academicJournals

Vol. 9(4), pp. 436-447, 23 January, 2014
DOI: 10.5897/AJAR2013.6991
ISSN 1991-637X ©2014 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Determination of method to evaluate parasitism and cover area for studies on *Cotesia flavipes* in sugarcane

Haroldo Xavier Linhares Volpe[1]*, José Carlos Barbosa[2], Silvio Rogério Viel[1], Roberto Marchi Goulart[1], Alessandra Marieli Vacari[1], Claudio Salas[1], Ana Carolina Pires Veiga[1] and Sergio Antonio De Bortoli[1]

[1]FCAV/Unesp - Departamento de Fitossanidade, Laboratório de Biologia e Criação de Insetos, Rodovia Paulo Donato Castellane, s/n, Bairro Rural, - 14884-900 - Jaboticabal, SP, Brasil.
[2]FCAV/Unesp - Departamento de Ciências Exatas, Rodovia Paulo Donato Castellane, s/n, Bairro Rural, - 14884-900 - Jaboticabal, SP, Brasil.

Accepted 4 December, 2013

The continual rearing of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in laboratories (for approximately 30 years) may have led to loss of genetic variability due to drift, selection, and crossing among siblings. This in turn may have compromised the biological characteristics of the insect, notably with respect to its activity level, thereby altering its parasitic and dispersal capacities. This study investigated methods that allowed the testing of parasitism and number of release points per hectare for *C. flavipes*. We tested the efficiency of different colored Moericke traps, yellow Moericke traps containing different concentrations of frass, stick yellow traps arranged at different heights and others containing sugarcane stems, with each stem containing a larva of the sugarcane borer, *Diatraea saccharalis* (Fabricius). Only the use of stems was efficient for measuring cover, aggregation, and geostatistics of its parasitism. The samples showed an aggregated distribution, and the maximum dispersal distance of *C. flavipes* was 25 m. Geostatistical analyses enabled the evaluation and mapping of the number of parasitized larvae. This method permits tests aimed at evaluating quality control during the biological control of *D. saccharalis* with *C. flavipes*.

Key words: Biological control, parasitoid, Hymenoptera, Braconidae, massive release, geostatistic analyses.

INTRODUCTION

Cultivation of sugarcane in large fields creates suitable conditions for pest occurrence and subsequent crop damage. This is particularly applicable to the key pest of sugarcane in Americas - the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) - on account of the frequency of its population surges, high biotic potential, and ability to protect itself from natural enemies (Posey et al., 2001).

The larva of *D. saccharalis* cause direct and indirect damage to sugarcane production. Direct damage is caused by the larvae building galleries in the sugarcane stalks, resulting in weight loss and plant mortality. Indirect damage is due to the invasion of the open galleries by microorganisms, which contaminate the sugarcane juice,

*Corresponding author. E-mail: hxlvolpe@ig.com.br. Tel: +55 16 32092643 Ext. 215.

there by reducing both sugar and ethanol production (Ogunwolu et al., 1991; Dinardo-Miranda et al., 2011).

The use of insecticides to control *D. saccharalis* is highly ineffective after the larva penetrate the sugarcane stalks, thus gaining protection from the insecticidal sprays. The control of *D. saccharalis* is essentially dependent on biological control, mainly by the use of the larval parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in Brazil (Rossi and Fowler, 2003a and b), Louisiana (Schexnayder Jr. et al., 2001; White et al., 2004; White and Wilson, 2012), Barbados (Alam et al., 1971), and south Texas (Fuchs et al., 1979). This species is native to Indo-Australian region and was introduced into Brazil in 1974 from Trinidad (Botelho, 1992; Kimani-Njogu and Overholt, 1997). Since then, most of the sugarcane fields in Brazil have received mass release of this parasitoid. The total area receiving *C. flavipes* release is 3×10^6 ha (Vacari et al., 2012). Although, biological control is practiced in a large area, there is currently a lack of methods for monitoring the efficacy of parasitoid release.

The success of biological control agents depends on their efficiency to search for and locate target hosts (Nordlund et al., 1988). In spite of the rearing of *C. flavipes* on a large scale since the 1970s, little is known about its quality components, which, according to Boller and Chambers (1977) are related to adaptability, mobility, sexual activity, reproduction, and colonization potential. Some characteristics, particularly flight are very restricted under laboratory conditions (van Lenteren, 1991).

The final steps in biological control, such as the release of natural enemies, are neglected in most of biological control programs, and only a few studies have aimed to determine the best release technique and the adaptations that it may require (Overholt et al., 1994). The continual rearing of *C. flavipes* in laboratories (for approximately 30 years) may have led to loss of genetic variability due to drift, selection, and crossing among siblings (Boller and Chambers, 1977). This in turn may have compromised the biological characteristics of the insect, notably with respect to its activity level, thereby altering its parasitic and dispersal capacities (van Lenteren, 2009).

Most works are carried out using larvae, which infest sugarcane. The disadvantage of using larvae from the fields is in that the distribution of this pest is aggregated. In this context, we aimed to test various methods to study the parasitism and cover area of *C. flavipes* and identify the method that is effective for field studies with this parasitoid in sugarcane field.

MATERIALS AND METHODS

Experiment one - Efficiency of colored Moericke traps to capture *C. flavipes*

The investigation was conducted in Jaboticabal, Sao Paulo, Brazil in a newly planted commercial sugarcane crop of the cultivar CTC-3 in 2009. The plants were 1 month-old (from planting to first harvest, 1.5 years), 0.5 m tall and spaced 1.5 m apart.

In the plot, we designed a concentric circle with a radius of 10 m from the central release point of *C. flavipes*, with an area of 314 m^2. The circumference was divided into four equal parts (resulting in four equal blocks). In each block, we placed equally spaced Moericke traps of different colors trade in the local marked on the edge of the circle (white, blue, yellow, light green, dark green, light blue, dark blue, and light pink randomly distributed in each block) distanced 1.97 m each other and subjected them to eight treatments with four replications. The traps were supplied with 350 ml experimental solution (70 g of salt and 3 ml of detergent per liter of water), and two commercial release receptacles used to trade *C. flavipes* containing together approximately 3,000 adults were released in the center of the circle. The traps were kept in the field for 3 days, and the trap color preference of *C. flavipes* was observed by counting the number of individuals present in the traps.

Experiment two - Efficiency of yellow Moericke traps containing different concentrations of frass

The arrangement, number and distance of traps followed the same pattern as that in “Experiment one”, but we added various concentrations of frass of *D. saccharalis* in the solution in the yellow traps since this color are known to attract many species of parasitoids (Hoelmer et al., 1998). We added 1, 2, 4, 6, 8, 10, and 12 g of frass per 350 ml of the solution since frass is a stimuli source that attracts *C. flavipes* (van Leerdam et al., 1985; Potting et al., 1997), and a control without frass. The traps were randomly placed in the area spaced 1.97 m apart each other, consisting of 8 treatments in 4 blocks, and approximately 3,000 parasitoids adults were released in the center of the circle. The traps were kept in the field for three days.

Experiment three - Effect of variation in the height of rectangular yellow sticky traps

The experimental setup was similar to that used in the earlier experiments, but we tested 3 heights for setting the sticky traps (0, 0.25, and 0.50 m) in each block. A randomized complete block design was used, comprised of three trap height treatments in each of four blocks, consisting of 3 treatments in 4 blocks and each trap was a replicate.

