Transgenic Bt maize does not affect the soil ant community

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Abstract – The objective of this work was to survey soil ants in Bt and non-Bt maize (*Zea mays*) crops, and to compare their effect on the soil ant community. Nine pitfall traps, 10 m apart, were installed in a central area (900 m^2) of each of the following treatments (2,500 m^2): conventional maize; maize modified with the Cry1F, Cry1Ab, and Vip3A proteins; and a native vegetation area. Fortnightly collections were conducted during four periods (complete producing cycles) of the crop, from 2011 to 2013. The number of ant species varied from 25 in Bt maize (Vip 3A) to 58 in Bt maize (Cry 1F). The treatment with conventional maize showed the highest Shannon-Wiener diversity index (H' = 2.60). Jaccard's index showed that there is dissimilarity between the cultivated maize areas and the native vegetation area in most treatments, and that Bt and non-Bt maize show similarity in their soil ant assemblages. The cultivation of Bt maize does not affect the soil ant community. The subfamily Myrmicinae shows the highest number of species in all the collection periods, with 57, 41, 47, and 50 species in the first, second, third, and fourth periods, respectively. The genus *Pheidole*, belonging to this subfamily, shows the greatest number of species.

Index terms: *Zea mays*, bioindicators, Formicidae, Myrmicinae, nontarget arthropods, soil biology.

Milho transgênico Bt não afeta a comunidade de formigas do solo

Resumo – O objetivo deste trabalho foi realizar um levantamento sobre formigas do solo, em cultivos de milho (*Zea mays*) Bt e não Bt, e comparar o efeito desses cultivos sobre essa comunidade. Nove armadilhas do tipo "pitfall" foram instaladas a intervalos de 10 m e distribuídas na área central (900 m^2) de cada um dos seguintes tratamentos (2.500 m^2): milho convencional; milho modificado com as proteínas Cry1F, Cry1Ab e Vip3A; e área de vegetação nativa. Foram feitas coletas quinzenais, durante quatro períodos (ciclos de produção completos) de cultivo, de 2011 a 2013. O número de espécies de formiga variou de 25 no milho Bt (Vip 3A) a 58 no milho Bt (Cry 1F). O tratamento com milho convencional apresentou o maior índice de diversidade de Shannon-Wiener (H' =2,60). O índice de Jaccard mostrou que há dissimilaridade entre as áreas cultivadas com milho e a área com vegetação nativa, na maioria dos tratamentos, e que os milhos Bt e não Bt apresentam similaridade quanto à composição de espécies de formigas do solo. O cultivo do milho Bt não afeta a comunidade de formigas de solo. A subfamília Myrmicinae apresenta o maior número de espécies em todos os períodos de coleta, com 57, 41, 47 e 50 espécies no primeiro, no segundo, no terceiro e no quarto períodos, respectivamente. O gênero *Pheidole*, pertencente a esta subfamília, apresenta o maior número de espécies.

Termos para indexação: *Zea mays*, bioindicadores, Formicidae, Myrmicinae, artrópodes não alvo, biologia do solo.

Introduction

Maize (*Zea mays* L.) is intensely cultivated throughout the world due to its importance for animal feed, especially poultry and swine, and for human food. As a result of the demand for this grain, larger areas are increasingly subjected to an intensive and competitive agriculture, which causes a lot of phytosanitary problems that should be overcome to obtain quality production. In 2015, maize was cultivated in Brazil in more than 15 million hectares (IBGE, 2016), from which 86.8% was maize genetically modified with *Bacillus thuringiensis* (Berliner, 1915) gene (Attie, 2016). This technology is used mostly to reduce damages caused by the attack of the fall armyworm [*Spodoptera frugiperda* (J. E. Smith, 1797)] that is considered the main pest in maize (Cruz et al., 2008).

The cultivation of genetically modified maize with *B. thuringiensis* (Bt) gene has several advantages, and the main one is the reduction of insecticide use (Roh et al., 2007). However, it is necessary to investigate the effects of this tactic on nontarget arthropods (Jensen et al., 2010; Cheeke et al., 2014), such as a range of insects present in the crop environment, which may eventually be affected. Insects perform many important environmental services such as plant pollination, nutrient recycling, decomposition, predation, parasitism, seed dispersal, which are even the main regulators of plant and other organism populations and are, therefore, indispensable for the environment functioning (Price et al., 2011).

The evaluation of ecosystem functionality can be done by monitoring biological indicators (Holt & Miller, 2010). Among these, insects have been frequently used, with ants having a prominent role (Oliveira et al., 2014). Ants are used in biomonitoring, since they are ubiquitous, permanently present as social organisms, have often wide geographic distribution, are sensitive to changes in the environment, and are easily sampled and identified at low cost (Alonso & Agosti, 2000).

