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Ant diversity (Hymenoptera: Formicidae) and predation by ants on the different stages of the sugarcane borer life cycle *Diatraea saccharalis* (Lepidoptera: Crambidae)

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Abstract. The sugarcane borer *Diatraea saccharalis* is an important pest of sugarcane and ants are one of its main predators. The practice of burning sugarcane straw in situ after harvest has been gradually replaced in Brazil by other practices. However, it is unknown whether ants can control the abundance of this borer in the presence of straw. In this study, we assessed the diversity and species composition of ants attacking different stages of the pest's life cycle. Specifically, we asked whether the species richness and abundance of ants varies during the course of day and a year. We established one-hectare plots at random locations in a sugarcane plantation. Once a month, we collected 20 samples of each stage of the *D. saccharalis* life cycle and randomly distributed these samples as bait on plants spaced 20 m apart within a plot. Ants were collected daily in the morning and afternoon over a period of 12 months. We identified several aspects of ant feeding behaviour that may affect their biological control of the borer: (1) the greatest number of ants were collected from baits consisting of the immature stages of the sugarcane borer, (2) ants were most active in the morning and (3) their activity varied from month to month. *Solenopsis saevissima* and morphotypes of *Crematogaster* sp.7 and *Pheidole* sp.35 are potentially important predators of borers in sugarcane crops in which the straw is not burnt.

INTRODUCTION

Ants have important functional roles in ecosystems (Risch & Jurgensen, 2008; McKey et al., 2010), including nutrient cycling (Hölldobler & Wilson, 1990; Folgarait, 1998) and biotic interactions (Schultz & McGlynn, 2000). Several studies have documented the diversity (Delabie et al., 2007; Philpott et al., 2008a; Mentone et al., 2009; Teodoro et al., 2011) and importance of ants in agroecosystems (Peck et al., 1998; Philpott & Armbrecht, 2006; Philpott et al., 2008b; Larsen & Philpott, 2010), including the cultivation of sugarcane (Rossi & Fowler, 2004; Souza et al., 2010).

The sugarcane borer, *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), is found in the West Indies and Central and South America (Pinheiro et al., 2008). It is one of the main pests of sugarcane (Gallo et al., 2002; Beuzelin et al., 2011) as it can cause a considerable reduction in sugar yields (White et al., 2008). The tunnels excavated by this borer in the stalks cause bud death and germination failure (Bortoli et al., 2003; Portela et al., 2010) and facilitate the invasion of plant pathogens, such as *Fusarium moniliforme* (Sheldon) and *Colletotrichum falcatum* (Went), which reduce the yield of sugar (Macedo & Botelho, 2002; Branco et al., 2010).

Ants and spiders are known to capture and kill the caterpillars of *D. saccharalis* before they burrow into the stems of sugarcane (Ali & Reagan, 1986; Reagan, 1986; Rossi & Fowler, 2004; Beuzelin et al., 2009) and larvae inside the plant are attacked by parasitoids (Botelho et al., 1999; Botelho & Macedo, 2002), especially *Cotesia flavipes* (Cameron, 1891; Hymenoptera: Braconidae).

Several studies have demonstrated that ants can be key generalist predators of crop pests (Majer, 1976; Way & Khoo, 1992; Symondson et al., 2002; El Keroumi et al., 2010; Fernandes et al., 2010; Sanders & Van Veen, 2011) because they (1) can respond to changes in the type and abundance of food sources via recruitment systems that amplify and enhance functional responses; (2) can persist as predators by attacking a wide range of different prey populations; (3) are efficient predators independent of their population size and (4) can be managed to maximize pest contact (Risch & Carrol, 1982; Way & Khoo, 1992).

In order to continue to meet the goals of economic development, sustainable resource use and environmental preservation the high productivity, quality and competitiveness of sugarcane crops require ongoing planning and technological advances. To this end, manual harvesting has been gradually replaced by a mechanical harvesting in Brazilian sugarcane plantations, because straw burning

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Table 1. The number of ants of different species recorded in the morning and afternoon at baits consisting of different stages of the life cycle of *Diatraea saccharalis*. Morphospecies numbers refer to the Formicidae collection of Alto Tietê.

