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Effects of parasitoid and host age on the parasitism of *Trichogramma pretiosum* on eggs of *Anticarsia gemmatalis*

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ABSTRACT

The ages of parasitoid females and their hosts can have substantial effects on parasitism and parasitoid efficacy. This relationship has been investigated over the last few decades and found to vary among species. To our knowledge, it was virtually unknown for *Trichogramma pretiosum* wasps that parasitize *Anticarsia gemmatalis* eggs. Therefore, the influence of parasitoid and host age on the parasitism of *T. pretiosum* on eggs of *A. gemmatalis* was here evaluated in three independent bioassays. Neither the number of parasitized eggs nor that of emerged parasitoids differed between *T. pretiosum* females of different age. The number of parasitoids per egg and the sex ratio were higher for 5-day-old females. Regarding host age, the number of parasitized eggs was highest for *A. gemmatalis* eggs aged 24 h (9.60), followed by eggs aged 48 h (1.00), while no parasitism was observed for eggs aged 72 h. The number of parasitoids per egg and sex ratio were not influenced by host age. Preference was significantly higher for host eggs aged 24 h (79.67%), followed by eggs aged 48 h (16.99%) and 72 h (3.33%). Accordingly to these results, the key challenge for a successful *T. pretiosum* release to control *A. gemmatalis* in the field is to synchronize the presence of up to 5-day-old adults with the most susceptible phase of the host eggs (1 day).

Soybean (*Glycine max* L. Merril) is one of the world's major crops, accounting for the majority of the global demand for oil and vegetable protein (Oerke and Dehne, 2004). Nevertheless, its yield might be seriously reduced by leaf feeders, especially improperly managed caterpillars (Hoffmann-Campo et al., 2003). The velvet bean caterpillar *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Eribidae) was previously the most important species of this group (Panizzi and Correa-Ferreira, 1997; Maruya et al., 2001) and remains the most abundant one during the early crop season of important soybean areas in Brazil (Conte et al., 2019). Despite the high efficacy of Bt cultivars in controlling this pest, as well as recent outbreaks and thus the increasing importance of the soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), which is more tolerant to insecticides (Stacke et al., 2019), some issues remain. Firstly, non-Bt soybean is still common in Brazil: during the 2017/2018 season, 13.7 millions of hectares were sowed with non-Bt soybean, and 7.2 millions of hectares of non-Bt soybean are predicted even at the future peak of Bt soybean adoption (CIB, 2019). Secondly,

A. gemmatalis is important during the early soybean season: for the first 30 days after soybeans emerge in the field, it is the most abundant species in non-Bt soybeans, accounting for 55.2% of all caterpillars in the 2018/2019 season in some of the important soybean-producing areas (Conte et al., 2019). A similar predominance of *A. gemmatalis* in those areas was previously recorded in all crop seasons between 2013 and 2018 (Conte et al., 2014, 2015, 2016, 2017, 2018).

A common tool used to control caterpillar outbreaks is the spraying of chemicals. Its frequent overuse by some soybean growers what can trigger important and undesirable side effects (Song and Swinton, 2009), among which the selection of resistant pest strains (Diez-Rodríguez and Omoto, 2001) and the death of biological control agents (Carmo et al., 2010; van Lenteren and Bueno, 2003) are most relevant. Therefore, a better alternative to the exclusive use of chemical pesticides in caterpillar pest management is urgently required (Bueno et al., 2011). Among possible options, biological control has produced good results in managing caterpillar outbreaks (Parra and Coelho Junior, 2019), especially in augmentative biological control (ABC). In ABC programs, egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are widely used because they are easily reared

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on factitious hosts (Haji et al., 1998) and are effective against target pest species (Bueno et al., 2009). Moreover, *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) was the first egg parasitoid commercially available to control Lepidoptera eggs in Brazilian soybean fields. However, the success of *T. pretiosum* releases relies on successful synchronization between the most susceptible age of the pest egg and the age at which the released adult parasitoids have the greatest parasitism activity (Cingolani et al., 2014). Both parasitoid and host age can have substantial effects on the efficacy of these entomophages (Pak, 1986; Polanczyk et al., 2007; Pizzol et al., 2012; Vargas et al., 2017; Laurentis et al., 2019).

