Sociobiology 61(2): 136-144 (June, 2014) DOI: 10.13102/sociobiology.v61i2.136-144

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Response of Ants to the Leafhopper *Dalbulus quinquenotatus* DeLong & Nault (Hemiptera: Cicadellidae) and Extrafloral Nectaries Following Fire

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Article History
Edited by
Gilberto M M Santos, UEFS, Brazil
Received 16 March 2014
Initial acceptance 24 April 2014
Final acceptance 16 May 2014

Keywords
Acacia pennatula, five-spotted gamagrass leafhopper, *Tripsacum dactyloides*, mutualism

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Abstract

Previous investigations of mutualistic associations between ants and plants bearing extrafloral nectaries (EFNs) or between ants and trophobiont leafhoppers have studied these relationships separately, but nothing is known on how ant abundance responds to these two food resources occurring in the same habitat when that habitat is disturbed by fire. The objectives of this study are to document ant abundance with the trophobiont five-spotted gamagrass leafhopper, *Dalbulus quinquenotatus* DeLong & Nault, and with EFNs on trees of *Acacia pennatula* (Schlecht & Cham.) Benth. (Fabaceae) that occur in the same habitat, and how ant abundance in both of these mutualisms is affected after disturbance by fire. This study was performed at several sites in central Mexico where the perennial gamagrass *Tripsacum dactyloides* L. (Gramminae) and *A. pennatula* both occur. More ants were collected in association with the leafhopper *D. quinquenotatus* than with EFNs of *A. pennatula*. At sites where dry season fire occurred, new green leaves were produced by both *T. dactyloides* and *A. pennatula* after the burn. On these new leaves after fire, significantly more ants tended *D. quinquenotatus* leafhoppers on *T. dactyloides* than visited EFNs on *A. pennatula*. In burned sites the ants *Anoplolepis gracilipes* Smith, *Brachymyrmex obscurior* Forel and *Pheidole* sp. live in association with the leafhoppers, whereas EFNs on *A. pennatula* were associated with the ants *A. gracilipes*, *B. obscurior*, *Camponotus* sp., *Crematogaster* sp. and *Solenopsis* sp.

Introduction

Ants (Hymenoptera: Formicidae) often live in mutualistic relationships with trophobiont insects that excrete honeydew, or with plants bearing extrafloral nectaries (EFNs) that produce nectar (Hölldobler & Wilson, 1990). Some species of aphids, whiteflies, scale insects, mealybugs, treehoppers, and leafhoppers (Hemiptera) live in facultative or obligatory mutualistic relationships with ants (Way, 1963; Buckley, 1987; Blüthgen et al., 2006; Gibb & Cunningham, 2009; Fagundes et al., 2013). In these associations, the insect provides honeydew, a sugary excretion of carbohydrates, amino acids, and water for the ants, whereas ants protect the hemipterans from natural enemies (Delabie, 2001; Heil & Mckey, 2003; Zhang et al., 2012; Zhang et al., 2013). Plants in over one hundred families bear EFNs that produce secretions rich in sugars, amino acids, and lipids that attract ants, and in return these ants protect those plants from herbivores (González-Teuber & Heil, 2009; Byk & Del-Claro, 2011; Marazzi et al., 2013; Weber & Keeler, 2013).

Ants are attracted to high quality sugar resources as food (Heil & McKey, 2003). Previous studies have shown that when both honeydew and extrafloral nectar are offered to ants, ants are more abundant at the honeydew rather than at exudates of EFNs (Fiala, 1990; Rashbrook et al., 1992; Del-Claro & Oliveira, 1993; Blüthgen et al., 2000; Katayama et al., 2013). Ants were more abundant tending the hemipterans, particularly when greater numbers of hemipterans are present because of the larger quantities of honeydew produced (Katayama & Suzuki, 2010). Blüthgen et al. (2000) found greater numbers of ants at honeydew resources as opposed to EFN resources because honeydew is apparently a higher quality

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

food resource, rich in amino acids. Moreover, Katayama and Suzuki (2003) demonstrated that if an aphid colony increases in size, ants stop using EFNs and strengthen their mutualistic association with aphids.