Various studies have already been carried out using ants as bioindicators in agroecosystems and natural habitats (Dias et al., 2008; Lacau et al., 2008; Delabie et al., 2009; Urrutia-Escobar & Armbrecht, 2013; Sanabria et al., 2014). Most of these studies correlate ant community composition with the degree of environmental anthropization.

In the maize agroecosystem, ants interact with crop in several ways: preying on nontarget herbivores that feed on maize plant, participating in the decomposition of crop remains, and insects that feed on certain plant structures, such as pollen, and even nesting and foraging in the soil. Even in these last two cases, they may come into contact with transgenic derived proteins, since, according to Borisjuk et al. (1999), the plants release exudates into the soil and, in the case of genetically modified plants, the toxic proteins can also be released with these exudates. In addition, contact with the protein can occur through leftovers on the soil after harvest (O'Callaghan et al., 2005). That is, ants are directly or indirectly susceptible to intoxication with Bt maize proteins. The null hypothesis was that transgenic maize (Bt) do not affect the community of soil ants, and the alternative hypothesis was that Bt maize may act positively or negatively on soil ant communities.

The objective of this work was to evaluate the effects of Bt and non-Bt maize crops on the soil ant community.

Materials and Methods

The study was conducted in the experimental fields of Embrapa Milho e Sorgo, in the municipality of Sete Lagoas, in the state of Minas Gerais, Brazil (19°27'57"S, 44°14'49"W). Areas of conventional maize and of maize genetically modified with *B. thuringiensis* (Bt) were used, as they show a history of intensive maize and sorghum cultivation; and an area of native vegetation (NV), characterized as Seasonal Forest Always Green (Costa et al., 2015). Ant samplings were carried out in four producing cycles (periods) of mayze crops grown at different times: the first one was performed in 2011 (May to October – 1st maize period); and the second one, in 2011/2012 (December 2011 to April 2012 - 2nd maize period). These areas were irrigated by sprinkling. Two other samplings were performed in 2012/2013: one from October 2012 to February 2013, in 3rd maize period; and the other in 2013, from June to October, in the 4th maize period; both last cultivations were irrigated by central pivot, generating a total of four replicates of the experiment. The treatments corresponding to each period were different with respect to the proteins present in the cultivated maize.

The experimental areas with maize were divided into four plots of 2,500 m^2. In the first period, two of the four plots were planted with transgenic maize (31F53Hx - Cry 1F, and DKB Yg - Cry 1Ab), and two plots with conventional maize (30F53 and DKB330). In the second period, three of the four plots had transgenic maize (30F35Hx- Cry 1F, 30F35YG - Cry 1Ab, and Impact Viptera - Vip3A), and a single plot had conventional maize (30F35). In the third and fourth periods, three of the four plots received transgenic maize (30F35Hx - Cry 1F, 30F35YG - Cry 1Ab and Impact Viptera - Vip3A), and a single had conventional maize (30F35). In the maize cultivation areas, the covering fertilization was carried out with 300 kg ha^{-1} of an N-P_2O_5-K_2O formulation 8-28-16+Zn, and 250 kg ha^{-1} of urea. The irrigation water depth for the maize areas was 20 mm, and the herbicides glyphosate (2.5 L ha^{-1} Roundup WG) and nicosulfuron (0.6 L ha^{-1}

Sanson) + atrazine (3.0 L ha^{-1}) were applied. There was no insecticide application in these plots.

During the four maize production periods, collections in the native vegetation (NV) area of 12.61 ha were also conducted. A plot of equal size as those grown with maize was delimited.

In each maize plot, a 900 m^2 (30x30 m) grid was laid out in the center of the plot. This grid was divided into nine quadrants of 10 m^2 where a pitfall trap (Bestelmeyer et al., 2000) was installed in the center. This resulted in an interval of 10 m between successive traps, totaling nine traps per plot. According to Bestelmeyer et al. (2000), the use of pitfall traps is efficient for collecting ants in places with little or no leaf litter, which is commonly observed in agricultural environments with conventional soil preparation.

Traps were installed in the field when maize was in the sixth full-size leaf stage (V6). Samplings were performed fortnightly, when the collecting vial was replaced. The collected material was properly labeled with the trap number, treatment, and collection date. The number of samples per cycle (period) varied according to crop development, with nine samples in the first growing period, eleven in the second one, and nine in each of the other periods. In the native vegetation area, the same number of samplings was carried out as in the maize cultivation areas.