The relationship between host age and parasitism as well as between parasitoid age and parasitism has been intensively investigated over the years (e.g., Marston and Ertle, 1969; Bragg, 1974; Pak, 1986; Hintz and Andow, 1990; Calvin et al., 1997; Queiroz et al., 2019) and found to vary between different species (Pak, 1986). For example, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) can parasitize *Plutella xylostella* (Lineu) (Lepidoptera: Plutellidae) eggs of all developmental stages (Miura and Kobayashi, 1998), while *T. pretiosum* parasitism was higher on 1-day-old eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Pratissoli and Oliveira, 1999). However, the emergence of parasitoid offspring was not affected by the age of parasitized eggs (Laurentis et al., 2019). The preference of *T. pretiosum* for young hosts appears to be related to the parasitoid's feeding behavior rather than the suitability of the egg for parasitoid development, since parasitoid preference subsided after contact with the first egg (Ruberson and Kring, 1993). However, as far as we know, there is no such information concerning *T. pretiosum* parasitism on *A. gemmatalis* eggs. The relationship between *A. gemmatalis* egg age and host acceptance behavior or host suitability of *T. pretiosum* females is virtually unknown. Therefore, the objective of this work was to evaluate the effect of both parasitoid and host age on the parasitism of *T. pretiosum* on eggs of *A. gemmatalis* as well as *T. pretiosum* preference for eggs of different ages.

Three independent bioassays were carried out under controlled conditions in climatic chambers (BOD) (ELECTROLab®, model EL 212, São Paulo, SP, Brazil) set at a temperature of 25 ± 2°C, relative humidity of 80 ± 10%, and a photoperiod of 14/10 h (L/ D). *Anticarsia gemmatalis* eggs and *T. pretiosum* females used in this study came from insect colonies kept at the laboratories of Embrapa Soja, State of Paraná, Brazil, according to the methodology described by Bueno et al. (2009).

In bioassay 1, we evaluated how the age of *T. pretiosum* females affected their parasitism on *A. gemmatalis* eggs. This experiment was carried out in a completely randomized design with five treatments (*T. pretiosum* females, 1 to 5 days old) and four replicates composed of five females each. Female *T. pretiosum* were individualized in plastic microtubes (12 mm diameter x 75 mm height) and sealed with PVC plastic film. For food, pure bee honey droplets were placed on the tube walls. Approximately 20 eggs of *A. gemmatalis* (≤ 24 h) were glued on white cardboard (2.5 cm x 5 cm) and assigned to their respective treatments. These cards were then added to the microtubes with individualized females and exposed to parasitism for 24 h, following the methodology described by Pratissoli and Oliveira (1999) and Laurentis et al. (2019). Subsequently, the females were removed from the microtubes, and the cards were kept in the same BOD until adult emergence. The biological parameters evaluated were the number of parasitized eggs, number of parasitoids per egg, number of emerged parasitoids (emergence %), and sex ratio of the progeny [sex ratio = number of females/(number of males + number of females)].

In bioassay 2, the influence of *A. gemmatalis* egg age on parasitism of *T. pretiosum* was evaluated. The trial was conducted in a completely randomized design with three treatments (*A. gemmatalis* eggs, aged 24, 48, and 72 h) and six replicates composed of five individualized

females each. Eggs of *A. gemmatalis* were collected daily and kept in BODs (temperature of 25 ± 2 °C, relative humidity of 80 ± 10% and photoperiod of 14/10 h L/D) for a period of 24, 48, and 72 h prior to exposure to parasitism. Subsequently, these eggs were glued on pieces of white cardboard (2.5 cm x 5 cm) and inserted into microtubes (12 mm diameter x 75 mm height) containing a newly emerged female (≤ 24 h) *T. pretiosum* previously fed with a bee honey droplet. Parasitism was allowed for 24 h. After parasitism, females were removed and discarded and the microtubes sealed with PVC film and kept in the same BOD until parasitoids emerged. Similar to bioassay 1, the number of parasitized eggs, parasitoids per egg, emerged parasitoids (emergence %), and sex ratio were evaluated.

