

# Dispersal of *Cotesia flavipes* in sugarcane field and implications for parasitoid releases

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## Abstract

*Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) is a major sugarcane pest in Brazil. The management of infested areas is based on the release of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), a parasitoid of *D. saccharalis* larvae, but there are doubts about the effectiveness of *C. flavipes*, primarily regarding its rate of dispersal in sugarcane fields. Thus, the objective of this study was to evaluate the dispersal of *C. flavipes* in a sugarcane field and suggest a release method that provides higher parasitoid efficiency. The study was carried out in four areas of approximately 1 ha, in which stalk pieces containing 20 *D. saccharalis* larvae were distributed in a rectangular grid, and 12,000 *C. flavipes* adults were released at four points, that were 50 m apart and 25 m from the field border. Three days later, the *D. saccharalis* larvae were recovered and kept in the laboratory until they reached pupal stage or *C. flavipes* emergence. Parasitism varied from 13.2% to 42.8%. The random distribution of parasitized larvae was found in one assay. In three areas, the parasitized larvae showed an aggregated distribution, with a range of 15 to 25 m. Since the parasite's success is directly linked to parasitoid dispersion, it would be interesting to move the release points to 30 m from each other because the dispersal may happen in a 15 m radius.

Key words: sugarcane borer, *Diatraea saccharalis*, biological control, aggregation, pest management.

## Dispersão de *Cotesia flavipes* em canavial e implicações para a liberação do parasitoide

### Resumo

*Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae) é uma das principais pragas da cana-de-açúcar no Brasil. O manejo de áreas infestadas é baseado na liberação de *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), um parasitoide de larvas de *D. saccharalis*, mas existem dúvidas a respeito da eficiência de *C. flavipes*, principalmente em relação à sua capacidade de dispersão em campo. Assim, o objetivo deste estudo foi avaliar a capacidade de dispersão de *C. flavipes* em canaviais e sugerir um método de liberação que propicie maior eficiência do parasitoide. O estudo foi conduzido em quatro áreas de aproximadamente 1 ha, e, em cada uma, pedaços de colmos contendo 20 larvas de *D. saccharalis* foram distribuídos em grid retangular e 12 mil adultos de *C. flavipes* foram liberados em quatro pontos, separados entre si por 50 m e a 25 m das bordaduras do campo. Três dias depois, as larvas de *D. saccharalis* foram removidas e mantidas em laboratório até se transformarem em pupas ou até a emergência de *C. flavipes*. O parasitismo variou de 13,2% a 42,8%. As larvas parasitadas se distribuíram ao acaso em uma área e em três áreas a distribuição foi agregada, com alcance de 15 a 25 m. Visto que o sucesso do parasitismo está diretamente ligado à dispersão do parasitoide, sugere-se que os pontos de liberação estejam distantes entre si 30 m, visto que o raio de dispersão pode ser de 15 m.

Palavras-chave: broca-da-cana, *Diatraea saccharalis*, controle biológico, agregação, manejo de pragas.

## 1. INTRODUCTION

The sugarcane borer, *Diatraea saccharalis* Fabr. (Lepidoptera: Crambidae), is one of the most important insect pests of sugarcane in Brazil due to the sharp reduction in sugarcane productivity and quality promoted by larvae attack (Botelho and Macedo, 2002; Dinardo-Miranda, 2008).

In Brazil, the management of infested areas is based on biological control, particularly those involving the larvae

parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), a gregarious endoparasitoid introduced from Trinidad, India and Pakistan at the beginning of the 1970s (Botelho and Macedo, 2002). Since then, *C. flavipes* has been reared in the laboratories of sugarcane mills and intensively released into the fields.

*C. flavipes* are released into infested fields throughout the year, although these releases are more frequent during

spring and summer when *D. saccharalis* populations increase. The released quantity of *C. flavipes* depends on the pest population, but is generally 6,000 adults (males and females) per hectare, one or more times per year (Botelho and Macedo, 2002; Dinardo-Miranda, 2008).

