

Impact of pesticides application on epigeic fauna in tomato cultivation

ABSTRACT

Our comprehension of the pesticides effects on the soil epigeic invertebrates, especially non-target organisms in tomato cultivation is still incipient. Our aim was to study the epigeic fauna in sites with and without insecticide application in Brejão municipally, Pernambuco, Brazil. The experiment was composed of three treatments: Two sites with higher input of insecticide in tomato production (SUPERA and TY10 tomato varieties); 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 statistic to find difference between treatments and multivariate statistic to verify the dissimilarity between treatments. A total of 2571 invertebrates were collected and distributed within 7 orders. Even among those, Coleoptera were more frequently sampled. The orders that most discriminated the areas were Coleoptera and Diptera. Within the order Coleoptera, the families Lycidae, Nitidulidae, Tenebrionidae and Cantharidae had important participation in the areas separation. The pesticides application had a strong effect on non-target organisms, reducing the Coleoptera family's richness. Coleoptera families that discriminated the most areas of study were more sensitive to the application of insecticides.

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 to several regions of the country (França et al., 2006; Carvalho et al., 2016). Regarding to the world's largest producers, Brazil occupies the eighth position, with approximately 63 thousand cultivated hectares and production reaching 3.5 million tons per year⁻¹ (ABCSEM 2016). However, in Brazil, tomato cultivation has been attacked by 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 (Franca et al., 2017; Gravena & Benvenega, 2003).

The use of synthetic pesticides in pest control is widely recognized as the most effective way, mainly in the STB control (Reis & Souza 1996; Franca et al., 2017). On the other hand, it is known that the indiscriminate use of pesticides can lead to several environmental problems with potential to reach and contaminate the soil, water, atmosphere, non-target organisms, including humans (Badji et al. (Gill & Garg 2014, Rigotto et al, 2014).

The epigeic fauna comprises invertebrates that live on soil surface and is very affected by soil and different crops and management (Brito et al., 2016). These arthropods have important ecosystem functions, since they mainly act in the predation and the processing of substrates, which facilitates the microbial attack during the process of decomposition of the organic residues (Lavelle et al. al. 2014).

Many studies have shown only the effect of soil management (i.e., conventional or no-tillage), and not pesticides, on the composition of the epigeic fauna (Araújo et al., 2004). 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 (Badji et al., 2007). However, the influence of the application of pesticides on tomato cultivation on epigeic fauna has never been studied, making this study necessary. Thus, Our aim was to study the epigeic fauna in sites with and without insecticide application in Brejão municipally, Pernambuco, Brazil.

2. MATERIAL AND METHODS

2.1 Experimental site and treatments

This study was carried out in Brejão (2010) (09°01'S, 36°34'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) (Raij 2001).

Briefly, we evaluated three areas, two placed in commercial tomato cultivation with insecticide application (SUPERA – Treatment 1; and TY 10 – Treatment 2) and a third 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 lactescens*, *Cordia sellowiana*, *Himatanthus phagedaenicus*, *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	Strobirulins	Fungicide	Low toxicity
Bravonil	Tetrachloroiso phthalonitrile	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

2.2 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. Eleven collections were carried out at 8, 12, 19, 27, 33, 40, 48, 54, 61, 69 and 75 days after seedlings transplanting. The samples were collected and stored in 90% ethanol solution, and then counted the total fauna number and 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 (Figure 1).

