

Can the absence of ants interfere in the arthropods abundance on corn plants (*Zea mays* L. - Poaceae)?

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Abstract

Alternative methods to reduce the chemical control in monoculture have been widely assessed in order to diminish the pesticide use. Thus, the efficiency of predator arthropods who work as population controllers in many agro-ecosystems has been tested. The current study aim to verify if the presence of ants interfere in the amount of arthropod herbivores and predators on corn plants, *Zea mays* L. (Poaceae). A total of 100 plants were assessed, out of them, 50 were isolated from ants and the other 50 were not. There were 25 taxa of arthropods on corn plants, in which 14 were herbivorous and 11 predators. The ants' free access to the plants negatively influenced the *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) amount, which is an important herbivorous plague in corn monoculture. It was also observed the greater amount of *Doru* sp.1 (Dermaptera: Forficulidae) on plants containing ants. Considering the assessed plants, the presence of ants did not influenced the abundance of other arthropods. These results suggest that the ant-plant-herbivore interactions in corn monoculture is a complex system, and the presence of predatory ants in plants not always influence the presence of other arthropods.

Keywords: agro-ecosystem, ant-plant interactions, biological control, herbivorous predatory insects

A presença de formigas pode interferir na abundância de artrópodes sobre plantas de milho *Zea mays* L. (Poaceae)?

Resumo

Métodos alternativos ao controle químico de pragas em monoculturas têm sido amplamente avaliados com o intuito de diminuir o uso de agrotóxicos. Neste sentido, a eficiência de artrópodes predadores que agem como controladores de populações nos mais diversos agroecossistemas vem sendo testada. Este estudo objetivou verificar se a presença de formigas interfere na abundância de artrópodes herbívoros e predadores sobre plantas de milho, *Zea mays* L. (Poaceae). Foram avaliadas 100 plantas, 50 isoladas de formigas e as outras 50 não isoladas. Foram observados 25 taxa de artrópodes sob plantas de milho sendo 14 de herbívoros e 11 de predadores. O livre acesso das formigas às plantas influenciou negativamente a abundância de *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae), um importante herbívoro praga da monocultura de milho. Nas plantas com formigas foi também observada maior abundância de *Doru* sp.1 (Dermaptera: Forficulidae), um importante inimigo natural de pragas em agroecossistemas. A abundância dos demais artrópodes não foi influenciada pela presença das formigas nas plantas avaliadas. Esses resultados sugerem que a interação formiga-planta-herbívoro em monocultura de milho é um sistema complexo, e que a presença de formigas predadoras sob plantas nem sempre interfere na presença de outros artrópodes.

Palavras chave: agroecossistema, controle biológico, herbívoros, insetos predadores, interação formiga-planta

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Introduction

Many studies have been developed in order to assess the efficiency of predatory arthropods as biological agents for biological control as an alternative method for chemical plague control in monocultures (Carvalho et al., 2013; Paredes et al., 2013;). In this context, ants stand out in this scenario due to their effectiveness as general predators, they influence the structure, composition and dynamics of arthropod communities in different environments (see Rico-Gray & Oliveira, 2007). In Europe, some studies have been made in order to assess the potential of ants as agents for biological control in pinus (Neuvonen et al., 2012) and olive orchards (Paredes et al., 2013) reforestation areas. Similar studies have been made in cotton (Kaplan & Eubanks, 2005; Wickings & Ruberson, 2011), corn (Knutson & Campos, 2008), cocoa (Philpott & Armbrrecht, 2006; Tadu et al., 2014), sugar cane (Souza et al., 2010) and orchards (Fernandes et al., 2012) in North and South America.

However, due to its aggressive behavior, ants can prey or repel other natural enemies of herbivorous and it causes negative indirect impact on plants protection against herbivorous (Kaplan & Eubanks, 2005; Powell & Silverman, 2010; Wickings & Ruberson, 2011). Therefore, results from these interactions do not always bring benefits to the involved plants and it shows limitations to the ant-plant interaction (Alves-Silva & Del-Claro 2016; Lange & Del-Claro, 2014).

Elements such as ant species features (Byk & Del-Claro, 2010; Calabuig et al., 2015), their density (De la Mora et al., 2015) and also herbivorous defense strategy against predation (Bächtold & Alves-Silva, 2013) have been pinpointed as a variance cause in plant-ant interaction results. Such fluctuation indicates that when species are placed inside a network of multitrophic interactions, it becomes harder to predict the ecological dynamics within these relationships (Jones et al., 2009).