Experiment four - Evaluation of *C. flavipes* parasitism by using sugarcane stems containing *D. saccharalis* larva under laboratory conditions

Twenty-seven stems of the cultivar SP80-3280 (susceptible to *D. saccharalis*) were cut using a circular saw. The cut sample comprised one full segment of the sugarcane stem and half of the segments above and below the cut, in order to prevent water loss and ensure continued nutrition and presence of larva inside the stem (Wiedenmann and Smith Jr., 2006). The stems were perforated using an electric drill with a 5/32 mm drill bit, and a single 1.5 cm long *D. saccharalis* larva was inserted into each opening.

Parasitism of *C. flavipes* was evaluated under the following conditions: (one) stems kept vertical, keeping the head of the larva facing upward and the larva on their back; (two) stems in the reversed position; and (three) stems lying prostrate (horizontal). The positions are changed to determine if the methods used to insert the larvae inside the stems or to fix the stems into the soil could compromise the parasitism during the field tests with sentinel caterpillars.

For each position, parasitism was evaluated with either frass lining the opening with frass completely obstructing the opening,

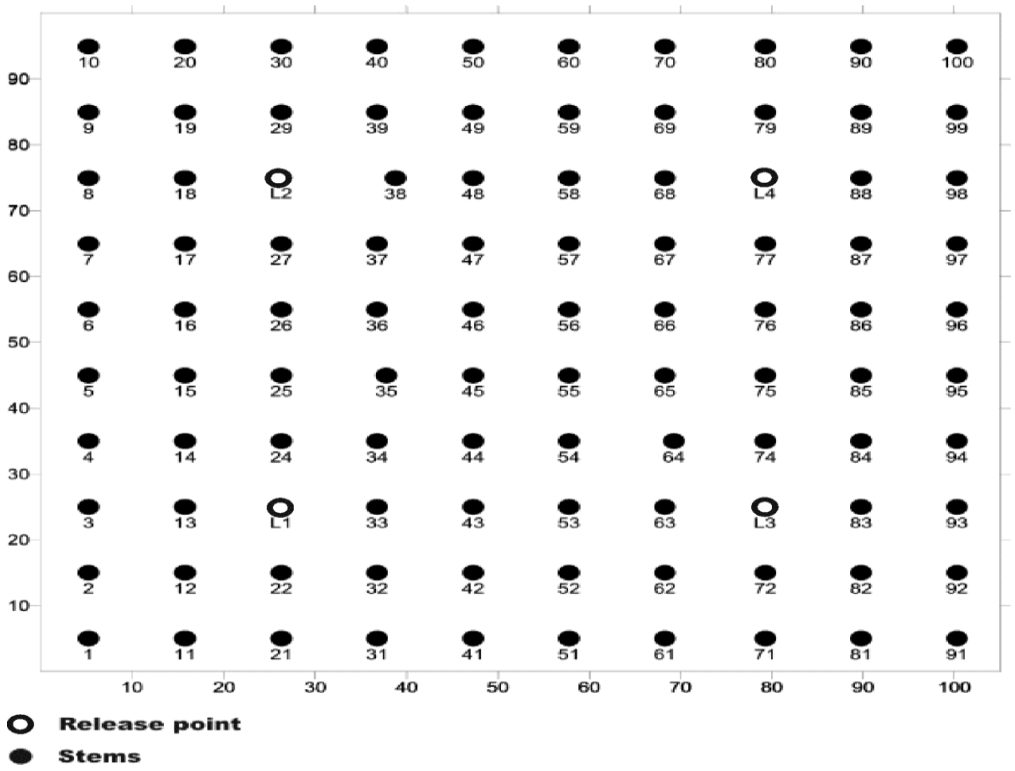

Figure 1. Schematic representation of the sugarcane sample area divided into 100 plots, showing the parasitoid release points and distribution of stems.

And without frass, for a total of nine treatments with three replications.

The stems were placed in a wooden cage (1.0 m × 0.5 m × 0.5 m) covered with the fabric "voile" and exposed to thirty females of *C. flavipes* after eight hours of emergence. After 2 days, the stems were cut longitudinally to retrieve the larvae, which were subsequently maintained in individual 6 cm Petri dishes labeled with the treatment. Each dish contained sufficient artificial diet for the larval development (produced by Sao Martinho mill, Pradopolis, Sao Paulo, Brazil) and was maintained at 25 ± 1°C, 70% ± 10% relative humidity, and a 12 h (L12:D12) photoperiod to evaluate the parasitism.

Experiment five - Determination of spatial distribution of *C. flavipes* by using sugarcane stems containing *D. saccharalis* larvae in field

The experiment was conducted in Jaboticabal, Sao Paulo, Brazil, in a commercial newly planted sugarcane crop of the cultivar CTC-3, covering an area of 10.65 ha. The plants were 7 months old from planting (normal lifecycle, 1.5 years), 2.2 m tall, spaced 1.5 m apart, and moderately resistant to *D. saccharalis*.

The data used to study the spatial distribution of *C. flavipes* parasitism were obtained in an area of 100 m × 105 m, which was divided into 100 plots of 10.5 m × 10.0 m each, considering a border of 10 m from the dirt road. Field sampling of *C. flavipes* populations can be difficult; in previous investigations, attempts to capture the insect by using Moericke traps and yellow adhesive traps have been unsuccessful. It was therefore necessary to count the number of parasitized larvae in each sample unit. The method using stems, which involved the placement of artificially infested sugarcane stems in the field was used as an alternative indicator of *C. flavipes* population distribution and its characteristics. We cut 1,000 stems of the cultivar SP80-3280 (7th harvest, susceptible to *D. saccharalis*), adopting the method described in "Experiment four". The lower segment was cut into a cone shape and inserted into the soil. A single *D. saccharalis* larva was inserted into each opening 1 day before the insertion of the stems into the soil in order to allow sufficient time for the larva to produce frass, because it is known that *C. flavipes* is attracted to the host excrement (Ngi-Song and Overholt, 1997).

In the center of each of the 100 plots, ten sugarcane stems, each containing a single *D. saccharalis* larva was inserted into the soil into the row of the planted sugarcane. The stems were placed in two lines, with five stems in each line and the lines spaced 20 cm apart. Care was taken to insert the stems in such a way that the head of each larva remained facing upward, duplicating the position naturally adopted by the larva.

The parasitoids were released at four points spaced 50 m apart and 25 m from the edge of the main field (Figure 1). One plastic 100 ml receptacle with lid, containing 8 h old parasitoids, was placed at each point. Each receptacle contained approximately 1,500 parasitoids (6,000 insects in total), and the release method was based on Botelho et al. (1980).

The larvae were exposed to *C. flavipes* for 3 days, which is the typical duration of the adult phase of the parasitoid, as has been shown in laboratory tests (Vacari et al., 2012). After this period, the stems were transported in labeled plastic bags [identified with the plot number (1-100)] the laboratory, where they were cut longitudinally to retrieve the larvae and subsequently maintained in individual 6 cm Petri dishes labeled with the plot number. Each dish contained sufficient artificial diet to complete larval development and was maintained at 25 ± 1°C, 70% ± 10% relative humidity, and a 12 h (L12:D12) photoperiod until the parasitoids emerged.

