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OVIPOSITION PREFERENCE OF *Plutella xylostella* (Lepidoptera: Plutelidae) AND PARASITISM BY *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) IN COLLARD GREEN

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ABSTRACT

The damage caused by the diamondback moth is a limiting factor in the production of brassicaceous plants worldwide and biological control by an egg parasitoid may be an effective way to control this pest. Thus, understanding oviposition preferences of pests and parasitoids in crop plants can be a key factor in optimizing biological control programs. The present study was aimed at determining the oviposition preference of *Plutella xylostella* and *Trichogramma pretiosum* in collard greens. Bioassays consisted of collard plants in cages and *P. xylostella* adults released inside to lay eggs on the leaves. After 24 hours, the adults were removed and *T. pretiosum* females released. In another experiment, cardboard strips containing *P. xylostella* eggs were offered to *T. pretiosum* released in cages with a collard greens plant inside and replaced every 2 hours for 24 hours. The results showed that *P. xylostella* has a preference for laying eggs on the leaves of the middle region of the plant, on the upper part of the adaxial surface. For *T. pretiosum*, no preference was observed, but mean parasitism rates were highest on the upper region of the adaxial surface, where most of the eggs were found. In the second experiment, the highest mean parasitism rates by *T. pretiosum* were observed at temperatures ranging from 25.9 to 37.4 °C and relative humidity between 42 and 78% in the photophase. No parasitism occurred in scotophase, indicating that field releases should be performed preferably in the early hours of the morning.

Keywords: Diamondback moth, Brassicaceae, *Brassica oleracea* var. *acephala,* egg parasitoids, photoperiod

PREFERÊNCIA DE OVIPOSIÇÃO DE *Plutella xylostella* L. (Lepidoptera: Plutelidae) E DE PARASITISMO POR *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) EM COUVE

RESUMO

A traça das crucíferas é um fator limitante na produção de brássicas em todo o mundo, e a utilização de parasitoides de ovos (*Trichogramma pretiosum* Riley, Hymenoptera: Trichogrammatidae) pode ser uma alternativa para o controle desta praga. Neste contexto, a compreensão da preferência de parasitismo na cultura a ser trabalhada pode ser um fator chave para a otimização de programas de controle biológico. O presente trabalho teve como objetivo determinar a preferência de oviposição de *P. xylostella* e de parasitismo de *T. pretiosum* em couve. Experimentos foram realizados com plantas de couve colocadas dentro de gaiolas, em temperatura ambiente e adultos de *P. xylostella* liberados no interior para que ovipositassem nas folhas. Após 24 horas, os adultos foram retirados e fêmeas de *T. pretiosum* liberadas. Em outro experimento, cartelas contendo ovos de *P. xylostella* foram ofertados a *T. pretiosum* liberados dentro de gaiolas e as cartelas foram substituídas a cada 2 horas por 24 horas. Os resultados mostram que *P. xylostella* tem preferência por ovipositar nas folhas da região média da planta, na parte superior da superfície adaxial. Para o parasitoide de ovos *T. pretiosum*, não se registrou preferência, mas as maiores médias de parasitismo foram registradas na parte superior da superfície adaxial, onde se encontravam grande parte dos ovos. No segundo experimento, *T. pretiosum* teve as maiores médias de parasitismo com temperaturas variando de 25,9 a 37,4 °C e umidade relativa entre 42 e 78% entre as 8 e as 18 horas (fotofase), e ausência de parasitismo no período noturno (escotofase), indicando que liberações em campo devem ser realizadas preferencialmente nas primeiras horas da manhã para que o parasitoide tenha toda a fotofase disponível para parasitismo.

Palavras-chave: Traça das crucíferas, Brassicaceae, *Brassica oleracea* var. *acephala*, parasitoide de ovos, fotoperíodo

INTRODUCTION

Among brassicaceous plants, the production of collard greens (*Brassica oleracea* var. acephala) has gradually increased in Brazil due to the demand for healthy foods and availability of information about its nutritional and medicinal properties (TRANI et al., 2010, 2015). Camargo

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Filho and Camargo (2010) reported a cultivated area of approximately 2000 hectares of collard greens in the state of São Paulo, with yields of over 55 tons.

