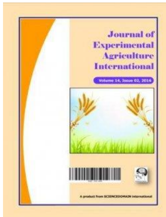

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Impact of Pesticides Application on Epigeic Fauna in Tomato Cultivation

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Authors' contributions

This work was carried out in collaboration between all authors. Author APAP did the data acquisition, data analysis, writing and editing. Author MRGZ did the data analysis (statistic support), writing results and discussion. Author PAMA was involved in data analysis (statistic support), writing, editing and English language revision. Author AJS performed the writing, edition (critical review), graphs and references standardization. Author CAB did the coordination, design of methodology, conceptualization, writing, critical review and conclusions. All authors read and approved the final manuscript.

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ABSTRACT

Our comprehension on the effects of the pesticide on soil epigeic invertebrates, especially non-target organisms in tomato cultivation is still incipient. We aimed to study the epigeic fauna from spots with and without insecticide application in Brejão municipally, Pernambuco, Brazil. The experiment was composed of three treatments: Two tomato crop production (two tomato varieties SUPERA and TY10) under high insecticide application; and a native fragment in Atlantic Forest without insecticide application. Epigeic fauna was evaluated using pitfall traps, sampled in eleven periods. They were identified at the level of order and, when possible, family. We used univariate

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statistic to find the difference between treatments, and multivariate statistic to verify the dissimilarity between treatments. We sampled 2571 invertebrates, distributed within 7 orders. Even among those, Coleoptera was more frequently sampled. The orders that mostly discriminated the areas were Coleoptera and Diptera. Within the order Coleoptera, the families Lycidae, Nitidulidae, Tenebrionidae and Cantharidae had greater contribution to the areas separation. The pesticides application had a strong effect on non-target organisms, reducing the Coleoptera family's richness when compared with the no-pesticides area (Treatment 3).

Keywords: Coleoptera richness; Pitfall traps; entomo-fauna; insecticides; insects.

1. INTRODUCTION

Brazilian agribusiness has become very attractive to tomato cultivation (*Lycopersicon esculentum*). This activity plays an economic and social importance for several regions of the country [1,2]. Regarding the world's largest producers, Brazil ranks as the eighth position, with approximately 63 thousand cultivated hectares and production reaching 3.5 million tons per year^{-1} [3]. However, in Brazil, tomato cultivation has undergone a production loss due to the high incidence of pests. In this case, the small tomato borer (STB) - *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) represent the most incident key-pest, increasing production costs [4,5].

The use of synthetic pesticides in pest control is widely recognized as the most effective way, mainly in the STB control [4,6]. On the other hand, it is known that the indiscriminate use of pesticides can lead to several environmental problems with the potential to reach and contaminate the soil, water, atmosphere and non-target organisms, including humans [7-9].

The epigeic fauna comprises of mostly arthropods, living on the soil's surface, that harbors important ecosystem functions such as the predation and the substrates degradation into de organic matter decomposition process [10]. In this context, the management practices for crop production has affect the diversity and composition of the invertebrates [11].

Many studies have shown only the effect of soil management (i.e., conventional or no-tillage) on the composition of the epigeic fauna, with limited information on the effects of pesticides to these organisms [12]. In crops such as maize, the negative influence of pesticide applications was observed, mainly in the reduction of the population of insects considered as natural enemies [7]. Thus, our objective was to study variation on the epigeic fauna community in sites with and without insecticide application in Brejão municipally, Pernambuco, Brazil.

2. MATERIALS AND METHODS

2.1 Experimental Site and Treatments

This study was carried out in Brejão (2010) (09º01'S, 36º34'W), a municipality located in the Southern Agreste of Pernambuco, Brazil. The region has a tropical rainy climate with dry summer and is (As') based in Köppen-Geiger climatic classification. The soil was classified as Quartzeneic Neosol (Brazil soil classification system), typically dystrophic, with pH = 4.8 and with low cation exchange capacity (<10 $mmol_c$ dm^{-3}) [13].

Briefly, we evaluated three areas, two planted with two commercial tomato varieties (SUPERA – Treatment 1; and TY 10 – Treatment 2) under insecticide application and an adjacent area without insecticide application characterized as Native Atlantic Forest (Native Forest - Treatment 3). This dense forest fragment was unchanged by anthropogenic activity and had predominantly: *Dialium guianensis*, *Parkia pendula*, *Pradosia lactescent*, *Cordia sellowiana*, *Himatanthus phagedenic*, *Xylopia frutescens* and other tree species. Due to the water restriction of the region, the tomato plants received water via a drip irrigation system.