Botelho et al. (1980)<sup>1</sup> estimated that the dispersion of *C. flavipes* adults varies from 25 to 48 m (34 m on average), thus, these wasps are usually released at four points per hectare, approximately 50 m apart from each other (Almeida et al., 1997)<sup>2</sup>.

For many years, *C. flavipes* has been reported as an efficient biological control agent for *D. saccharalis* (Botelho and Macedo, 2002; Dinardo-Miranda, 2008). However, reports of areas with high borer populations have been frequent despite the continuous release of the parasitoid. One of the primary reasons for this growth in the sugarcane borer population is that newly released cultivars are more susceptible to the pest than are the older ones. However, there are doubts about the effectiveness of *C. flavipes* in relation to pest control, mainly regarding the rate of its dispersal in a sugarcane field.

Thus, the objectives of this study were to evaluate the dispersal of *C. flavipes* in sugarcane fields, by examining the distribution of parasitized hosts, and to suggest a release method that provides greater parasitoid efficiency.

## 2. MATERIAL AND METHODS

The study was carried out in Ribeirão Preto (SP), Brazil (21°12'56"S and 47°52'38"W, at an altitude of 630 m) in an area of approximately 50 ha of 7-month-old sugarcane field (RB857515 cultivar). During previous years, this area had been cultivated with pasture and *C. flavipes* had never been released. In this area, four fields of approximately 1 ha (94.5 × 100 m) each that were separated from each other by at least 200 meters were demarcated. An experiment was conducted in each delimited area (study field).

Stalks of the IACSP95-5094 sugarcane cultivar were cut into five internodal pieces. In each of the four superior internodes, a hole that was 0.5 cm in diameter was made using a drilling machine. A 14-day-old and 2-cm-long *D. saccharalis* larva from the laboratory rearing was put into each hole. Thus, each stalk contained four larvae. Because *C. flavipes* responds strongly to the odor of the frass generated by *D. saccharalis* larvae (Van Leerda et al.,

1986), the stalks remained in the laboratory for one day after their preparation to allow for larval establishment and frass production.

Each field of approximately 1 ha was divided into 100 plots measuring 10.5 × 10.0 m. A group of five stalks containing 20 larvae was placed in the center of every plot. The stalk pieces were placed together in the sugarcane row, standing between the crops stalks. Therefore, the *D. saccharalis* larvae were distributed in a rectangular grid measuring 10.5 × 10.0 m, except in field 2. In a portion of field 2 (10% of the field), the stalk pieces were distributed in a rectangular grid measuring 21.0 × 10.0 m and in a rectangular grid measuring 10.5 × 10.0 m in another portion of field 2. Thus, in fields 1, 3, and 4, 2,000 larvae were distributed, while in field 2, 1,800 larvae were distributed.

Experiments on fields 1, 2, 3, and 4 were initiated on January 13, 2011, February 2, 2011, February 15, 2011, and March 15, 2011, respectively.

Just after the stalks containing *D. saccharalis* larvae had been distributed in the experimental fields, 12,000 *C. flavipes* adults were released at four points (3,000 adults in each point) that were 50 m apart and 25 m from the field border. The *C. flavipes* were released at 10 a.m. on a cloudy morning (fields 1 and 3), at 10 a.m. on a sunny morning (field 2), and at 4 p.m. on a sunny afternoon (field 4).

The parasitoids were obtained from a large laboratory rearing in which the sugarcane borer was used as a host. When the parasitoids were in the pupal stage, they were transferred to plastic glasses at the rate of 1,500 individuals per glass. The glasses were capped and maintained in the laboratory at 25 °C for 1 or 2 days, until approximately 80% of adults had emerged (Cano et al., 2006). Since in commercial sugarcane fields in Brazil, *C. flavipes* are released into fields when approximately 80% of adults had emerged (Botelho and Macedo, 2002), the same criterion was used in these experiments.

The borers were exposed to the *C. flavipes* parasite for three days, after which all stalks were collected, identified and taken to the laboratory, where the stalks were carefully opened to remove the larvae. Each recovered larva was transferred to a plastic Petri dish containing an artificial diet described by King and Harley (1985).