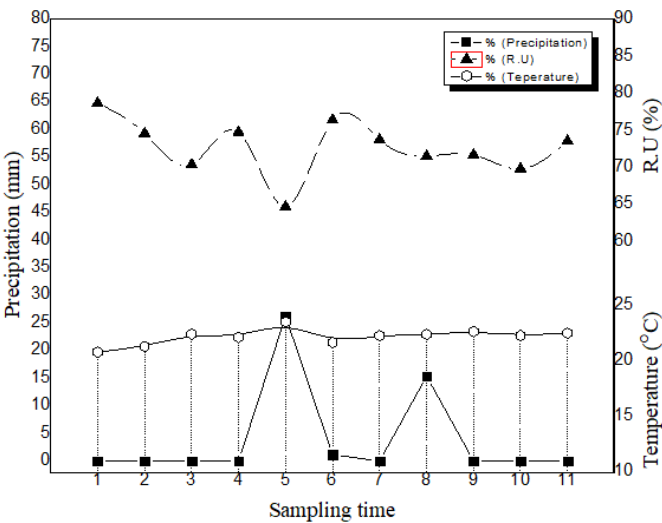


Figure 1. Climatic data on the intervals of collection of soil fauna. Values of temperature (°C) and relative humidity (%) coincide with the day of collection. Precipitation values (mm) refer to the cumulative volume of rainfall between each collection.

2.3 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 (Baretta et al., 2008). Also, the epigeic mesofauna community diversity was determined using the Simpson Index ($1/(1-D)$), where D is the dominance expressed by $\sum p_i^2$, where p_i represents the relative abundance of each taxonomic group sampled. The closer the result to 1, higher the diversity of the system, because the dominance is lower (Magurran, 2004). Richness was determined by mean number of invertebrate orders and Coleoptera families sampled in each plot along the sampling period.

3. RESULTS AND DISCUSSION

A total of 2571 invertebrates were collected, distributed in 7 orders. Among these, Coleoptera was found more frequently in all treatments (Table 2).

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

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

There was greater abundance of Coleoptera and greater total abundance of invertebrates in TY10 than in the other treatments. In turn, the Diptera was more abundant in treatment 1 than in the other treatments (Table 2). In 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 for causing fruit aromas (Wang et al., 2017). It may have contributed to the greater total abundance of invertebrates in tomato crops, since these organic precursors have great palatability to the soil fauna (Lavelle 1997). 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 (Baldwin et al., 2000), which does not occur in the present study, in which rainfall was scarce (Figure 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, as well as those belonging to the Coleoptera order 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 (Figure 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 infestation of the pest