After sampling, the vials were taken to the Laboratório de Zoologia e Entomologia Geral of the Universidade Federal de São João del-Rei (UFSJ), Sete Lagoas Campus, in Sete Lagoas, MG, Brazil, where the material was screened with the aid of a stereoscopic microscope. Next, the Formicidae were fixed, assembled, and identified with the help of taxonomic keys (Bolton, 2014), and by comparison with species reference collection of the Laboratório de Zoologia e Entomologia Geral (UFSJ) and Laboratório de Mirmecologia (Cepec/Ceplac), in Ilhéus, BA, Brazil.

Collected data allowed of the production of a list of species per treatment. The Shannon-Wiener diversity index was determined (Magurran, 1988), and comparison was made between the Shannon-Wienner index means using the Scott-Knott test, at 5% probability. Similarity dendrograms between the studied areas were also drawn with the Jaccard index, using the R statistical software (R Core Team, 2014).

Results and Discussion

A total of 145 species of ants were found (Table 1). In the first sampling period, 104 species were identified, in the second one, 66, and in the third and the fourth ones, 83 species in each period. The number of species between the maize treatments (conventional or Bt) varied from 25 (Impact Viptera / Vip3A, second period) and 58 (31F53Hx / Cry 1F, first period); and, in native vegetation, the number of species varied from 40 (second period) to 56 species in the first and fourth periods.

The subfamily Myrmicinae was represented with the highest number of species in all the collection periods, with 57, 41, 47, and 50 species in the 1st, 2nd, 3rd and 4th periods, respectively. The genus *Pheidole*, belonging to this subfamily, showed the greatest number of species (Table 1), which is usual in studies on ant communities in the Neotropical region (Camacho & Vasconcelos, 2015; Silva et al., 2017).

From maize treatments, that with most species (58) in the first period was Bt maize with the protein Cry 1F (31F53Hx). In the second period, two treatments showed the highest number of species, which were: maize crop with the protein Cry 1F (30F35Hx, with 32 species), and maize crop with Cry 1Ab (30F35YG, with 33 species). In the third period, maize with the Cry 1F protein (30F35Hx) was also the treatment with the highest number of species (48). Only in the fourth period, the conventional maize treatment (30F35) showed a higher number of species than the other treatments (47 species) (Table 2).

The highest diversity index values were found in conventional maize in the first (30F53, C, H' = 2.60) and in the fourth periods (30F35, H' = 2.45), and in the Bt maize containing the Cry 1F in the third period (H' = 2.44). The lowest values occurred in native vegetation, in the second (H' = 0.81) and fourth periods (H' = 0.74) (Table 2). The treatments showed a significant difference (Table 2) and, in the first period, the diversity index of the native vegetation area did not differ from the value found in conventional maize (DKB330). Except for this case, in the other periods the diversity of the native vegetation differed statistically from other treatments. In the second period, maize treatments did not differ statistically, but showed distinct diversity in the native vegetation area; and, in the last periods (third and fourth), the majority of the treatments were statistically different. It was also

Table 1. Ant species collected with pitfall traps in areas of native vegetation (NV), conventional maize 30F53 (C), DKB330 (C'), and 30F35 (C"), transgenic maize 31F53 Hx/Cry 1F (T1), DKBYG/ Cry 1Ab (T2), 30F35Hx/Cry 1F (T3), 30F35YG/ Cry 1Ab (T4), and Impact Viptera/Vip3A (T5), in the four sampling periods, the first one being from May to October, 2011, the second one from December 2011 to April 2012, the 3rd one from October 2012 to February, 2013, and 4th one from June to October, 2013.