2.2 Pesticides Application

Pest control was carried out every day, from the beginning to the end of the tomato cultivation cycle. The pesticides applications were carried out manually, with the aid of a costal sprayer, without the previous monitoring of the pest density population, being carried out even when the level of action to control the potential pests was not reached. Table 1 describes the pesticides used during the experiment time.

Table 1. Pesticides used in pest control in tomato cultivation during the experiment

Commercial name	Active ingredient	Chemical group	Use	Toxicological class
Actara	Thiamethoxam	Neonicotinoid	Insecticide	Moderately toxic
Amistar	Azoxystrobin	Strobilurins	Fungicide	Low toxicity
Bravonil	Tetrachloroisophthalonitrile	Isophthalonitriles	Fungicide	Extremely toxic
Orthene	Acefato	Organophosphorus	Insecticide	Low toxicity
Kasumin	Casugamicina	Antibiotic	Fungicide	Moderately toxic
Lannate	Methomyl	Carbamate	Insecticide	Extremely toxic
Lorsban	Cloropirifós	Organophosphorus	Insecticide	Moderately toxic
Polytrin	Profenós	Pyrethroid	Insecticide	Moderately toxic
Pirate	Clorfenapir	Pirazol	Insecticide	Moderately toxic

2.3 Epigeic Community Sampling

For the evaluation of the epigeic fauna, pitfall traps were installed in each treatment, covering all experimental areas (4 traps per treatment), which remained in the field during the interval between one collection and another. Each treatment had 4 replicates. The samples were obtained in a temporal scale 8, 12, 19, 27, 33, 40, 48, 54, 61, 69 and 75 days after seedlings transplantation. The samples were collected and stored in 90% ethanol solution, and then the total fauna was counted regarding the order level classification. The beetles were separated at the family level. The precipitation, temperature and air humidity were variable along the sampling period. The peaks of precipitation occurred in the fifth and eighth sampling (Fig. 1).

2.4 Statistical Analyses

Kolmogorov-Smirnov normality test was performed to test the normality of the data. The analysis of variance (ANOVA) was carried out, followed by Tukey's test ($p < 0.05$) for invertebrate abundance according to the orders found and later for collected Coleoptera families, in order to find differences between the treatments and sampling times. The composition of epigeic fauna was submitted to Discriminant Canonical Analysis (DCA) to identify which orders of invertebrates and/or Coleoptera families were more responsive to dissimilarities between studied areas [14]. Also, the epigeic mesofauna community diversity was determined using the Simpson Index (Is = 1-D), where D is the dominance expressed by Σpi^2,

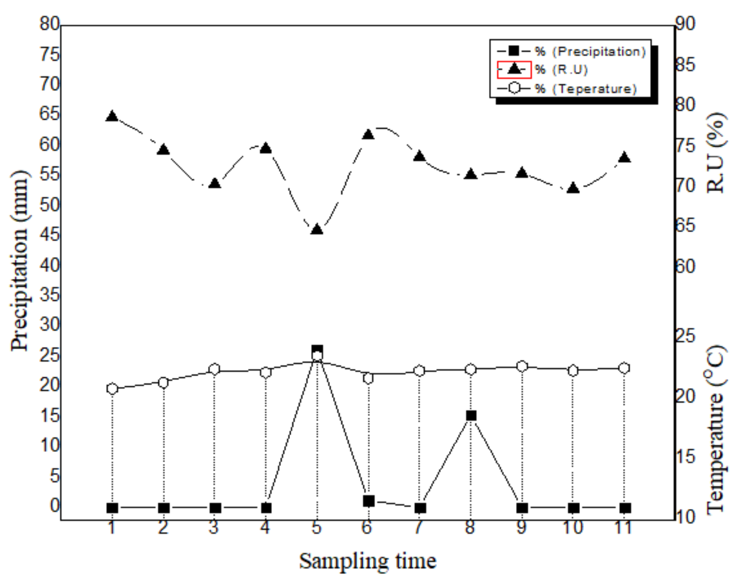

Fig. 1. Climatic data on the intervals of a collection of soil fauna. Values of temperature (ºC) and relative humidity (%) coincide with the day of collection. Precipitation values (mm) refer to the cumulative volume of rainfall between each collection

pi represents the relative abundance of each taxonomic group sampled. The Simpson index vary between from 0 to 1 and high values means that the system harbors a high diversity of organisms, in contrast to the low dominance of groups [15]. Richness was determined by a mean number of invertebrate orders and Coleoptera families sampled in each plot along the sampling period.