Despite the importance of understanding the ecological potential features of generalist predatory insects – as in the case of some predatory ant species - in comparison to other populations, so far, studies are away from covering the whole context in which those interactions

happen. These studies may be considered rare, mainly in agro-ecosystem environments, and such fact makes harder to understand the real efficiency of ants as biological plague control agents. Thus, the current study aims to verify if the presence of ants on corn plants influences the amount of herbivorous and other predatory arthropods on these plants. It is expected that not only herbivorous, but also predators, are influenced by the presence of ants on plants; however, some species may be more influenced than others.

Material e Methods

Studied Area

The study was conducted in Dourados County, MS, Brazil (22°13'16"S and 54°48'20"W), from January to March 2005 in a 3.000 m² area of *Zea mays* L. (Poaceae) monoculture, using the no-tillage system. The climate in the area is Cwa (Dry humid subtropical climate, which has humid summer and dry winter) according to the Köppen classification (Fietz & Fisch, 2008).

Data collection

In this study were established 10 transects with 10 plants each. Each transect was composed of five plants from the control group, and five from the treatment group, interleaved. All plants were isolated by keeping one meter of distance between plants. In the treatment group plants, a 3 cm band of TJEATON'S® glue was placed on the main stem, 10 cm from the soil, to avoid ants foraging upon them. The glue was weekly renewed and always kept in the recommended condition to avoid ants passing on the top of the plants. Ants that were eventually found on the glue-isolated plants were manually removed. Ants were removed from the treatment group after the plants reached 30 cm height. During the assessment period, no herbicide or insecticide was applied to the studied area.

Data were weekly collected throughout a seven-week period, maintaining 50 plants of the control group (with access to ants) and 50 in the treatment group (without access to ants), totaling 100 sample units. All collections were performed between 04:00 and 06:00 pm. The number of individuals from each arthropod

species found on the plant was recorded, even the ants on plants in which the removal had not been performed yet. Only one individual of each ant specie/morphospecies was collected and put in alcohol 70% for further identification. These species samples were stored in the etymological collection of the insect ecology laboratory at Universidade Federal da Grande Dourados (UFGD). Efficiency data of the assessed plants were not collected due to the Psittaciformes infestation that took place days before harvest.

Data analysis

The mean amount of individuals found on each plant, during the seven assessments, were compared to that of plants with and without ant culture (n=50 to each group of plants). It was done to assess the influence of ants' presence on the amount of arthropods on corn. Subsequently, the arthropod communities were separated in two functional groups – predators (except ants) and herbivorous – and they were also compared to those of plants with and without ants. The

Mann-Whitney test (the U test) was used for these comparisons, since data did not present regular distribution – according to the normality test of Lilliefors, 5% level. It was also observed a correlation between the amount of herbivorous and predators and the amount of ants in the assessments – plants were the sample units (n=50 to each correlation) and Spearman was used. Results were considered statistically significant whenever they presented probability lower or equal to 5%.

Results

Ten (10) morphospecies of predatory ants – belonging to seven genus and four subfamilies (Table 1) - were collected during the whole assessment period. Among them, genus *Solenopsis* and *Brachymyrmex* were more frequent and represented 78.3% of ant frequency. The morphospecies *Solenopsis* sp.1 showed the highest relative frequency (56.6%) and it was followed by the genus *Brachymyrmex* sp.1 (18.9 %) (Table 1).

Table 1. Relative frequency of ant species found on the top of corn plants, *Zea mays* L. (Poaceae), in Dourados, MS, Brazil, from January to March 2005.

Subfamilies	Morphospecies	Relative frequency (%)
Myrmicinae	<i>Solenopsis</i> sp.1	56.6
	<i>Pheidole</i> sp.1	9.5
	<i>Pheidole</i> sp.2	1.9
	<i>Crematogaster</i> sp.3	1.9
	<i>Brachymyrmex</i> sp.1	18.9
Formicinae	<i>Brachymyrmex</i> sp.2	2.8
	<i>Camponotus crassus</i> Mayr, 1862	0.9
	<i>Camponotus</i> sp.2	3.8
Dolichoderinae	<i>Dorymyrmex</i> sp.1	2.8
Pseudomyrmicinae	<i>Pseudomyrmex</i> sp.1	0.9
Total		100

Twenty five (25) arthropod taxa (except ants) were found on the studied plants. Fourteen (14) of them were herbivorous insects that belong to five insect orders: Lepidoptera (2 taxa), Hemiptera (6 taxa), Coleoptera (4 taxa), Hymenoptera (1 taxon), and Orthoptera (1 taxon). The other 11 taxa were predators from six insect orders: Hemiptera (4 taxon), Coleoptera (2 taxa), Hymenoptera (1 taxon), Diptera (1 taxon),

Dermaptera (1 taxon), Neuroptera (1 taxon), and an order which belongs to the class Arachnida (See the identification of the arthropods on table 2).

