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Dispersal Capacity of *Trichogramma* for the Management of *Duponchelia fovealis*

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Abstract: The European pepper moth (*Duponchelia fovealis*) is predominant in the main strawberry production areas, including Brazil, as an important invasive pest and causes substantial damage to the crop. This damage is mainly attributed to the lack of effective management strategies. A promising alternative for managing this pest is implementing biological control through releasing natural enemies. This study determined the dispersal capacity of *Trichogramma pretiosum* for the management of *D. fovealis* in a strawberry crop in a low tunnel system 24, 48, and 72 h after parasitoid release. The experiments were carried out on strawberry farms in the mountainous region of Espírito Santo. Tunnels measuring 1.20 m wide by 50 m long were selected, in which artificial infestations of 30, 60, 90, and 120 eggs of *D. fovealis* were made on both sides of the strawberry tunnel at distances of 3, 7, 11, and 15 m, respectively, from the central point where the parasitoids were released. After the initial 24 h, new eggs were placed to replace the infested ones; the replacement was repeated at 48 and 72 h. The results indicated that, in strawberry plantations, *T. pretiosum* was efficient in parasitism. There was no difference between parasitism 24 and 48 h after parasitoid release, but parasitism was substantially lower after 72 h. The dispersion capacity of *T. pretiosum* was 14.21 linear meters, corresponding to an area of 17.05 m^2. It is recommended that 93,000 *T. pretiosum* females be released per hectare every three days.

Keywords: egg parasitoid; Trichogrammatidae; biological control; strawberry

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1. Introduction

Strawberry production is of great social and economic importance in Brazil. However, the crop is highly sensitive and frequently attacked by harmful agents, such as pests and diseases [1–3].

The European pepper moth, *Duponchelia fovealis* Zeller, 1847 (Lepidoptera: Crambidae), is a polyphagous insect that was introduced into Brazil in 2007, quickly established itself, and is now widespread in the country's main strawberry production fields. Adults of *D. fovealis* lay eggs on the abaxial surface of the plant's low-lying leaves close to the ground. After hatching, the caterpillars, from neonates to final instars, feed on the leaves, causing significant damage to strawberry crops, mainly due to the lack of efficient management methods [1,2,4,5].

For this reason, one way to control this pest is to integrate existing management methods. A viable management alternative is using biological control through the release

of natural enemies. Parasitoids of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are natural enemies used with significant frequency in biological pest control and are distributed worldwide [6–8].

The origin of the species *T. pretiosum* is unknown, but it is naturally recurrent in the Americas and is the most commercialized species in Brazil for inundative release. The distribution of this species is recorded in 10 of the 12 countries of South America, and, in Brazil, it is found naturally in 15 of the 26 Brazilian states [9]. This parasitoid species is widely known as an alternative for controlling pests of agricultural importance, being reported to parasitize the eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) [10] and *Heraclides astyalus* (Godart, 1819) (Lepidoptera: Papilionidae) in *Citrus* sp. [11] and has high potential for use in *D. fovealis*.

Arguments in favor of using these parasitoids include their ease of rearing in alternative hosts at a lower cost than natural hosts, wide geographic distribution, specialization, and efficiency in parasitizing [11–13]. Several factors play a role in the efficacy of these parasitoids in controlling pests in the field [14]. These factors are mainly related to the foraging and dispersal ability of the species/strain used (which are most often influenced by the host), the number of insects released, the density of the target pest, the number and season of releases, the method of field distribution, and the phenology of the plant, which can influence the path of the parasitoid on the plant [8,12,15–18].

Dispersal ability is a particularly important factor because it directly affects the release techniques used, the potential effect of non-target pests, and the time required for *Trichogramma* to efficiently colonize the crop area [8,12,14,15].

The dispersion capacity is fundamental in inundative biological control techniques, as it determines the ideal number of parasitoid release points in the field to achieve homogeneous coverage [8].

Therefore, this study aimed to evaluate the parasitism behavior of *T. pretiosum* in the field within 72 h of release, determine the dispersal ability of *T. pretiosum* in strawberry production fields, establish the ideal release distance in low tunnel cultivation, and estimate the number of *T. pretiosum* to be released per hectare.

