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AGRARIAN SCIENCES

Dispersal of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) in cabbage, cucumber, and sweet corn

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Abstract: The dispersion capacity is fundamental to establish a biological control program with parasitoids. This information is used to determine the efficiency and the number of release points. Thus, the objective of this work was to determine the dispersion and to estimate the number of release points of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), in sweet corn, cucumber and cabbage in the Ceará State. The experiments were carried out in areas of maize with four leaf pairs (V4) and eight leaves pair (V8), stacked and cabbage. Unviable eggs of an alternative host were distributed in concentric circles of radius 2.5; 5.0; 9.0 and 12.0 m. Mean dispersal distance in the V4 stage maize was 4.7 m with a dispersion area of 48.6 m^2, parasitism index of 18.4%, requiring 206 points/ha. In the V8 stage maize, the mean distance was 5.9 m, dispersion area of 60.3 m^2, mean parasitism index of 22.7% and 166 release points/ha. For the cucumber culture the mean distance was 6.0 m, dispersion area 62.2 m^2, mean parasitism index of 21.1% and 161 release points/ha. For cabbage the mean distance was 5.6 m, dispersion area of 56.8 m^2, mean parasitism index of 22.1% and 176 release points/ha.

Key words: biological control, dispersion, egg parasitoid, release.

INTRODUCTION

Egg parasitoids of the *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are the natural enemies most produced and used in the world in applied biological control programs, only in Brazil, in the 2018 growing season, in sugarcane fields alone about 2 million ha are being 'treated' with *Trichogramma galloi* Zucchi (Hymenoptera: Trichogrammatidae), in order to control *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) (Parra & Coelho Jr. 2019). *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) is other *Trichogramma* spp. used efficiently in biological control programs to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato (Pratissoli et al. 2005). However, this specie shows a great potential to be used in others biological control programs, such as to control *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize (Cruz 1995), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) in cabbage (Pluke & Leibee 2005) and *Diaphania nitidalis* Stoll (Lepidoptera: Pyralidae) in cucumber (Gonring et al. 2003). For the use of *T. pretiosum* reach the success in other crops, even as occur with tomato, is necessary the development of a series of studies, such as evaluating the enemy's, search ability by their hosts and dispersal ability (Fournier & Boivin 2000, Pratissoli et al. 2005, Canto-Silva et al. 2006).

Dispersal ability of a parasitoid is fundamental for determining effectiveness in control because, based on this information, the number of points for release per unit area can be determined so that distribution is as uniform as possible (Pinto & Parra 2002, Parra 2010, Pereira et al. 2010, Pastori et al. 2013). However, this parameter is difficult to estimate in the laboratory (Kolliker-Ott et al. 2004). In the field, since they are of reduced size, *Trichogramma* disperse at short distances with reduced dispersal rates in relation to distance from the point of release (Geetha & Balakrishnan 2011). In spite of the importance of dispersal, information regarding the mobility of these biological control agents in the field is still lacking (Corbett & Rosenheim 1996).

The lack of information regarding dispersal ability of *Trichogramma* in the cucumber, cabbage, and sweet corn crops in the state of Ceará induces the farmer to use information pre-established for other crops in different regions, and that may affect the results of control since the plant architecture and climate conditions of the location are totally different from the location where the study was carried out (Molina et al. 2005). Nevertheless, for practical reasons and lack of knowledge of the variables mentioned, in many crops, dispersal ability and the number of parasitoids per area end up being pre-established (Parra 2010).

Thus, the aim of this study was to determine dispersal ability, as well as estimate the number of points of release of a local lineage of *T. pretiosum* in sweet corn, cucumber, and cabbage crops in the state of Ceará.

MATERIAL AND METHODS

Description of the experimental area

The experiment was conducted from March to April 2017 in commercial crops of cucumber, cabbage, and sweet corn in the municipality of "Guaraciaba do Norte", in the region of the "Serra da Ibiapaba", in the northwest of Ceará State, Brazil, at a distance of 320 km from the capital Fortaleza (4°10'S, 40°44'W, altitude: 902.4 m). The experiment was conducted in four distinct areas, the first being a commercial crop of cucumber in an area of approximately 1 ha, at approximately 50 days after transplanting. The second area was a cabbage crop planted in an area of approximately 0.8 ha, at approximately 40 days after transplanting. The last two areas were of the hybrid maize, the first measuring 2 ha in the V8 development stage, with approximately 2.0 m height and 50 days after planting, and the second measuring 1 ha in the V4 development stage, with approximately 1.0 m height and 35 days after planting. In the period of conducting the experiment, no insecticides for control of insect pests were applied.