One of the limiting factors to its cultivation is the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), which causes losses of US$ 4 billion per year worldwide (TALEKAR & SHELTON, 1993; ZALUCKI et al., 2012). Chemical control is the main method used to control this pest. However, the damage caused by the consumption of collard green leaves by *P. xylostella* larvae, as well as the negative impacts associated with insecticide use, can be minimized with the use of egg parasitoids such as *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (PARRA &ZUCCHI, 2004).

To be feasible, studies on the preferences of pests and natural enemies are essential. Oviposition preference by insects is influenced by several intrinsic and extrinsic factors and egg parasitoids of the genus *Trichogramma* are no different. Factors such as habitat location, host recognition and acceptance are critical to parasitoid efficacy (BADENES-PEREZ et al., 2006; FARIA et al., 2008; ZAGO et al., 2010). Preferences of *P. xylostella* have also been reported in studies conducted with cabbage, with the highest oviposition rates occurring on the adaxial surface, in the upper region of younger leaves of cabbage (ZAGO et al., 2010).

In addition to preference, environmental factors such as variations in temperature, humidity, photoperiod, wind speed, chemical compounds, food availability and plant structure directly affect parasitoid effectiveness in the field (VINSON, 1997; SHIOJIRI & TAKABAYASHI, 2003; ROMEIS et al., 2005; OLSON & ANDOW, 2006; ZAGO et al., 2010). Therefore, knowledge on the effect of these factors on *Trichogramma* parasitism is essential before implementing a biological control program to ensure positive field results.

The present study evaluated the oviposition site preference of *P. xylostella* and parasitism of *T. pretiosum* in collard greens as a contribution to future field studies.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Entomology at the Center for Agroenvironmental Research Studies, and Development, State University (CPEDA) in Tangará da Serra, Mato Grosso State, Brazil.

Plutella xylostella rearing

Larvae were maintained in 5-L plastic containers with openings on the lid and sides (Ø = 15 cm) covered with thin fabric (voil) for ventilation and fed daily with leaves of collard green cultivated in a greenhouse (Figure 1 A). Upon reaching the pupal stage (Figure 1 B), larvae (Figure 1 C) were transferred to an acetate cage (Ø = 10 cm and height = 20 cm) (Figure 1 D) (MARCHIORO & FOERSTER, 2011).

The upper part of the cage (lid) had an opening of 2.5 cm² where collard green leaves were placed to stimulate oviposition (Figure 1 E). Cages were internally lined with filter paper and externally, a second lid overlapped the collard green leaf, which was then fitted between the two lids. Oviposition was performed on filter paper around the leaf, which was changed every two days for a new lid. The filter paper containing the eggs was placed on a collard green leaf in the plastic containers mentioned above and a new cycle began. Adults were not fed and were kept in a climate controlled room at 25 ± 2 ºC, 70 ± 10% RH, and 12:12 h photoperiod (D:L).