2. Materials and Methods

2.1. Rearing of D. fovealis

Rearing of *D. fovealis* took place in the Entomology Sector of the Center for Scientific and Technological Development in Pest and Disease Phytosanitary Management (NUDEMAFI) of the Center for Agricultural Sciences of the Federal University of Espírito Santo (CCA-UFES), Brazil.

Insects were maintained in the laboratory under conditions of 25 ± 1 °C, 70 ± 10% relative humidity, and 14 h of photophase. Newly hatched adults were transferred to cages (20 × 20 cm) made of PVC pipe, lined internally with bond paper, and sealed at the bottom with Styrofoam, which was also coated with bond paper. The end of the cage was closed with voile fabric to prevent insects from escaping. The adults were offered a 10% solution of honey soaked in cotton as food. The eggs were collected daily by changing the paper covering the cages, and they were immediately placed in Gerbox® acrylic boxes (11 × 11 × 3.5 cm).

The paper fragments containing the egg masses were transferred to flat-bottomed glass tubes (8.5 × 2.5 cm) containing an artificial diet [19]. After the third day of the pupal stage, these were removed from the tubes and transferred to rearing cages to obtain the adults.

2.2. Maintenance and Multiplication of Parasitoids

The maintenance and multiplication of parasitoids of the genus *Trichogramma* was also performed in NUDEMAFI, CCA-UFES. The species used was a commercial strain of *T. pretiosum*, Koppert®.

For the maintenance and multiplication of *Trichogramma*, eggs from stock breeding at NUDEMAFI were used, derived from the alternative host *Ephestia kuehniella* (Lepidoptera:

Pyralidae). The eggs of *E. kuehniella*, after being made non-viable under a germicidal lamp, were fixed with 20% gum arabic on blue cardboard (8.0 × 2.5 cm). These cartons were transferred to flat-bottomed glass tubes (8.5 × 2.5 cm) containing newly hatched adult parasitoids. The tubes were then sealed with PVC plastic film to prevent the escape of the parasitoids [20].

2.3. Evaluation of the Dispersal of T. Pretiosum in Strawberry Plants under Low Tunnel Cultivation for Parasitism of D. fovealis Eggs

To determine the displacement distance (dispersal ability) in linear meters and the number of release points within a low tunnel cultivated with strawberry plants, dispersal experiments were carried out on farms in the district of São João do Garrafão, municipality of Santa Maria de Jetibá, mountainous region of Espírito Santo (coordinates 1: 20°09′30″ S; 40°59′05″ W; coordinates 2: 20°08′09″ S; 40°56′53″ W). Plants of the Albion cultivars were used, planted with a spacing of 0.3 m between rows and 0.3 m between plants in beds with three planting rows during the productive period (approximately four months after planting) in a low tunnel system, with one location per tunnel. The experiment was installed under field conditions, that is, the climatic and management conditions were those typically found in the agricultural operation in question, with no additional control or monitoring of environmental conditions.

Four strawberry tunnels measuring ~1.20 m wide and 50 m long were selected. The low tunnels were built following the recommendations of Santos and Medeiros [21]. The structure was made with galvanized wire No. 6 arches at a minimum height of 80 cm, and the spacing between the arches was between 1.20 and 1.50 m. The structure was covered entirely with nonwoven fabrics (TNT). From the center of the tunnel (~25 m), plants were artificially infested at 3, 7, 11, and 15 m intervals on both the left and right sides with three, six, nine, and twelve cartons, respectively, each containing ten eggs of *D. fovealis* between 24 and 48 h old (Figure 1A,B).

The number of eggs was increased based on distance to preserve the turgidity of the more distant eggs and avoid their unavailability due to parasitism. After artificial infestation, approximately 2400 female individuals of *T. pretiosum* were released early in the day, immediately after hatching and in the middle of the bed, in each tunnel according to its treatment. The average radius of action and the dispersion area of the parasitoid were based on the methodology of Dobzhansky and Wright [22] with adaptations for the cultivation conditions found in Espírito Santo. Parasitoids were released in the tunnel's center at the recommended ratio of 1:4 (egg/parasitoid) using cartons containing the parasitoid species.