Changes were not evident (U = 1188; p = 0,671 - Figure 1) when the mean amount of arthropods (except ants) was compared among plants with and without ants. Plants from the control group (with ants) presented mean

Table 2. The mean abundance (\pm standard error) of each arthropod taxon, except for the ants found on the top of plants with and without ants on the monoculture of *Zea mays* L. (Poaceae), in Dourados, MS, Brazil, from January to March 2005.

Functional groups/ Species	Stage	Without ants	With ants	Mann-Whitney	
				Test-value	p-value
Herbivores					
<i>Spodoptera frugiperda</i> (Smith, 1797)	Adult	-	-	-	-
	Larvae	0.04 \pm 0.014	0.049 \pm 0.019	1245	0.964
<i>Helicoverpa zea</i> (Boddie, 1850)	Larvae	0.063 \pm 0.23	1.1 \pm 0.08	1231	0.864
<i>Deois flavopicta</i> (Stal, 1854)	Adult	-	0.011 \pm 0.006	-	-
<i>Dalbulus maidis</i> (Delong & Wolcott, 1923)	Adult	0.689 \pm 0.095	0.689 \pm 0.093	1243	0.964
<i>Rhopalosiphum maidis</i> (Fitch, 1856)	Adult	15.260 \pm 3.702	9.566 \pm 2.075	1208	0.774
<i>Leptoglossus zonatus</i> (Dallas, 1852)	Adult	0.077 \pm 0.025	0.046 \pm 0.014	1153	0.373
	Nymph	0.017 \pm 0.008	0.063 \pm 0.024	1140	0.210
<i>Euschistus heros</i> (Fabrius, 1794)	Adult	-	0.003 \pm 0.003	-	-
<i>Nezara viridula</i> (Linnaeus, 1758)	Adult	0.003 \pm 0.003	0.014 \pm 0.008	1199	0.305
Lagriidae sp.1	Adult	-	0.003 \pm 0.003	-	-
<i>Sitophilus</i> sp.1	Adult	0.237 \pm 0.37	0.160 \pm 0.036	990.0	0.061
<i>Diabrotica speciosa</i> (Germar, 1824)	Adult	0.171 \pm 0.023	0.100 \pm 0.022	880.0	0.006*
<i>Astylus variegatus</i> (Germar, 1824)	Adult	0.017 \pm 0.008	0.023 \pm 0.009	1201	0.553
<i>Apis mellifera</i> Linnaeus, 1758	Adult	0.003 \pm 0.003	0.003 \pm 0.003	1250	0.989
Orthoptera sp.1	Adult	0.003 \pm 0.003	0.006 \pm 0.004	1225	0.568
Predators					
<i>Geocoris</i> sp.1	Adult	0.106 \pm 0.019	0.140 \pm 0.025	1117	0.326
<i>Orius</i> sp.1	Adult	0.246 \pm 0.033	0.183 \pm 0.029	1053	0.161
Reduviidae spp.	Adult	0.023 \pm 0.009	0.031 \pm 0.011	1219	0.730
<i>Nabis</i> sp.1	Adult	0.011 \pm 0.006	0.017 \pm 0.007	1200	0.511
	Adult	0.103 \pm 0.023	0.060 \pm 0.012	1138	0.372
Coccinellidae spp.	Nymph	0.037 \pm 0.019	0.006 \pm 0.006	1174	0.165
	Adult	0.029 \pm 0.010	0.037 \pm 0.012	1201	0.617
<i>Cycloneda sanguinea</i> (Linnaeus, 1763)	Adult	0.249 \pm 0.036	0.274 \pm 0.045	1248	0.989
<i>Telemonus</i> sp.1	Adult	0.014 \pm 0.006	0.006 \pm 0.004	1175	0.245
Syrphidae spp.	Adult	0.014 \pm 0.006	0.006 \pm 0.004	1175	0.245
<i>Doru</i> sp.1	Adult	0.754 \pm 0.080	1.023 \pm 0.095	930.5	0.027*
Chrysopidae spp.	Nymph	0.011 \pm 0.011	0.009 \pm 0.005	1202	0.330
Araneae	Adult	0.046 \pm 0.010	0.026 \pm 0.009	1079	0.107

* taxa with significantly different volume among the different plant groups ($p < 0.05$).

arthropods amount 18.21 ± 3.708 (\pm standard error) and plants from the treatment group (without ants) presented 12.59 ± 2.072 . Changes were also not evident ($U = 1160$; $p = 0.537$ - Figure 2a; $U = 1053$; $p = 0.174$ - Figure 2b) when the average of herbivorous and other predators were compared among plants with and without

ants. However, plants without ants had almost twice more herbivorous than plants with ants (116.04 ± 25.89 , 75.42 ± 14.49 , respectively). On the other hand, the average of predators was similar between plants with and without ants (12.68 ± 0.66 ; 11.10 ± 0.80 , respectively).