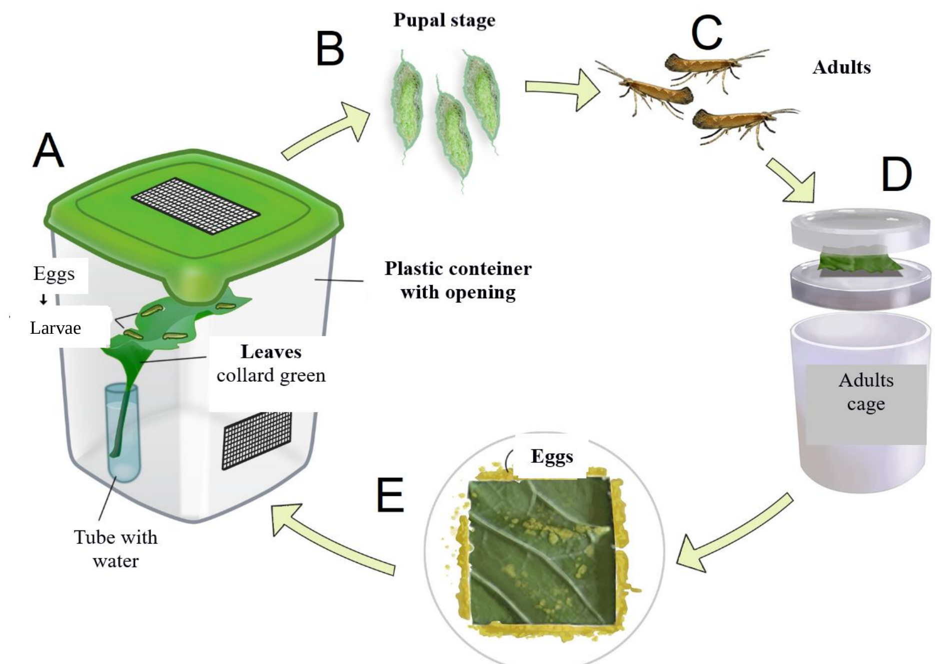

Figure 1. (A) caterpillars cage; (B) *P. xylostella* pupae removed from the cages; (C) Emerged *P. xylostella* adults; (D) adult cage with cap adapted for oviposition (Marchioro and Foerster, 2011); (E) Lid containing *P. xylostella* eggs that are packed in the cage (A). Tangará da Serra, Mato Grosso State, 2018.

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Trichogramma pretiosum rearing

Parasitoids were obtained from soybean fields in Tangará da Serra, MT, during the 2015/2016 crop season and maintained in the Laboratory of Entomology at CPEDA.

Parasitoids were maintained and mass reared using eggs of the alternative host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae). Adults of *A. kuehniella* were kept in cages that consisted of plastic polyvinyl chloride (PVC) tubes (Ø = 10 cm and height = 20 cm), with the ends covered by thin fabric (tulle). Eggs were collected in petri dishes at the base of the cage, and a portion of these eggs was used as hosts to rear *T. pretiosum*. Eggs were sterilized with a germicidal lamp (Osram®, 15W, G13) for one hour and then spread on a rectangular glass tray (5.1 cm x 39.4 cm x 23.9 cm) with strips of honey to feed the parasitoids and covered with plastic film. Parasitoids were kept in an incubator under the same conditions mentioned for *P. xylostella*. To maintain the *Trichogramma* colony, ca. 180,000 eggs were parasitized daily.

The remaining eggs were used for the maintenance of *A. kuehniella* in the laboratory. Fertile eggs were inoculated in the proportion of 0.3 g of eggs to 1 kg of flour-based diet, consisting of 60% fine corn flour, 37% whole wheat flour, and 3% beer yeast. Trays were kept in rearing rooms at 25 ± 2 °C, 70 ± 10% relative humidity (RH), and 24:00 h photoperiod (D:L), where larvae and pupae were kept until the first moths emerged, which were then transferred to PVC cages with the aid of an adapted vacuum cleaner to start a new production cycle (PARRA et al., 2014).

Collard greens growth

For the maintenance of *P. xylostella* rearing and conducting bioassays, plants of *Brassica oleracea* var. acephala (Brassicaceae) were grown in a greenhouse according to Filgueira (2007).

Seeds were distributed in seedling trays and when plants reached 10 cm in height, they were transplanted to individual 10-L plastic pots containing soil amended for collard greens. The pots were kept on a bench in a greenhouse at temperatures between 25 and 30ºC; the plants were irrigated twice a day with approximately 3 mm of water. Forty days after seedling transplantation, plants were used in the experiments.