Fire affects the growth of plants because some perennial species such as grasses and plants bearing EFNs quickly re-grow after disturbance occurs. New leaves formed after the plants burn are ready to be colonized directly or indirectly by ants, often attracted to food resources such as the honeydew produced by leafhoppers that feed on young grasses (Moya-Raygoza, 1995) or from nectar produced by EFNs (Alves-Silva & Del-Claro, 2013). Ants respond to burned plants with EFNs or hemipterans in the same way. The abundance of ants increased on the shrub *Banisteriopsis campestris* (A. Juss.) which bears EFNs after fire, mainly because of concentrated extrafloral nectar (Alves-Silva & Del Claro, 2013). Alves-Silva (2011) and Koptur et al. (2010) also found a richer ant community guarding plants from herbivory after fire because of the production of extrafloral nectar. Similarly, higher numbers of ants were found tending the honeydew-producing fivespotted gamagrass leafhopper, *Dalbulus quinquenotatus* DeLong & Nault, after its host plant, the perennial gamagrass *Tripsacum dactyloides* L. (Gramminae), was burned (Moya-Raygoza, 1995).

Mutualisms between ants and EFNs-bearing plants and ants and trophobiont hemipterans have been investigated separately after disturbance by fire, but little is known how ant abundance responds to these two food resources when present in the same habitat. This study was performed in central Mexico, where the perennial gamagrass *T. dactyloides* hosts *D. quinquenotatus* leafhoppers and trees of *Acacia pennatula* (Schlecht & Cham.) Benth. (Fabaceae) with EFNs occur together in the same habitats (Fig. 1a). These sites often are accidentally burned, and the fire often kills or drives away insects living on those plants. *Dalbulus quinquenotatus* lives on the basal leaves of *T. dactyloides* in an obligatory mutualism with tending ants (Larsen et al., 1991). Ants tending *D. quinquenotatus* receive honeydew and protect this leafhopper from natural enemies (Moya-Raygoza & Nault, 2000). In contrast, *A. pennatula* have EFNs and live in a mutualistic relationship with ants (Moya-Raygoza, 2005), providing nectar for the ants in return for protection from herbivory.

Fire is an important abiotic factor in mutualisms because it affects plant re-growth and the abundance of ants that depend on exudates produced indirectly by trophobiont insects and directly by EFNs. When fire consumes the foliage of both *T. dactyloides* and *A. pennatula*, the mutualisms involving ants with both species are temporarily disrupted. However, only a few days after being burned, new leaves of both plant species begin to re-grow (Fig. 1b) and are soon colonized by *D. quinquenotatus* and ants in the case of *T. dactyloides*, or by ants visiting EFNs in the case of *A. pennatula*. Measuring the total abundance of ants collecting honeydew from *D. quinquenotatus* and visiting EFNs resources before and after the host plants are burned helps us understand the ecological importance of mutualisms that can be strong driving forces for community organization (Wimp & Whitham, 2001). The objectives of this study are to document ant abundance with *D. quinquenotatus* leafhoppers and EFNs in the same habitat, and how ant abundance in both of these mutualisms is affected after disturbance to their habitat by fire.

Materials and Methods

Study system

Nine field sites containing both *T. dactyloides* and *A. pennatula* were chosen for this study. Each site had both species of plant present and covered an area of 0.05-0.25 ha. All sites were in the state of Jalisco in Central Mexico. The sites were: 1) El Arenal: 1,501 m elev, 20°46.032´N, 103°40.766´W; 2) Los Chorros: 1,371 m elev, 20°41.211´N, 103°41. 558´W; 3) San Isidro: 1,266 m elev, 20°49.014´N, 103°20. 262´W; 4) Agua Caliente: 1,385 m elev, 20°25.770´N, 103°41.485´W; 5) Cocula: 1,273 m elev, 20°25.595´N, 103°44.601´W; 6) San Agustin: 1,638 m elev, 20°30.682´N, 103°28.796´W; 7) La Mimila: 1,649 m elev, 20°44.411´N, 103°37.686´W; 8) El Molino: 1,608 m elev, 20°23.938´N, 103°32.760´W; and 9) Zapopan: 1,631 m elev, 20°44.283´N, 103°30.805´W (Fig. 1c). The closest sites were 5.45 km apart (Agua Caliente and Cocula) while the most distant sites (San Isidro and Cocula) were 60.44 km apart. All sites had similar habitat characteristics and belong to pine-oak ecosystem (Rodríguez-Trejo & Myers, 2010). Plants of both species live on steep slopes or beside roadways and grow on limestone soils (Wilkes, 1972). The sites had similar vegetation consisting of a plant community containing *T. dactyloides* interspersed with *A. pennatula* trees and few other plants such *Lysilona* sp. Each *T. dactyloides* population was composed of scattered clumps consisting of clusters of stems. *Tripsacum dactyloides* can use rhizomes to spread across the landscape and does not possess extrafloral nectaries. Moreover, ants are present on *T. dactyloides* only when the plants are hosts for *D. quinquenotatus* leafhoppers as compared with plants without *D. quinquenotatus* (Larsen, et al. 1991).