Rearing of *T. pretiosum*

The *T. pretiosum* line used in the experiment was collected in the municipality of "Guaraciaba do Norte", Ceará State, Brazil (Oliveira et al. 2020) and was maintained and reared in the "Laboratório de Entomologia Aplicada (LEA)". Celestial blue paperboard (8.0 x 2.5 cm) containing eggs of the alternative host *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) was used to prepare sheets. Eggs were placed on the sheets containing gum arabic (30%) with the aid of a moistened brush. The eggs were made unviable through exposure to a germicidal lamp for 50 min. The sheets were placed in glass tubes (8.5 × 2.5 cm) containing parasitoids. On the inner wall of the tubes, a drop of pure honey was inserted to serve as food for the adult parasitoids. The tube was closed with plastic PVC® film. After a period of 24 hours, the sheets were transferred to new tubes, where they remained until emergence of the next generation of parasitoids.

Preparation of "parasitism units"

About 40 *A. kuehniella* eggs (of up to 24 hours) were placed with gum arabic on celestial blue paperboard (4.0 x 2.5 cm) and these sheets were then placed in cloth bags (8.0 × 4.0 cm) to later be fastened in the field.

Experimental development

In each experimental area, parasitism units with unviable of *A. kuehniella* eggs were distributed in concentric circles with radius of 2.5, 5.0, 9.0, and 12.0 m, distributing 4, 8, 16, and 32 parasitism units, respectively. A completely randomized experimental design was adopted, with six replications for each area evaluated.

In the center point of each experimental area, 5,000 newly-emerged parasitoids were released; this quantity was calculated based on a release rate of 100,000 parasitoids/ha distributed over 20 points. A control area was demarcated at a distance of 50 m from the experimental plots in which six parasitism units were set up in each crop to detect the occurrence of natural parasitism. Over the time of the experiment, mean temperature ranged from 18 to 28°C, relative humidity from 60 to 80%, and mean rainfall of 8 mm.

Exposure to parasitism in the field was 48 hours. After that, the sheets with *A. kuehniella* were removed and placed in Styrofoam boxes and taken to the "LEA". There, they were isolated in glass tubes (8.5 × 2.5 cm) that were previously identified and they were kept under climate-controlled conditions of 25 ± 2°C, 70 ± 10% relative humidity, and 12 h photoperiod up to evaluation of the parameters.

To calculate the parasitism rate for each distance from the central point of release of the parasitoids, the following formula was used: [%P= (number of parasitized eggs / total number of eggs contained on the sheets) × 100], at where parasitized eggs were considered to be darkened eggs that showed emergence of *T. pretiosum*.

Linear regression analysis was used to evaluate the relationship between the parasitism rate and the circumferences of the radii using the software SAS®.

The mean dispersal distance (MDD) and the dispersal area (S^2) of the parasitoid in the areas were determined by the model proposed by Dobzhansky and Wright (1943), in accordance with:

$$\text{MDD} = \frac{\sum r^2 . \frac{i}{a}}{\sum r . \frac{i}{a} + \frac{c}{2\pi}}$$

$$S^2 = \frac{\sum r^3 . \frac{i}{a}}{\sum r . \frac{i}{a} + \frac{c}{2\pi}}$$

in which:
MDD= mean dispersal distance (m) of the parasitoid during the experimental period;
S^2= dispersal area (m^2) during the experimental period;
r= distance (m) from the center to the parasitism units;
a= number of points per circle;
c= mean number of parasitoids in the center circle;
i= percentage of parasitism.