In bioassay 3, the preference of *T. pretiosum* among *A. gemmatalis* eggs of different ages was evaluated. The experiment was conducted in a completely randomized design with three treatments (*A. gemmatalis* eggs aged 24, 48, and 72 h) and 15 replicates composed of an arena adapted from Thuler et al. (2007), made with polyethylene bottles (4 cm high and 2 cm in diameter) and six plastic microtubes (75 mm high and 12 mm in diameter) arranged equidistantly in the lower part of the bottle (Queiroz et al., 2017). In each arena, approximately 20 eggs of *A. gemmatalis* aged 24, 48, and 72 h were glued on white cardboard (2.5 cm x 5 cm) and were later introduced into the microtubes on opposite sides of each arena. A female *T. pretiosum* (≤ 24 h) was released at the top of the arena following the methodology used by Laurentis et al. (2019). Parasitism was allowed for 24 hours. Afterwards, the microtubes containing the eggs were removed, sealed with plastic PVC film, and kept in the same BOD until the emergence of adults. The evaluated parameter was the number of parasitized eggs for each host age and then calculated parasitism preference (%) for each age (parasitism preference% = number of eggs parasitized of each age/total number of parasitized eggs x 100).

All results were evaluated for normality of residuals (Shapiro and Wilks, 1965) and homogeneity of variance among treatments (Burr and Foster, 1972) for ANOVA, followed by a Tukey test ($p \le 0.05$). When the data did not follow a normal distribution (number of parasitized eggs and number of emerged parasitoids), the Kruskal-Wallis test, a non-parametric analysis of variance was used, followed by Chi-Square (χ^2) mean comparisons ($p \le 0.05$) (SAS Institute, 2009).

The numbers of parasitized eggs (Kruskal-Wallis ANOVA, $\chi^2 = 3.6305$, df = 17, $p = 0.4583$) and emerged parasitoids (Kruskal-Wallis ANOVA, $\chi^2 = 2.1604$, df = 17, $p = 0.7063$) were unrelated to the age of *T. pretiosum* females. The parasitism on *A. gemmatalis* eggs observed in this trial was similar to the results found by Bueno et al. (2012), who reported seven to nine *A. gemmatalis* eggs parasitized by *T. pretiosum* on the first day. In contrast, the number of parasitoids per egg (ANOVA, df = 17, $p = 0.0057$; F = 5.62) and sex ratio (ANOVA, df = 17, $p = 0.0083$; F = 5.70) were higher for 5-day-old *T. pretiosum* females. Even though the sex ratio was lower (ANOVA, df = 17, $p = 0.0083$; F = 5.70) for eggs parasitized by 1-day-old *T. pretiosum* females (0.96), it is important to note that the sex ratio was always higher than 0.90 for all evaluated age groups of parental *T. pretiosum* (Table 1).

In contrast, the age of host eggs appeared to have a greater impact on parasitism. *Trichogramma pretiosum* preferred eggs at early developmental stages (ANOVA, df = 19, $p < 0.0001$; F = 55.82) and also parasitized a higher number of these eggs. The number of parasitized eggs was highest for *A. gemmatalis* eggs of 24 h (9.60), followed by eggs of 48 h (1.00). Eggs of 72 h were not parasitized (Kruskal-Wallis ANOVA, $\chi^2 = 14.8750$, df = 19, $p = 0.0006$). This resulted in a higher number of emerged parasitoids from host eggs aged 24 h (10.00) compared to eggs of 48 h (1.93) (Kruskal-Wallis ANOVA, $\chi^2 = 14.8587$, df = 19, $p = 0.0006$) despite 100% emergence in all parasitized eggs. However, the number of parasitoids per egg (ANOVA, df = 19, $p = 0.5857$; F = 0.34) and sex ratio

(ANOVA, df = 19, p = 0.7428; F = 0.12) did not differ between evaluated age groups of host eggs (Table 2). Preference was significantly higher for eggs of 24 h (79.67%), followed by those of 48 h (16.99%) and 72 h (3.33%) (ANOVA, df = 19, p <0.0001; F = 55.82) (Table 2).