One day after the release (24 h), the cartons containing the eggs of *D. fovealis* were collected and replaced by others. This procedure was repeated for two more consecutive days (at 48 h and 72 h). All collected samples were taken to the laboratory, where the cartons were placed in plastic bags (5 × 23 cm), separated adequately according to the distance they were arranged with respect to the point of release and treatment, and placed in climate-controlled chambers at 25 ± 1 °C, relative humidity of 70 ± 10%, and photophase of 14 h, where they remained until parasitism was assessed by observing the darkening of the eggs, and confirmed after the emergence of the individuals. (Figure 1C).

A randomized block design with 4 tunnels was used. Each tunnel was considered a block, and the blocks were selected at a distance of >15 m between them. Regression analyses were performed between parasitism indices as a function of parasitoid density and species released. The mathematical relationship to obtain the average displacement, the number of release points, and the parasitoid dispersal area in strawberry cultivation in a low tunnel system was calculated based on the quadratic fit of the data; the optimal release point was determined from the derivatives, thus optimizing the intersection points between the parasitism curves.

The ExpDes. package of the R computer application [23] was used to perform the analysis.

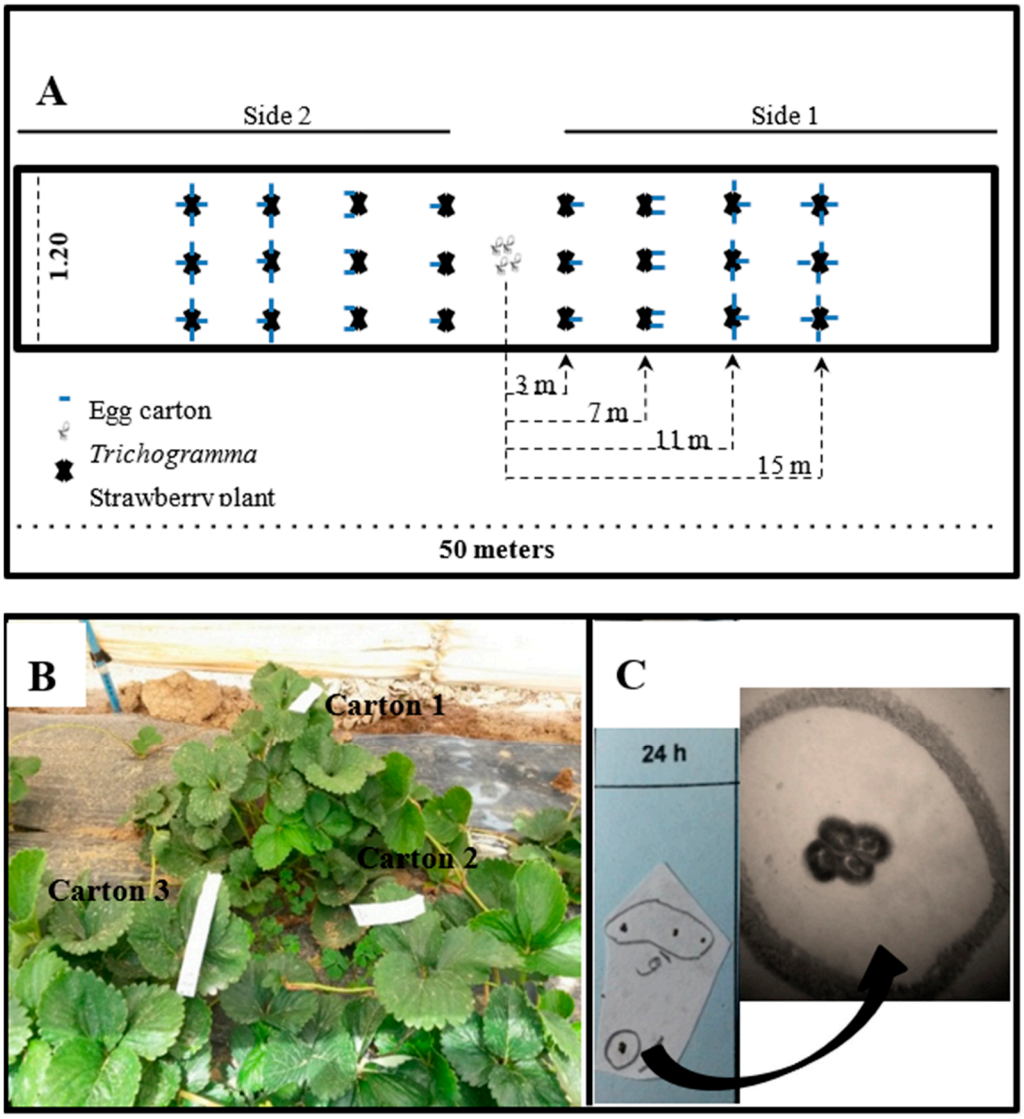


Figure 1. (**A**) The distribution of cartons and parasitoid release in a strawberry production field in a low tunnel system; (**B**) cartons with *Duponchelia fovealis* eggs distributed in the field to determine parasitoid dispersal ability; (**C**) parasitized eggs from the dispersal ability experiment after seven days.

3. Results

3.1. Release Times and Parasitism

Regarding the release time and parasitism of *T. pretiosum* in *D. fovealis* eggs, an F-test revealed a significant difference at the 5% probability level between parasitism at 24, 48, and 72 h after parasitoid release (Table 1). The efficiency of parasitism of *T. pretiosum* did not differ after 24 and 48 h. However, it was substantially lower at 72 h after release under strawberry cultivation conditions in the mountainous region of Espírito Santo (Table 2).

Table 1. Parasitism of *Trichogramma pretiosum* within 15 m from the release point, at a density of one *Duponchelia fovealis* egg for four parasitoids (1:4), at 24, 48, and 72 h after release in strawberry production fields in a low tunnel system.