The Petri dishes were kept in a climatized room (25 ± 1 °C, 70 ± 10% relative humidity, 12 h photophase) until the emergence of *C. flavipes* or the *D. saccharalis*. The larvae that were parasitized in each sampling grid point were counted. In fields 2, 3 and 4, *C. flavipes* males and females emerged from each parasitized borer were counted.

During each assay carrying out, some weather parameters (air temperature, air relative humidity, total solar radiation an rainfall volume) were recorded.

The data from the parasitized borers per point, in each field, were initially analyzed by descriptive statistics; the mean, standard deviation, variation coefficient, maximum

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and minimum values, skewness and kurtosis were calculated. To verify the hypothesis of the normality of the data, the Shapiro and Wilk (1965) test was carried out. The Morisita dispersal indexes and the variance/mean ratio were calculated, as described by Silveira Neto et al. (1976).

Subsequently, geostatistical analyses of the data were completed using a semivariogram and kriging interpolation to construct maps, as described by Vieira et al. (1983). The semivariogram analyses were conducted using the Geostat 1.0 software (Vieira et al., 1983) and they were fitted to the model which gave the best coefficient of determination ( $R^2$ ). 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 ( $C_0 + C_1$ ), 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. The  $C_1$  value represents the structured spatial variability of the data.

The ratio between nugget effect and sill ( $C_0/C_0+C_1$ ), expressed in percent, was used in order to classify the spatial dependence of the studied properties. According to Cambardella et al. (1994), the ( $C_0/C_0+C_1$ ) ratio can be used to classify the spatial dependence in strong (ratio < 25%), moderate (26% < ratio < 75%) and weak (ratio > 75%).

Based on the models fitted to the semivariograms, the jackknifing test was used to verify whether the estimates of the semivariogram parameters were adequate and to estimate the number of neighbors that should be used in the kriging interpolation (Vieira, 2000). Once the parameters for the model were validated and the adequate number of neighbors was determined, the values were interpolated by the kriging method for the locations that were not measured using Geostat (Vieira et al., 2002). The kriging-estimated values were used in the Surfer 7.0 software (Golden Software, 1999) to construct maps of the *D. saccharalis* parasitized larvae.

### 3. RESULTS AND DISCUSSION

Although 1,800 larvae (field 2) or 2,000 larvae (fields 1, 3, and 4) were distributed in each field, no more than 50% of these larvae were recovered (Table 1); the remaining

larvae died or were lost. Poor handling of the larvae may have resulted in the death of many of them. Several earwigs and ants were found in the holes in the stalks, suggesting that a portion of the borers placed in those holes had been predated. In sugarcane, the natural enemies, including earwigs and ants, play an important role in reducing the population of *D. saccharalis* by predated its eggs and larvae (Degaspari et al., 1987).

Based on the recovered larvae from the field, parasitism varied from 13.2% (field 2) to 42.8% (field 3) (Table 1). These values are similar to those observed by other researchers under Brazilian conditions. Almeida et al. (1997), who studied several sugarcane areas from 1981 to 1996, verified that the average parasitism of *D. saccharalis* by *C. flavipes* ranged from 7% in 1984 to 37% in 1996. Macedo and Araújo (2000) found parasitism by *C. flavipes* to be from 0 to 30% in a sugarcane field evaluated over two consecutive years. Botelho and Macedo (2002), upon analyzing data from the Barra sugarcane mill from 1975 to 2000, found that average parasitism was lower than 10% from 1975 to 1985, while from 1989 to 2000, it was between 40 and 60%.

According to Botelho and Macedo (2002), parasitism values smaller than 20% are considered low. Thus, in this work, parasitism was considered low in 2 of 4 assays, despite a twofold increase in the quantity of parasitoids released compared to that of commercial operations.