3. RESULTS AND DISCUSSION

We sampled 2571 epigeic invertebrates, distributed in 7 orders. Among these, Coleoptera was found more frequently in all treatments (Table 2).

There was a higher total abundance of invertebrates specially regarding the abundance of Coleoptera in TY10 compared to the other treatments. On the other hand, the Diptera was more abundant in treatment 1 than in the other treatments (Table 2). During the tomato maturation periods, there is a large increase in the concentrations of various fatty acids, carotenoids and amino acids, which are precursors of aldehydes, ketones, alcohols and volatile esters responsible resulting in fruit aromas [16]. It might have contributed to the greater total abundance of invertebrates in tomato crops since these organic precursors seems to be palatable attractive to the soil fauna [17]. In addition, the greatest abundance of invertebrates in the area with tomato cultivation may be associated with the addition of poultry litter (8-10 t ha^{-1}), increasing the presence of easily degradable C source.

The occurrence of large amounts of rainfall dilutes the volatile compounds [18], which does not occur in the present study, in which rainfall was scarce (Fig. 1). Thus, there may have been a higher concentration of these compounds, which may have attracted large numbers of invertebrates in tomato crops, even though pesticides may be highly applied in these areas.

In general, there was a greater abundance of invertebrates in the first collections, reaching an average count of 34 and 42 individuals in the fourth collection for TY10 and SUPERA tomato varieties, respectively. From the sixth collection, there was a marked reduction in the abundance of invertebrates (Fig. 2A and B). Possibly, this happened due to the intensification of the control practices of the STB, since this collection period coincided with the phase of emission of the first floral buds of the tomato, constituting a critical point for an infestation of the pest.

The Coleoptera order had an increase in the count of individuals, with average values varying from 7 to 11 individuals in the TY10 variety between the fourth and seventh collection, while the number of beetles in the SUPERA variety was reduced from 7 to 5 individuals in the same collection interval (Fig. 2A and B). After the seventh collection, there was a gradual decrease in the abundance of this order. In the native forest area adjacent to the crops, there was lower abundance for both total invertebrates and members of the order Coleoptera (Fig. 2C). In the total invertebrate count, the native forest area presented a smaller number of individuals than the tomato cultivation sites (Treatments 1 and 2) (Table 2, Fig. 2C).

Table 2. Average abundance and diversity of epigeic fauna orders collected in areas with pesticide application in the tomato crop (SUPERA and TY10) and in areas without pesticides application (Native Forest)

Order	Abundance and Diversity (Mean ± Standard Deviation)		
	Treatment 1 (SUPERA)	**Treatment 2 (TY10)**	**Treatment 3 (Native Forest)**
Coleoptera	103.8 ± 32.4	153.5 ± 12.5 *	103.5 ± 22.9
Lepidoptera	1.5 ± 3.0	3.5 ± 2.5	1.0 ± 1.2
Heteroptera	3.3 ± 5.3	3.8 ± 4.5	2.5 ± 2.5
Hymenoptera	21.8 ± 2.2	29.8 ± 4.3	31 ± 7.9
Acarina	21.8 ± 9.9	35.8 ± 17.3	26.8 ± 19.9
Diptera	16.8 ± 5.5 *	12.0 ± 3.7	4.3 ± 2.8
Orthoptera	20.0 ± 7.1	14.8 ± 5.1	32.0 ± 16.7
Total	188.8 ± 24.4	253.0 ± 30.5 *	201.0 ± 63.4
Richness	5.8 ± 0.9	6.5 ± 0.6	6.0 ± 0.8
Simpson	0.65 ± 0.09	0.58 ± 0.07	0.65 ± 0.05

