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Dispersion of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) in kale and cabbage fields

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Abstract

Parasitoids belonging to *Trichogramma* are utilized for pest management worldwide. The dispersal ability of these insects in crops determines the number of release points necessary for satisfactory results in terms of parasitism and consequently, pest management. To determine the dispersal capacity of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) in kale and cabbage crops, a 26 × 26 m area was demarcated, in which cards containing *Anagasta kuehniella* eggs were distributed in a concentric and equidistant manner. At the center of each area, 100,000 parasitoids were released and parasitism was allowed for 24 h. The experimental design was completely randomized, with five treatments (distance) and four replications. The average dispersal distance was 6.27 m in kale and 6.07 m in cabbage, whereas the dispersal area was 81.23 m^2 in kale and 79.03 m^2 in cabbage, thus determining 123 and 126 release points for kale and cabbage, respectively.

Keywords: biological control program, *Brassica oleracea,* integrated pest management, inundative release

Introduction

Parasitoids belonging to *Trichogramma* are the most widely used for biological control programs worldwide (Zang et al., 2021). In Brazil, 25 *Trichogramma* species have been described, with *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) being the most abundant and one of the most used species, as it is easy to reproduce them in the laboratory. In addition to possessing numerous hosts, this species can be used for the management of lepidopteran pests such as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae), and *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Oliveira, et al., 2020; Bueno et al., 2011; Parra & Coelho Júnior, 2019).

In vegetable crops, the use of egg parasitoids, such as *T. pretiosum*, is an important tool to manage difficult-to-control pests such as *P. xylostella*, the main pest of brassicas worldwide (Wang et al., 2020). However, studies that guide the implementation of biological control programs for brassicas are needed. Controlled release studies are necessary to understand the dispersal ability of microwasps and determine the number of release points for pest management in a given crop.

Although *T. pretiosum* is a well-studied species, its biological characteristics such as flight activity may vary from one lineage to another. In addition to factors intrinsic to insects, the characteristics related to culture, such as phenological stage, height, and protection capacity can be highlighted, and climatic factors such as wind, rain, and temperature, which can limit parasitoid dispersion. Thus, evaluation of dispersal capacity in a given crop becomes an important parameter to implement biological control programs to establish the number of release points per hectare, a factor implied in the cost of releases. Other studies on *T. pretiosum*

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dispersion were conducted in staked tomato, cabbage, sweet corn, and cucumber, where it was found that the dispersion was 138.72 m², 56.8 m², 60.3 m², and 62.2 m², respectively. Therefore, dispersion of the same species varies depending on the crop or even the strain used (Pratissoli et al., 2005; Oliveira et al., 2020).

The objective of this study was to evaluate the dispersal capacity of a *T. pretiosum* strain in kale (*Brassica oleracea* var. *acephala*) and cabbage (*B. oleracea* var. *capitata*) cultivation to determine the number of release points of the biological control agent per hectare, aiming to implement biological control programs for the major lepidopteran pests in brassicas.

Material and Methods

Obtaining insects

Production of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae)

To produce the alternative host *Anagasta kuehniella*, plastic pots of 4.5 L containing 1 kg of diet comprising whole wheat flour (97%) and yeast (3%) were used. Each pot was inoculated with 0.36 g of fresh *A. kuehniella* eggs, sealed with plastic tape, and kept in an acclimatized room with a temperature of 18 ± 2°C and a photoperiod of 14 h for larval development. The pots remained in the same room until the first adult emergence, after which they were transferred to the adult rearing room with temperature at 25 ± 2°C and a photoperiod of 14 h, where they were collected daily.

To collect, rear, and release *T. pretiosum, A. kuehniella* eggs were made unviable by keeping them 10 cm away from a UV (ultra violet) germicidal light source for 50 min.

Production of *T. pretiosum* strains

The parasitoids were multiplied in the eggs of *A. kuehniella*, the most suitable alternative host for rearing under laboratory conditions (Gomes & Parra, 1998). For packaging the adult insects, 3.5 L transparent plastic pots were sealed with plastic PVC film. For feeding, pure honey was made available in thin fillets on the inner side of the pots. The containers with adult *T. pretiosum* lineages were kept in an acclimatized room with a temperature of 25 ± 1°C, relative humidity of 70 ± 10%, and a photoperiod of 14 h for parasitoid development.