The number of parasitized larvae was counted, together with the

number of males, and females that emerged in each plot. The mean, variance, and aggregation indices were determined using the data on parasitized larvae. Subsequently, the data obtained were analyzed using Poisson and negative binomial models. A chi-square adherence test was used to compare the observed and expected frequencies, as described by Anscombe (1949). The indices used to calculate the aggregation and study the spatial distribution of the parasitoids are described as follow:

Aggregation indices

Variance/mean ratio: This index was first used by Clapham (1936) as cited by Perry and Mead (1979). It is also called a dispersion index, and according to Rabinovich (1980), it can be used to measure the deviance of an array of random conditions. Values equal to one indicate a random spatial array; smaller than one, a uniform array; and significantly greater than one, an aggregated array. According to Southwood (1971), the application of this index is affected by the sample unit size and the number of observed individuals. The index is estimated by the following formula:

$I = s^2/m$,

where s^2 is sample variance and m is sample mean.

Morisita's index: This index was developed by Morisita (1959, 1962), with the objective of developing an index independent of the mean sample size and the total number of individuals. Values equal to one indicate a random distribution; more than one, an aggregated distribution; and lesser than one, a uniform distribution. A limitation of the Morisita index is that it may be influenced by sample size (N). Thus, if the index is to be relied upon for accurate data, it is necessary to ensure that the number of sample units is the same in each area being compared. The index is represented by the following formula:

$$I_\delta = N\frac{\sum x^2 - \sum x}{(\sum x)^2 - \sum x},$$

where N is sample size and x_i is the number of insects in the i^{th} sample unit.

The removal of randomness can be tested using the following formula:

$$X_\delta^2 = I_\delta(\sum x_i - 1) + n - \sum x_i \sim \chi^2_{(n-1)} .$$

If $X_\delta^2 \geq \chi^2_{(n-1g.l.;0.05)}$, the hypothesis of randomness of distribution should be rejected.

Green's coefficient

Green's coefficient can be used to compare aggregated distributions. Negative values indicate a uniform pattern, whereas positive values indicate an aggregated pattern (Green, 1966). The coefficient is based on the ratio of distribution variance to mean and is expressed by the following formula:

$$C_x = \frac{(s^2/\hat{m}) - 1}{\sum_{i=1}^{n} x_i - 1},$$

where m is sample mean, s^2 is sample variance, and x_i is the number of insects in the i^{th} sample unit.

The k exponent of negative binomial distribution

According to Anscombe (1949), k is estimated for the moment method by equating the first two moments of distribution with the estimated sample moments, resulting in the following expression:

$$k = \frac{m^2}{s^2 - m},$$

where m is sample mean and s^2 is sample variance.

Negative values indicate a uniform distribution. A low positive value (k < 2) indicates a highly aggregated distribution; moderate positive value (k = 2-8), moderate aggregation; and a high positive value (k > 8), a random distribution (Elliott, 1979).

Probabilistic models

Poisson distribution: The Poisson distribution is characterized by variance being equal to the mean ($\sigma^2 = \mu$). The formulas used to calculate the series of probabilities are as follows:

$P(0) = e^{-m}$

$$P(x) = \frac{m}{x} \cdot P(x-1)$$

, parameter x = 1, 2, 3,...,

where e is the base of the Napierian logarithm (e = 2.718282...), $P(x)$ is the probability of finding individual x in the sample unit, and m is sample mean.

Negative binomial distribution

The negative binomial distribution is characterized by variance being larger than the mean, indicating aggregated distribution. This index has two parameters: mean (μ) and parameter k (k > 0), and the probabilities are calculated using recurring formulas shown below:

$$P(0) = \left(1 + \frac{m}{k}\right)^{-k}$$

, to x = 0

$$P(x) = \frac{k + x - 1}{x} \cdot \frac{m}{m + k} \cdot P(x-1)$$

, parameter x = 1, 2, 3,...,

where k is the k exponent of the negative binomial distribution and m is sample mean.

Chi-square goodness of fit test

Chi-square goodness of fit test was used to adjust the data to each probability distribution, using the following expression:

$$X^2 = \sum_{i=1}^{N_c} \frac{(FO_i - FE_i)^2}{FE_i}$$

where N_c is the number of classes of the frequency distribution, FO_i is the observed frequency in the i^{th} class, and FE_i is the expected frequency in the i^{th} class.

The models show a good adjustment to the original data when observed and expected frequencies are reasonably close.

Geoestatistical analyses

The semivariograms were constructed using spherical model for parasitism and Gaussian model for number of males, females and total of emerged adults. Krigeage maps were generated using the data from the semivariograms with the objective of estimating the necessary interpolations to construct isolines and three-dimensional parasitism maps for *C. flavipes.*

Further, geostatistical analyses were conducted using the data on parasitized larvae and the number of males and females emerged in the first generation, in relation to the physical coordinates of the collected samples. Thus, each sample was analyzed in light of the variable value or the number of emerged parasitoids and the coordinates of each point where data were collected, using GPS Garmin Etrex Vista. The Surfer 7.0 software was used to construct a map of the observed values, a semivariogram, and a two-dimensional representation by using isolines and three-dimensional graphics.

RESULTS

Experiment one

C. flavipes was not attracted by the colors of receptacles used in "Experiment one". However, two males were caught in two light-blue traps (one male in each trap), one male each in light-green and yellow traps, and one female in pink trap. Thus, this test provided evidence that *C. flavipes* is not attracted by color.

Experiment two

C. flavipes was not attracted to the frass dissolved in the solution contained in the traps, showing that the traps used were inefficient in capturing the parasitoids and thus cannot be used in determining spatial distribution.

Experiment three

In this experiment, 60, 25, and four *C. flavipes* adults were captured at the heights of 0, 25, and 50 cm, respectively. The low catch of parasitoids using the traps in experiment one, two and three did not allow performing statistical analyses of data, but showed that Moericke and adhesive traps are not a good tool to catch *C. flavipes.*

Experiment four

D. saccharalis larvae in all the treatments were parasitized, independent of the position of the stem in the cage or the addition of frass in the opening made for the insertion of the larvae.

Experiment five

In this experiment, we recovered 622 of 1,000 *D. saccharalis* larvae from the manually infested sugarcane stems of which 87 were parasitized (13.98% parasitism).

Aggregation indices

We found that the variance values were larger than the mean values for all the evaluated parameters (parasitism, number of females, number of males, and total number of insects). Accordingly, the dispersion index (I) was greater than 1, indicating an aggregated spatial distribution of parasitism, emerged males, emerged females, and total emerged insects (first generation). However, the variance/mean ratio showed that values describing the number of males, number of females, and total number of adults were considerably larger than those describing parasitism. This indicates that aggregation of the first generation of insects was greater than that of the parasitism of the insects initially released in the crop (Table 1).

The values obtained using Morisita's index (I_δ) were greater than one, confirming aggregated distribution for the evaluated parameters. In fact, X^2_δ values obtained for the parasitism, number of males, number of females, and total numbers of adults (first generation) confirm higher aggregation for these parameters (Table 1).

The aggregated distribution pattern was further confirmed by calculating Green's coefficient (C_x). The C_x values obtained were more than zero, which, according to Davis (1993), indicates an aggregated distribution (Table 1).