Species / Morphospecies	Morning			Afternoon		
	Egg	Immature	Adult	Egg	Immature	Adult
Dolichoderinae						
Dorymyrmex brunneus (Forel, 1908)	1	2	1	–	26	–
Linepithema humile (Mayr, 1868)	–	147	–	–	12	–
Formicinae						
Brachymyrmex incisus (Forel, 1912)	–	–	–	2	–	–
Camponotus crassus (Mayr, 1862)	2	2	–	1	2	–
Camponotus melanoticus (Emery, 1894)	2	2	–	–	–	–
Camponotus novogranadensis (Mayr, 1870)	13	2	45	3	6	–
Camponotus rufipes (Fabricius, 1775)	–	–	2	–	–	–
Camponotus sp.19	1	–	–	–	–	–
Nylanderia fulva (Mayr, 1862)	2	63	18	–	25	–
Myrmicinae						
Crematogaster sp.7	136	845	330	13	85	21
Pheidole sp.35	80	748	270	6	362	11
Solenopsis saevissima (Smith F., 1855)	16	43	26	–	137	4
Ponerinae						
Ectatomma brunneum (Smith F., 1858)	–	3	–	–	–	–
Pseudomyrmecinae						
Pseudomyrmex sp.3	2	–	–	–	–	–
Pseudomyrmex sp.7	1	–	–	1	–	-
Number of species of ants	11	10	7	6	8	3
Total number of ants	256	1,857	692	26	655	36
Total richness			15			
Overall total number of ants			3,522			

is environmentally damaging, especially for insects (Araújo et al., 2005). The permanent presence of straw provides a favourable habitat for the predators of *D. saccharalis*. Thus we assessed the diversity and species composition of ants feeding on the different stages of the pest. Specifically, we asked whether the species richness and abundance of ants varies temporally, both daily and annually. Our results will help in the development of more effective ways of reducing the abundance of sugarcane borer in crops where straw is present.

MATERIAL AND METHODS

Study area

The study area is a sugarcane plantation at the Research Station of the Sugarcane Technology Center – "Estação Experimental do Centro de Tecnologia Canavieira" (22°41′50.9″S; 47°33′21.4″W), in Piracicaba (SP, Brazil). In this area, we planted the CTC15 variety of sugarcane at the beginning of the year after preparing the soil using conventional techniques (i.e., removing the surface layer and fertilizing at the time of planting). The experiment began five months after the first sugarcane was cut, which was at the tillering phase. During the experiment the area was not treated chemically, harvesting was performed mechanically and the straw was left on the ground.

Sample design

We established one-hectare plots at random sites within the plantation**.** Each month, 20 baits consisting of individuals at each stage in the life cycle of *D. saccharalis* were distributed in each plot. We distributed the individuals at random on the leaves of plants spaced 20 m apart (Bacaro et al., 2011). This procedure was continued for 12 months. We produced different stages of the borer's life cycle at the Sugarcane Technology Center as follows: (1) eggs – four egg masses containing 30 eggs each were placed in a perforated Falcon tube (diameter = 2 mm) the perforations of which resemble those made by the borer; (2) immature borers – two third-instar larvae and one pupa were placed in a perforated Falcon tube; (3) adults – two fertilized female borers were placed in a small cage built from 2 mm mesh netting, which was attached to a plant for 24 h.

We collected ants attacking eggs and immature individuals twice a day, at a particular time in the morning and afternoon, for seven days. However, the collection of ants attacking adult borers was limited to two days. We only collected ants that were tearing off small pieces or had a remnant of integument of adult sugar borers in their jaws. Eggs, immatures and adults, were replaced when damaged or eaten by ants. Ants were preserved in 70% ethanol until identified.

Identification of ants

We initially classified the samples into subfamilies following Bolton (2003) and then identified them to genus following Bolton (1994), except for the group *Prenolepis*, for which we followed the classification of Lapolla et al. (2010). We then sorted the ants to morphospecies/species, comparing them with the Formicidae in the collection of Alto Tietê. The numbering of taxa follows this collection. Vouchers were deposited at the University of Mogi das Cruzes (SP) and Museum of Zoology, University of São Paulo.