Typically, in commercial sugarcane fields, approximately 6,000 adults per ha of *C. flavipes* are released when the *D. saccharalis* population is at most 3,000 suitable larvae per ha (Almeida et al., 1997; Botelho and Macedo, 2002). In this study, twice the typical quantity of *C. flavipes* adults was released because the pest also attacked the field in which the assays took place. This way, the released wasps could also parasitize the borers in the sugarcane field. Additionally, a higher number of parasitoids was released in an attempt to induce the insect to disperse farther in search of non-parasitized hosts because, according to Campos-Farinha et al. (2000), *C. flavipes* females prefer to lay eggs in larvae that have not yet been parasitized.

Several factors must have interfered with parasitoid efficiency during the assays, including the climatic conditions at the moment of release. According to Pinto and Parra

**Table 1.** Experimental field descriptive parameters

Parameters	Field				
	1	2	3	4	
Larvae recovered in the area	999	803	794	947	
Larvae recovering (%)	50.0	44.6	39.7	47.4	
Parasitized larvae	165	106	340	358	
Parasitism (%)	16.5	13.2	42.8	37.8	
<i>Cotesia flavipes</i> emerged from each parasitized larvae	Total	*	44.7	42.5	33.9
	♀	*	31.9±2.1	32.1±1.2	26.6±0.9
	♂	*	12.8±1.5	10.4±0.6	7.3±0.4

\*without estimation.

(2002), temperature and humidity that diverge from those that are optimum to the parasitoid can reduce the lifespan of *C. flavipes* and, consequently, its parasitism capacity. Zhou and Overholt (2001) reported variations in *C. flavipes* establishment in different regions of Africa and attributed those variations to the climate differences between the areas, among other factors. Emaná et al. (2004) and Emaná (2007), working under controlled conditions with two populations of *C. flavipes*, one from India and another from Pakistan, found that, although there were differences between the populations, temperatures generally between 25 and 28 °C and a relative humidity from 60% to 70% resulted in high survival, greater female fecundity, and a greater number of descendants. Sétamou et al. (2002), studying the influence of temperature on *C. flavipes* behavior, concluded that better parasitoid performance under field conditions would be obtained when temperatures were between 26 and 30 °C. Thus, possibly the average temperature and humidity conditions during the four assays (Table 2) were suitable for *C. flavipes* parasitism. However, while assay 2 was being carried out, the maximum absolute temperature was higher than the temperatures observed during the other assay. These high temperatures may have contributed, at least in part, to the lower parasitism observed in this field.

In field 2, the higher maximum absolute temperature is directly related to higher solar radiation during the three days in which the *D. saccharalis* larvae were exposed to *C. flavipes* parasitism (Table 2). In this assay, the *C. flavipes* release was performed at 10:00 a.m. on a sunny day with clear skies; the sky had few clouds during the following days, while in the other assays, the releases were performed under cloudy skies (fields 1 and 2) or in the afternoon (field 4). In field 2, the total solar radiation was 17%, 17% and 43% higher than observed in fields 3, 4, and 1, respectively.

Despite the difference between the solar radiation values during the experiments, solar radiation may not be the direct cause of the low parasitism in field 2. Several studies indicate that high luminosity favors parasitoid dispersal and host localization, factors that would favor an increase in parasitism. For example, Elzen et al. (1987) worked with *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) and found that parasitoid fly frequency, in response to host stimuli (frass), was affected by light intensity or luminosity. Likewise, Gu and Dorn (2001), working under controlled conditions, found that the quantity of flights of *Cotesia*

*glomerata* (L.) to infested plants was greater when the light intensity was also greater, suggesting that parasitoid performance could be reduced on cloudy days. Thus, in the present work, the higher total radiation during field 2 most likely had a positive, direct effect on parasitism. However, it is important to consider that solar radiation may have indirectly affected parasitism by altering the temperature; higher solar radiation resulted in a higher temperature. In assay 2, it is likely that the indirect effect of solar radiation increasing the temperature and reducing parasitism was more pronounced than the direct effect.

In field 1, the low parasitism found is most likely related to precipitation because in the first two days after the release, precipitation was from 21.6 and 30.5 mm (totaling 52.1 mm). The rain may have hindered the spread of the parasitoid and, hence, reduced the parasitism. The effect of rain on parasitoid efficiency was evaluated by Weisser et al. (1997), who found that the number of aphid colonies visited by *Aphidius rosae* (Haliday) (Hymenoptera: Aphidiidae) decreased when the duration of the rain increased because long rainy periods would hinder or even decrease the demand by the host.