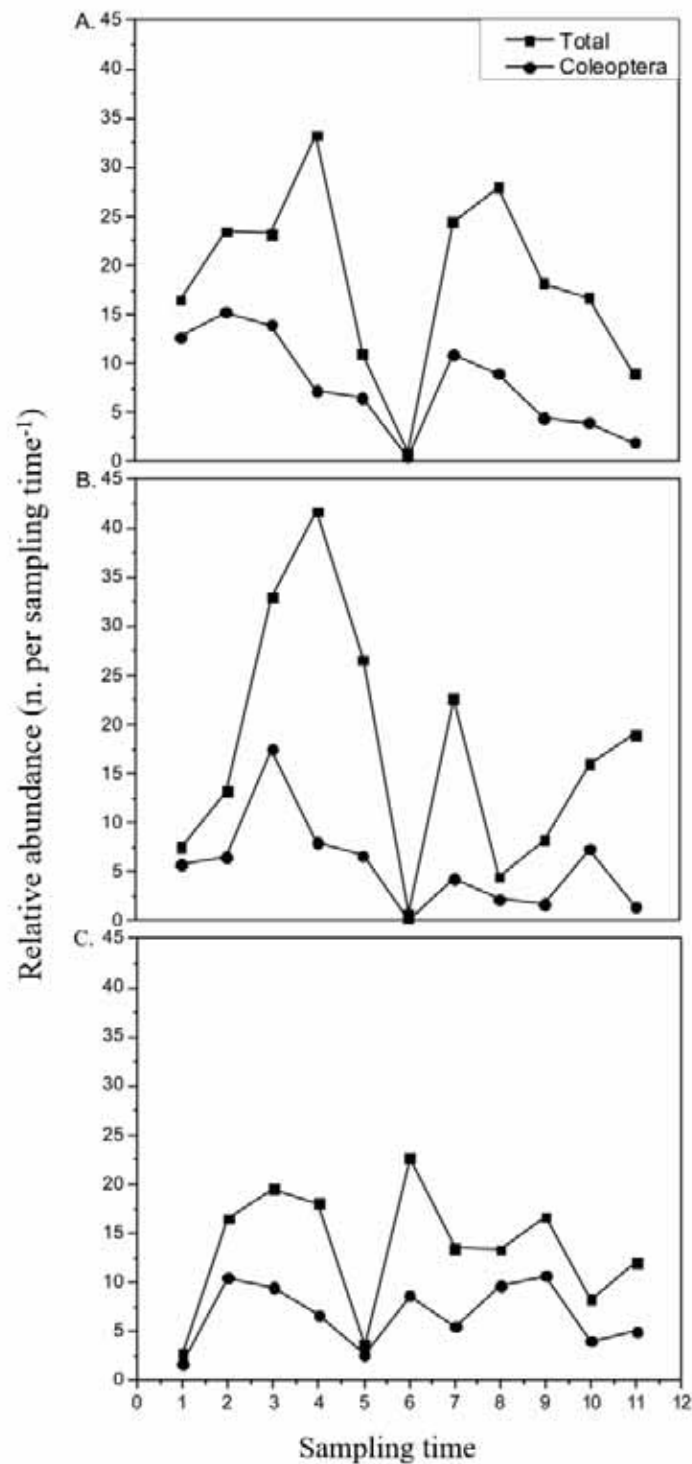


Figure 2. Influence of pesticides and seasonality on the non-target epigeal fauna of the applications. Figure A indicates the variety TY10; Figure B, indicates the variety SUPERA, while figure C, refers to the Native Forest area.

The 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 (Figures 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 (Figure 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, Figure 2C).

The impact of the use of pesticides on non-target epigeal fauna, especially from repeated applications, has been of great concern (Badji et al., 2007), 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 (Lavelle et al., 2006; Gill & Garg, 2014). 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 (Howell et al., 2014).

In a recent study, Nare et al. (2017) 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 the last study, in the treatments of organic fertilization, such as (bovine manure, swine manure and compound of vegetal residues + bovine manure) there was an increase in the abundance of earthworms and termites, even in the presence of pesticides (Nare et al., 2017).

The lowest total abundance of invertebrates, as well as of Coleoptera observed in the native forest, was possibly due to the low volume of precipitation between collection intervals (Figure 1), with 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. Associated with this, crop irrigation management may have favored higher counts of invertebrates in tomato crops, since the soil invertebrates, especially Coleoptera larvae are favored by soil water content (Zagatto et al., 2017). 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) (Figure 3).

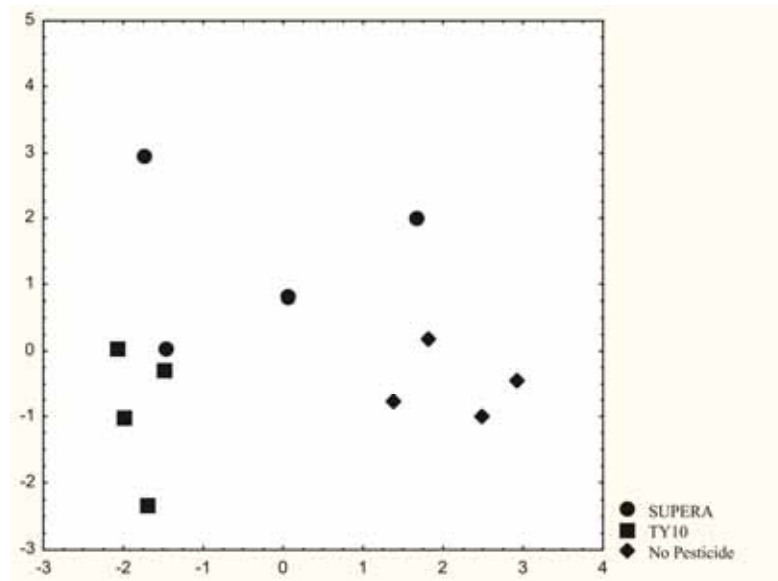


Figure 3. Discriminant Canonical Analysis (ACD) for the orders of invertebrates associated to the separation of the areas of tomato cultivation with intensive application of pesticides (SUPERA and TY10) and the Native Forest, without application of pesticides.