** Averages with an asterisk differ from one another by Tukey's test ($p<0.05$)*

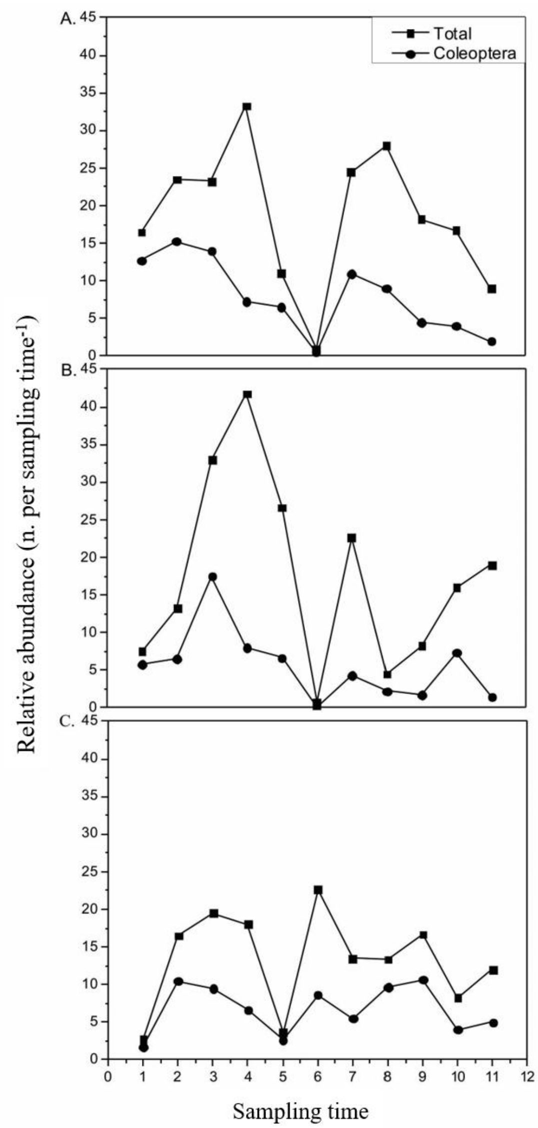

Fig. 2. Influence of pesticides and seasonality on the non-target epigeal fauna of the applications. Figure A indicates the variety TY10; Figure B, indicates the variety SUPERA, while Figure C, refers to the Native Forest area

The impact of pesticides on non-target epigeal fauna, especially from repeated applications, has been of great concern [7], considering the valuable role that these organisms play in the soil, acting in the structuring, soil aggregation and aeration, nutrient cycling, organic matter mineralization and pest control and disease suppression [8,19]. Effects on these organisms may result from the direct toxicity of the pesticide or the effect caused by the removal and/or increase in the abundance of certain species [20].

In a recent study, Nare et al. [21] observed a strong effect of the lambda-cyhalothrin and chlorpyrifos insecticides used to control tomato pests on soil fauna. In this study, the authors reported a greater effect of pesticides on soil macrofauna members (worms, termites, beetles, ants, bedbugs, spiders, and spiders). In addition, Nare et al. [21] showed in the treatments of organic fertilization, such as (bovine manure, swine manure and a compound of vegetal residues + bovine manure) there was an increase in the abundance of earthworms and termites, even in the presence of pesticides [21].

The lowest total abundance of invertebrate, including the low abundance of Coleoptera observed in the native forest, might be related to the low volume of precipitation between collection intervals (Fig. 1), and the occurrence of precipitation in the fifth, sixth and eighth collections, with volumes of 26.20, 1.20 and 15.60 mm accumulated in the respective intervals. In addition, the higher counts of invertebrates in tomato crops might be related to the crop irrigation management, since the soil invertebrates, especially Coleoptera are favored by soil water content [22]. In spite of the effect exerted by the insecticides used in the chemical control of BPT, the greater abundance of invertebrates, especially the order Coleoptera in the crops, can indicate the avoidance of invertebrate fauna from the forest to the crops, especially in dry periods, as well characterized throughout the experiment.

Discriminant canonical analysis (ACD) indicated a separation between treatments, mainly in the area without pesticide application (native forest) (Fig. 3).

The orders of epigeic fauna that contributed to the treatments discrimination in ACD analysis were: Coleoptera (p <0.02) and Diptera (p <0.04). Among the insects that inhabit the soil and the litter, the order Coleoptera is the most diverse, with 350,000 to 370,000 species described [23]. These invertebrates present the most diverse feeding habits, such as rhizophagia, detritivorous, fungivory and predation, which allows them to develop in the most varied environments [23], so much that in the present study, (Table 2), possibly attracted by volatile compounds exhaled by tomato plants [16].

Likely, the great dispersal power contributed to the Diptera order discriminating areas of study (Fig. 3). Hardly any winged Diptera collected in pitfall traps, especially those of the Phoridae family, present larvae stage in the soil, due to the fact that they are parasites. In addition, such larvae require very specific conditions to develop and then pupate in the soil [24].