For the parameter *k*, the estimated number of males, number of females, and total number of emerged adults were slightly more than zero, indicating high levels of aggregation. For parasitism, the *k* value was larger than that obtained for the number of males, females, and total of emerged insects, indicating that parasitism is less aggregated than the number of insects emerged in the first generation (Table 1).

Probabilistic models

Data adjustment for parasitism, number of males, number of females, and total number of adults emerged in the first generation was conducted to study the probabilistic models that explain the spatial distribution of *C. flavipes*, that is, Poisson and negative binomial distributions (Figure 2). The Poisson distribution did not adjust the data for parasitism, number of males, number of females, or total number of insects emerged in the first generation.

Further, the chi-square value was significant with 99% probability, indicating that the distributions of parasitism and number of insects emerged in the first generation were not random. Since the variance was larger than the mean, the data adjustment was tested for negative

Table 1. Means, variances, and dispersion indices for *Cotesia flavipes* in sugarcane crop.

Index	Parasitism	Females	Males	Total insects
m	0.8700	28.7700	12.8300	41.6000
s^2	1.6092	2,289.3304	696.6476	4,972.6869
I = s^2/m	1.8496	79.5735	54.2983	119.5357
I_δ	17.7500	3.7047	5.1159	3.8216
X^2_δ	1,824.1385**	7,877.70**	5,375.50**	11,834.00**
d.f.	4	9	28	39
$k_{moments}$	1.0239	0.3662	0.2407	0.3509
C_x	0.0405	0.0273	0.0416	0.0285

m = sample mean, s^2 = sample variance, I = variance/mean ratio, I_δ = Morisita's index, X^2_δ = Chi-square adherence test for the calculated values of Morisita's index, ** = significance level of 1% probability, $k_{moments}$ = k calculated by the moment method, C_x = Green's coefficient.

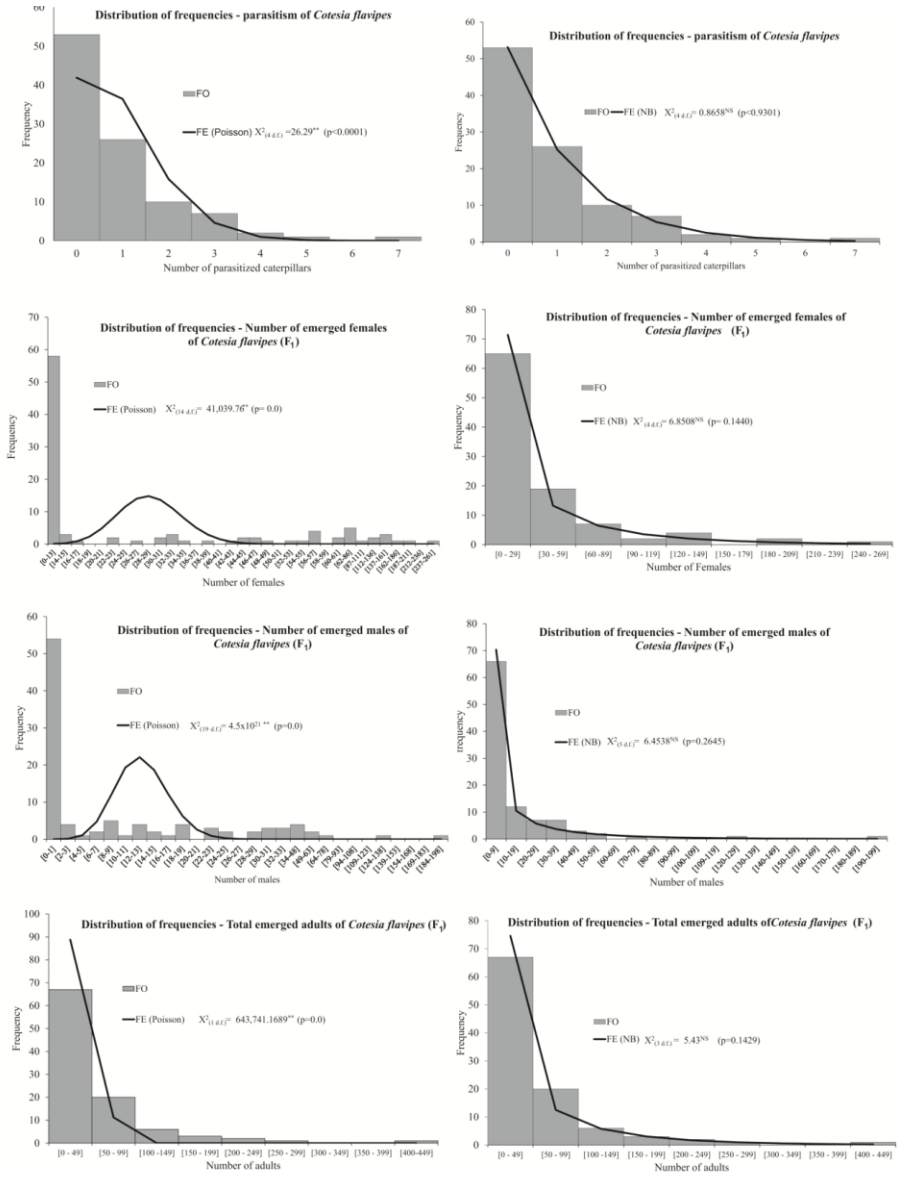

Figure 2. Graphs showing the observed frequencies and data adjustment to Poisson and negative binomial distributions for *C. flavipes* parasitism, number of males, number of females, and total number of adults emerged in the first generation.