FV [1]	Df [2]	SQ [3]	QM [4]	Fc [5]	P > F [6]
Block	3	1401.90	467.30	1.025	0.446
Treatment	2	13,789.40	6894.70	15.119	0.004 *
Residue	6	2736.20	456.00		
Total	11	17,927.50			
CV [7] (%)	20.33%				

[1] FV: Source of variation; [2] Df: degree of freedom; [3] SQ: sum of squares; [4] QM: mean squares; [5] Fc: observed F value; [6] P > F: F value in relation to the observed *p*-value; [7] CV: coefficient of variation. * Significant values at the 5% significance level.

Table 2. Mean accumulated parasitism of *Duponchelia fovealis* eggs by *Trichogramma pretiosum* at 1:4 density in the four replications and four distances from the release point after the initial release time in strawberry production fields in a low tunnel system.

Time after Release	Accumulated Parasitism [1]
24	129.1 a*
48	128.9 a
72	57.1 b

* Means followed by the same letter do not differ from each other at the 5% probability level using the Tukey test. [1] Mean accumulated parasitism in the proportion 1:4, in the four repetitions and four distances from the release point.

3.2. Estimation of Dispersal Ability and Number of Release Points of T. pretiosum in Strawberry Plants Cultivated in a Low Tunnel for Parasitism of D. fovealis Eggs

The results indicate that parasitism of *D. fovealis* eggs by *T. pretiosum* decreases as the sampled points move away from the parasitoid release point, with statistical differences at all distances analyzed (Table 3).

Table 3. Mean values (±standard error) of parasitism of *Duponchelia fovealis* eggs by *Trichogramma pretiosum* Koppert® commercial strain after 72 h, at different distances from the central point of release in the strawberry crop in a low tunnel system in the district of São João do Garrafão, municipality of Santa Maria de Jetibá-ES.

Distance (m)	Parasitism (%)
3	32.64 ± 2.84 a[1]
7	14.03 ± 0.08 b
11	5.74 ± 0.23 c
15	0.11 ± 0.06 d
CV [2]	20.94

[1] Means followed by the same letter do not differ from each other at the 5% probability level using the Tukey test. [2] CV: coefficient of variation.

Based on the proposed model, the mean distance of action and dispersal area of *T. pretiosum* in the strawberry crop in a low tunnel system to control *D. fovealis* eggs was 14.21 m (linear) and 17.05 m^2, respectively (Table 4). Therefore, the number of release points of *T. pretiosum* to control *D. fovealis* eggs was determined by dividing the length of tunnels by the average action of the parasitoid (14.21 linear m) (Figures 2 and 3).