Subfamily/species	Occurrence, 1st period					Occurrence, 2nd period					Occurrence, 3rd period					Occurrence, 4th period				
	NV	T1	C	T2	C'	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5
Amblyoponinae																				
Fulakora sp.1	X															X				
Dolichoderinae																				
Dorymyrmex brunneus (Forel, 1908)	X	X	X	X	X		X	X	X			X	X				X	X	X	X
Linepithema pulex (Wild, 2007)											X		X		X	X				X
Linepithema micans (Forel, 1908)	X				X	X					X					X				
Tapinoma sp. 1																			X	
Dorylinae																				
Eciton rapax (Smith, 1855)				X	X															
Eciton sp. 1		X	X		X				X											
Labidus coecus (Latreille, 1802)	X				X						X					X				X
Labidus praedator (Smith, 1858)	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X		
Labidus sp. 2	X	X		X																
Neivamyrmex sp. 1																		X		
Ectatomminae																				
Ectatomma permagnum (Forel, 1908)										X			X							X
Ectatomma suzanae Almeida Filho, 1986					X															
Gnamptogenys moelleri (Forel, 1912)	X	X	X		X	X					X	X	X	X	X	X			X	X
Gnamptogenys sulcata (Smith, 1858)		X			X					X	X		X			X	X	X	X	
Formicinae																				
Brachymyrmex admotus (Mayr, 1887)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Brachymyrmex sp.2		X						X	X	X			X	X	X		X	X	X	
Brachymyrmex sp.3															X					
Brachymyrmex sp.4													X							
Camponotus atriceps (Smith, 1858)														X				X		
Camponotus crassus (Mayr, 1862)	X			X	X															
Camponotus lespesii (Forel, 1886)	X																			
Camponotus melanoticus (Emery, 1894)	X		X	X	X	X		X		X				X		X	X	X		
Camponotus renggeri (Emery, 1894)												X	X							X
Camponotu ssenex (Smith, 1858)											X									
Camponotus (*Tanaemyrmex*) sp. 1	X										X					X				
Camponotus sp. 1	X	X	X				X		X	X	X	X	X	X		X				
Camponotus sp. 2						X														
Camponotus sp. 3	X								X							X				
Camponotus sp. 4						X					X									
Camponotus sp. 6		X					X		X				X							
Camponotus sp. 7		X																		
Camponotus sp. 8			X								X				X					
Camponotus sp. 9	X																			
Camponotus sp. 10	X										X									
Camponotus sp. 12											X									
Camponotus sp. 13	X		X	X	X									X						
Paratrechina longicornis (Latreille,1802)		X																		
Myrmicinae																				
Acromyrmex balzani (Emery, 1890)													X							
Acromyrmex rugosus (Smith, 1858)		X			X						X					X	X	X	X	X
Acromyrmex subterraneus (Forel, 1893)	X	X	X		X		X	X									X	X	X	X
Apterostigma sp. 1	X																			

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Subfamily/species	Occurrence, 1st period					Occurrence, 2nd period					Occurrence, 3rd period					Occurrence, 4th period				
	NV	T1	C	T2	C'	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5
Atta laevigata (Smith, 1858)	X					X														
Atta sexdens (Linnaeus, 1758)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cardiocondyla minutior (Forel, 1899)		X	X	X			X		X								X			
Cardiocondyla obscurior (Wheeler, 1929)	X	X	X	X	X			X					X	X	X		X			
Carebara urichi (Wheeler, 1922)		X				X														
Cephalotes jheringi (Emery, 1894)						X														
Cephalotes minutus (Fabricius, 1804)											X					X				
Cephalotes pusillus (Klug,1824)	X										X					X				
Cephalotes sp. 1	X				X	X														
Cephalotes sp. 3	X																			
Crematogaster acuta (Fabricius, 1804)	X	X	X		X	X	X	X	X	X		X	X	X	X		X	X	X	X
Crematogaster abstinens (Forel, 1899)				X		X		X					X	X	X		X	X	X	
Crematogaster erecta (Mayr, 1866)		X		X								X	X							
Crematogaster victima (Smith, 1858)				X										X			X	X	X	
Crematogaster sp. 3		X																		
Crematogaster sp. 5										X										
Crematogaster sp. 6			X																	
Crematogaster sp. 7											X									
Cyphomyrmex transversus (Emery, 1894)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyphomyrmex sp. 1		X						X		X										
Cyphomyrmex sp. 2		X																		
Mycetarotes parallelus (Emery, 1906)																	X		X	
Mycocepurus goeldii (Forel, 1893)		X		X	X						X		X						X	X
Mycocepurus smithii (Forel, 1893)		X			X												X	X	X	X
Pheidole aberrans (Mayr, 1868)				X	X															
Pheidole diligens (Smith, 1858)	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X		X	X
Pheidole gr. *fallax* sp. 2	X	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X		X
Pheidole gr. *fallax* sp.4	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Pheidole gr. *fallax* sp. 6															X	X	X		X	X
Pheidole fimbriata (Roger, 1863)	X	X			X	X			X	X	X	X				X				
Pheidole gr. *flavens* sp.1	X					X			X		X		X		X	X		X		X
Pheidole gr. *flavens* sp. 2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pheidole gertrudae (Forel, 1886)		X			X			X	X				X		X	X	X	X	X	X
Pheidole jeannei (Wilson, 2003)					X									X	X		X	X	X	X
Pheidole jelskii (Mayr, 1884)							X		X											
Pheidole laevinota (Forel, 1908)	X	X										X	X	X	X	X	X	X	X	X
Pheidole midas (Wilson, 2003)											X			X		X				
Pheidole oxyops (Forel, 1908)	X				X						X	X	X	X	X	X	X	X	X	X
Pheidole radoszkowskii (Mayr, 1884)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pheidole rufipilis (Forel, 1908)	X	X	X		X	X	X	X			X	X	X	X	X	X	X			X
Pheidole prox. *subaberrans* (Kusnezov, 1952)												X	X	X	X	X	X	X	X	X
Pheidole subarmata (Mayr, 1884)														X	X		X	X	X	X
Pheidole gr. *tristis* sp. 1																			X	
Pheidole gr. *tristis* sp. 3	X	X	X	X	X	X	X	X	X	X				X	X		X	X	X	X
Pheidole gr. *tristis* sp. 4	X				X						X	X	X		X	X	X	X	X	X
Pheidole gr. *tristis* sp. 5	X	X			X		X		X			X	X		X	X	X	X		X
Pheidole gr. *tristis* sp. 7																	X	X	X	X
Pheidole sp. 3						X														
Pheidole sp. 6							X													
Pheidole sp. 7					X	X		X	X	X										
Pheidole sp. 12		X																		
Pheidole sp. 13		X															X			
Pheidole sp. 14		X																		
Pheidole sp. 24																	X			