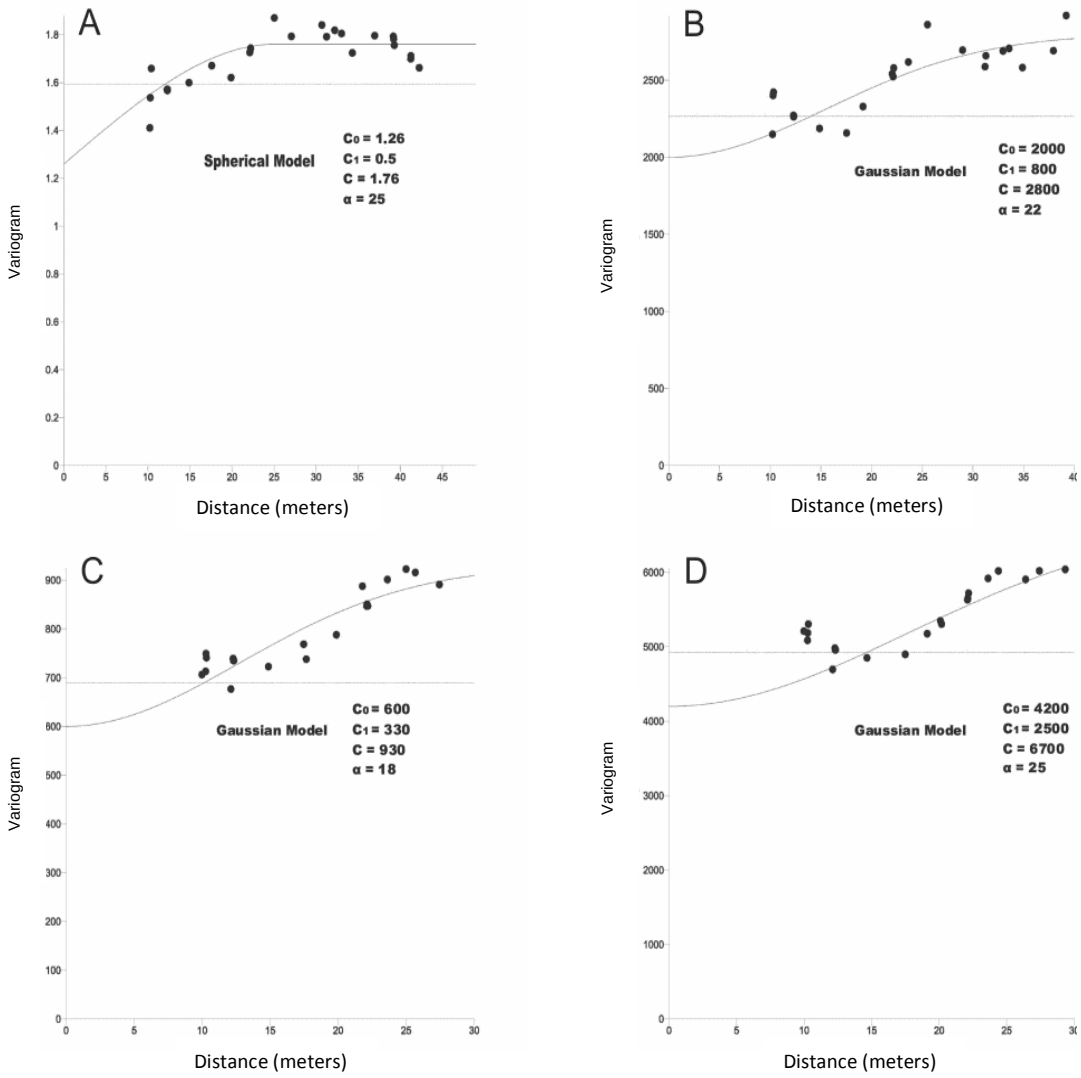

Figure 3. Semivariograms. (A) Number of parasitized larvae by *C. flavipes*. (B) Number of *C. flavipes* males that emerged in the second generation. (C) Number of *C. flavipes* females that emerged in the second generation. (D) Total number of *C. flavipes* adults that emerged in the first generation.

binomial distribution (Figure 2).

Fits of the negative binomial distribution to the data did not show a significant difference between the expected and observed frequencies, indicating that the spatial distribution of parasitism and number of insects emerged in the first generation was aggregated, independent of the sex of the parasitoids (Figure 2).

According to all the dispersion indices examined, the parasitism, number of males, number of females, and total number of emerged adults of *C. flavipes* indicated aggregated distribution, which was confirmed by adjustments to the frequency distribution data of the negative binomial distribution (Figure 2). Thus, the distribution of *C. flavipes* parasitism on *D. saccharalis* larvae was aggregated, indicating that the parasitoid did not disperse uniformly in the release area.

Geostatistical analyses

The semivariogram for *C. flavipes* parasitism showed a range of approximately 25 m. The range distance, defined as the distance between pairs, increased until a certain level (Figure 3A). The range indicates the flight distance of the parasitoid, or in other words, its dispersal potential. In this study, we found that *C. flavipes* parasitizes *D. saccharalis* larvae at distances up to 25 m from the release point (Figure 3A). This 25 m distance was the distance between the release point and the edge of the aggregation area.

Krigeage maps were generated using the data from the semivariograms with the objective of estimating the necessary interpolations to construct isolines and three-dimensional parasitism maps for *C. flavipes* (Figure 4A).

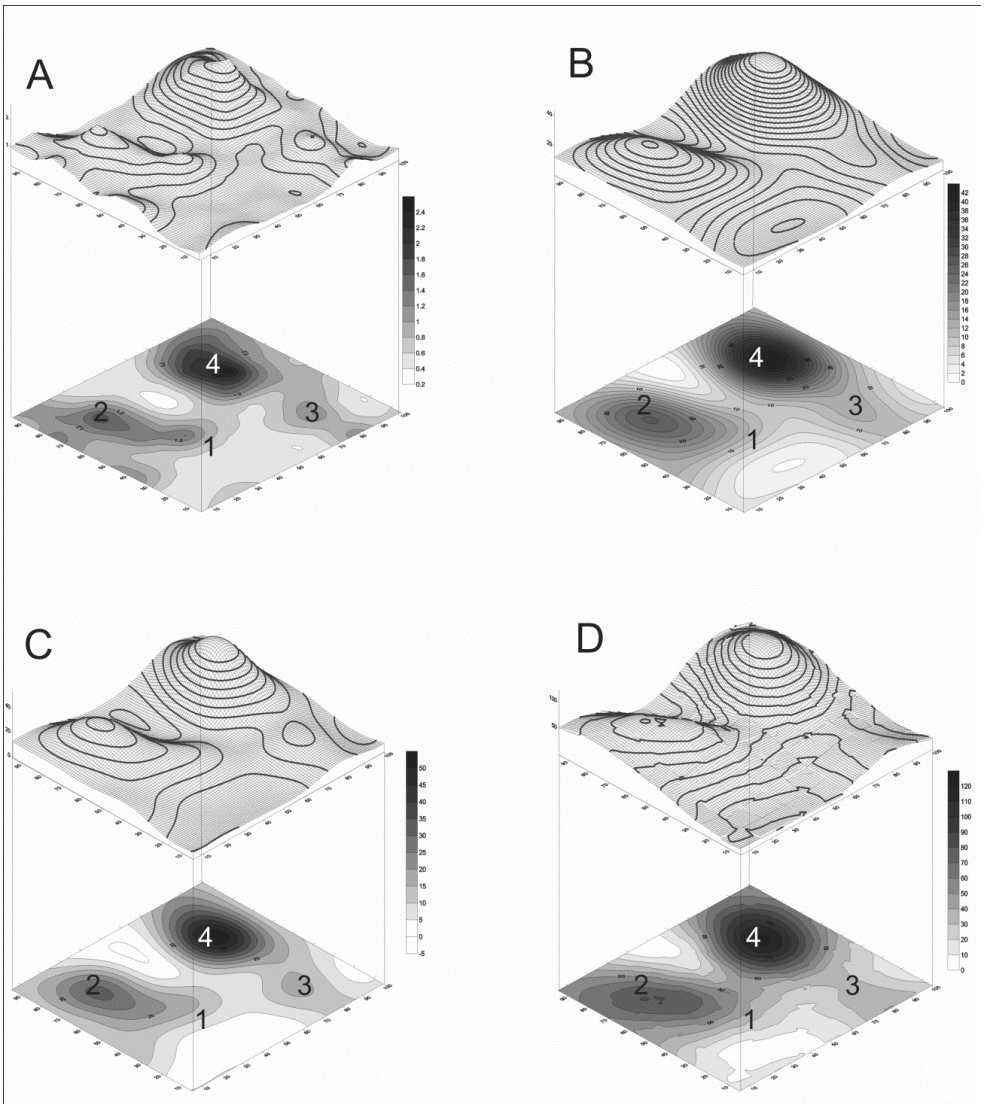

Figure 4. Krigeage maps. (A) Number of parasitized larvae by *C. flavipes*. (B) Number of *C. flavipes* males that emerged in the second generation. (C) Number of *C. flavipes* females that emerged in the second generation. (D) Total number of *C. flavipes* adults that emerged in the first generation.