All sites were sampled to determine the numbers of ants when leafhoppers and EFNs were available. *Acacia pennatula* has actively secreting extrafloral nectaries on young leaves primarily from April to June (McVaugh, 1987; Moya-Raygoza, 2005), whereas leafhoppers are present on *T. dactyloides* primarily during the wet season from June to September (Moya-Raygoza, 1995) when these habitats are not burned. We observed that when the habitats were burned, both plant species started to produce new green leaves within several days, and this altered the food resources available for visiting ants. Fires generally occur from March to May towards the end of each dry season. The dry season in Jalisco generally occurs from October to May and is characterized by lower rainfall, lower temperatures and shorter days as compared with the wet season which typically lasts from June to September (Mosino-Aleman & Garcia, 1974). After burning, both honeydew and EFN nectar food resources for ants are found in May and June within the same plant community. The highest nectar secretion rates have been documented from EFNs on young leaves of damaged plants (Heil et al., 2004), while high numbers of *D. quinquenotatus* leafhoppers have been found on *T. dactyloides* after fire (Moya-Raygoza, 1995). No data were collected between October and April because ants do not visit either of these food resources during that time. The wet season begins in June, and no fires occur once the rains begin to fall.

Fig 1. Tritrophic interaction trophobiont five-spotted gamagrass leafhopper-Ants-*Acacia pennatula*. A) Hillside in Jalisco, Mexico late in the dry season covered with *T. dactyloides* hosting *D. quinquenotatus* leafhoppers, interspersed with trees of *A. pennatula* bearing EFNs in unburned site. B) Young green leaves growing on *A. pennatula* (left) and *T. dactyloides* (right) several days after being burned by fire. C) Location of field sites containing both *T. dactyloides* and *A. pennatula* from the state of Jalisco in central Mexico.

Sampling

We confirmed the presence of ants associated with EFNs of *A. pennatula* and *D. quinquenotatus* at each site. Once these fires took place, we sampled ants on burned and unburned sites. We selected *A. pennatula* trees at each site and neighboring clumps of *T. dactyloides*. Ten terminal branches on each selected *A. pennatula* tree and one basal leaf from each of ten different *T. dactyloides* clumps were randomly selected. Terminal branches of *A. pennatula* were selected because the highest concentration of EFNs occurs on these branches, whereas basal leaves of *T. dactyloides* were selected because this is where the highest numbers of *D. quinquenotatus* are found. We collected all nymphs and adults of *D. quinquenotatus* leafhoppers and all tending ants from the basal 10 cm of each selected *T. dactyloides* stem.

All EFNs were counted and ants collected from the terminal 10 cm of each selected *A. pennatula* branch. Therefore ant abundance at each resource was quantified on one stem or branch for each of 10 separated plants of *T. dactyloides* and *A. pennatula* by site. We selected the same 10 cm surface on both plant species to have approximately the same area of food resource available for the ants. Sampling at all sites was performed between 09:00 and 14:00 h, one site per day during the last week of May 2007, first week of June 2012, and the second week of September 2012. The Arenal and Los Chorros sites were burned in May 2007, while the Zapopan and Los Chorros sites were burned in June 2012. *Dalbulus quinquenotatus*, EFNs and ants were sampled approximately one month after each fire. Ants were sampled at these times because both extrafloral nectar produced by *A. pennatula* and honeydew produced by *D. quinquenotatus* was present. All collected insects were stored in 70% ethanol and returned to the lab for identification.