Dispersal ability of *T. pretiosum* in kale and cabbage

The experiment was conducted in a commercial *Brassica* plantation area (23°0'41"S, 48°3'80"W), located in the Pardinho - SP municipality. The experimental area was 26 × 26 m, divided into four quadrants, and each quadrant was marked at concentric distances of 3, 5, 8, 10, and 13 m from the central point. Each quadrant received cards containing unviable *A. kuehniella* eggs that were equidistantly distributed (Table 1).

Table 1. Distance, area, and number of *Anagasta kuehniella* egg cartons used to determine *Trichogramma pretiosum* dispersion in kale and cabbage.

Distance (m)	Area (m²)	Number of cards
3	9	4
5	25	12
8	64	28
10	100	52
13	169	68

Sentinel cards measuring 0.5 × 1.5 cm, containing 160 ± 20 unviable *A. kuehniella* eggs, were fixed on the abaxial side of the leaves and distributed at determined points, spaced equidistantly from each other. At the center of each dispersal area, approximately 100,000 previously fed parasitoids with a maximum of 24 h of emergence were released, with dispersion being allowed for 24 h (Figure 1).

At the time of release, the air temperature was 24 ± 1°C and the wind speed and direction (recorded with a digital anemometer model Tan 100 Incoterm T-ANE-0010) was 0.02–0.021 m/s east. In addition to the demarcated experimental area, sentinel traps containing eggs were placed 100 m away from the experimental site to assess natural parasitism, allowing for the correction of parasitism

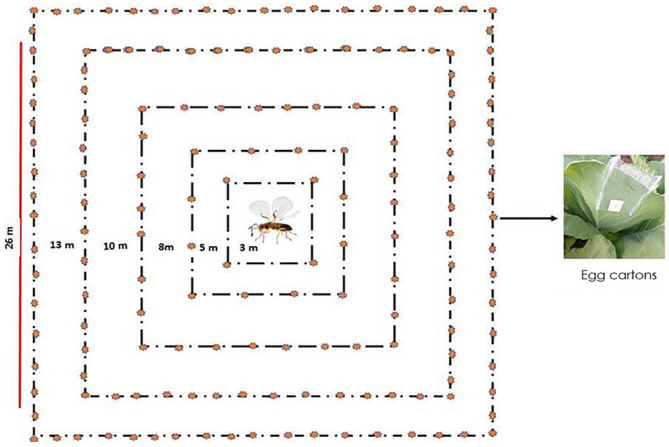

Figure 1. Schematic representation of the experimental area of *Trichogramma pretiosum* dispersion in kale and cabbage commercial cultivation areas.

based on Abbot (1925). The experimental plots were at least 100 m apart between cultures.

After 24 h, the cards were collected and placed individually in plastic bags (2 × 22 cm) filled with oxygen and were evaluated after parasitoid emergence and death. The total number of eggs (in the cards) and number of parasitized eggs (characteristic dark colored eggs) were evaluated by dissecting the eggs using a pointed stylet. The mean scattering distance (MD) and scattering area (s^2) of *T. pretiosum* were determined according to formulas adapted from Dobzhansky & Wright (1943), as follows:

$$MD = \frac{\sum\left(r2 * \frac{i}{a}\right)}{\sum\left(r * \frac{i}{a}\right) + \hat{C}/2\pi}$$

$$S2 = \frac{\sum\left(r3 * \frac{i}{a}\right)}{\sum\left(r * \frac{i}{a}\right) + \hat{C}/2\pi}$$

where s^2 = dispersion area (m^2), *MD* = mean dispersion distance (m), *r* = distance (m) from the center to the infestation points, *a* = number of infestation points per circle, $\check{C}$= average percentage of parasitized egg masses in the central circle, and *i* = total number of parasitized egg masses in each circle. We created concentric circles around the marked points to calculate the *MD* and s^2.

Statistical procedures

The experimental design was completely randomized, with five treatments (distance) and four replicates for each culture. The results were subjected to normality analysis (Shapiro & Wilk); and the average parasitism percentage in kale was transformed as $X = \sqrt{x}$ (normality value: 0.91106, *p*-value: 0.06676), whereas the average parasitism in cabbage was transformed as X = log□(x) (normality value: 0.95414, *p*-value 0.43426). The transformed means were subjected to analysis of variance (ANOVA) and a means comparison test using Tukey's test at 5% significance using ASSISTAT® 2017 software (Silva & Azevedo, 2016). Subsequently, linear regression analysis was performed using SigmaPlot® 2016 software.