Experiment 1 – Oviposition site preference of *P. xylostella* and parasitism of *T. pretiosum*

The entire experiment was conducted in a greenhouse with an overhead watering system. Twelve pots each containing a collard green plant, 40 days after transplantation, were individually placed in wooden cages (40 cm x 40 cm x 60 cm), covered with thin (voile) fabric. One side of each cage was fitted with a "sleeve" that allowed access to the interior. The bottom of the cages was sealed with expanded polystyrene plates (Isopor®).

Two pairs (2 males and 2 females) of one-day-old *P. xylostella* were released in each cage, where they remained for 24 hours to lay eggs on any part of the plant. After the oviposition period, the adults were removed. Plants were manually watered, and forty females of *T. pretiosum* were released inside. This number was chosen because each *P. xylostella* female lays an average of 15 eggs per day (CHAGAS FILHO et al., 2010) while each *T. pretiosum* female parasitizes 10 eggs per day on average (PASTORI et al., 2007; PEREIRA et al., 2007). Parasitoids were in contact with eggs for 24 hours and after this period, cages were disassembled and the plants were taken to the laboratory for evaluation.

In the laboratory, plants were cut at the base, each leaf was numbered from the bottom to the top (Figure 1) and removed from the stem. Leaves were then classified according to surface (abaxial or adaxial) and region (upper, middle or lower) (Figure 2).

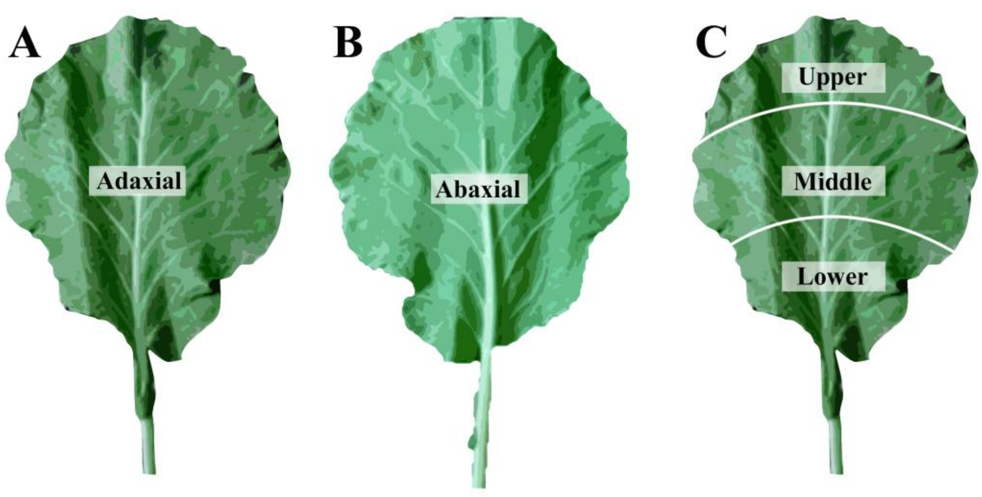

Figure 2. Collard green view: A - Adaxial surface; B - Abaxial Surface; C - Leaf regions (Upper, Middle, Lower). Tangará da Serra, Mato Grosso State, 2018.

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With the aid of a stereomicroscope, leaves were examined for the presence of *P. xylostella* eggs in each of these areas. When found, eggs were removed with a brush and fixed on a cardboard strip identified with the part of the plant from which they were removed. The cards were isolated in plastic test tubes sealed with plastic film and kept in a B.O.D. incubator at 25 ± 2 ° C, and 12:12 photoperiod (D:L). After 10 days, the eggs were examined for signs of parasitism.

P. *xylostella* egg distribution and *T. pretiosum* parasitism were evaluated for: leaf surface (abaxial or adaxial) (Figure 2 A and B), leaf region (upper, middle or lower) (Figure 2 C), and leaf number from the base to the top (Figure 3).

Figure 3. Numbering model of collard green leaves (side view of the plant). Tangará da Serra, Mato Grosso State, 2018.