In field 4, although the rain volume was similar to that in assay 1, the weather was mild in the two days after the release (totaling 13 mm), with a higher volume (45.2 mm) only on the third day when most insects had most likely already dispersed and found a host.

An average of 33.9 (field 4) and 44.7 (field 2) *C. flavipes* adults emerged from every parasitized larvae, which is similar to the results of Potting et al. (1997), who found that the emergence of approximately 40 individuals was common. Campos-Farinha et al. (2000), Campos-Farinha and Chaud-Neto (2002) and Scaglia et al. (2005) also observed adults emerging from each parasitized larvae in similar numbers in laboratory studies.

Among the emerged adults, females were predominant (sexual ratio = 0.75). This female predominance among the *C. flavipes* descendants that emerged from *D. saccharalis* was also observed by several other researchers, such as Campos-Farinha and Chaud-Neto (2000), Sétamou et al. (2005) and Yamaushi et al. (1997). These results indicate that, even though twice the standard quantity of *C. flavipes* adults had been released, there was no superparasitism.

According to Suzuki and Iwasa (1980), when the host/parasitoid ratio is low, more than one female can lay eggs in the same host, characterizing superparasitism, which generally

**Table 2.** Weather parameters during each assay carrying out

Field	Air temperature (°C)			Air relative humidity (%)		Total solar radiation (MJ m <sup>-2</sup> )	Rain (mm)
	Maximum absolute	Minimum absolute	Average	at 7:00 a.m.	at 1:00 p.m.		
1	30.6	19.4	24.0	92.0	81.6	48.05	63.3
2	31.8	19.1	25.0	94.0	25.2	68.49	32.3
3	29.8	19.3	24.7	94.3	60.0	58.63	10.9
4	30.5	19.4	24.9	93.7	63.9	58.37	58.2

increases the proportion of males in the progeny. Campos-Farinha et al. (2000) also observed that in *D. saccharalis* that were parasitized more than once by *C. flavipes*, there was an increase in the male progeny.

In the four fields, the coefficients of variation were very high, with large differences between the maximum and minimum values of parasitized larvae at each point, indicating that there was variability in the parasitism ratio in the fields (Table 3).

The variance was greater than the mean in all fields (Table 3); thus, the variance/mean ratio was greater than 1, indicating an aggregated spatial distribution. However, it was observed in assay 1 that the variance/mean ratio value calculated (1.13) was less than that calculated in the other assays. According to Perecin and Barbosa (1994), variance/mean ratio values smaller than 1.20 indicate either less contagious or a random distribution. However, those same authors affirmed that when averages are too low, it is difficult to separate the distributions of contagious from random distributions. Thus, in field 1, the parasitized larva distribution was random. However, it is important to consider that the lack of detection of aggregation in this assay might have been due to low parasitism in the assay.

The random distribution of parasitized larvae in field 1 was confirmed by the Morisita index (Table 3): in this assay, the calculated variance/mean ratio value (1.07) was not significantly greater than 1, indicating a non-contagious distribution. In the remaining fields, the Morisita index greater than 1 indicates that the parasitized larvae were distributed in an aggregated manner in the fields.

Skewness values near 0 and kurtosis near 3 indicate a normal distribution of the data. Field 2 had a lower discrepancy in these values (Table 3). As expected for this type of enumeration, data normality was rejected by the Shapiro and Wilk (1965) test, but, according to Cressie (1991), it is not necessary that data present a normal distribution to use geostatistical analysis; it is merely convenient that the distribution curve does not indicate a long tail, which would endanger the results. Thus, considering the previous analysis of these data, the studied variable had an appropriate distribution for geostatistical analysis.

Among the four calculated semivariograms (one for each field), the semivariogram for field 1 did not fit to any model (pure nugget effect, Table 4), suggesting that there was no aggregation in this case. This result supports the variance/mean ratio and the Morisita index (Table 3).