Analysis of Deviance, using R.3.1.0 for Windows (R Project), was performed to evaluate the interaction (resource for ants, honeydew-extrafloral nectar × disturbance, fire-without fire) on the number of ants. This comparison included the ant abundance obtained on the three sampling dates. Furthermore, the total number of ants tending *D. quinquenotatus* on *T. dactyloides* was compared vs the total number of ants on *A. pennatula* bearing EFNs with a Wilcoxon test using SPSS 12 for Windows. Therefore a comparison of ant abundance at leafhoppers vs EFNs was conducted when combining both burned and unburned resources in the three sample dates. Average and standard error were determined for the number of *D. quinquenotatus* nymphs and adults, tending ants, and EFNs for each burned and unburned site.

Results

Ant species collected from burned *T. dactyloides* associated with the leafhopper *D. quinquenotatus* were *Anoplolepis gracilipes* Smith, *Pheidole* sp., and *Brachymyrmex obscurior* Forel. Ants found on unburned *T. dactyloides* tending *D. quinquenotatus* included *A. gracilipes* and *B. obscurior*. Greater ant species richness was associated with *A. pennatula*. *Anoplolepis gracilipes*, *B. obscurior*, *Camponotus* sp., *Crematogaste* sp. and *Solenopsis* sp. were found at EFNs when *A. pennatula* was burned. Ant taxa found visiting the EFNs of unburned *A. pennatula* included *A. gracilipes*, *B. obscurior*, *Crematogaster* sp., *Dorymyrmex* sp. and *Pheidole* sp. We observed these ants differed in body size and likely collect and store honeydew or extrafloral nectar differently.

New green leaves were produced by both *T. dactyloides* and *A. pennatula* after they were burned. Disturbance by fire does not have the same effect on the numbers of ants tending *D. quinquenotatus* and visiting EFNs. We found an interaction between fire and plant species, and significantly more ants were found tending *D. quinquenotatus* leafhoppers on *T. dactyloides* than visiting EFNs (Z = 7.63; *P* = 0.001). Rapid colonization of new growth on *T. dactyloides* by ants and leafhoppers was observed after burning in the last week of May 2007. At this time only adult leafhoppers were observed in the two burned sites tended by a great number *B. obscurior* ants, while EFNs were visited by few ants of *Solenopsis* sp. at the two burned sites (Table 1 and Fig. 2). In June 2012, leafhoppers were tended by *Pheidole* sp. and a great number of nymphs were tended by great numbers of *B. obscurior* ants at the two 2012 burned sites (Table 2 and Fig. 3). Near the end of the wet season in September 2012, four months after the June fire, a large number of leafhopper nymphs were tended by larger numbers of *B. obscurior* ants, while low numbers of *A. gracilipes*, *Camponotus* sp., *Crematogaster* sp. and *B. obscurior* ants visited the EFNs at the two burned sites (Table 3 and Fig. 4).

The number of ants tending leafhoppers was significantly higher than the number of ants found visiting EFNs of *A. pennatula* when combining both burned and unburned resources in the three sample dates (Wilcoxon = 299.50; Z = 3.04; *P* = 0.002). Leafhoppers and ants were found together at the end of the dry season in May 2007 on the six unburned sites, while only in two of the six unburned sites ants visited the EFNs of *A. pennatula* (Table 1 and Fig. 2). In June 2012, at the end of the dry season, no ants or leafhoppers were found on the leaves of unburned *T. dactyloides* plants that were dried out (Table 2 and Fig. 3). In September 2012, at the end of the wet season, only in one of the four unburned sites ants visited the EFNs of *A. pennatula*, whereas in these four unburned sites ants tended the leafhoppers (Table 3 and Fig. 4).

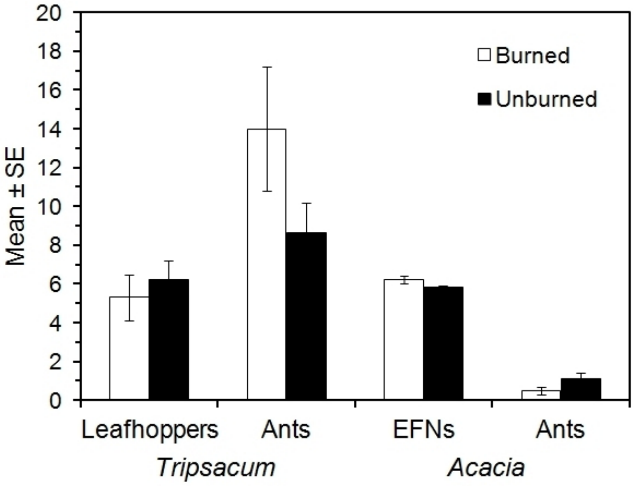

Fig 2. Average number (± standard error) of leafhoppers, ants tending *D. quinquenotatus* leafhoppers on *T. dactyloides*, EFNs, and ants visiting EFNs on *A. pennatula* from burned and unburned sites in Jalisco, Mexico in the last week of May 2007.