The results presented here indicate that one of the most important challenges in ABC programs that use *T. pretiosum* to control *A. gemmatalis* is to synchronize the release of parasitoid females with *A. gemmatalis* eggs recently laid in the field, since parasitism seems to be more efficient on *A. gemmatalis* eggs of 24 h. The decrease in parasitism with progressing host egg development is probably caused by changes in external and internal characteristics of the egg. Parasitoid females are known to assess their hosts, and their behavior is based on perceived cues during probing (Godfray, 1994; Vinson, 1997) or visual cues (Ruberson and Kring, 1993), among other mechanisms (Outreman et al., 2001; Desneux et al., 2009). Eggs of different age groups may differ in surface, size, and structure of the chorion and other characteristics, such as a change in color during embryonic development. All these characteristics and their variation can influence not only *Trichogramma* handling time and exploitation but also the suitability of hosts for parasitism and parasitoid development (Cônsoli et al., 1999). In addition, changes in the external characteristics of eggs may be related to hardening of the chorion during embryonic development, thus limiting the penetration capacity of the ovipositor (Pak et al., 1986). Parasitoid females have more difficulties penetrating the chorion of older eggs (Ruberson and Kring, 1993). Furthermore, as a host embryo's development advances, its nutritional value is reduced, which also influences the host acceptance behavior of the parasitoid (Navarajan, 1979). Thus, the consistent preference of *T. pretiosum* for recently laid (≤ 24 h) eggs of *A. gemmatalis* compared with older eggs (48 and 72 h) might be due to the better nutritional value of young eggs (Molina et al., 2005). This corresponds to the optimal foraging theory, which predicts that organisms should find, assess, and exploit the resources that contain the most energy in order to maximize their lifetime fitness. Numerous studies have confirmed this theory by demonstrating that when food is abundant, consumers tend to avoid items yielding low net energy return and specialize in more energetically rewarding items (Stephens and Krebs, 1986). Ruberson and Kring (1993) likewise observed that *T. pretiosum* preferred feeding on young hosts. Therefore, the selection of young hosts for initial acceptance by parasitoid females will ensure

not only that adult parasitoids will obtain nutrients from their hosts but also that at least some of their offspring will develop in highly suitable hosts.

In contrast to host egg age, parasitoid age (1 to 5 days old) seemed to have little influence on parasitism. This finding allows the conclusion that it is possible to store parasitoids for up to 5 days with low fitness costs before releasing them in the field. The lack of impact of *T. pretiosum* females' age on their parasitism is possibly related to their ability to adjust egg production according to host availability (Hougardy et al., 2005). The absence of hosts can increase the fecundity index of the parasitoid, maintaining its rate of parasitism even after some days of host deprivation.

It is important to mention that according to Pratissoli et al. (2004), a higher rate of egg resorption is prevalent in older *Trichogramma* spp. during the absence of hosts. This could lead to a reduction in parasitism, although this was not observed in our trials. Thus, the number of parasitized eggs did not differ among *T. pretiosum* females up to 5 days of age. However, a higher number of parasitoids per egg emerged after parasitism by older *T. pretiosum* females (5 days old). This could be detrimental to pest control because an increase in the number of parasitoids per egg may reduce control efficiency. This is due to the smaller number of parasitized eggs when females repeatedly parasitize the same egg (Beserra and Parra, 2004). Moreover, superparasitism in a single host individual can generate smaller and deformed individuals (Moreira et al., 2009), which may compromise the survival of subsequent generations of the parasitoid in the field. This should be analyzed in future studies.

Vinson (1997) pointed out that host quality is the major factor affecting the sex ratio in *Trichogramma* spp. This might be due to the capacity of *Trichogramma* females to differentiate host quality prior to laying male or female eggs (Bueno et al., 2012). Despite the ability of *Trichogramma* females to differentiate *A. gemmatalis* eggs of different ages and their preference for fresh eggs, the sex ratio was similar for all age groups tested in our study and in all cases greater than 0.90. A sex ratio above 0.60 is considered adequate in biological control programs (van Lenteren and Bueno, 2003); therefore, egg and parasitoid ages did not impact this biological characteristic.