The spatial distribution of parasitism was calculated by geostatistical analyses of the data using a semivariogram and kriging interpolation to construct maps, as described by Vieira et al. (1983). The semivariogram analyses were conducted using the GS + software (Gamma Design Software 2015) and they were fitted to the model which gave the best coefficient of determination (R^2), and to the data interpolated by the ordinary kriging method to spatially characterize the parasitism (Vieira et al. 1983). From the fitted models, the following semivariogram parameters were taken: nugget effect (C_0), which represents the random variability being an indicative of shorter distance variability; sill (C_0+C), which is the semivariance value in which the semivariogram curve stabilizes; range (a), the distance at which the sill is reached which defines the spatial dependence limit and the C value represents the structured spatial variability of the data (Vieira et al. 1983). The kriging-estimated values were used in the Surfer 8.0 software (Golden Software 2017) to construct the map layers. It was used, with the x and y variables representing the local coordinates and z representing the parasitism value in each sampling unit.

RESULTS AND DISCUSSION

Dispersion hability of *T. pretiosum*

The maximum estimated parasitism in all assays was 22.7%. Indeed was a low parasitism percentage, although this may be related to the number of parasitoids released in relation to the number of plants, where the number of parasitoids being insufficient to achieve a greater percentage of parasitism. Researches has shown that the number of parasitoids to be released is variable as a function of phenology of the plant, of the species and lineage of the parasitoid, as well as the dynamics of the host posture (Pratissoli et al. 2005, Pereira et al. 2010). For this crops there were not in the literature recommendations about the number of parasitoids to be released, so a recommendation of 100,000 parasitoids was used as a starting point. In potato fields, for achieve 98,0% of the parasitism was released 500,000 *Trichogramma ostriniae* Pang & Chen (Hymenoptera: Trichogrammatidae) for control *Ostrinia furnicalis* Guenée (Lepidoptera: Crambidae) (Chapman et al. 2009). Furthermore, the natural presence of others lepidopteran eggs in the experimental areas may have attracted the released *T. pretiosum* to areas outside study plots, and reduced the calculated parasitism rate.

There was linear reduction between increasing distance from the point of release and the intensity of parasitism by *T. pretiosum* for all the crops evaluated. Reduction of parasitism in the traps most distant from the release point may be related to the fact of few parasitoids making long flights, as they tend to establish themselves and deposit eggs near the point of release (Suverkropp et al. 2009).

In the sweet corn crop in the V4 stage, the parasitism observed was 22.1 and 8.2% for the distances of 2.5 and 12 m, respectively (Figure 1). The parasitism rates at the points nearest (2.5 m) suggests that it is easy for the *T. pretiosum* 'Guaraciaba' line to locate the hosts in the lowest radius due host density and chemical and physical signals associated with the hosts and its host plant (Heimpel & Casas 2008, Colazza et al. 2010).

In the V8 stage, the variation was 34.5 and 10.7% for the distances of 2.5 and 12

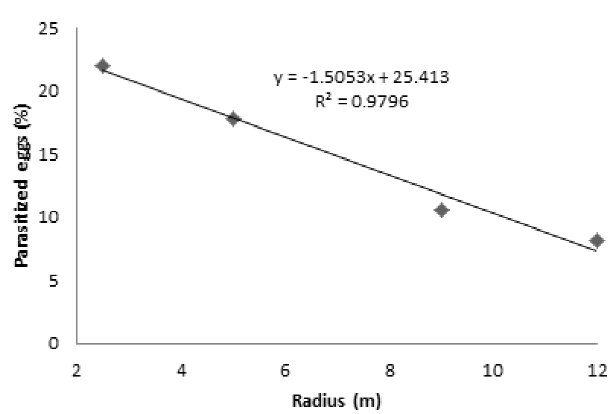

Figure 1. Scattering radius of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and parasitism on eggs of the alternative host *Anagasta kuehniella* (Lepidoptera: Pyralidae) in maize V4 stage (F= 96.44 and P= 0.0102).