Table 1. Average number (± standard error) of *Dalbulus quinquenotatus* nymphs, adults, and tending ants (and species of tending ant), *Acacia pennatula* EFNs, and ants on 10 stems and 10 branches of *T. dactyloides* and *A. pennatula* respectively in burned (in May 2007) and unburned sites at locations in Jalisco, Mexico at the end of the dry season in May 2007.

Site	Ant/Leafhopper interaction on *Tripsacum dactyloides*				Ant/*Acacia* interaction		
	Dalbulus quinquenotatus		Ants	Ant species	*A. pennatula*	Ants	Ant Species
	Nymphs	Adults			EFNs		
Both resources burned							
1. Arenal	0	9.2 ± 1.7	7.7 ± 1.4	*B. obscurior*	5.6 ± 0.1	1.0 ± 0.4	*Solenopsis* sp.
2. Los Chorros	0	1.4 ± 0.3	20.4 ± 5.7	*B. obscurior*	6.9 ± 0.3	0	-
Both resources unburned							
3. San Isidro	3.9 ± 2.2	1.7 ± 0.7	15.1 ± 5.7	*B. obscurior*	5.8 ± 0.2	0	-
4. Agua Caliente	0.7 ± 0.5	0.5 ± 0.4	1.7 ± 1.3	*B. obscurior*	5.4 ± 0.1	1.0 ± 0.2	*Pheidole sp.*
5. Cocula	10.2 ± 1.2	1.2 ± 0.4	4.3 ± 0.8	*B. obscurior*	5.4 ± 0.1	0	-
6. San Agustin	6.5 ± 3.3	1.9 ± 1.1	13.0 ± 5.1	*B. obscurior*	6.6 ± 0.1	0	-
7. La Mimila	3.7 ± 1.9	1.1 ± 0.5	5.7 ± 4.3	*B. obscurior*	5.5 ± 0.2	0	-
8. El Molino	2.0 ± 0.6	3.9 ± 1.2	12.1 ± 2.9	*B. obscurior*	6.6 ± 0.1	5.8 ± 1.4	*B. obscurior*

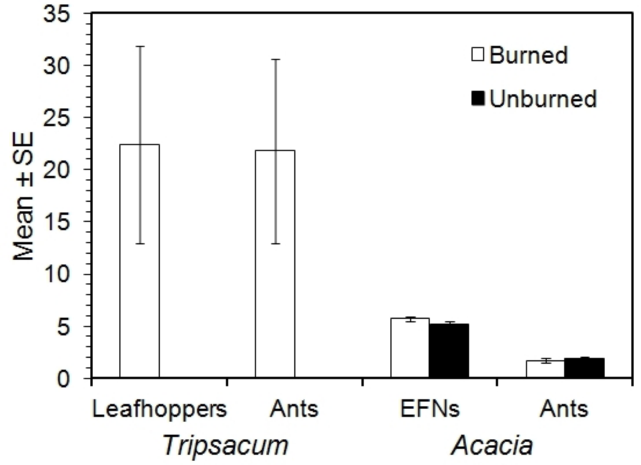

Fig 3. Average number (± standard error) of leafhoppers, ants tending *D. quinquenotatus* leafhoppers on *T. dactyloides*, EFNs, and ants visiting EFNs on *A. pennatula* from burned and unburned sites in Jalisco, Mexico in the first week of June 2012.