Experiment 2 – Time of parasitism

The experiment was conducted in an open area to evaluate the effects of temperature, relative humidity and photoperiod on *T. pretiosum* parasitism over a 24 h period. Ten collard greens plants, 40 days after being transplanted to 10-L pots, were placed inside cages as described in the previous experiment. In each cage, 120 24-hours old *T. pretiosum* females, were released, which had previously been fed pure honey.

Ten-24 hour-old eggs of *P. xylostella* were sterilized under a germicidal lamp and glued on light blue cardboard strips (1 cm x 5 cm) and placed on each plant. The strips were changed every 2 hours to record parasitism rate as a function of time. The strips were replaced at: 8, 10, 12, 14, 16, 18, 20, 22, 00, 2, 4 and 6 hours. The strips removed from the plant were then isolated in vials sealed with a plastic film and kept in a incubator to observe parasitoid emergence under the same environmental conditions previously described. Climate variables (temperature and relative humidity) were monitored every 2 hours using a digital thermohygrometer.

Statistical Analysis

Data obtained from both experiments were analyzed with an ANOVA and the results were compared with the Scott-Knott test (SCOTT & KNOTT, 1974). The influence of temperature and relative humidity on parasitism success was tested with regression linear models (LM). Data normality was evaluated using the Shapiro-Wilk test and results were also graphically evaluated with the fit of residues of generalized linear models (GLM) using graphs to adjust quartiles predicted by the model, with the data (QQ Plot), and with the residue histograms. All analyzes were performed using the R Studio statistical software version 3.4.1 (R CORE TEAM, 2017) and the ScottKnott package (JELIHOVSCHI et al., 2014).

RESULTS AND DISCUSSION

Distribution of eggs

Females of *P. xylostella* preferred to oviposit on the leaves of the middle part of the plants, where the highest number of eggs was recorded (Table 1). For *T. pretiosum*, no preference was observed for eggs regarding the position of leaves in the plant, as means were not significantly different (Table 1).

When evaluating oviposition and parasitism on the surface of the collard greens leaves, *P. xylostella* and *T. pretiosum* preferred the adaxial surface, with a mean of 21.00 eggs, of which 13.22% were parasitized, significantly differing from the abaxial surface (Table 2).

Regarding the region of collard greens leaf in the plant, the highest number of *P. xylostella* eggs was recorded in the upper part of the plant, with an average of 0.80 eggs and 2.68% of them were parasitized by *T. pretiosum* (Table 3).

Table 1. Number of leaves evaluated (N), maximum and mean number (± standard deviation) of *Plutella xylostella* eggs per leaf and mean number of eggs parasitized by *Trichogramma pretiosum* according to the position of the leaves in collard green plants. Tangará da Serra, Mato Grosso State, 2018.

Leaf	N	Oviposited		Parasitized	
		Maximum	Mean	Maximum	% Mean
F1	12	10	1.50 ± 3.03 b[1]	4	0.80 ± 1.78 a
F2	12	17	1.92 ± 4.81 b	5	1.20 ± 2.16 a
F3	12	27	2.92 ± 7.65 b	16	3.20 ± 7.15 a
F4	12	11	1.83 ± 3.56 b	8	3.25 ± 3.59 a
F5	12	29	**6.41 ± 9.66 a**	18	8.16 ± 6.55 a
F6	12	32	**7.83 ± 10.42 a**	22	6.43 ± 7.97 a
F7	12	6	1.33 ± 2.01 b	2	0.80 ± 1.09 a
F8	12	40	**5.4 ± 12.55 a**	24	5.80 ± 10.30 a
F9	12	8	0.83 ± 2.32 b	1	0.50 ± 0.70 a
F10	10	6	1.60 ± 2.27 b	3	1.75 ± 1.25 a
F11	7	1	0.25 ± 0.46 b	1	0.50 ± 0.70 a
F12	3	1	0.25 ± 0.50 b	0	0.00 ± 0.00 a
Core	12	0	0.00 ± 0.00 b	0	0.00 ± 0.00 a
Stem	12	18	2.75 ± 6.06 b	4	2.00 ± 2.30 a
p			< 0.01		0.49

[1] Means followed by different letters in the column differ significantly according to the Scott-Knott test at 5%.