The development and emergence of more than one parasitoid per egg is related to host egg volume and nutritional quality (Vinson, 1997).

Table 1
Influence of the age of *Trichogramma pretiosum* females on parasitism of *Anticarsia gemmatalis* eggs (bioassay 1). Assay performed under controlled conditions with a temperature of 25 ± 2°C, relative humidity of 80 ± 10%, and photoperiod of 14/10 h [L/D].

Female parasitoid age (days)	Parasitized eggs[a]	Parasitoids[b].egg^{-1}	Parasitoids emerged[a] (Emergence %)	Sex ratio[b]
1	7.65 ± 1.85 a	1.45 ± 0.08 b	10.80 ± 2.24 a (100)	0.96 ± 0.01 b
2	8.20 ± 1.39 a	1.47 ± 0.06 b	11.95 ± 2.33 a (100)	0.99 ± 0.01 a
3	5.40 ± 1.71 a	1.25 ± 0.13 b	7.60 ± 2.72 a (100)	1.00 ± 0.00 a
4	5.75 ± 0.90 a	1.45 ± 0.11 b	8.95 ± 2.05 a (100)	0.99 ± 0.01 ab
5	5.00 ± 1.29 a	2.03 ± 0.20 a	9.74 ± 2.04 (99.61)	1.00 ± 0.00 a

[a]*Means ± SEM followed by the same letter within a column did not differ statistically (Kruskal-Wallis ANOVA, test χ^2 test p≥0.05).* [b]*Means ± SEM followed by the same letter within a column did not differ statistically (ANOVA, Tukey test p≥0.05).*

Table 2
Biological characteristics (bioassay 2) and preference of parasitism (bioassay 3) of *Trichogramma pretiosum* in eggs of *Anticarsia gemmatalis* of different ages (hours). Tests performed under controlled conditions with a temperature of 25 ± 2ºC, relative humidity of 80 ± 10%, and photoperiod of 14/10 h [L/D].

Host egg age (h)	Parasitized eggs[a]	Parasitoids[b].egg^{-1}	Parasitoids emerged[a] (Emergence %)	Sex ratio[b]	Parasitism distribution - preference (%)[b]
24	9.60 ± 0.20 a	1.29 ± 0.07^{ns}	10.00 ± 1.95 a (100)	0.65 ± 0.04^{ns}	79.67 ± 6.82 a
48	1.00 ± 0.07 b	1.46 ± 0.33	1.93 ± 0.77 b (100)	0.63 ± 0.07	16.99 ± 5.61 b
72	0.00 ± 0.00 c	-	0.00 ± 0.00 c (-)	-	3.33 ± 3.33 b

[a]*Means ± SEM followed by the same letter within a column did not differ statistically (Kruskal-Wallis ANOVA, test χ^2 test p≥0.05).* [b]*Means ± SEM followed by the same letter within a column did not differ statistically (ANOVA, Tukey test p≥0.05).* ^{ns}ANOVA not significant. -Data nonexistent.

Consequently, the high amounts of nutrients available in *A. gemmatalis* eggs allow the development of more than one *T. pretiosum* individual per egg (Parra, 1997). Also, embryonic development inside the egg did not significantly reduce the volume and nutritional quality of the host, therefore allowing for the development of more than one parasitoid per egg. Overall, according to the results, the key challenge for a successful *T. pretiosum* release to control *A. gemmatalis* is to synchronize the most susceptible phase of the host eggs (1-day-old) with the presence of adults up to 5 days old in the field. Moreover, adults of *T. pretiosum* can be stored (25°C) for up to 5 days until release without any apparent fitness costs.

Acknowledgments

Conflict of interest

The authors declare there is no conflict of interest in publishing this article.

Author contribution statement

AP Queiroz, AF Bueno and CO Costa contributed to the study design and wrote the paper. AP Queiroz and CO Costa conducted the sampling. AF Bueno and GV Silva contributed reagents/ material/ analysis tools. AP Queiroz, BM Favetti, CO Costa conducted the experiments. All authors have read and approved the manuscript.

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