Results and Discussion

Parasitism decreased as the distance from the release point increased. At 3 m away, approximately 30% parasitism of sentinel eggs was obtained for both cultures (Table 2). By parasitizing the closest cards, the chances of females parasitizing eggs farther are reduced. To reduce this effect, only four cards are used at 3 m; the greater the distance, the more cards are available. In addition, the presence of lepidopteran pest eggs in the experimental area may have contributed to reduced parasitism rates in sentinel cards when compared to those reported in other studies, such as in staked tomato, where parasitism ranging from 53.1% to 87.3% was reported (Pratissoli et al., 2005).

Table 2. Dispersal capacity and percentage of parasitism in *Anagasta kuehniella* eggs by *Trichogramma pretiosum* in kale and cabbage crops.

Distance (m)	Kale*	Cabbage**
	Parasitism (%)	
3	32.3 ± 1.9 a	31.0 ± 4.4 a
5	24.0 ± 3.9 a	15.4 ± 4.5 b
8	9.0 ± 2.8 b	8.2 ± 2.4 c
10	6.2 ± 2.6 bc	5.0 ± 1.4 c
13	2.5 ± 1.4 c	2.7 ± 0.4 d
CV%	12.5	11.1

Means followed by the same letter in the column do not differ by the Tukey's test ($p \leq 0.05$) of probability. (*Means transformed as $X = \sqrt{x}$) (**Means transformed as $X = \log(X)$).

In cabbage, Oliveira et al. (2020) obtained approximately 30% parasitism by *T. pretiosum*; therefore, the results can be attributed to the species or even to the insect/plant combination. Numerous interactions between plants and herbivorous insects also involve three trophic levels: plant/herbivore/parasitoid, and predators. Thus, the combination of cabbage plant and release of *T. pretiosum* obtained similar results, although they were conducted in different regions with different parasitoid lineages.

Under natural infestation, trophic interactions occur in which the infested plant signals that it is being attacked through release of volatiles (Turlings & Erb, 2018). The release of volatiles guides the parasitoids to the host (Hilker & Fatouros, 2015), which promotes competition between the pest eggs present in the crops and the eggs of sentinel cards.

As one moves away from the central point of release, there is an increase in the number of plants and in the area to be covered to reach the distributed eggs, with a smaller number of parasitoids per area, which makes foraging difficult. Additionally, plant architecture, leaf size, phytochemical compounds, and the presence of floral nectar influence the dispersion of insects (Chen, 2008; Moraes et al., 2008), which seek shelter, food, and substrates for offspring survival.

Plants of the family Brassicaceae are herbaceous. Kale (*B. oleracea* var. *acephala*), cabbage (*B. oleracea* var. *capitata*), broccoli (*B. oleracea* var. *italica*), and kale flowers (*B. oleracea* var. *botrytis*) have the same ancestor and are practically identical up to 50 days after sowing. They have very similar characteristics in terms of plant height, leaf size, and phytochemical components, thus

providing the same protective substrate for parasitoids. Among the chemical compounds, glucosinolates, which act in defense of *Brassica* plants, are associated with resistance against herbivory (Kos et al., 2012). Production of volatiles such as jasmonic acid signals herbivory and serves as an attractant for parasitoids (Aljbory & Chen, 2018).

The dispersion radius decreased linearly for both kale and cabbage. Parasitism was estimated to be close to zero after 13 m (Figure 2).