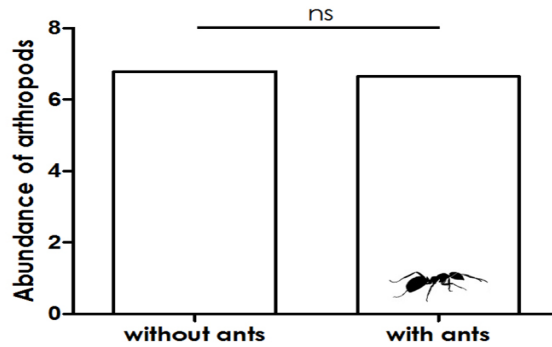


Figure 1. Mean abundance median of arthropods found on plants with and without ants in the monoculture of *Zea mays* L. (Poaceae), in Dourados, MS, Brazil, from January to March 2005. ($U = 1188$; $p = 0.671$)

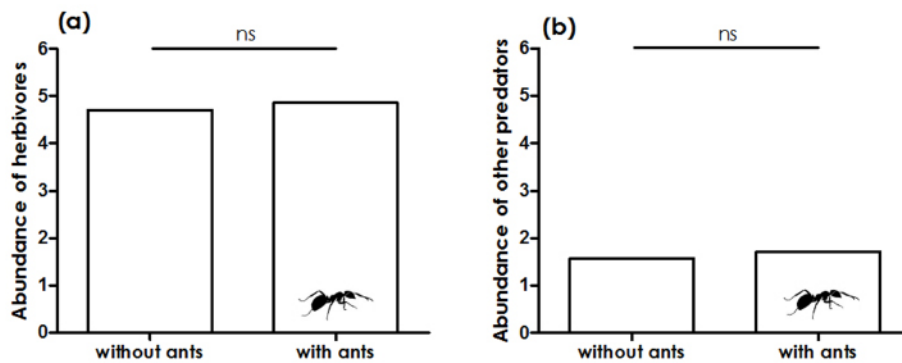


Figure 2. Mean abundance median of herbivorous (a) and of other predators (b) found on plants with and without ants in the monoculture of *Zea mays* L. (Poaceae), in Dourados, MS, Brazil, from January to March 2005. ($U = 1160$ $p = 0.537$ - Figure 2a; $U = 1053$ $p = 0.174$ - Figure 2b)

A non-significant and negative connection was observed ($R_s = -0.1635$ $p = 0.256$) between mean abundance of herbivorous and ants on plant with ants. A similar result was presented when the connection between the mean abundance of other predators and ants on plants with ants was assessed ($R_s = -0.1981$ $p = 0.172$).

When the mean abundance of each taxon was separately compared among plants with and without ants, the species *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) was the only one from the functional group of herbivorous that presented significantly different values among the plant groups (see Table 2). Therefore, plants with ants presented greater abundance of *D. speciosa* individuals than plants without them.

Trophobiosis cases between Hemiptera species and ants were observed during the study performance. The following hemipterous presented such behavior: *Deois flavopicta* (Stal, 1854) (Hemiptera: Cercopidae), *Dalbulus maidis* (Delong & Wolcott, 1923) (Hemiptera: Cicadellidae) and *Rhopalosiphum maidis* (Fitch,

1856) (Hemiptera: Aphididae). Nevertheless, changes in the mean abundance of these organisms among plants with and ants were not noticed (see Table 2).

Among the taxa in the group of predators, *Doru* sp.1 (Dermaptera: Forficulidae) was the only one that presented abundance levels significantly different among plants with and without ants. Thus, a greater abundance of that specie was observed on plants with ants.

Discussion

Only two species of arthropods (*Diabrotica speciosa* and *Dorus* sp.1) were negatively influenced by the presence of ants on plants of *Z. mays*. The herbivorous *D. speciosa* was found in greater abundance in plants without ants. According to Kalsi et al. (2014), *D. speciosa* is a polyphagous insect that is considered to be a plague in monocultures with great economic importance, such as corn, soy, bean and rice. *Diabrotica speciosa* adults basically feed on leaves and stand on the ground. The larvae damage the root system of the plants and affect the absorption of water and nutrients (Marodim et al., 1998). According to the results of this

study, individuals from this herbivorous species prefer plants without ants or, on the other hand, ants would be preying these herbivorous on corn plants. Regardless the presented result, it is possible to assert that, in this case, ants that patrol the corn plants work as their plants' protectors and it leads to decrease in the presence of *D. speciosa* on them.