Table 2. Maximum and mean number (± standard deviation) of eggs of *Plutella xylostella* and eggs parasitized by *Trichogramma pretiosum* on the adaxial and abaxial surface of collard green. Tangará da Serra, Mato Grosso State, 2018.

Surface	Oviposited[1]		Parasitized[1]	
	Maximum	Mean	Maximum	% Mean
Abaxial	30	6.67 ± 9.38 b	13	5.71 ± 4.60 b
Adaxial	47	**21.00 ± 18.46 a**	30	**13.22 ± 9.79 a**
p		< 0.001		0.06

[1] Means followed by different letters in the column differ significantly according to the T-test at 5%.

Table 3. Maximum and mean number (± standard deviation) of eggs of *Plutella xylostella* and parasitized by *Trichogramma pretiosum* in the upper, middle and lower region of collard green. Tangará da Serra, Mato Grosso State, 2018.

Region	Oviposited[1]		Parasitized[1]	
	Maximum	Mean	Maximum	% Mean
Upper	32	**0.80 ± 3.23 a**	22	2.68 ± 4.60 a
Middle	12	0.27 ± 1.28 b	8	1.50 ± 2.28 a
Lower	24	0.42 ± 2.08 b	16	2.04 ± 3.55 a
p		0.03		0.53

[1] Means followed by different letters in the column differ significantly according to the Scott-Knott test at 5%.

Time of parasitism

Parasitism of *T. pretiosum* only took place during daytime (photophase), with mean parasitism rates ranging between 34.21% and 57.65%, which significantly differed from that of the nocturnal period (scotophase) (Table 4).

At 2pm, a significant decrease was observed in the percentage of parasitized eggs due to rainfall, which caused a drop in temperature and an increase in relative air humidity (Table 4). The linear regression correlating climatic variables and parasitism success revealed that temperature ($F = 111.1$, $R^2 = 0.48$, $p < 0.001$) and relative humidity ($F = 127.72$, $R^2 = 0.52$, $p < 0.001$) (Figure 4) had a significant influence.

Table 4. *Trichogramma pretiosum* parasitism rate in *Plutella xylostella* eggs, temperature (°C) and relative humidity (%) during the semi-field experiment. Tangará da Serra, Mato Grosso State, 2018.

Time	Parasitism rate (%)	Temperature (°C)	RH (%)
8h	57.65 ± 31.02 a[1]	26.10	78.00
10h	46.80 ± 21.03 b	34.90	55.00
12h	40.48 ± 21.81 b	37.40	42.00
14h	9.87 ± 14.25 c	23.50	88.00
16h	34.21 ± 19.33 b	25.90	77.00
18h	39.99 ± 32.61 b	26.20	71.00
20h	4.67 ± 9.19 c	22.40	85.00
22h	4.73 ± 6.97 c	21.00	92.00
00h	0.53 ± 2.29 c	22.00	90.00
2h	0.00 ± 0.00 c	21.90	88.00
4h	0.00 ± 0.00 c	21.90	89.00
6h	0.00 ± 0.00 c	21.40	90.00
p	2.00^{-16}		

[1] Means followed by different letters in the column differ significantly according to the Scott-Knott test at 5%.