In the other fields, the larvae parasitized by *C. flavipes* populations showed spatial dependence in all criteria: semivariogram, variance/mean ratio and Morisita index. The spherical model was best fitted to the semivariograms in fields 2 to 4 (Figure 1, Table 4). Although the observed  $r^2$  values were lower than 0.75, the parameters estimated for the spherical model ( $C_0$ ,  $C_1$ ,  $a$ ) were endorsed by the jackknifing test because the mean values for the reduced errors were near zero and the values for the variance of reduced errors were near 1 (Table 4). The jackknifing test also revealed the ideal number of neighbors to be used in kriging. As a result, kriging was performed using 16 neighbors in assays 2 and 4 but 8 neighbors in assay 3. The maps constructed based on the data are presented in the figure 2.

**Table 3.** Statistical parameters of data of *Diatraea saccharalis* larvae parasitized by *Cotesia flavipes*

Field	Mean (parasitized larvae per point)	Standart deviation	Variance	Coefficient of variation (%)	Minimum value	Maximum value
1	1.65	1.37	1.87	82.8	0	6
2	1.18	1.45	2.10	123.1	0	7
3	3.40	2.25	5.05	66.1	0	9
4	3.58	2.63	6.95	73.6	0	11
Field	Skewness	Kurtosis	Shapiro Wilk test	Variance/mean ratio	Morisita index	
1	0.73	-0.02	0.89**	1.13	1.07 <sup>NS</sup>	
2	1.72	3.42	0.78**	1.78	1.67**	
3	0.38	-0.56	0.94**	1.49	1.48**	
4	0.68	0.01	0.94**	1.94	1.94**	

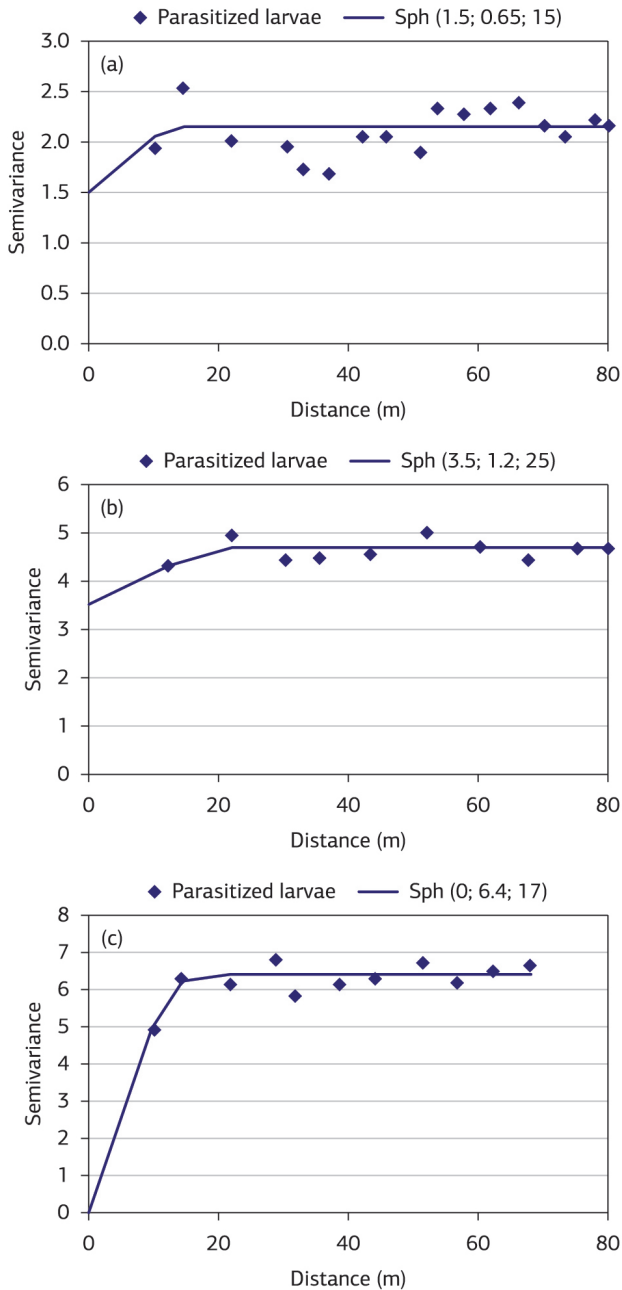
<sup>NS</sup> = non significant, \*\* = significant at 1%.

