always higher than one. The highest mean number of parasitoids emerging per egg occurred at 33ºC (2.0 individuals/egg), while the smallest figures were obtained at 24 and 30ºC (1.3 and 1.5 individuals/egg, respectively). At the remaining temperatures studied, the figures varied from 1.7 and 1.9 individuals/egg. The temperature can cause alterations in the physiology and development of the insects, and the suitability and the behavioral and functional responses of the insects to certain environmental conditions clearly vary between individuals (BOIVIN, 2010; CHOWN; NICHOLSON, 2004; GULLAN; CRANSTON, 2010; SPEIGHT et al., 2008).

The host directly influences the development of the parasitoids because it is the source of food as well as shelter (ÖZDER; KARA, 2010). Therefore, the size of the host egg not only influences the number of eggs deposited by the parasitoid female but also the size of the adult *Trichogramma*, which will depend of the nutritional resources available for the development of the larva (VINSON, 1997). Molina and Parra (2006) have reported similar results obtained with eggs of *Gymnandrosoma aurantianum* Lima (Lepidoptera: Tortricidae) in which they found a variation between 1.4 and 1.8 individuals/egg at 25ºC. The values found in this study, however, are higher than the figures determined for other species of pest lepidopterans such as *Bonagota cranaodes* Meyrick (Lepidoptera: Tortricidae) and *Chrysodeixis includens* Walker (Lepidoptera: Noctuidae) (BUENO et al., 2009; FONSECA et al., 2005). These authors reported values of 1.3 and 1.0 individuals/egg, respectively, at 25ºC. Similarly, Pratissoli and Parra (2000) verified that on two different hosts, the highest number of parasitoids per host egg was obtained at 25ºC. Another study by Pastori et al. (2008) reported a higher number of descendants per egg at 22 and 30ºC.

There were statistically significant differences in the viability parameter at different temperatures (Table 1). At all temperatures, however, the values observed were above 88%. Several other authors have reported similar values (MELO et al., 2007; MILANEZ et al., 2009; PRATISSOLI; PARRA, 2000), demonstrating that the cabbage looper *T. ni* is a potentially suitable host for the egg parasitoid *T. pretiosum*.

Conclusion

Temperature heavily influences the parasitism rate of *T. pretiosum* reared on eggs of *T. ni*.

The egg parasitoid *T. pretiosum* has suitable characteristics for the control of the cabbage looper *T. ni* within a thermal range between 24 and 27ºC.

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