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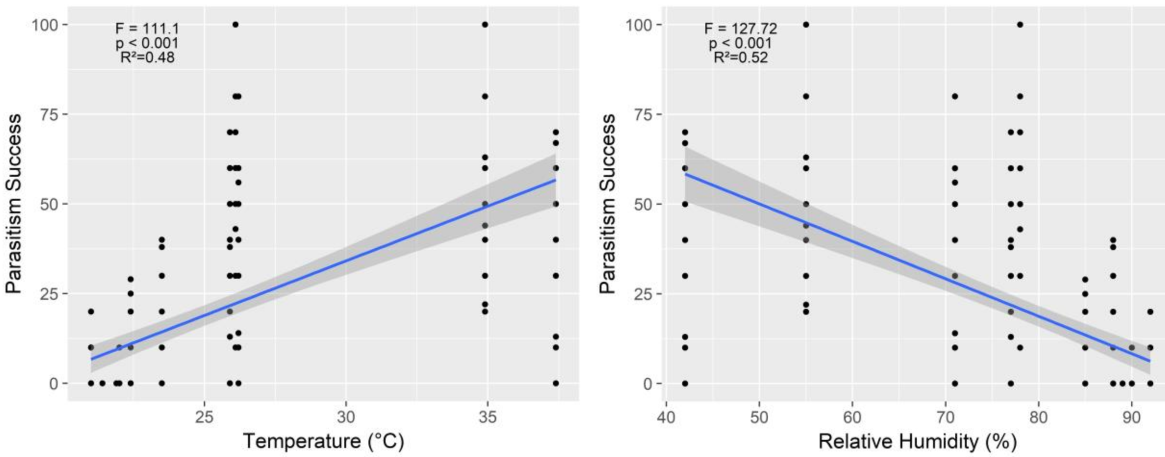

Figure 4. Linear regression of *Trichogramma pretiosum* parasitism rate in *Plutella xylostella* eggs for 24 hours with Temperature (°C) and Relative Humidity (%). Tangará da Serra, Mato Grosso State, 2018.

Female lepidopterans prefer ovipositing on certain areas of the leaf, therefore information about this behavior allows the planning of more effective strategies for monitoring and control, and for evaluating natural enemies (GINGRAS et al., 2003; HAMILTON et al., 2005; FARIA et al., 2008). Females of *P. xylostella* preferred to ovipositon on middle leaves, while oviposition on older and very young leaves was low. In addition, the highest number of eggs was recorded on the adaxial leaf surface. Similar results were obtained by Badenes-Perez et al. (2006) in a mixed experiment comparing cabbage and bittercress [*Barbarea vulgaris* (Brassicaceae)], where *P. xylostella* laid 99% of the eggs in *B. vulgaris*, and preferred younger leaves, laying on average 3.7 times more eggs than on old leaves. Our results also corroborate those by Zago et al. (2010) that observed the preference of *P. xylostella* for ovipositing on the adaxial surface, in the upper region of younger leaves of cabbage.

Other lepidopterans such as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops, showed a preference for oviposition on adaxial leaf surfaces, on the first leaves from top to bottom, and were absent at the top, corroborating our results (FARIA et al., 2008).

Two factors may be associated with this preference for middle leaves in the present study. The first is the presence of saponins in the leaves, which tend to increase with plant age and negatively affect pest development (CARLSSON, 1980). The second is that the top leaves are smaller in size and more exposed to chemicals that can be applied to foraging natural enemies (SHIOJIRI & TAKABAYASHI, 2003; ZAGO et al., 2010).

Regarding parasitism by *T. pretiosum*, no significant differences were observed for leaf age (old → young) and leaf region. However, there was a significant difference in leaf surface, with the highest mean parasitism recorded for the adaxial surface. Faria et al. (2008) observed similar results, as *T. pretiosum* did not parasitize eggs at the top of tomato plants, preferring the adaxial surface of leaves. In corn, eggs of *Ostrinia nubilalis* (Lepidoptera: Pyralidae) stapled on leaves in the upper third of the plant were much less parasitized by *T. ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae) than those in the middle and lower third of the plant (WANG et al., 1997), demonstrating that egg parasitoids of the genus *Trichogramma* have preferences for eggs laid in the middle and lower third of the plants.