**Table 4.** Parameters of fitted semivariogram and jackknifing, coefficient of determination ( $R^2$ ) and  $C_0/(C_0+C_1)$  ratio of each field

Field	Semivariogram parameters			Jackknifing parameters (reduced errors)		$R^2$	$C_0 / (C_0 + C_1)$
	$C_0$	$C_1$	a (m)	mean	variance		
1				Pure nugget effect			
2	1.5	0.65	15	0.0099	0.9660	-0.007	0.70
3	3.5	1.2	25	0.0092	1.0012	0.14	0.74
4	0	6.4	17	0.0181	0.9461	0.67	0

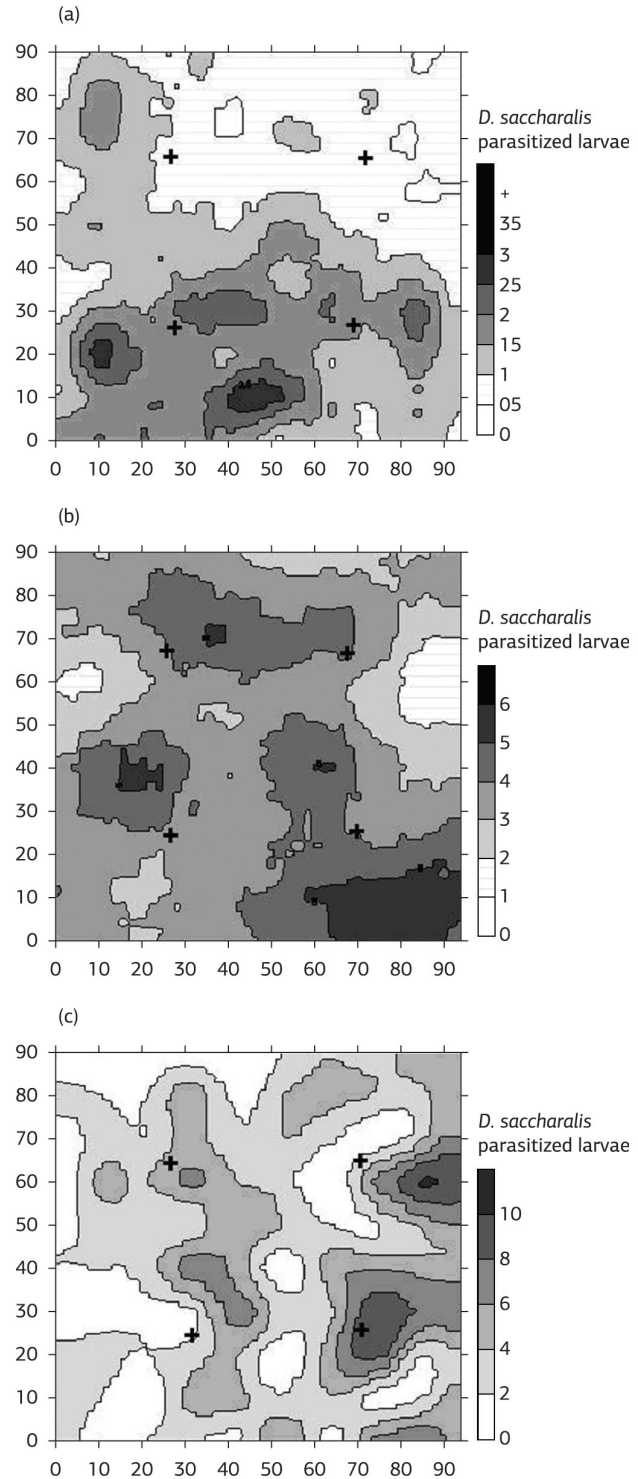
The portion of variability attributed to the spatial dependence, given by the  $C_0/(C_0+C_1)$  ratio, varied from 0 to 0.74 (Table 4). These values indicated a strong spatial dependence between the samples in assay 4 and moderate spatial dependence between fields 2 and 3 (Cambardella et al., 1994).

