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Can Bt maize change the spatial distribution of predator *Cycloneda sanguinea* (L.) (Coleoptera: Coccinelidae)?

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Cultivation of Bt crops is an important tactic in integrated pest management. The effect of Bt maize on arthropod predators needs to be investigated because of the important role of these natural enemies in the absence of target pests. The objective of the present study was to generate information on the distribution model of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinelidae) in Bt and non-Bt maize. A sampling field of 2.500 m^2 area, divided into 100 plots, was used in this study. Five plants per plot, totaling 500 plants in each field, were studied. We counted the total number of adults every week, totaling six samples for each field (Bt and non-Bt). The aggregation index (variance/mean ratio, Morisita index, and exponent k of the negative binomial distribution) and Chi-square fit of the observed and expected values to the theoretical frequency distribution (Poisson, binomial, and negative binomial positive) revealed that, in both cultivars, the adults of *C. sanguinea* were distributed according to the random distribution model, which fits the pattern of Poisson distribution.

Key words: Spatial distribution, natural enemy, lady beetle, Poisson.

INTRODUCTION

Since its commercial release in 2008, the use of Bt crops containing *Bacillus thuringiensis* (Berliner) genes for insect resistance is being increasingly adopted by Brazilian farmers (James, 2011), especially as it decreases the need of insecticide application for targeted pest such as *Spodoptera frugiperda* (Brookes and Barfoot, 2008; Mendes et al., 2011). However, the widespread use of this technology may have unknown effects on the non-targeted species as well (Dutton et al., 2002; Sanvido et al., 2009).

Bt crops may introduce changes that can directly influence beneficial insects which come in direct contact of toxins in prey and hosts, called as tri-trophic interactions (Lumbierres et al., 2011; Dutra et al., 2012; Mota et al., 2012). In addition, Bt crops may have indirect effects because of possible changes in the behavioral aspects of natural enemies of pests in insufficient target technology (Zwahlen et al., 2000; Stephens et al., 2012). For example, coccinellid species are important polyphagous predators within agroecosystems, as *Cycloneda sanguinea* (L.) is a voracious predator of pests such as aphids, mealybugs, and eggs of Lepidoptera (Bruck and Lewis, 1998; Smith et al., 2004). These natural enemies are reported to be affected by the toxins of Bt cotton, for example, Funichello et al. (2012) reported changes in the biological parameters of aphids when fed from Bt cotton.

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The spatial dispersion of a population in a given ecosystem or agroecosystem basically corresponds to three models: Aggregate (or contagious), random (or by-chance), or uniform (or regular) (Young and Young, 1998). To determine the spatial arrangement pattern of a given species, it is necessary to obtain numerical data of individuals in the ecosystem to be studied, and the ecosystem in question must allow sampling (Fernandes et al., 2003). These samples may be used to infer the sample distribution pattern or the characteristics of distribution (Young and Young, 1998). Indices of aggregation and frequency distributions are utilized for description of the distribution patterns of a population.

Studies on the frequency distributions of different insect species in different crops are important to understand the real spatial distribution of these individuals by adopting appropriate sampling criteria to estimate population parameters (Barbosa et al., 1992). According to the study of Kuno (1991), the area under the study must first be divided into several units or grids of same size and then the occupation model of the area must be described by individuals in the population as a frequency distribution of the individuals observed in each grid.

As the spatial distribution of *C. sanguinea* related to a targeted field population (Hagen, 1962), we tested the hypothesis that lack of *S. frugiperda* in maize Bt would influence the spatial distribution of adult *C. sanguinea* as compared with conventional maize populations infested with *S. frugiperda*.

Material and Methods

The experiment was conducted during the autumn-winter season of 2012 as a part of Fazenda Experimental by Universidade Federal da Grande Dourados (UFGD) in Dourados, Mato Grosso do Sul, Brazil. Fields were planted with Bt transgenic corn (event MON89034) expressing the toxins Cry1A.105 and Cry2Ab2 and with non-Bt commercial field corn. Each field was divided into 100 plots of 25 m^2 (5 × 5 m) area, and, five plants were tested in each plot, totaling 500 plants per field. We counted the total number of *C. sanguinea* adults every week to total six samples for each field (Bt or non-Bt).