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Subfamily/species	Occurrence, 1st period					Occurrence, 2nd period					Occurrence, 3rd period					Occurrence, 4th period				
	NV	T1	C	T2	C'	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5	NV	C"	T3	T4	T5
Pheidole sp. 27														X			X	X	X	X
Pheidole sp. 30			X		X															
Pheidole sp. 31					X															
Pheidole sp. 32					X															
Pogonomyrmex naegelii (Emery, 1878)		X																		
Procryptocerus sp. 1																X				
Rogeria sp. 1															X					
Solenopsis globularia (Smith, 1858)	X	X	X	X	X	X	X	X	X	X			X	X	X					
Solenopsis invicta (Buren, 1972)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Solenopsis saevissima (Smith, 1855)	X																			
Solenopsis substituta (Santschi, 1925)														X	X			X	X	X
Solenopsis sp. 1	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Solenopsis sp. 2	X	X	X	X	X	X	X				X		X	X	X	X	X	X	X	X
Solenopsis sp. 3		X		X		X		X				X	X	X		X	X	X		
Solenopsis sp. 4		X	X					X								X		X		X
Solenopsis sp. 5		X						X			X		X	X						
Solenopsis sp. 6		X						X												
Solenopsis sp. 7	X					X			X		X			X		X		X	X	X
Solenopsis sp. 8		X				X	X													
Solenopsis sp. 10	X															X	X	X		
Stegomyrmex sp. 1						X					X					X				
Strumigenys appretiata (Borgmeier, 1954)											X									
Strumigenys denticulata (Mayr, 1887)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Strumigenys gundlachi (Roger, 1862)											X	X	X	X	X	X	X		X	X
Strumigenys louisianae (Roger, 1863)								X												
Strumigenys sp. 2		X			X															
Strumigenys prox. *ogloblini* (Santschi, 1936)																X				
Trachymyrmex sp.1																X				
Wasmannia rochai (Forel, 1912)																X				
Ponerinae																				
Anochetus diegensis (Forel, 1912)			X	X	X										X					X
Anochetus targionii (Emery, 1894)		X			X		X	X	X	X		X	X	X						
Hypoponera sp. 1			X		X	X		X				X				X				X
Hypoponera sp. 2	X	X	X			X										X		X	X	
Hypoponera sp. 3				X																
Hypoponera sp. 4				X					X	X			X							
Hypoponera sp. 5	X	X		X					X		X	X	X	X	X	X	X	X	X	X
Hypoponera sp. 6	X										X		X		X	X		X		X
Hypoponera sp. 7			X								X	X	X	X		X	X	X	X	
Hypoponera sp. 8				X							X							X		
Hypoponera sp. 9	X			X																
Odontomachus chelifer (Latreille, 1802)	X					X					X					X				
Odontomachus meinerti (Forel, 1905)	X										X			X		X				
Odontomachus sp. 1						X		X												
Pachycondyla striata (Smith, 1858)	X	X	X		X	X					X		X		X	X	X			
Pachycondyla harpax (Fabricius, 1804)	X				X	X					X				X	X				
Pachycondyla sp. 2		X																		
Pachycondyla sp. 4					X				X											
Pseudomyrmecinae																				
Pseudomyrmex sp. 1		X																		
Pseudomyrmex termitarius (Smith, 1855)													X							
Total of species/treatament	56	58	35	34	51	40	27	32	33	25	49	30	48	41	41	52	47	46	42	44
Total of species per sampling period			104					66					83					83		
Total of ant species collected										145										

observed that the diversity of native vegetation areas was lower than in most treatments, especially in the second and fourth periods (Table 2).