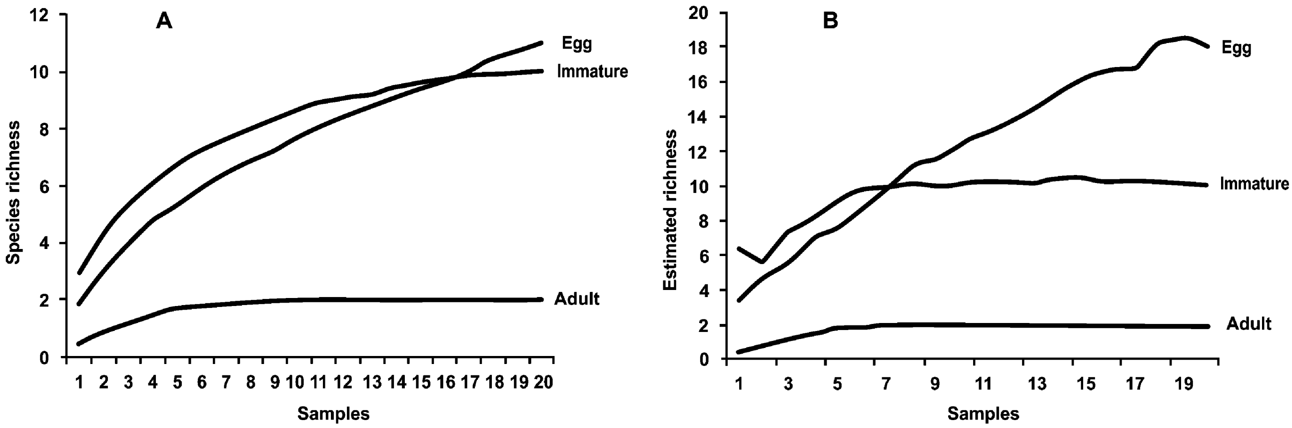

Fig. 1. Curves of species accumulation and estimated richness for different stages of the sugarcane borer.

Data analysis

Species richness was measured as the number of species recorded, abundance as the total number of individuals collected at baits. Relative frequency was used to classify ant species as uncommon (< 25% of all ant individuals), common (25 to 75%) and very common (> 75%) (Tanaka & Pereira, 1990).

Patterns of species richness of ants attacking egg, immature and adult baits were compared using sample-based rarefaction curves constructed using EstimateS version 7.5.2 (Colwell, 2005). Matrices of species by stage were constructed, and filled with occurrence data (presence = 1, absence = 0). One occurrence was defined as presence of at least one individual in each stage, no matter how many individuals were present. Estimated species richness was calculated for each stage using EstimateS version 7.5.2, using Chao2 (Colwell, 2005).

We tested whether species richness and abundance of ants attacking eggs and immature *D. saccharalis* varied during the day or from month to month during a year using Mann-Whitney U-tests (Ayres et al., 2007) and G-tests (Sokal & Rohlf, 1981). For the analysis of similarities between stages, we constructed a presence/absence matrix using the data from the first two days of the experiment. The species composition analysis was conducted using Bray-Curtis dissimilarity index (Oksanen et al., 2009).