The range (a), which represents the distance up to which there is spatial dependence between samples, varied from 15 m (field 2) to 25 m (field 3, Table 4). Therefore, the parasitized larvae were concentrated in a 707 to 1,965 m<sup>2</sup> area. In this



**Figure 1.** Semivariograms for populations of *Diatraea saccharalis* parasitized larvae by *Cotesia flavipes* in field 2 (a), 3(b) and 4 (c). Numbers in parenthesis are nugget effect value ( $C_0$ ),  $C_1$  and range (a) of spherical model (Sph).

study, the range (a) represented the dispersal capability of most *C. flavipes* adults, although several specimens may have presented greater dispersal capabilities. This finding is consistent with the work of Sallam et al. (2001), who studied *C. flavipes* dispersal in maize fields in Kenya and



**Figure 2.** Kriging maps of spatial distribution of *Diatraea saccharalis* larvae parasitized by *Cotesia flavipes*, in fields 2 (a), 3 (b) and 4 (c). The (+) marks the releasing point.

found that even though the parasitized larvae were found up to 64 m away from the releasing point, most of them were within a distance of 15 m.

However, the dispersal capability of *C. flavipes* estimated in this work differs from the capability found by Botelho et al. (1980) in a work carried out in Brazilian sugarcane fields. These authors estimated the average flight of *C. flavipes* to be 34 m, with the most of them from 25 to 48 m, although in several cases, parasitized larvae have been found up to 140 m away from the release point.

Although weather conditions might interfere with insect dispersion, as discussed above, the reduction of insect flight capacity from 34 m (Botelho et al., 1980) to 15-25 m, as observed in the present work, is significant and could be attributed to genetic degeneration due to continuous rearing of the insect in the laboratory.

*C. flavipes* was introduced in Brazil in 1971 (Teran, 1975, cited by Botelho and Macedo, 2002). However, due to problems in rearing the species in the laboratory and low success observed in the field during that time, the studies were abandoned. In 1974, *C. flavipes* was reintroduced in the country (Mendonça Filho et al., 1977, cited by Botelho and Macedo, 2002), and since then, it has been intensively reared in the laboratory to be released into infested sugarcane fields. Thus, mating between relatives has occurred, and it is likely that the populations have lost great a deal of their original genetic variability due the high inbreeding levels, resulting in a reduced flight ability. This hypothesis is confirmed by Gu and Dorn (2000), who worked with several *C. glomerata* populations in the field and the laboratory and found differences among them regarding flight capacity and the ability of females to find hosts. The authors also found that these behavioral characters were inherited. Thus, reproductive isolation during several generations of the Brazilian *C. flavipes* populations may have resulted in a reduction in flight and the capacity to search for hosts, impairing parasitoid efficiency in the field.

The Kriging maps showing the spatial distribution (Figure 2) confirm that the regions of higher parasitized larvae are near the *C. flavipes* release points. Despite releasing 12,000 parasitoids, and not the typical 6,000, several fields presented low parasitism, such as in the upper quadrant on the right side of assay 2. Because parasitism success is directly linked to the parasitoid dispersion and its ability to find a host, one of the tools to increase parasitism is to improve parasitoid distribution in the field.

Botelho et al. (1980) estimated that the dispersion of *C. flavipes* adults varies from 25 to 48 m, and these wasps are frequently released at four points per hectare, approximately 50 m from each other (Almeida et al., 1997; Botelho and Macedo, 2002). However, the current study revealed that *C. flavipes* has a smaller dispersal capacity, even when released in a higher quantity than recommended. Possibly, it would be interesting to move the release points to 30 m from

each other because according to our data the dispersal may happen in a 15 m ray. Thus, parasitoids would be released in approximately nine points per hectare.

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