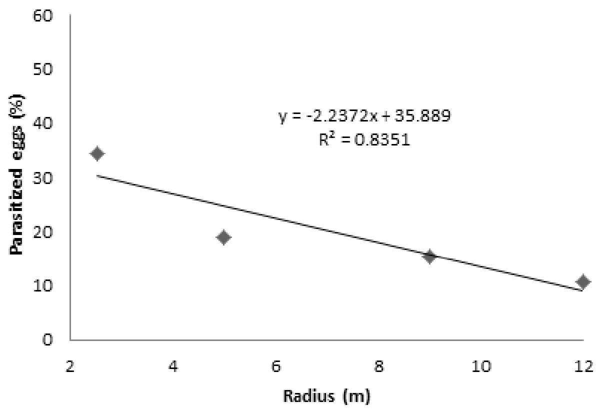

Figure 2. Scattering radius of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and parasitism on eggs of the alternative host *Anagasta kuehniella* (Lepidoptera: Pyralidae) in maize V8 stage (F= 10.14 e P= 0.0861).

m, respectively (Figure 2). The result was to simulate corn in the V4 stage and proves that the parasitism unit became more distant, parasitism decreased in a linear manner. This decline may be related to the larger amount of energy spent and longer time for the parasitoid to locate hosts in a larger area from the release point (Turchin 1998, Chapman et al. 2009, Barbosa et al. 2019).

For the cucumber crop, the parasitism rate ranged from 31.4 to 8.8% at the distances of 2.5 and 12 m, respectively (Figure 3). The dispersal of *Trichogramma* spp. usually is about 10 m as observed in the cucumber crop (Sá et al. 1993, Pratissoli et al. 2005, Chapman et al. 2009, Geremias & Parra 2014), but variations from this mean value can be attributed to the intrinsic characteristics of the parasitoid and of the crop (Pratissoli et al. 2005) or as well as to climatic factors, such as rainfall and wind direction and speed, because this variables in the parasitoid dispersal process may influence flight dynamics and the distance traveled in the field (Pastori et al. 2008, Pereira et al. 2010).

For the cabbage crop, the parasitism percentage ranged from 29.4 to 12.6% at the distances of 2.5 and 12 m, respectively (Figure 4). In the case of cabbage, the results can be

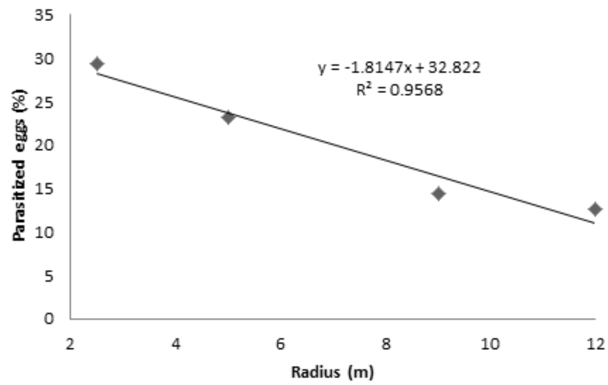

Figure 3. Scattering radius of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and parasitism on eggs of the alternative host *Anagasta kuehniella* (Lepidoptera: Pyralidae) in cucumber (F= 44.37 e P= 0.027).

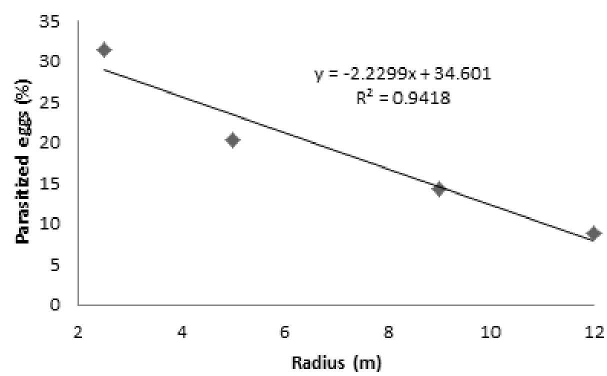

Figure 4. Scattering radius of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) and parasitism on eggs of the alternative host *Anagasta kuehniella* (Lepidoptera: Pyralidae) in cabbage (F= 32.38 e P= 0.029).

attributed to lower protection against the wind and rain provided by the simple architecture of cabbage plants, which led to decreased dispersal for the parasitoid, and parasitism centered on the release points (Fournier & Boivin 2000). Field studies, though in small number, suggest, for example, that *Trichogramma* avoids dewy conditions (Keller et al. 1985), extreme temperatures (Wanjeberg & Hassan 1994), intensely lighted areas (Wanjeberg & Hassan 1994) and heavy rains (Kot 1979).