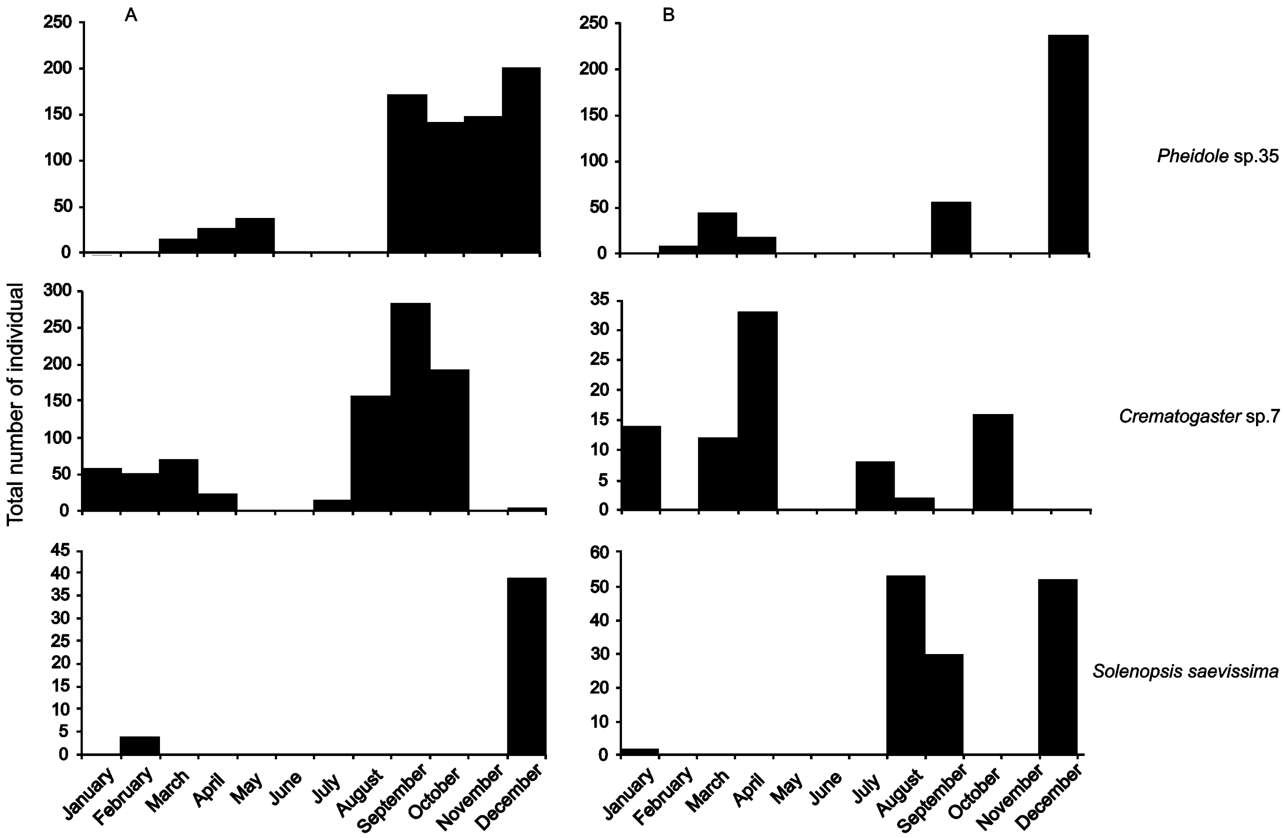

Fig. 2. Seasonal activity of three ants species foraging for different stages of the sugarcane borer in different months and at different times of a day (A – morning; B – afternoon).

TABLE 2. Co-occurrence of different species of ants recorded at baits of eggs, immatures or adults of the sugarcane borer.

	Camponotus rufipes	*Nylanderia fulva*	*Crematogaster* sp.7	*Pheidole* sp.35	*Solenopsis saevissima*
Dorymyrmex brunneus		1* immature		1 immature	1 immature
Linepithema humile				1 immature	
Camponotus crassus				1 immature	
Camponotus melanoticus			1 immature		1egg
Camponotus novogranadensis	1 adult	1 immature	1 egg 3 immature 2 adult	1 immature 1 adult	1 egg
Nylanderia fulva			1 immature	3 imature	
Crematogaster sp.7				9 imature 4 adult	1 adult
Pheidole sp.35					2 immature

* Number of times when the species co-occurred at baits consisting of a particular stage in the life cycle of the sugarcane borer.

TABLE 3. Abundance and species richness of ants collected at baits of eggs and immatures of *Diatraea saccharalis* at different times of the day.

Stage	Abundance			Richness		
	Morning	Afternoon	*p*	Morning	Afternoon	*p*
Eggs	258	24	0.0043*	11	6	0.0531^{ns}
Larvae	1,857	655	0.0531^{ns}	10	8	0.6442^{ns}
Total	2,115	679	0.0179*	14	10	0.3263^{ns}

*Significant difference (Mann-Whitney; $p < 0.05$); ^{ns} not significant.