From the map, we observed that each release point showed different parasitic behavior of *C. flavipes*. For better understanding, the release points are discussed separately. Release points one and three were less efficient than points two and four because they were located close to a dirty road used to transport agricultural machines. The low efficiency can be explained by the fact that the part of the site containing release points one and three was less protected by the crop. The release points closer to the road were thus less efficient than those that were protected by the crop. The dirty road cannot be avoided when releasing the parasitoids since it provides necessary access to the release area (Figure 4A).

The parasitoids released at point two showed higher parasitism aggregation than those released at the points located nearest to the road (release points one and three); the number of parasitized larvae ranged between 1.2 and 1.4 near the release point. However, this number decreased by 50% at a distance of 10 m from the release point and reached a value of 0.2 at a distance of 15 to 25 m from the release point (Figure 4A).

The parasitoids released at point 4 showed the highest parasitism ratio, ranging from 2.2 to 2.4 parasitized larvae near the release point. However, at 15 m from the release point, this value decreased by 50%, ranging from 1.0 to 1.2, and beyond this distance, the parasitism ratio was 0.8 - 0.4 parasitized larvae (Figure 4A).

Thus, despite the activity radius of *C. flavipes* (25 m)

being comparable to the distance between the release points of the parasitoids (50 m), the number of parasitized larvae rapidly decreased, reducing to 50%, when the distance from the release point reached 10 m (Figures 3A and 4A).

A three-dimensional map for the number of males that emerged in the first generation was constructed on the basis of the corresponding semivariogram, and it showed that the emerged males had a range of 22 m (Figures 3B and 4B). From this map, we could also observe that there were differences among the number of male parasitoids that emerged at the four points. These differences can be explained in terms of the differences in the number of parasitized larvae at the same points. The points with a smaller number of parasitized larvae had fewer emerged males (Figure 4B). Since the number of parasitized larvae decreased with increasing distance from the release point, there was a corresponding decrease in the number of emerged male parasitoids. Near release point 4, we observed the emergence of 40 males; however, at a distance of more than 10 m from the release point, this number decreased by 50% (Figure 4B).

The semivariogram for the number of females that emerged in the first generation showed that the females had a range of 18 m (Figure 3C). However, as the parasitoids dispersed further from the release point, there was a corresponding decrease in the number of parasitized larvae and consequently in the number of emerged females, similar to the emerged males (Figure 4C).

Comparison of the krigeage maps for the male and female parasitoids showed that the number of emerged males and females was similar, with a sex ratio of approximately 1:1 (Figures 4B and 4C).

Considering the total number of *C. flavipes* in the first generation (Figures 3D and 4D), the semivariogram showed that the adults had a range of 22 m (Figure 3D) and the distribution and density of the adults (Figure 4D) followed the same behavior showed to males (Figure 4B) and females (Figure 4C), but were higher when compared to males and females, since this figures shows a sum of the emerged males and females. However, the distribution of this generation is an import tool to know the contribution of the emerged adults to control the next generation of *D. saccharalis* in the area.

DISCUSSION

Tests with traps - Experiments one to four

Attraction to color has been demonstrated in insects (Romoser, 1981). Yellow is more attracted than other colors to *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae) (Udayagiri et al., 1997) and in general, yellow color attracts parasitoids (Hoelmer et al., 1998).

In a previous study using traps to capture Braconidae insects, Noyes (1989) tested five methods of sampling Hymenoptera (sweep-netting, Malaise trapping, yellow pan trapping, flight intercept trapping and canopy fogging), demonstrating that for most groups of this order, sweeping was the most effective single method of sampling. Malaise traps were also very effective in most habitats. Flight intercept traps were found to be an ineffective means of sampling populations of Hymenoptera. However, there are no reports on the effectiveness of this method for the collection of *Cotesia* species.

We tested the influence of frass dissolved in the solution used in Moericke traps on the attraction of the parasitoids, but obtained negative results. During foraging, parasitoids utilize plant volatiles to locate their host habitat (Finidori-Logli et al., 1996). This technique was observed by Boethel and Eikenbary (1986). They commented that parasitoids are initially oriented in response to the stimuli provided by the plant and then in the second stage respond to the stimuli provided by the host. Similarly, the frass dissolved in the solution could serve as a stimulus for attracting the parasitoids to the traps, but the traps themselves were not effective in attracting these parasitoids.

In the experiment using sticky traps, it was observed that the insects were not attracted by the color of the traps. The observation that different numbers of insects were collected in the traps set at three different heights and that the traps placed at the ground level were more efficient evidently shows that *C. flavipes* individuals were collected by trapping and that they flew at a low height.

With the method using stems, with each stem containing a single larva, we observed 100% parasitism in “Experiment four” (laboratory test), regardless of the position of the stem in the cage or the addition of frass in the opening made for the insertion of the larva. Considering this result, we adopted the same method to test spatial distribution (Experiment five), using stems inserted upright in the ground, with the head of the larvae facing upward, and without the addition of frass in the opening made for the insertion of the larva.

The method of the use of larva with their head facing upward and no addition of frass was adopted to mimic the natural behavior of *D. saccharalis* larva and to prevent attraction by frass in the opening, the factor that can mask the results, interfering with the insect behavior.

Experiment 5

Aggregation indices and probabilistic models

Some parasitoids aggregate in patches with a high host density. Positive and negative relationships between parasitism ratios and host densities in different patches indicate direct and indirect relations of the patterns of parasitism with spatial density, respectively. When parasitoids and hosts are not spatially related, the parasitism pattern is independent of the spatial density

(Hassell, 2000). Parasitism pattern that is directly dependent on density is very important as a stabilizing factor for the host population, because it can reduce host population densities (Giles et al., 2000).

This differential spatial distribution of natural enemies and their hosts has been described by Kring and Gilstrap (1983). They demonstrated that the aphids *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (Linnaeus) show an aggregated distribution in wheat crops in the United States, whereas their parasitoids *Lysiphlebus testaceipes* (Cresson), *Diaeretiella rapae* (M'Intosh), and *Aphelinus nigritus* (Howard) disperse randomly in the crops. However, in the aforementioned study, the parasitoids were not released but were natural populations with low population densities, which explain the random distribution pattern.

In the field, different natural hosts are present per sample point. The method of artificial infestation of the host, as adopted in this study, eliminates the variation in the host density per sample point because of the uniform distribution of the hosts.

Spatial distribution of *D. saccharalis* is aggregated and tended to be weaker (more random) at densities near the threshold spray in Florida (Hall, 1986) and ramdomly in sugarcane in Loissiania (Schexnayder Jr. et al., 2001). In the present study, the parasitism of *C. flavipes* was aggregated, but its host *D. saccharalis* was uniformly distributed in the field. This indicates that *C. flavipes* parasitism does not depend on host density.

Thus, the method using artificial introduction of *D. saccharalis* allows studies on the dispersion of the adult parasitoid only, without any interference of the host, thereby eliminating the interference of this variable.

Geostatistical analyses

Since determining the density of released parasitoids, number of release points and distribution of the releases is a very difficult problem (van Lenteren and Tommasini, 2003). There is no information about distribution of *C. flavipes* to determine number of releases. In the present study, a distance of 25 m from the original release point represented the limit of successful *C. flavipes* parasitism (Figure 4A). This distance is smaller than that reported by Botelho et al. (1980), who concluded that *C. flavipes* has the potential to disperse approximately 34 m. Further, it has been reported that this parasitoid can disperse to a distance of 64 m to find a host (Sallam et al., 2001).