The MDD for sweet corn in the V4 stage was estimated at 4.7 m and S^2 at 48.6 m^2 and mean parasitism of 18.4% ha. For sweet corn in the V8 stage, the MDD was estimated at 5.9 m and S^2 at 60.3 m^2 and mean parasitism of 22.7% (Table I). The results of this experiment were similar to those obtained by Sá et al. (1993) in which dispersal of *T. pretiosum* in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) eggs of 36 hours after release was 6.3 m and 9.4 m in two different areas, with a S^2 from 80 to 102 m^2. The lower dispersal of *T. pretiosum* in this experiment compared to the experiment of Sá et al. (1993) may be related to the location, to the variety, and to the spacing of the crop, which may have reduced the dispersal ability of the parasitoid. During unfavorable climate conditions, such as high wind or rainfall, *T. pretiosum* may seek refuge and, consequently, unexpected intraspecific variations can arise in dispersal behavior, even within the same species (Fournier & Boivin 2000). For the staked cucumber crop, the MDD was estimated at 6.0 m, S^2 at 62.2 m^2 and parasitism of 21.1% (Table I). This distance and S^2 is similar to the results reported by Pastori et al. (2008) who worked with *T. pretiosum* for control of *Bonagota salubricola* (Meyrick) (Lepidoptera: Tortricidae) in apple and obtained dispersal ability of 61.1 m^2. In the cabbage crop, the MDD was 5.6 m, S^2 was 56.8 m^2, and parasitism was 22.1% (Table I).

In the cabbage crop, low mean distance was observed compared to the study of Pratissoli et al. (2005) in *T. absoluta* eggs in tomato, in which 24 hours after release, there was dispersal from 7.4 to 7.9 m and S^2 from 120.2 to 138.7 m^2, and that can be attributed to lower protection against the wind and rain provided by the simple architecture of cabbage plants, which led to decreased dispersal for the parasitoid, and parasitism centered around the release points (Fournier & Boivin 2000).

For all crops the release points per ha was superior 160 releasing 100,000 parasitoids (Table I). In this case, the number of release points was higher than results obtained for the tomato crops (Pratissoli et al. 2005). However, each crop has specific architecture and phenology, therefore the importance of determine this information for achieve a higher efficiency in the parasitism rate. It is necessary development methodology to improve release and reduce the walking through considering the high number of releasing points required per hectare recommended (Barbosa et al. 2019). For example, use of unmanned aerial vehicles (UAV) has been considered a convenient approach to be pursued aiming release of natural enemies in large areas (van Lenteren et al. 2018). Moreover, several laboratory studies showed promising results related to spraying of eggs parasitized by *Trichogramma* spp., because boom sprayers already are widely used on farms and would be a much simpler application method for farmers (Dionne et al. 2018).

In addition, fitted semivariogram was according to model indicating strong spatial dependence from the releasing point in all crops, with shorter distance resulting in greater parasitism (Table I; Figs. 5, 6, 7 and 8). The range

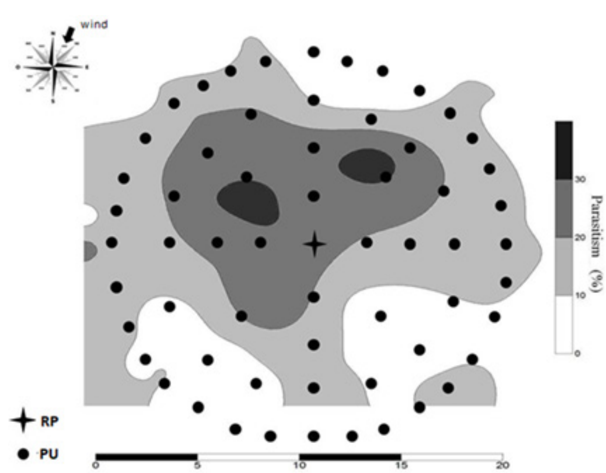

Figure 5. Spatial arrangement of parasitism units (PU), parasitoid release point (RP), wind direction (NE and 2.2 m/s) and interpolation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) parasitism on eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae) in maize in the V4 stage.