RESULTS

We collected a total of 3,522 ants, belonging to 5 subfamilies, 10 genera and 15 species (Table 1). Curves of species accumulation and estimated richness indicated a plateau, except for those attacking the eggs of the sugar borer (Fig. 1).

The most abundant subfamily was Myrmicinae with 3,133 individuals, and the most species-rich was Formicinae with seven species. Most (morpho-) species were uncommon. *Pheidole* sp.35 was common at both periods of the day sampled, and C*rematogaster* sp.7 was observed only in the morning. These species were the most abundant predators of all stages of the *D. saccharalis* life cycle (Table 1).

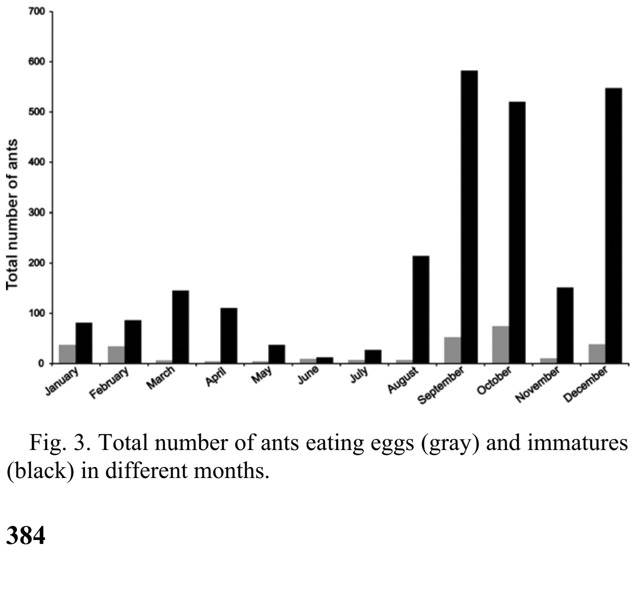

Fig. 3. Total number of ants eating eggs (gray) and immatures (black) in different months.

The immature stages of the life cycle attracted the largest number of ant workers, primarily of *Pheidole* sp.35, C*rematogaster* sp.7 and *Solenopsis saevissima* (Smith F., 1855) (Table 1). A comparison of the numbers of ants at baits consisting of immature stages of the borer suggests that *Pheidole* sp.35 and *Crematogaster* sp.7 occur relatively constantly throughout the year, and both are more abundant in the morning. *S. saevissima* is more abundant in the afternoon but occurs sporadically throughout the year. These species clearly forage at different times of the day (Fig. 2). *Pheidole* sp.35 and *Crematogaster* sp.7 were often recorded in the same samples, especially those collected from baits consisting of immature individuals (Table 2).

On egg baits, ants were most abundant in the morning and on those with immature individuals equally abundant in the morning and afternoon (Table 3). Unlike species richness (G = 1.14; $p > 0.05$), the number of ants collected at baits consisting of eggs and immature borers varied throughout the year (G = 85.92; $p < 0.0001$). We recorded a large number of worker ants feeding on immature borers, particularly between August and December (Fig. 3). Species compositions of ants at baits consisting of eggs and immature individuals of *D. saccharalis* were more similar to each other than those feeding on adult *D. saccharalis* (Fig. 4).

DISCUSSION

This study of ants feeding on eggs and immature borers of *D. saccharalis* revealed that, in plantations where the sugarcane straw is left on the ground in the crop, the immature stages of the borer prior to boring into the stems of sugarcane are at risk of being eaten by ants as:

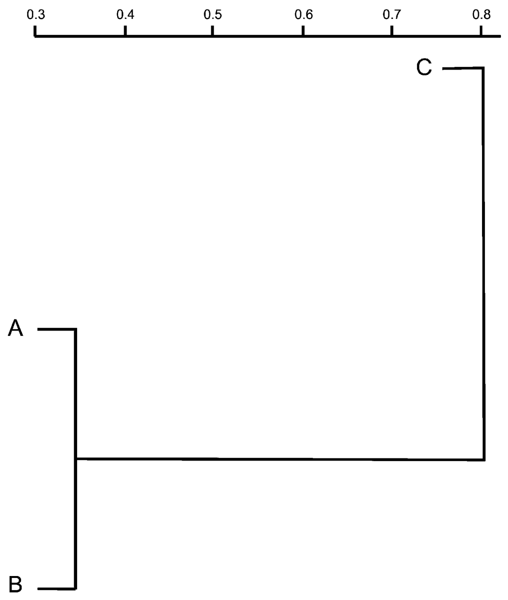

Fig. 4. Bray-Curtis similarity dendrogram based on the species composition of ants collected at baits made up of eggs (A), immature individuals (B) and (C) adults of the sugar cane borer, *Diatraea saccharalis*.