In the analysis of similarity between treatments, the formation of two distinct groups was observed. Except for the first period, in which one of the groups was formed by Bt maize (T4) and the other group was formed by the other treatments, the first group of other periods was composed by the native vegetation area, and the second group was formed by the other treatments together (Figure 1). In the first period, one of the subgroups was formed by conventional maize treatments. In the second period, one of the subgroups was formed by Bt maize (T4) and its isoline. In the third and fourth periods, both subgroups were formed by treatments T3 and conventional maize (C").

As to the most frequent ant species, the subfamily Myrmicinae stood out in the following treatments: conventional maize (first period), native vegetation, and T5 (second period), and innative vegetation (third and fourth periods). In the first period, the most frequent ant species in Bt maize were *Pheidole* gr. *flavens* sp. 2 (T1) and *P. radoszkowskii* Mayr, 1884 (T2). The first mentioned species was also the most frequent in non-Bt maize, besides *Labidus praedator* (Smith, 1858) (C and C'). In the second and third periods, *P.* gr. *flavens* sp. 2 was the most frequent in non-Bt (C") maize; and, in Bt maize, the most frequent species were: *Atta sexdens* (Linnaeus, 1758) (T3, second period), *P.* gr. *flavens* sp. 2 (T4 second period, and T3 and T5 in the third period), and *L. praedator* (T5 second period). In the third period, only *P.* gr. *flavens* sp. 2 was the most frequent in C", T3 and T5, and *Crematogaster abstinens* (Forel, 1899), in T4. In the fourth period, *Pheidole oxyops* (Forel, 1908) was the most frequent species in all nest treatments (Bt and non-Bt). In the native vegetation area, the leafcutter ant *A. sexdens* was the most frequent in the first period, together with *L. praedator*; and, in the other periods, *L. praedator* was the most frequent species, with frequency superior to that of the other species.

The number of species varied among the treatments, even when comparing the same treatments in the different collection periods, as, for instance, the native vegetation area and the conventional maize in periods 2, 3, and 4. There was also variation in the total number of species collected per period (Table 1). However, the total number of species found in the work is similar to that of other studies carried out with the same collection method, in agricultural and native vegetation environments (Delabie et al., 2009; Sanabria et al., 2014; Silva et al., 2017).

The subfamily Myrmicinae, known for its high number of species found in different types of environments (Hölldobler & Wilson, 1990), also showed a high number of species in the present study (60%, that is 87 species). A total of 47 species were identified as belonging to the genera *Pheidole* and *Solenopsis*, which are characterized as aggressive and

Table 2. Mean and standard deviation (SD) of the Shannon-Wienner index for ant species (Formicidae) in native vegetation environments, and in soil under cultivation with conventional maize (30F53, 30F35, and DKB330), Bt maize (31F53Hx, DKBYG, 30F35Hx, and 30F35YG), and 'Impact Viptera', for the periods 1, 2, 3, and 4[(1)].

Treatment	1st period		2nd period		3rd period		4th period	
	Number of species	Mean±SD	Number of species	Mean±SD	Number of species	Mean±SD	Number of species	Mean±SD
Native vegetation	56	2.18±0.10c	40	0.81±0.13b	49	2.04±0.05c	52	0.74±0.01 d
30F53	35	2.60±0.01a	-	-	-	-	-	-
DKB330	51	2.04±0.03c	-	-	-	-	-	-
30F35	-	-	27	2.12±0.01a	30	2.17±0.01b	47	2.45±0.02 a
31F53Hx	58	2.34±0.10b						
DKBYG	34	2.28±0.01b						
30F35Hx	-	-	32	2.19±0.02a	48	2.44±0.01a	46	2.25±0.01 c
30F35YG	-	-	33	2.22±0.01a	41	1.84±0.07d	42	2.30±0.01 c
Impact Viptera	-	-	25	2.07±0.01a	41	2.53±0.01a	44	2.37±0.02 b

[(1)]Means followed by equal letters do not differ significantly, by the Scott-Knot test, at 5% probability. Periods: 1, from May to October, 2011; 2, from December 2011 to April 2012; 3, from October 2012 to February 2013; 4, from June to October, 2013.

generalist in the control of food resources (Silvestre et al., 2003).