In our study, release points two and four were more efficient because they were protected by the sugarcane crop in all directions, which provided shelter to the parasitoids. Further, these points were located far from the road, which could be a means by which the parasitoids could leave the sugarcane crop (Figure 4A).

Considering density of infestation of *D. saccharalis* around 3,000 larvae per hectare, generally found in sugarcane fields in the same area, there would be approximately one larva every two linear meters. Based in this study, releasing *C. flavipes* using the method with four points per hectare and 6,000 parasitoids, the parasitism performance will reach less than one parasitized larva (Figure 4A) and in this way, the control of *D. saccharalis* will not be satisfactory.

The fact that the *C. flavipes* strains used by Botelho et al. (1980) dispersed 34 m from the release point indicates that in current times, after 30 years, *C. flavipes* may have lost the genetic characteristics associated with aggressiveness and dispersal potential. This finding warrants additional dispersal tests to identify a release method using which the parasitoids could cover the entire area of 1 ha.

Assefa et al. (2008) identified genetic differences between *C. flavipes* strains, when comparing one from Ethiopia with other strains from different parts of the world, including a Brazilian strain from Piracicaba, Sao Paulo, Brazil. They identified nine different haplotypes and concluded that the African strains are similar to those from Piracicaba and Jamaica (haplotype I). The haplotypes could differ because of reproductive isolation over several generations and could differ with respect to the host-searching capability, parasitism, and flight range, which are important characteristics of biological control agents. Thus, we believe that the genetic differences between wild populations and the *C. flavipes* strains actually used, due to reproduction isolation and genetic degeneration can be linked to the aggressiveness of the *C. flavipes* strains.

Comparison of the krigeage maps for the emerged males and females showed that the number of emerged males and females was similar, with a sex ratio of approximately 1:1. Studies conducted by Suzuki and Iwasa (1980), Werren (1980) and Godfray (1994) show that sex ratio is a valuable index when investigating the hypothesis of local competition for copulation. Parasitoid females deposit lesser eggs in already parasitized larva and that the sex ratio of the offspring tends to become skewed toward a higher proportion of males. This suggests that when a female finds a host that is not adequate for the development of its offspring, it tends to produce male progeny. As adults, these males would copulate with many females (given that they are polygamous), increasing the female progeny responsible for superparasitism in the next generation.

This information and the finding that the observed proportions of the males and females in the present study were similar suggest the absence of superparasitism, as also indicated by the sex ratio close to 1:1. The lack of superparasitism indicates that the *C. flavipes* populations that occur naturally in the field (that is, insects that are not present because of releases) are either small or non-existent.

It appears that the results of the present study were not influenced by the presence of naturally occurring

C. flavipes in the area. This is because the presence of these parasitoids would tend to have produced a sex ratio skewing toward more males than females and also because the observed parasitism was aggregated. We believe that if there was a natural population of *C. flavipes* in the field, parasitism would have been observed outside the aggregation area. Interestingly, a mean of one to six *C. flavipes* releases are performed in the same area every year, implying that *C. flavipes* does not persist in the ecosystem after release.

C. flavipes parasitism on *D. saccharalis* larvae showed an aggregated distribution in the sugarcane plot used in this study. The aggregation radius of *C. flavipes* was 25 m. The method of using the natural host of *C. flavipes* in sugarcane stems proved to be efficient for assessing parasitoid dispersion. It is suggested that geostatistical analyses, which allow the evaluation and mapping of the number of parasitized larvae and the numbers of male and female parasitoids that emerged in the next generation can be useful for any study aimed at determining an efficient method for *C. flavipes* release.

The methods of the use of Moericke traps of different colors, addition of frass, and use of adhesive traps were not effective for capturing the parasitoids and therefore not appropriate for studies about cover area of *C. flavipes*.

The method using stems is appropriate to be adopted for sugarcane crops, allowing measuring the parasitism and studies on cover area of the parasitoid, its qualitative characteristics, number of release points per unit area, and other parameters. On the other hand, *C. flavipes* did not show any direct density-dependent relationship with *D. saccharalis* densities (Rossi and Fowler, 2003b). Sugarcane plants can support different cuts, or be destined for irrigation or seedling production. The harvest adopted extends over approximately 5 months per year. In this way, plants would not be of the same age across the smaller spatial scales during the growing period and all these factors may lead to different levels of *D. saccharalis* infestation, either spatially or temporally (Mailafiya et al., 2010; van Lenteren et al., 2003). In this way, studies to find a release method under different field conditions may contribute to manage this pest.

REFERENCES

Alam M, Bennett F, Carl K (1971). Biological control of *Diatraea saccharalis* (F.) in Barbados by *Apanteles flavipes* Cam. and *Lixophaga diatraea*. Entomophaga 16:151-158.

Anscombe FJ (1949). The statistical analysis of insects counts based on the negative binomial distribution. Biometrics 5:165-173.

Assefa Y, Mitchell A, Conlong DE, Muirhead KA (2008). Establishment of *Cotesia flavipes* (Hymenoptera: Braconidae) in sugarcane fields of Ethiopia and origin of founding population. J. Econ. Entomol. 101:686-691.

Boethel DJ, Eikenbary RD (1986). Interactions of plant resistance and parasitoids and predators insects, Ellis Horwood, Chichester.

Boller EF, Chambers DL (1977). Quality aspects of mass reared insects. In Ridgway et al. (eds) Biological control by augmentation of natural enemies, Plenum Press, New York. pp. 219-235.

Botelho PSM (1992). Quinze anos de controle biológico da *Diatraea saccharalis* utilizando parasitoides. Pesq. Agrop. Bras. 27:255-262.

Botelho PSM, Macedo N, Mendes AC (1980). Aspects of the population dynamics of *Apanteles flavipes* (Cameron) and support capacity of its host *Diatraea saccharalis* (Fabr.). Proc 17th Congress of the International Society of Sugar Cane Technologists, Manila, Philippines, pp. 1736-1745.

Davis PM (1993). Statistics for describing populations. In: Pedigo L, Buntin GD, (eds) Handbook of sampling methods for arthropods in agriculture, CRC Press, Boca Raton, pp. 33-54.

Dinardo-Miranda LL, Fracasso JV, Perecin D (2011). Variabilidade espacial de populações de *Diatraea saccharalis* em canaviais e sugestão de métodos de amostragem. Bragantia 70:577-585.

Elliott JM (1979). Some methods for the statistical analysis of sample benthic invertebrates, Freshwater Biological Association, Ambleside.

Finidori-Logli V, Bagneres AG, Clement JL (1996). Role of plant volatiles in the search for a host by parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) J. Chem. Ecol. 22:541-558.

Fuchs T, Fuffman F, Smith J (1979). Introduction and establishment of *Apanteles flavipes* (Hymenoptera: Braconidae) on *Diatraea sacharalis* (Lepidoptera: Pyralidae) in Texas. Entomophaga 24:109-114.

Giles KL, Roye TA, Elliott NC, Kindler SD (2000). Development and validation of a binomial sequential sampling plan for the greengug (Homoptera: Aphididae) infesting winter wheat in the southern plains. J. Econ. Entomol. 93:1522-1530.

Godfray HCJ (1994). Parasitoids behavioral and evolutionary ecology. Princeton University Press, New Jersey.