Statistical analyses to determine the spatial distribution pattern of the insect population considered the means and variances of the number of individuals found in each plot from the two working areas, and the following dispersion indices were used for the same:

Variance/Mean Ratio Index (Southwood 1966)

This ratio (I) is an index that measures the deviation of a random data arrangement. $I = 1$ indicates random or by chance spatial arrangement, $I < 1$ indicates regular or uniform spatial arrangement, and $I > 1$ indicates aggregated or contagious distribution (Rabinovich, 1980). According to (Southwood, 1966), the limitation of this index is in the influence of the size of the sampling unit on the number of individuals observed, which is significantly affected by the provisions of infection.

Morisita index

The Morisita index ($I\delta$) is relatively independent of the mean and number of samples. $I\delta = 1$ indicates a random distribution, $I\delta > 1$ indicates a contagious distribution, and $I\delta < 1$ indicates a regular distribution.

The limitation of this index is that it is significantly influenced by the sample size (N), as, for safe use, the number of sampling units must be the same for all fields being compared.

Exponent k of the Negative Binomial Distribution

The exponent k is a suitable dispersion index when the size and numbers of sample units are the same in each sample; this is often influenced by the size of the sampling units. This parameter is an inverse measure of the degree of aggregation. In this case, negative values indicate a normal or uniform distribution, positive values near zero indicate an aggregated arrangement, and higher values up to eight indicate a random distribution (Southwood,1966; Elliot, 1977). Considering this aspect, (Poole, 1974) utilized a different interpretation, where $0 < k < 8$ indicates an aggregated distribution, and $0 < k > 8$ indicates a random distribution.

The theoretical frequency distributions used to assess the spatial distribution of the observed species are as follows (Young and Young, 1998):

Poisson distribution

Also known as random distribution, it is characterized by presenting variance equal to the mean.

Negative binomial distribution

It shows greater variance than the mean, thereby indicating an aggregated distribution, and it has two parameters: the mean (m) and the parameter k (k > 0).

Chi-square test

To verify the fit test of the collected data to theoretical distributions of frequency, the Chi-square test for adherence was used for comparing the total number of frequencies observed in the sample area with the expected frequencies (Young and Young, 1998), where these frequencies were defined by the product of the probabilities for each class and the total number of sampling units used. In the present study, Chi-square test was used to fix the minimum expected frequency equal to one.

RESULTS AND DISCUSSION

Dispersion indices

The Chi-square test on the data related to mean-variance index indices and Morisita gave values equal to one in three samples collected from Bt corn, with the three remaining values much greater than one. In conventional farming, only the fourth sample showed a value of Chi-square, indicating that the calculated indices were much greater than one and the remaining equaled one. Parameter values set K aggregation in four samples for both the cultivars, as it was the sixth sampling adjusting uniformity (Table 1).

Table 1. Statistical analysis (sample mean and variance) and dispersion indexes for *Cycloneda sanguinea* (Coleoptera: Coccinelidae) on Bt and non-Bt maize, Dourados, MS, 2012.

Cultivar	Index	Sampling Period (D.A.E)					
		7	14	21	28	35	42
Bt	$\hat{m}$	0.31	0.53	0.49	0.63	0.72	0.43
	S^2	0.418	0.615	0.515	0.842	0.729	0.672
	I	1.349	1.161	1.051	1.336	1.012	1.562
	$I\delta$	2.151	1.306	1.105	1.536	1.017	2.326
	K	0.889^{ag}	3.295^{ag}	9.585^{al}	1.876^{ag}	58.32^{al}	0.765^{ag}
	X^2	133.516*	114.924^{ns}	104.061^{ns}	132.238*	100.222^{ns}	154.674*
Nbt	$\hat{m}$	0.39	0.62	0.8	1.12	0.6	0.27
	S^2	0.48	0.62	0.93	1.48	0.67	0.26
	I	1.238	1.003	1.162	1.322	1.111	0.962
	$I\delta$	1.619	1.005	1.203	1.287	1.186	0.855
	K	1.64^{ag}	211.42^{al}	4.95^{ag}	3.481^{ag}	5.4^{ag}	-7.076^{un}
	X^2	122.538^{ns}	99.290^{ns}	115^{ns}	130.857*	110^{ns}	95.22^{ns}

$\hat{m}$ = sample mean of adults per sampling unit; S^2 = variance; I= variance/mean relationship; $I\delta$ = Morisita index; K = K exponent; X^2*= significant at 5% probability and ^{ns} non-significative in chi-square test; **ag** = aggregated; **al** = random; **un** = uniform; **D.A.E** = days after plant emergence.