The orders of epigeic fauna that contributed with the treatments separation 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 (Brown et al., 2015). 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 (Brown et al., 2015), so much that in the present study, (Table 2), possibly attracted by volatile compounds exhaled by tomato plants (Wang et al., 2017).

It is likely that the great dispersal power contributed greatly to the Diptera order discriminating areas of study (Figure 3). Hardly any winged Diptera collected in pitfall traps, especially those of the Phoridae family, present their larvae 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 (Frouz 1999).

The analysis of variance with Tukey's test ($p < 0.05$) for the families of the order Coleoptera found 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 allows colonization by more families than more simplified systems, especially to detritus and predators (Yekwayo et al., 2017).

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

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

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

The ACD revealed that the families of Coleoptera that separated most of the study areas were Lycidae ($p < 0.001$), Nitidulidae ($p < 0.008$), Tenebrionidae ($p < 0.01$) and Cantharidae ($p < 0.03$) (Figure 4).

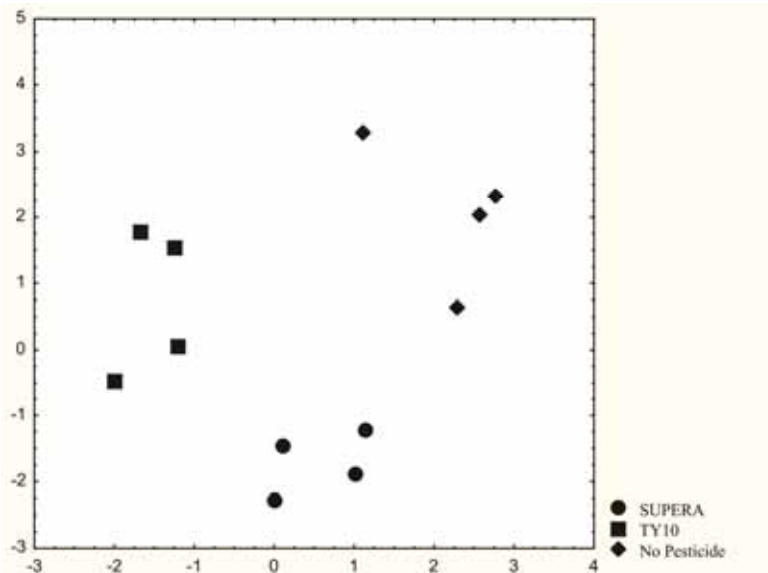


Figure 4. Discriminant Canonical Analysis (ACD) for the families of Coleoptera associated with the separation of tomato cultivation areas with intensive application of pesticides (SUPERA and TY10) and Native Forest, without application of pesticides.

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, where they dig galleries and deeply alter the soil structure (Lescano et al., 2017).

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 (Zaragoza-Cabalero & Perez-Hernandez 2014).

Another family of beetles that contributed with treatments separation was Nitidulidae (Figure 4). The main function of these insects is the pollination and the detritivore, being able to be vector of yeasts and pathogenic bacteria in industrial fruits. Studies have shown that pyrethroids are effective in combating this vector (Eickermann et al., 2011), so that these insects were absent in the areas cultivated with tomatoes (Table 3). However, the indiscriminate use of this compound alone can cause resistance to the insect (Heimbach & Muller 2013).

The other coleopteran families sampled, cosmopolitan and less sensitive to applied insecticides, showed similar abundances and were not significantly different among the areas (Table 3) in both tomato and native forest. However, the impact of such pesticides on tomato cultivation and the identification of species-level beetles is not known. Possibly, the sensitivity to pesticides would be much higher and the impact of these on the epigeic invertebrate community would be more marked than that presented in the present study.

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 the crop irrigation when compared to the native forest. As

the taxonomic resolution was increased, it was possible to observe more significant differences, so much so that 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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