Factors such as leaf surface area and plant structural complexity can interfere with parasitoid efficacy. In one experiment, the relationship between the structure of cruciferous plants with different degrees of complexity and mean parasitism rate by *Trichogramma evanescens* Westwood and *T. pretiosum* was investigated in cabbage, broccoli and Brussel sprouts (low, medium, and high complexity, respectively). Cabbage had the highest mean parasitism rate for both parasitoid species, while Brussel sprouts with high complexity leaves had the lowest (GINGRAS et al., 2003), demonstrating the importance of understanding the influence of the plant structure on parasitoid species.

Studies also demonstrated that characteristics of the surface of host plants can hinder or slow *T. pretiosum*'s search speed, since females look for eggs in the plant by walking and host eggs are recognized at a distance of about 4 mm (SCHMIDT, 1994).

Therefore, collard greens have favorable physical characteristics, such as well-developed, rounded limb with long petiole, prominent ribbing and absence of trichomes, which hinder the search for host eggs by parasitoids (FILGUEIRA, 2007; TRANI et al., 2015).

Other studies have emphasized that factors such as plant surface area, plant density and structural complexity of the plant and habitat should also be taken into account in parasitoid release programs (KANOUR & BURBUTIS, 1984; SMITH, 1988; ANDOW & PROKRYM, 1991; LUKIANCHUK & SMITH, 1997; ROMEIS et al., 2005).

Regarding climate conditions, different *Trichogramma* species have preferences for certain relative humidity and temperature ranges, as both influence life table parameters and parasitism activity (PAK & OATMAN, 1982; SMITH et al., 1986; BIEVER, 1972; BOLDT, 1974; FORSSE et al., 1992; BOURCHIER & SMITH, 1996; ROMEIS et al., 2005).

In the present study, the highest mean parasitism rates were recorded from 8 to 18 hours, a period of intense luminosity, temperatures ranging from 25.9 to 37.4°C, and relative humidity between 42 and 78%. The mean daytime temperature was 25.4°C, similar to those reported by several laboratory studies, as the most appropriate for *T. pretiosum* (PRATISSOLI & PARRA, 2000; PEREIRA et al., 2004). According to Pratissoli and Parra (2000), these variations in temperature can be explained by biological parameters that not only depend on the temperature used, but also on the adaptation of the species or lineage and the host used. Thus, parasitoids used in the present experiment are adapted to the high temperatures observed in Mato Grosso.

Other field studies have also reported the influence of climate conditions on parasitism effectiveness. Wang et al. (1997) released *T. ostriniae* Pang et Chen (Hymenoptera: Trichogrammatidae) in corn fields for the control of the European corn borer, *O. nubilalis*. These authors found that higher mean temperatures negatively affected parasitism during warmer periods, while lower temperatures (<17 °C) reduced egg parasitism during the colder periods of the season. They concluded that *T. ostriniae* parasitizes more eggs between 20 and 28°C than at temperatures below 17°C and above 30°C, and the best temperature for foraging behavior was between 22 and 25°C. Bourchier and Smith (1996) demonstrated that temperature alone can explain 75% of the variation in *T. minutum* Riley parasitism rates under field conditions, thus showing the importance of information on species activity at the site where parasitoids will be used.

Based on our findings, climate conditions and plant architecture must be taken into account to optimize parasitism rates. In addition, other aspects cited by Romeis et al. (2005), such as surface area, plant density and spacing, structural complexity, and habitat should also be included in parasitoid release programs.

CONCLUSIONS

We conclude that *P. xylostella* has a preference for ovipositing on the adaxial surface of leaves in the middle region of the collard greens. The highest mean parasitism rates of *T. pretiosum* were recorded in the upper part of the adaxial surface, with no preference for the position of the eggs on the leaf or in the plant. Parasitism by *T. pretiosum* was highest at temperatures ranging from 25.9 to 37.4°C, relative humidity between 42 and 78% and between 8 and 18 hours (photophase). No parasitism was recorded at night (scotophase), indicating that field releases should preferably be performed early in the morning to provide the entire photophase to parasitoids.

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