The dispersion indexes indicated random distribution of adult *C. sanguinea* occurring in non-Bt corn. However, these indices were inconclusive when observing the behavior of adults in Bt corn, because the Chi-square values calculated both the aggregate distributions as random (Table 1). Thus, it was necessary to calculate the indices of frequency, which is standard in studies of spatial distribution of arthropods (Maruyama et al., 2002; Fernandes et al., 2003; Martins et al., 2010; Rodrigues et al., 2010).

Theoretical frequency distributions

Tests frequency calculated from the data of class number of individuals in the field did not indicate a good fit to the negative binomial distribution of spatial arrangement of individuals in the population of *C. sanguinea* in both the cultivars of maize (Bt and non-Bt) (Table 2). After adjusting to the Poisson distribution, four samplings were well adjusted to the test in Bt maize, whereas all conventional maize samples had a good fit to this frequency distribution.

From the results obtained by fitting the data to the field frequency indexes, adult *C. sanguinea* was found to present random distribution. Furthermore, the absence of larvae of *S. frugiperda* in cultivating Bt maize did not contribute to the spatial distribution of the predator in both the cultivars with similar results. Therefore, we assumed that indirect factors related to the absence of prey in Bt corn would not influence the spatial distribution of *C. sanguinea*. For example, Funichello et al. (2012) observation that the biological characteristics of *C. sanguinea* are negatively influenced when these predators are fed prey from Bt crops do not interfere in the behavioral aspects of the population, at least with regard to their spatial distribution in cotton.

Guerreiro et al. (2005) reported that spatial distribution of adults and nymphs of *Doru luteipes* (Scudder, 1876) (Dermaptera: Forficulidae) occurs in the aggregate and is dependent on the spatial distribution of prey *S. frugiperda*. However, they found that the hypothesis changed these results in the absence of a preferred prey, as in the present study. Other factors that influences the behavior of *C. sanguinea*, for example, the local landscape and liking for preys such as aphids and scale insects (Elliott et al., 1999; Garcia et al., 2004), and it not being considered as a target of Bt maize (Lumbierres et al., 2004) in both the cultivars used in this study may be related to the distribution of horizontal type randomly.

As the spatial distribution of both the genotypes of *C. sanguinea* adult baker is random, the absence of prey *S. frugiperda* did not influence the result. Thus, the assumption that the spatial distribution of predators is related to the distribution of prey was not confirmed to *C. sanguinea* in maize.

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Table 2. Chi-square test to *Cycloneda sanguinea* (Coleoptera: Coccinelidae) (Poisson and Negative Binomial) on Bt and non-Bt maize cultivars in Dourados, MS, 2012.

Cultivar	Sampling Period (D.A.E)	Poisson		Negative Binomial	
		X^2	DF(nc-2)	X^2	DF(nc-3)
	7	6.076*	1	2.589^{ns}	1
	14	0.707^{ns}	2	1.136^{ns}	1
	21	7.461*	2	7.068**	1
Bt	28	1.630^{ns}	2	0.374^{ns}	2
	35	3.840^{ns}	2	3.981*	1
	42	2.325^{ns}	1	6.979*	1
	7	1.448^{ns}	1	2.187^{ns}	1
	14	1.154^{ns}	2	1.1479^{ns}	1
	21	1.189^{ns}	2	2.731^{ns}	3
Nbt	28	3.734^{ns}	2	8.41*	3
	35	1.402^{ns}	2	2.774^{ns}	1
	42	0.010^{ns}	1	0.027^{i}	-

^{ns} – non-significative at 5% probability, * - significative at 5% probability, ** - significative at 1% probability, ^{i} = insufficient of classes, X^2 – value of the calculated chi-square, DF – degree of freedom, nc – number of classes observed at field; **D.A.E** = days after plant emergence.

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