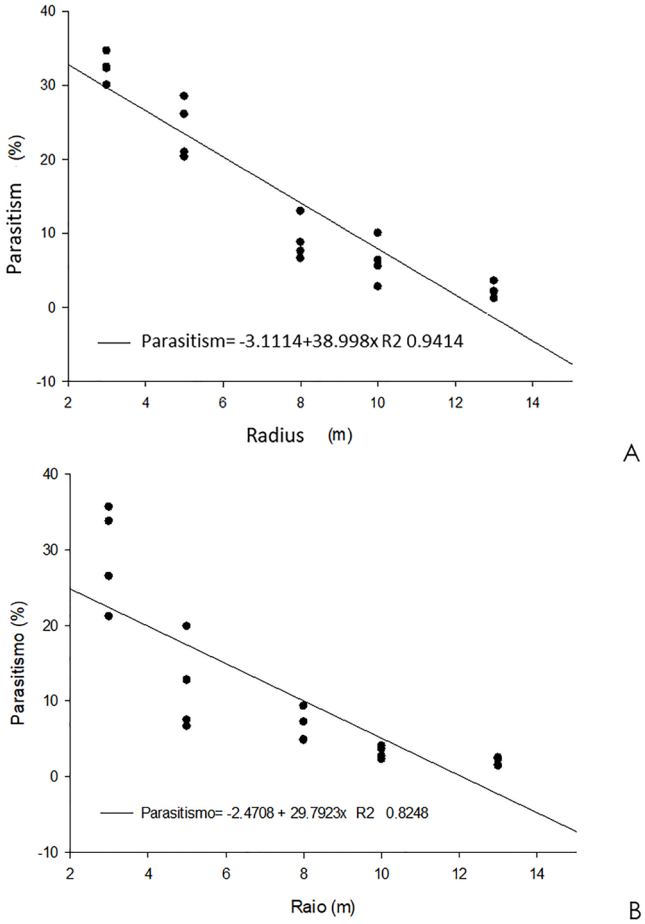

Figure 2. *Trichogramma pretiosum* dispersion radius and parasitism in *Anagasta kuehniella* eggs in (A) kale (F = 140.1 e P = 0.08) and (B) cabbage (F= 38.3 e P= 0.51).

Most parasitoids do not disperse over long flights, foraging for targets close to the point of release or emergence (Suverkropp et al., 2009). Insects with small bodies, such as *T. pretiosum*, disperse a few meters in 24 h; however, the dispersion radius can reach hundreds of meters with the action of the wind (Bueno et al., 2011). Wind is the climatic factor greatly influencing dispersion (Mahrughan et al., 2015). At the time of parasitoid release, the wind speed was measured so that its influence was an additional justification for the dispersed distance. However, the speed was very low (0.02–0.021 m/s) and so was the influence of wind on this experiment, considering that the parasitism observed in the sentinel cards decreased further away from the central point of the release.

The average dispersion distance was 6.26 m for kale and 6.07 m for cabbage; the dispersion area was 81.23 m^2 and 79.03 m^2 for kale and cabbage, respectively (Table 3). From these data, we can establish 123 and 126 release points/ha for kale and cabbage, respectively, for the strain used in this study. This reinforces the importance of satisfactory distribution of parasitoids in the cultivation area, as the maximum *T. pretiosum* parasitism was observed 72 h after emergence.

Table 3. Mean dispersion distance (MD), dispersion area (S^2), and equation for *Trichogramma pretiosum* lineage in *Anagasta kuehniella* eggs on kale and cabbage.

	MD	S^2	R^2	Equation
Kale	6.26	81.23	0.941	y = -3.1114 + 38.9980x
Cabbage	6.07	79.03	0.824	y = -2.4708 + 29.7923x

High values of the coefficient of determination (R^2) indicated the reliability of the results. The dispersion distance of *Trichogramma* species is close to 10 m (Sá et al., 1993), which may vary with the species used as well as the lineage and stage of development of the crop, with adjustments being necessary for each situation. The results obtained were close to the release points determined for *T. pretiosum* in soybean, established by Zachrisson & Parra (1998), whereas Oliveira et al. (2020) studied the dispersion of the same parasitoid and determined 176 release points/ha in cabbage. To date, no study has reported the dispersion of *T. pretiosum* in kale.

The plant canopy also influences parasitoid dispersion. In tomato, the dispersion in 24 h was 120.2–138.7 m^2, depending on the stage of plant development, which represented 75 release points/ha (Pratissoli et al., 2005). Management of *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) in maize cultivation requires 100 release points/ha (Sá et al., 1993). Thus, the number of release points varies depending upon the location, species or line used, and culture in which the biological control agent is used.

Conclusions

The dispersal ability of *T. pretiosum* in *A. kuehniella* eggs 24 h after release, reached a radius of 6.26 m and 6.07 m, corresponding to a dispersal area of 81.23 m^2 and 79.03 m^2 in kale and cabbage, respectively. A total of 123 and 126 release points per hectare were determined for kale and cabbage, respectively.

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