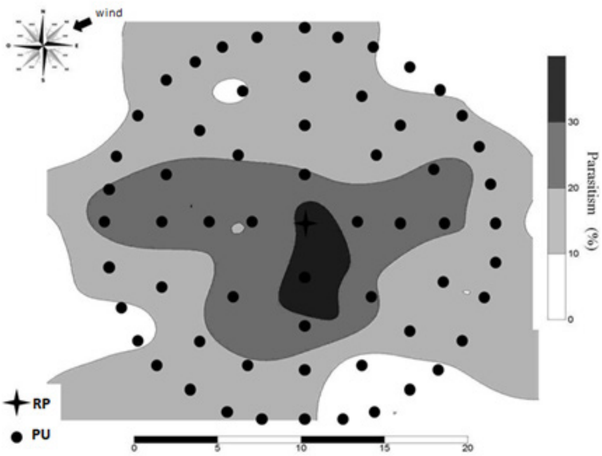

Figure 6. **Spatial arrangement of parasitism units (PU), parasitoid release point (RP), wind direction (NO and 1.2 m / s) and interpolation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) parasitism on eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae) in maize V8 stage.**

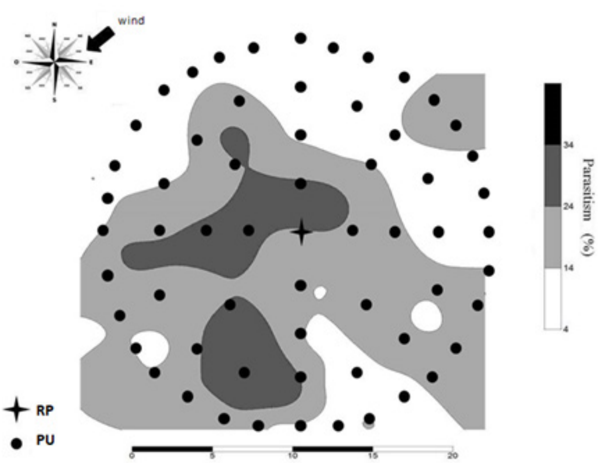

Figure 7. **Spatial arrangement of parasitism units (PU), parasitoid release point (RP), wind direction (NO and 1.4 m/s) and interpolation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) parasitism on *Anagasta kuehniella* eggs (Lepidoptera: Pyralidae) in cucumber.**

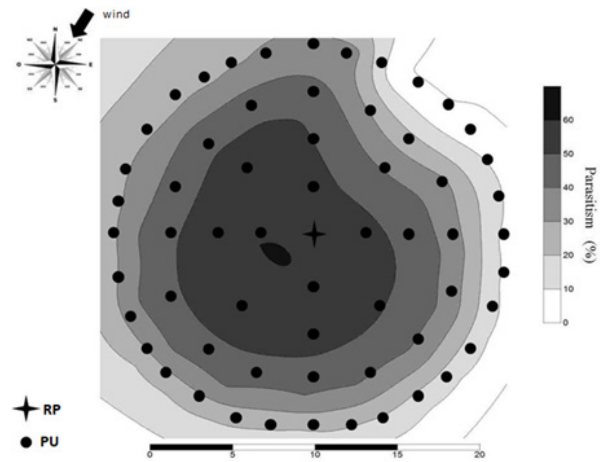

Figure 8. **Spatial arrangement of parasitism units (PU), parasitoid release point (RP), wind direction (NO and 0.6 m/s) and interpolation of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) parasitism on *Anagasta kuehniella* eggs (Lepidoptera: Pyralidae) in cabbage culture.**

(a), which represents the distance up to which there is spatial dependence between samples, varied from 5.4 m (maize V4) to 31.3 m (cabbage, Table I). Therefore, the parasitized larvae were concentrated in a 48.8 to 56.8 m² area. In this study, the range (a) represented the dispersal capability of most *T. pretiosum* adults (Zappala et al. 2012).

Finally, in all studies, no natural parasitism was observed in *A. kuehniella* in the control plots. Thus, the parasitism obtained in the parasitism unit was a result from released parasitoids.