Table 4. Mean distance (linear meters) and dispersal area (m^2) with the respective model and determination coefficient (R^2) for *Trichogramma pretiosum* Koppert® commercial strain in eggs of *Duponchelia fovealis* in a strawberry crop in a low tunnel system in the district of São João do Garrafão, municipality of Santa Maria de Jetibá-ES.

Parameters	Strawberry Crop
Average distance (linear m)	14.21
Dispersal area (m^2)	17.05
Mathematical model	$y = 63.267 - 8.307x + 0.275x^2$
R^2	0.9985
CV [1]	20.94

[1] CV: coefficient of variation.

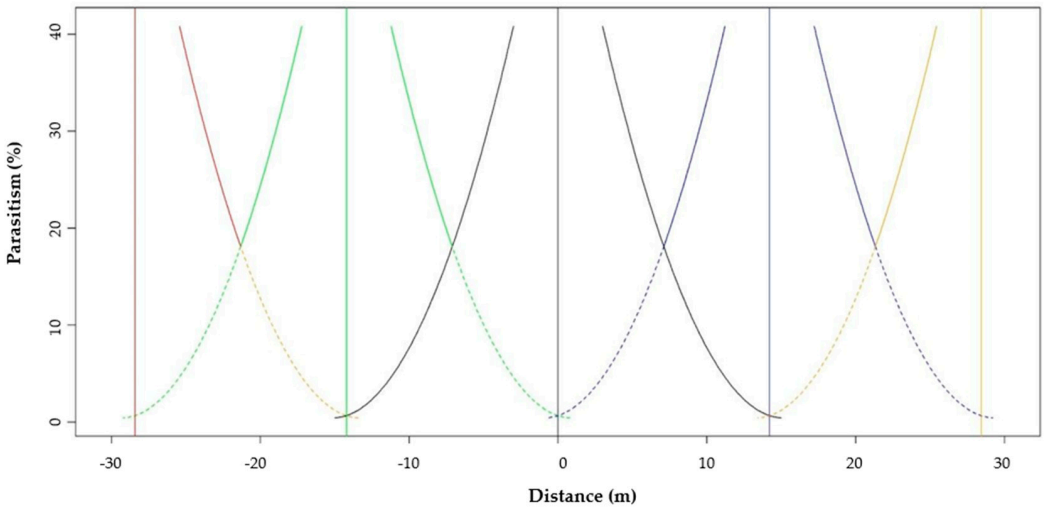


Figure 2. Estimation of the parasitism behavior of *Duponchelia fovealis* eggs by *Trichogramma pretiosum* using the model proposed for strawberry cultivation in a 50 × 1.20 m low tunnel system. Continuous vertical lines represent the release point of the parasitoids. Curves represented by solid lines correspond to the area of parasitism covered by the releases.

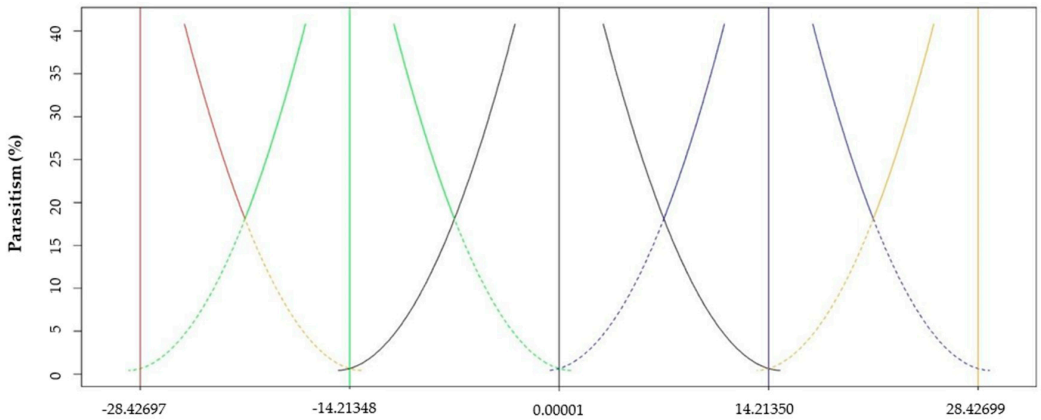


Figure 3. Parasitism of *Duponchelia fovealis* eggs by *Trichogramma pretiosum* considering the parasitoid release points every 14.21 linear meters in strawberry cultivation in a low tunnel system. Continuous vertical lines represent the release point of the parasitoids. Curves represented by solid lines correspond to the area of parasitism covered by releases.