The current study also confirmed that plants with ants had higher mean abundance of the predatory species *Dorus* sp.1. This result may be due to generalist habits of species of the order Dermaptera. Similarly to ants, Dermaptera species also are predators. Therefore, the presence of ants may have attracted Dermaptera individuals to plants of control group. Even though there was not an evidence of ants predation by *Dorus* sp.1 individuals, the presence of these two predator groups on plants may have caused stress on both. In contrast, the presence of these predators or their free access to plants may have increased the protection of these plants against herbivorous. According to Figueiredo et al. (2006), in a study performed with *Dorus luteipes* (Scudder, 1876) (Dermaptera: Forficulidae), these insects may be important *Spodoptera frugiperda* infestation (J.E. Smith, 1797) (Lepidoptera: Noctuidae) – an important plague in corn culture - controller when they are put together with other predators. Other study showed that Dermaptera of genus *Dorus* are efficient herbivorous predators in corn monoculture (see Cruz 1991). Additionally, Buschman et al. (1977) observed that Formicidae and Dermaptera were the most frequent predators on plants in soy monocultures. Both predators worked as plague insect population controllers. Therefore, according to authors, some predator species – as those in the genus *Dorus* – may be influenced by the presence of ants. However, further studies should be performed in order to verify the predation taxa among these two predator groups (Formicidae and dermapterous of genus *Dorus*).

According to the present study, the mean abundance of herbivorous on plants with ants was higher in comparison to plants without ants, although this difference was not significant. Therefore, ants helped to decrease the amount

of herbivorous on corn plants evaluated in this study. This result can be applied to the herbivorous *Rhopalosiphum maidis* and *Sitophilus* sp.1 – besides *D. speciosa*, as mentioned before. On the other hand, predatory arthropod communities in general were not influenced by the presence of ants on the plants. However, some species such as *Orius* sp.1 and *Coccinelidae* spp. presented higher mean abundance on plants without ants. *Dorus* sp.1 presented higher abundance on plants with ants, as it was previously mentioned. Such results showed the uniqueness among species in the same guild.

Evident negative correspondence was not shown among ants, herbivorous and the amount of predators on plants. These results show that the abundance of ant species found on the studied plants was not a key factor to influence the presence of other arthropods. Likely, other factors, such as species identity, herbivorous and predators - and their predation and defense strategies - may have been important.

Some studies have shown that the result of ant-plant interaction is dependent of the species of predatory ants involved interaction (Byk & Del-Claro, 2010; Calabuig et al., 2015) and of the strategies of herbivorous against predation (Bächtold & Alves-Silva, 2013; Carita et al., 2006; Styrsky & Eubanks, 2010). Nevertheless, little is known about the behavior between pairs (predator-prey) determining plant protection.

The two most frequent ant species on plants in the current study were *Solenopsis* sp. 1 and *Brachymyrmex* sp.1. The first one belongs to a genus of efficient predatory ants (Styrsky et al., 2006). However, according to Silvestre et al. (2003), the foraging activity of the species *Solenopsis* happens under the soil. This species belongs to a guild of soil dominant omnivorous. In contrast, species of the genus *Brachymyrmex* belong to an opportunistic guild on the soil and on the vegetation. Species in this genus do not present dominant aggressive behavior regarding food resources (Silvestre et al., 2003). Other ant species found in the present study presented frequency lower than 10%. It may reflect *Solenopsis* prevalence on soil and on the plants, it may have prevented that other ant species from climbing on the plants. The aggressiveness of ant

species, such as *Solenopsis* genus may generate hierarchy dominance, when some ant species dominate over others. This scenario create the *ant mosaic* (sensu Ribeiro et al., 2013), and it is considered to be one of the basic factors for ant communities (Blight et al., 2014).

Conclusion

According to the results, is possible to observe the complexity of the ant-plant-herbivore interaction. Although not significant, the mean herbivorous abundance on plants without ants was high. On the other hand, many herbivorous were not influenced by the absence of ants on the plants. A similar result was shown for predatory arthropods and some of them may have been attracted by the presence of ants. Therefore, the complexity for this multitrophic interaction does not allow to state that ants are efficient agents for biological control. Further studies on this pair-to-pair interaction among the species involved in these process need to be performed to better understand the features and behaviors of these species that could influence the results of these ecological connections.

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