Spatial distribution of parasitism for *T. pretiosum*

The semivariogram fitted showed strong spatial dependency among the samples, showing that parasitism varies in accordance with the distance of the location of release of the *T. pretiosum* in relation to parasitism unit. This approach is used to characterize the dispersal of parasitoids, additionally the parameters of the semivariogram model using the kriging analysis allow estimating unsampled areas by interpolation techniques and construction of

Table I. **Mean distance, dispersal area, expected parasitism, parameters of the fitted, and cross validation of the interpolated data by the ordinary Kriging method for parasitism of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) on *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs in the sweet corn, cucumber and cabbage, "Guaraciaba do Norte", Ceará State.**

Estimated parameters	**Crops**			
	Maize V4[1]	**Maize V8[2]**	**Cucumber[3]**	**Cabbage[4]**
Mean Dispersal Distance (MMD)	4.7 m	5.9 m	6.0 m	5.6 m
Dispersion area (S^2)	48.6 m^2	60.3 m^2	62.2 m^2	56.8 m^2
Expected parasitism	18.4%[1]	22.7%[2]	21.1%[3]	22.1%[4]
No of points per ha	206	166	161	176
Estimated parameters	**Cross validation**			
Pure nugget (C_0)	3.8	4.5	29.4	35.7
Structural variance (C)	19.9	91.2	54.6	113.2
Level (C_0 + C)	23.8	95.7	84.1	148.9
Range (a)	5.4	22.6	11.3	31.3
R^2	0.49	0.81	0.48	0.63

[1]Expected parasitism calculated by means of the mean distance in the model $\hat{y}$= 25.41-1.50x. [2]Expected parasitism calculated by means of the mean distance in the model $\hat{y}$= 35.89-2.23x. [3]Expected parasitism calculated by means of the mean distance in the model $\hat{y}$= 32-1.82x. [4]Expected parasitism calculated by means of the mean distance in the model $\hat{y}$= 34.6-2.23x.

specific maps (Vieira et al. 1983, Zappala et al. 2012).

Wind speed and direction are also important variables in the parasitoid dispersal process (Machtinger et al. 2015, Barbosa et al. 2019), with a tendency to concentrate parasitism in the predominant direction of the winds since the wind contributes to passive dispersal when the insect begins its flight, such as occurred in in the sweet corn, cucumber, and cabbage crops areas (Mahecha & Manzano 2016, Barbosa et al. 2019). The rainfall impacted the dispersion of the parasitoid, given that it was concentrate close the releasing point, which resulted in greater aggregation of parasitism in that area.

The Kriging maps showing the spatial distribution (Figure 5, 6, 7 and 8) confirm that the regions of higher parasitized larvae are near the *T. pretiosum* release points.

The spatial disposition pattern of parasitism for sweet corn in the V4 stage showed greater aggregation in the northwest direction, with a higher percentage of parasitism near 9 m (Figure 5). In their studies, Pinto & Parra (2002) released *T. galloi* in different manners and techniques for control of *D. saccharalis* eggs, and they observed that climate conditions affected dispersal and parasitism; rains that occurred on the days of releases or soon after carried the pupae of the parasitoids and decreased the effectiveness of parasitism.

Sweet corn in the V8 stage had a higher dispersal pattern in the central-west region (Figure 6), with a higher parasitism rate near the release point. For sweet corn in the V8 stage, dispersal ability was less affected by wind and rainfall, aggregating more in the central-west region, near the point of release. The higher values of mean distance and S² compared to sweet corn in the V4 stage are related to the protection provided by the higher fresh matter content, reducing the effects of wind and rain.

In the cucumber crop, the greatest aggregation was in the west direction, with a higher parasitism rate up to 9 m (Figure 7). There was higher aggregation in the west region of the cucumber area due to the influence of wind on the arrangement of the trained cropping system.

For cabbage, the dispersal pattern was homogeneous (Figure 8). Whereas in cabbage, dispersal was centered on the release point due to low protection against environmental factors provided by low plant architecture. Although the parasitism occur, attention must be paid to the effect of the increase in parasitoid density and to the time allowed a better dispersal in whole area.

Acknowledgments

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