(1) baits of immature borers attract a greater number of ants; (2) ants are most abundant in the morning, both at baits consisting of eggs and immature individuals; (3) ant abundance varies from month to month and (4) *Crematogaster* sp.7, *Pheidole* sp.35 and *S. saevissima* are potentially important predators, given that they are commonly used to control pests (Way & Khoo, 1992; Van Mele & Cuc, 2000; Dejean et al., 2003).

Solenopsis (Way & Heong, 2009; Pereira et al., 2010), *Crematogaster*, *Pheidole* (Fowler, 1993; Pereira et al., 2004; Philpott et al., 2008c; Schatz et al., 2008), *Ectatomma*, *Gnamptogenys* and *Dorymyrmex* species control *D. saccharalis* populations (Sousa-Silva et al., 1992; Rossi & Fowler, 2004). However, this study shows that *Dorymyrmex brunneus* (Forel, 1908) is of little importance in the biological control of borers, as it was infrequently observed attacking the different stages of the *D. saccharalis* life cycle.

S. invicta is considered to be an important predator of *D. saccharalis* (Reagan, 1986; Rossi & Fowler, 2004; Beuzelin et al., 2009). However, it can also prevent the establishment of *C. flavipes* (White et al., 2004), a microhymenopteran species that is very effective in controlling the borer (Lv et al., 2011). *S. invicta* also causes serious allergies among plantation workers (Fernández-Meléndez et al., 2007; More et al., 2008). Identification of other ant species that may potentially prey on *D. saccharalis* may therefore be important for the management of pests of sugarcane.

The high levels of foraging by most species of ants recorded in the morning may be related to abiotic factors (Kaspari & Weiser, 2000), primarily temperature and relative humidity (Hölldobler & Wilson, 1990; Kaspari & Valone, 2002; Sabu et al., 2008). *Crematogaster* sp.7 and *Pheidole* sp.35 may have similar environmental requirements, as they forage at similar times each day and throughout the year, unlike *S. saevissima*. Furthermore, *Crematogaster* sp.7 and *Pheidole* sp.35 were often both present at the same baits. As the three most abundant genera are territorial (Silvestre et al., 2003) temperature may limit the dominance of one taxon over another at a particular food source (Hunt, 1974; Fellers, 1989; Moutinho, 1991).

In southeastern Brazil, the caterpillars of *D. saccharalis* are most abundant between September and October, if sugarcane is planted at the beginning of a year (Botelho et al., 1993; Botelho & Macedo, 2002). However, the greatest numbers of ants attacking immature *D. saccharalis* were recorded between August and December. This may be determined by the population dynamics of *D. saccharalis*, which varies depending on the variety of sugarcane (Botelho & Macedo, 2002). Regardless of the month of the year, ants visited more baits consisting of immature stages of the borer, but eggs are the stage most frequently attacked in crops with straw burning (Botelho & Macedo, 2002).

Ants play an important role in reducing the numbers of eggs and immatures in sugarcane crops over a period of one to two weeks. During this period, eggs and immatures are more exposed to natural enemies. Inside the stem of sugarcane the percentage mortality of the immature stages is much lower, especially during the pupal stage (Botelho & Macedo, 2002).

Given the importance of *D. saccharalis* as a pest and the problems inherent in producing and efficiently applying chemical control agents (Gallo et al., 2002) we favour control methods that use natural predators. The results of this study show that ants are potential predators of the sugarcane borer, particularly the immature stages. However, it is necessary for better crop management to (1) better understand the processes determining ant community structure in sugarcane crops where the straw is not burnt and (2) identify the morphospecies of ants present.

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