4. Discussion

The high activity in the first 48 h after release could be due to the energetic support provided by the *T. pretiosum* eggs. After this period, with the decrease in energy supply, foraging and parasitism ability were substantially affected. A decline in parasitism over the lifetime of the parasitoid after hatching is common, whether well-fed parasitoids are involved or not [8,24].

The main factor in this decline in parasitism over time may be related to the food supply affecting *Trichogramma* activity, as the quality of the available carbohydrates is essential for increasing the parasitoid's reproductive ability. Longevity and potential for parasitism are maximized when adult *Trichogramma* are fed regularly [25–27]. Therefore, the supply in the field may have been insufficient.

Therefore, based on the data obtained, it can be concluded that the *T. pretiosum* strain used in this study did not show any difference in parasitism on *D. fovealis* eggs in the first 48 h (two days) after release, indicating that the release frequency should not be reduced.

Dispersal ability can be influenced by several factors. The lower parasitism of eggs at greater distances from the initial release point may have been influenced by the biological characteristics of the *Trichogramma* species/strain released and the crop itself. In this case, the strawberry plant was a physical barrier to parasitoid dispersal. Other authors have also studied the inverse correlation between distance and the parasitism capacity of *Trichogramma* [28–30].

In our study, the lowest rates of parasitism were associated with the biological characteristics of the parasitoid and the architectural characteristics of the plant and were observed at greater distances from the release point. These results are usually observed as there is a dependent relationship between release distance and parasitism rate, with a reduction in parasitism rate by *Trichogramma* usually observed at greater distances from the release point [30,31].

Lower parasitism rates due to greater distances from the release point have also been observed for *T. pretiosum* in soybean [15], maize [32], apple [33], tomatoes in a greenhouse [31], and tomatoes in the field [28,29]. The same pattern was observed in rice for *T. chilonis* and *T. japonicum* [34].

The turgidity of the egg of *D. fovealis* may also have affected parasitism at greater distances. Eggs located at a greater distance from the parasitoid release point may lose their turgidity because they are more exposed to adverse environmental conditions, such as high temperature and low humidity, and when found by *Trichogramma* eggs, they are in a condition unsuitable for parasitism [15]. Adverse factors may have been maximized because the tunnels remained closed throughout the period in which the experiments were conducted.

The mean radius of action and dispersal area of the parasitoid are other important parameters [22]. Based on the proposed estimates of the dispersal ability of *T. pretiosum* for control of *D. fovealis*, it is recommended that 93,000 females of the parasitoid be released at three-day intervals for each hectare of strawberry plants.

Control efficiency is directly related to the uniform distribution of the parasitoid [8,26]. Therefore, the results aimed to satisfy this condition. The calculated mean distance and the dispersal area provided greater homogeneity in the parasitoid's coverage of the area and, consequently, greater efficiency in controlling *D. fovealis* by *T. pretiosum*.

The recommendation for parasitoid release may change depending on the crop type, planting density, and intensity of pest infestation in the field [8,26,30]. For fruit trees, they can vary from 70,000 to 3.8 million parasitoids per hectare [35,36]. For staked tomatoes, the recommendation is 576,000 *Trichogramma* released every eight days [37], while in Europe, the recommendation is 150,000 to 300,000 *Trichogramma* released every seven days in maize [38].

5. Conclusions

In conclusion, in strawberry plantations, *T. pretiosum* was efficient in the parasitism of *D. fovealis* eggs. The parasitism rate was similar between 24 and 48 h after parasitoid release, but could be substantially lower after 72 h. The dispersion capacity of *T. pretiosum* was 14.21 linear meters, corresponding to an area of 17.05 m^2. It is recommended that 93,000 *T. pretiosum* females be released per hectare every three days in regions with similar climate characteristics.

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