Green RH (1966). Measurement of non-randomness in spatial distributions. Res. Pop. Ecol. 8:1-7.

Hall DG (1986). Sampling for the sugarcane borer (Lepidoptera: Pyralidae) in sugarcane. J. Econ. Entomol. 79:813-816.

Hassell MP (2000). The spatial and temporal dynamics of host-parasitoid interactions. Oxford University Press, Oxford.

Hoelmer KA, Roltsch WJ, Chu CC, Henneberry TJ (1998). Selectivity of whitefly traps in cotton for *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), a native parasitoid of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environ. Entomol. 27:1039-1044.

Kimani-Njogu SW, Overholt WA (1997). Biosystematics of the *C. flavipes* complex (Hym.: Braconidae), parasitoids of gramineous stemborers. Insect Sci. and Its Appl. 17:119-130.

Kring TJ, Gilstrap FE (1983). Within-field distribution of greenbug (Homoptera: Aphididae) and its parasitoids in Texas winter wheat. J. Econ. Entomol. 76:57-62.

Mailafiya DM, Ru BPL, Kairu EW, Calatayud PA, Dupas S (2010). Geographic distribution, host range and perennation of *Cotesia sesamiae* and *Cotesia flavipes* Cameron in cultivated and natural habitats in Kenya. Biol. Control. 54:1-8.

Morisita M (1959). Measuring of the dispersion of individuals and analysis of the distributional patterns. Mem. Fac. Sci. 2:215-235.

Morisita M (1962). Id-index, a measure of dispersion of individuals. Res. Pop. Ecol. 4:1-7.

Ngi-Song AJ, Overholt WA (1997). Host location and acceptance by *Cotesia flavipes* Cameron and *C. sesamiae* (Cameron) (Hymenoptera: Braconidae). Parasitoids of African gramineous stemborers: Role of frass and other host cues. Biol. Control 9:36-142.

Nordlund DA, Lewis WJ, Altieri MA (1988). Influence of plant produced allelochemicals on host-prey selection behavior of entomophagous insects. In: Barbosa P, Letourneau DK (eds) Novel Aspects of Insect-Plant Interaction, Wiley, New York, pp. 65-90.

Noyes JS (1989). A study of five methods of sampling Hymenoptera (Insecta) in a tropical rainforest, with special reference to the Parasitica. J. Nat. Hist. 23:285-298.

Ogunwolu EO, Reagan TE, Flynn JL, Hensley SD (1991). Effects of *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae) damage and stalk rot fungi on sugarcane yield in Louisiana. Crop Prot. 10:57-61.

Overholt WA, Ochieng JO, Lammers PM, Ogedah K (1994). Rearing and field release methods for *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borers. Insect Sci. Its Appl. 15:253-259.

Perry JN, Mead R (1979). On the power of the index of dispersion test

to detect spatial pattern. Biometrics 35:613-622.
Potting RPJ, Overholt WA, Danso FO, Takasu K (1997). Foraging behavior and life history of the stemborer parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae). J. Insect Behav. 10:13-29.
Posey FR, White WH, Reay-Jones FPF, Gravois K, Salassi ME, Leonard BR, Reagan TE (2001). Integrated pest management in sugarcane. LA Agriculture 44:16-18.
Rabinovich JE (1980). Introducción a la ecologia de poblaciones animales. Continental, Mexico.
Romoser WS (1981). The science of entomology. Mcmillan, New York.
Rossi MN, Fowler HG (2003a). Temporal patterns of parasitism in *Diatraea saccharalis* Fabr. (Lep., Crambidae) populations at different spatial scales in sugarcane fields in Brazil. J. Appl. Entomol. 127:501-508.
Rossi MN, Fowler HG (2003b). The sugarcane borer *Diatraea saccharalis* (Fabr.) (Lep., Crambidae) and its parasitoids: a synchrony approach to spatial and temporal dynamics. J. Appl. Entomol. 127:200-208.
Sallam MN, Overholt WA, Kairu E (2001). Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. Entomol. Exp. Appl. 98:211-217.
Schexnayder Jr. HP, Reagan TE, Ring DR (2001). Sampling for the sugarcane borer (Lepidoptera: Crambidae) on sugarcane in Louisiana. J. Econ. Entomol. 94:766-771.
Southwood TRE (1971). Ecological methods. Chapman and Hall, London, UK.
Suzuki Y, Iwasa YA (1980). Sex ratio theory of gregarious parasitoids. Res. Pop. Ecol. 22:366-382.
Udayagiri S, Mason CE, Pesek JD (1997). *Coleomegilla metadata*, *Coccinella septempunctata* (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Neuroptera: Chrysopidae), and *Macrocentrus grandii* (Hymenoptera: Braconidae) trapped on colored sticky traps in corn habitats. Environ. Entomol. 26:983-988.
Vacari AM, De Bortoli SA, Borba DF, Martins MIEG (2012). Quality of *Cotesia flavipes* (Hymenoptera: Braconidae) reared at different host densities and the estimated cost of its commercial production. Biol. Control 63:102-106.
van Leerdam MB, Smith Jr. JW, Fuchs TW (1985). Frass-mediated, host-finding behavior of *Cotesia flavipes*, a braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 78:647-650.
van Lenteren JC, Babendreier D, Bigler F, Burgio G, Hokkanen HMT, Kuske S, Loomans AJM, Menzler-Hokkanen I, van Rijn PCJ, Thomas MB, Tommasini MG (2003). Regulation of import and release of mass-produced natural enemies: a risk-assessment approach. In van Lenteren JC (ed) Quality control and production of biological control agents. Theory and testing procedures. CABI Publishing, Cambridge, pp. 191-204.
van Lenteren JC (1991). Quality control of natural enemies: Hope or illusion? In: Bigler F (ed) Quality control of mass reared arthropods. Proc 5th Workshop of the IOBC global working group OILB/IOBC, Wageningen, pp. 1-14.
van Lenteren JC (2009). Testes para o controle de qualidade de agentes de controle biológico comercializados. In Bueno VHP (ed) Controle Biológico de Pragas: produção massal e controle de qualidade. 2 ed. UFLA, Lavras, P. 430.
van Lenteren JC, Tommasini MG (2003). Mass production, storage, shipment and release of natural enemies. In Van Lenteren JC (ed) Quality control and production of biological control agents. Theory and testing procedures. CABI Publishing, Cambridge, pp. 181-189.
Werren JH (1980). Sex ratio adaptations to local mate competition in a parasitic wasp. Sci. 208:1157-1159.
White WH, Reagan TE, Smith Jr. JW and Salazar JA (2004). Refuge releases of *Cotesia flavipes* (Hymenoptera: Braconidae) into the Louisiana sugarcane ecosystem. Environ. Entomol. 33:627-632.
White WH, Wilson LT (2012). Feasibility of using an alternative larval host and host plants to establish *Cotesia flavipes* (Hymenoptera: Braconidae) in temperate Louisiana sugarcane ecosystem. Environ. Entomol. 41:275-281.
Wiedenmann RN, Smith Jr. JW (2006). Use of ablated stalks to assess field rates of parasitism of *Diatraea saccharalis* (Lepidoptera: Crambidae) by *Cotesia flavipes* (Hymenoptera: Braconidae). Biocontrol Sci. Techn. 16:37-48.