The number of ant species varied in the sampled areas, and no species occurrence patterns was observed among the treatments. When evaluating the diversity, significant differences among the treatments were found; however, in the second period, Bt and non-Bt maize treatments, showed similar diversity. This result suggests that Bt maize does not interfere with the soil ant community. In the first period, conventional maize DKB330 (C) showed a number of species close to and a diversity similar to that of the native vegetation, which is a result distinct from the diversity pattern reported by Delabie et al. (2009) and Braga et al. (2010). Also in relation to this period, it was observed that in the Bt treatments the number of species was similar, and in those of conventional maize, it was different. In the third and fourth periods, conventional maize was different from the Bt maize and native vegetation.

As to diversity, it was observed that in some treatments the high number of species did not provide a high H' value, as for instance in the native vegetation in all periods. In the fourth period, the number of species found was 52 in the native vegetation, which represents the third largest number of species in the present study, but with H' = 0.74 only, which is the lowest among treatments (Table 2). Probably, the low value of H' is related to the high abundance of *L. praedator*, a species that occurred with a high amount of individuals in several samples. Ants of the genus *Labidus* are nomadic, have legionary behavior, and are aggressive (Gotwald Jr., 1995). The *L. praedator* species was the most frequent in the collections in the native vegetation area, thus

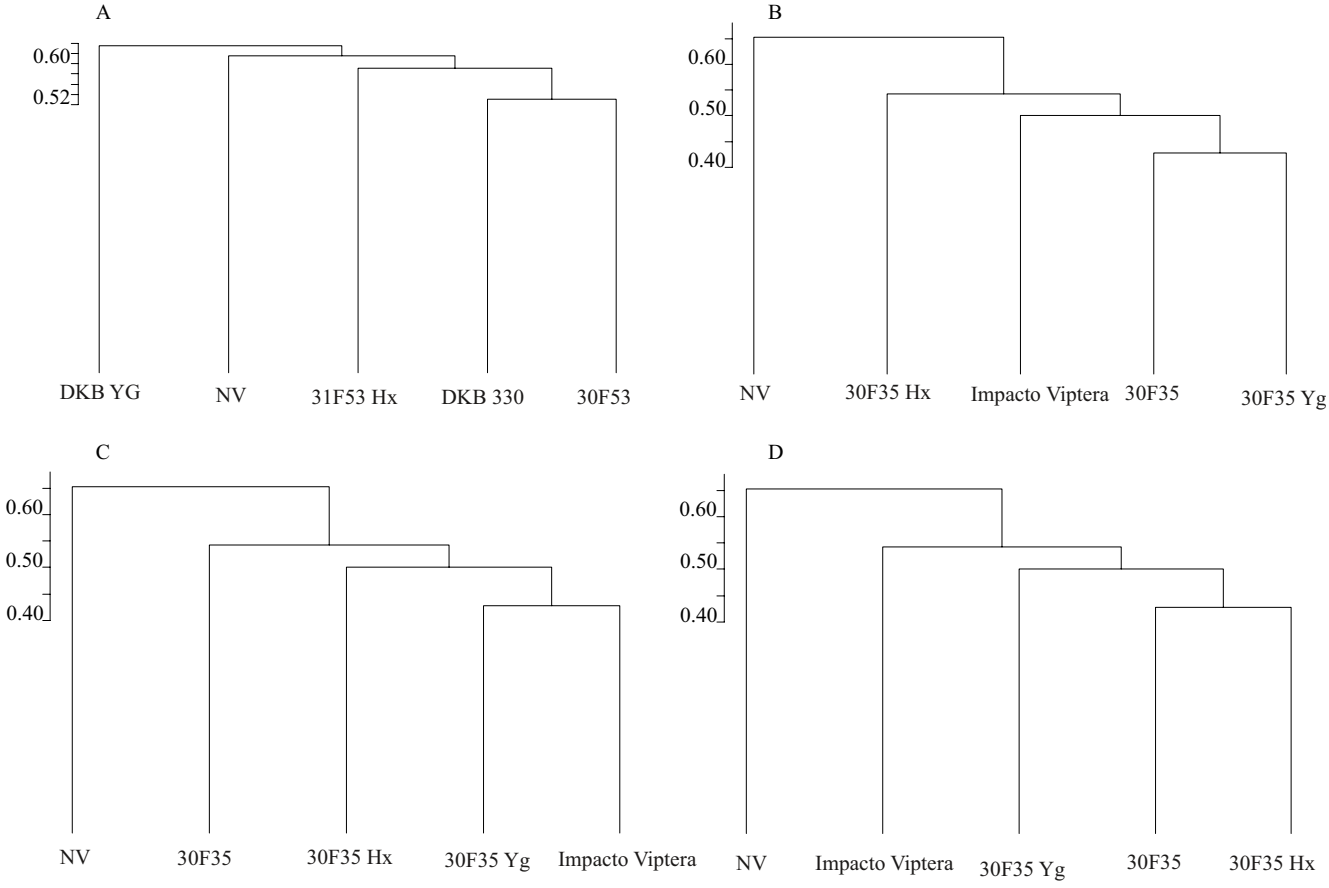

Figure 1. Dendrogram of similarity of ant species (Formicidae) for native vegetation environments (NV), and cultivation of conventional maize (30F53, 30F35, and DKB330), Bt maize (31F53Hx, DKBYG, 30F35Hx, 30F35YG), and Impact Viptera, for periods 1 (A), 2 (B), 3 (C), and 4 (D).