The analysis of variance with Tukey's test (p <0.05) for the families of the order Coleoptera found the only significant difference for the variable Richness. The native forest area obtained a larger number of families than the other treatments (Table 3), considering that the order Coleoptera performs several functions in the ecosystem and the forest presents a great diversity of niches that allow colonization by more families than more simplified systems, especially to detritus and predators [25].

The ACD revealed that the families of Coleoptera that contribute to the study areas separation were Lycidae (p <0.001), Nitidulidae (p <0.008), Tenebrionidae (p <0.01) and Cantharidae (p <0.03) (Fig. 4). These invertebrates presented low abundance (Table 3) but were sampled more frequently in the forest area. The Tenebrionidae family is widely distributed in places with arid climate. But as a result of management, significant changes can be found in the community, especially at the species level, affecting the ecosystem services provided, since these beetles are found predominantly inside the soil, digging galleries and changing the soil structure [26].

Lamparydae, Cantharidae and Lycidae belong to the superfamily Elateroidea. Most beetles of the Lycidae and Cantharidae families are day-old and have a short life cycle, while the Cantharidae live about 3 weeks, the Lycidae, remain alive in the environment for a few hours. These beetles are predominant in forests, inhabiting mainly flowers and logs. Due to the hostile environment found in such habitats, they have developed a slightly sclerotized body, which allows the development of glands and differentiated staining patterns that protect the invertebrate from adversities, especially predators [27].

Another family of beetles that contributed with treatments discrimination in the ACD analysis was Nitidulidae (Fig. 4). The main function of these insects is the pollination and the detritivore,

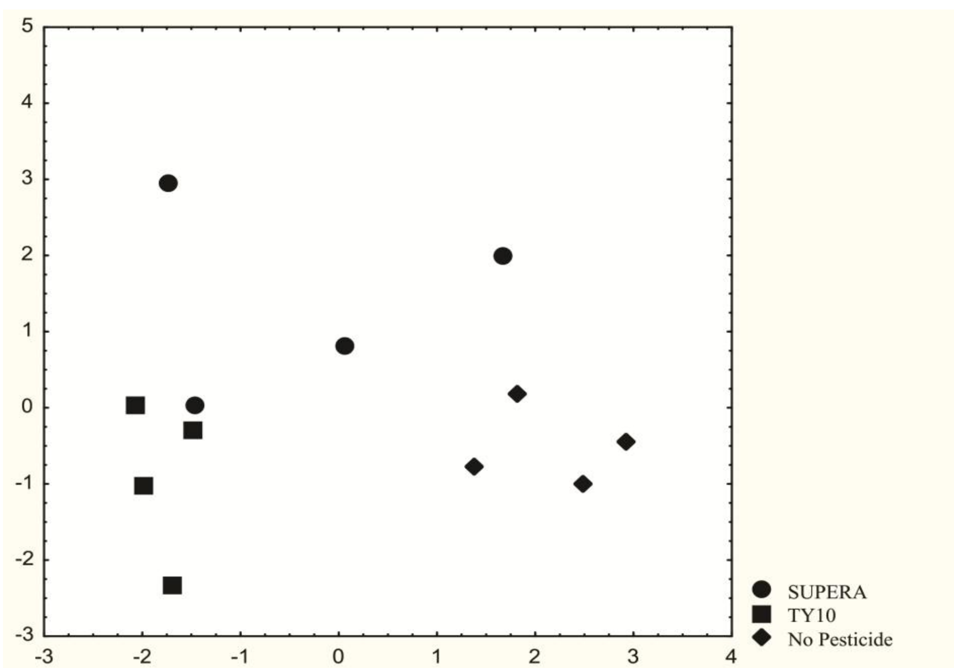

Fig. 3. Discriminant Canonical Analysis (ACD) for the orders of invertebrates associated to the separation of the areas of tomato cultivation with intensive application of pesticides (SUPERA and TY10) and the Native Forest, without application of pesticides

Table 3. Average abundance of individuals and diversity of Coleoptera families collected in tomato crops and in Native Forest

Famílies	Abundance and Diversity (Mean ± Standard variation)		
	Treatment 1 (SUPERA)	Treatment 2 (TY10)	Treatment 3 (Native Forest)
Coccinelidae	46.3 ± 22.2	59.3 ± 15.9	60.0 ± 23.8
Staphylinidae	6.5 ± 2.5	3.3 ± 3.3	4.5 ± 3.7
Crysomelidae	1.5 ± 1.9	1.0 ± 1.41	1.0 ± 1.41
Elateridae	2.8 ± 1.5	3.3 ± 1.9	3.3 ± 3.4
Lagriidae	2.3 ± 2.6	2.8 ± 3.2	1.3 ± 2.9
Curculionidae	4.0 ± 2.9	9.3 ± 7.3	6.8 ± 7.5
Carabidae	1.0 ± 1.41	0.5 ± 0.5 A	1.8 ± 0.9
Lycidae	0.0 ± 0.0	1.0 ± 1.5	1.5 ± 1.58
Lamparydae	0.3 ± 0.50	0.0 ± 0.0	0.3 ± 0.50
Nitidulidae	0.0 ± 0.0	0.2 ± 0.5	2.0 ± 1.4
Cantharidae	0.0 ± 0.0	1.0 ± 1.4	2.0 ± 1.4
Tenebrionidae	0.0 ± 0.0	4.3 ± 8.5	3.0 ± 3.2
Richness	5.8 ± 0.9	6.8 ± 0.9	9.0 ± 0.2 *
Simpson	0.48 ± 0.07	0.48 ± 0.12	0.47 ± 0.21

** Means followed by asterisks differ from one another by the Tukey test (p <0.05)*

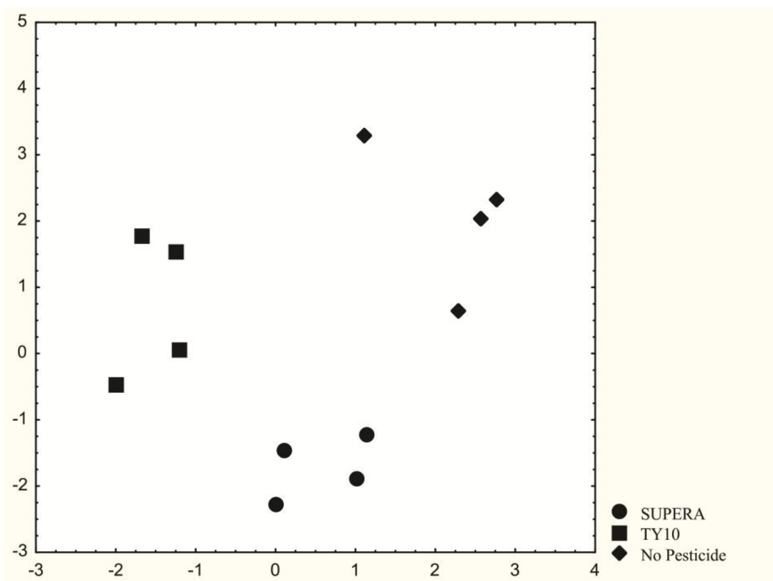

Fig. 4. Discriminant Canonical Analysis (ACD) for the families of Coleoptera associated with the separation of tomato cultivation areas with an intensive application of pesticides (SUPERA and TY10) and Native Forest, without application of pesticides

being able to be a vector of yeasts and pathogenic bacteria in industrial fruits. Studies have shown that pyrethroids are effective in combating this vector [28], besides the non-occurrence of these insects in the tomatoes cultivated areas (Table 3). However, the indiscriminate use of this compound alone can cause resistance to the insect [29].

The other coleopteran families sampled, were classified as cosmopolitan and less sensitive to insecticides application as we observed similar abundances and not significantly difference among the areas (Table 3). The impact of such pesticides on tomato cultivation and the identification of species-level beetles is not known.

4. CONCLUSION

The application of pesticides had an effect on the total community of invertebrates and the Coleoptera order, reducing Coleoptera family richness, but this effect did not necessarily affect the abundance of the epigeic invertebrates. The low precipitation favored greater abundance of invertebrates in tomato areas possible due to the crop irrigation when compared to the native forest. As the taxonomic resolution was increased, it was possible to observe more significant differences. In general, only two orders discriminated the study areas, whereas four families of Coleoptera were significant to discriminate such areas. Coleoptera families that discriminated the most areas of study (Lycidae, Nitidulidae, Tenebrionidae and Cantharidae) were more sensitive to the application of insecticides and showed higher affinity for the forest area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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