determining the low Shannon-Wienner diversity index values for a local, in which the highest diversity values was expected to be found due to greater plant diversity. According to Perfecto & Snelling (1995), more structured environments, such as native vegetation areas, provide better nesting conditions for ants, and this consequently promotes high diversity.

Another aspect was that ants of the Myrmicinae subfamily were the most frequent, highlighting *Pheidole* gr. *flavens* sp. 2, which was the most frequent in seven of the twenty treatments, followed by *L. praedator* (Dorylinae subfamily), the most frequent in five of the treatments, and *P. oxyops*, which was more frequent in four treatments. The genus *Pheidole* comprises omnivorous species, which are widely distributed in the environment and have generally large subterranean nests, and some species are favored by disturbed environments (Silvestre et al., 2003). According to Fowler (1987), *P. oxyops* shows a wide nest opening, which promotes the capture of its prey still alive, supplementing the diet with dead or injured insects, and for that reason it is considered an important predatory ant.

The predatory ant *Gnamptogenys moelleri* (Forel, 1912) was among the five most frequent in three sampling periods in the native vegetation area, but it did not show an expressive frequency in maize treatments, regardless of whether it was Bt or non-Bt. Being a predatory ant, it needs nesting sites with food availability in the proximity, which is favored by areas with greater plant heterogeneity, such as the native vegetation area. This aspect deserves to be highlighted, since only in the first period did the native vegetation area show a different result from the others.

Information on diversity can also be complemented by similarity analysis, in which two distinct groups were formed in all periods. As expected in periods two, three, and four, one of the groups was formed by the native vegetation, and the other group by Bt and non-Bt treatments. However, in the first period, the native vegetation unexpectedly remained in a group together with Bt (Cry 1F) and non-Bt (DKB330 and 31F53). In periods two and four, for instance, there was the formation of subgroups containing Bt maize and its isoline. That is, the ant assemblage composition in these treatments is similar. In this case, if ant assemblages in Bt and non-Bt maize parcels are similar, it is suggested that genetically modified plants represent little or no risk to the soil ants community.

Field studies carried out to evaluate the effects of genetically modified crops, such as cotton and maize, on nontarget arthropods have shown little risk to these organisms, according to Naranjo et al. (2005). These authors argue that most studies suggest that the use of broad spectrum insecticides was potentially more harmful than Bt crops to nontarget organisms (this case was not yet tested in the present study). Carrière et al. (2009) suggest even a positive effect of this technology on ants and beetles in transgenic cotton. In another study carried out on mayze crops, Arias-Martín et al. (2016) also concluded that the continuous cultivation of Bt maize does not negatively affect soil microarthropods.

Considering the data presented here, and also the known efficiency of ants as bioindicators of environmental quality, the biomonitoring may be carried out frequently in agricultural environments aiming to evaluate if transgenic plants are able to affect the soil organisms otherwise than conventional crops. It is noteworthy that any kind of agriculture strongly affects the native ant community, but it is still important to create tools which are able to point out unexpected deleterious effects of any change in a crop cultivation that can have a genetic or managing origin. It is indispensable the monitoring of the use of genetically modified plants of economic interest as for their effects on soil ants because the transgenic technology is constantly modified, including sometimes more than one event (protein for example) in the same plant, which could affect soil ant activities and, consequently, promote effects different from those reported here.

Conclusions

1. The cultivation of Bt maize (*Zea mays*) containing the Cry 1F, Cry 1Ab, and Vip3A proteins does not affect significantly the soil ant community able to live in conventional maize crop.

2. The conventional maize shows the highest Shannon-Wiener diversity index (H' = 2.60); and the Jaccard index shows that there is dissimilarity between the cultivated maize areas in relation to the native vegetation, in most treatments, despite the

similarity between Bt and non-Bt maize for their soil ant assemblages.

3. The subfamily Myrmicinae shows the highest number of species in all the collection periods with 57, 41, 47 and 50 species in the 1st, 2nd, 3rd and 4th periods, respectively; the genus *Pheidole*, belonging to this subfamily, shows the greatest number of species.

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