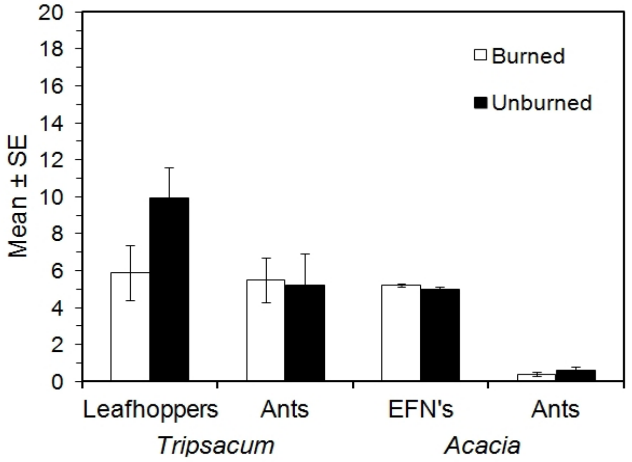

Fig 4. Average number (± standard error) of leafhoppers, ants tending *D. quinquenotatus* leafhoppers on *T. dactyloides*, EFNs, and ants visiting EFNs on *A. pennatula* from burned and unburned sites in Jalisco, Mexico in the second week of September 2012.

Discussion

The exudates honeydew and extrafloral nectar are key factors determining the abundance of ants when both food resources for ants are present (Buckley, 1983; Fiala, 1990; Rashbrook et al., 1992; Del-Claro & Oliveira, 1993; Blüthgen et al., 2006; Katayama et al., 2013). Considering the abundance of ants tending the leafhopper *D. quinquenotatus* compared with the abundance of ants visiting EFNs, more ants were collected in association with *D. quinquenotatus* than with EFNs on *A. pennatula*. This finding is similar to the results of other studies (Fiala, 1990; Rashbrook et al., 1992; Del-Claro & Oliveira, 1993; Blüthgen et al., 2000; Katayama & Suzuki, 2003; Katayama & Suzuki, 2010; Katayama et al., 2013) comparing ant abundance at honeydew-producing insects with plants with EFNs in non-disturbed conditions. In the rainforest canopy, ants are usually more abundant at honeydew than extrafloral nectar, as honeydew is apparently a more valuable resource to ants than nectar from EFNs (Blüthgen et al., 2000). Ants (*Camponotus* sp.) also did not stop tending the honeydew-producing membracids (*Guayaquila xiphias* Fabricius) when an alternative EFN sugar source was available on *Didymopanax vinosum* (Cham. & Schltdl.), their host plant (Del-Claro & Oliveira, 1993). Recently Katayama et al. (2013) demonstrated that the ant *Lasius japonicus* Santsci switches from visiting EFNs on the bean plant *Vicia faba* L. to the aphid *Aphis craccivora* Koch, because the density and total food reward to ants from the aphids exceed that from EFNs.

We ascribe the difference in abundance between ants visiting the leafhopper *D. quinquenotatus* and EFNs on *A. pennatula* to several factors. First, *D. quinquenotatus* leaf-

Table 2. Average number (± standard error) of *Dalbulus quinquenotatus* nymphs, adults, and tending ants (and species of tending ant), *Acacia pennatula* EFNs, and ants on 10 stems and 10 branches of *T. dactyloides* and *A. pennatula* respectively in burned (in June 2012) and unburned sites at locations in Jalisco, Mexico in June 2012.

	Ant/Leafhopper interaction on *Tripsacum dactyloides*				Ant/*Acacia* interaction		
Site	*Dalbulus quinquenotatus*		Ants	Ant species	*A. pennatula* EFNs	Ants	Ant Species
	Nymphs	Adults					
<u>Both resources burned</u>							
1. Zapopan	4.4 ± 2.3	2.4 ± 0.7	4.8 ± 1.8	*Pheidole* sp.	5.6 ± 0.1	1.9 ± 0.2	*A. gracilipes* *Camponotus* sp.
2. Los Chorros	37.0 ±17.9	1.1 ± 0.5	38.9 ± 16.2	*B. obscurior*	5.8 ± 0.3	1.5 ± 0.4	*Crematogaster* sp. *B. obscurior*
<u>Both resources unburned</u>							
3. San Isidro	0	0	0	-	6.5 ± 0.4	2.5 ± 0.5	*Dorymyrmex* sp. *Crematogaster* sp.
4. San Agustin	0	0	0	-	6.2 ± 0.4	0.3 ± 0.1	*B. obscurior*
5. La Mimila	0	0	0	-	1.4 ± 0.7	1.1 ± 0.5	*A. gracilipes*
6. El Arenal	0	0	0	-	6.3 ± 0.4	3.3 ± 0.9	*A. gracilipes*

Table 3. Average number (± standard error) of *Dalbulus quinquenotatus* nymphs, adults, and tending ants (and species of tending ant), *Acacia pennatula* EFNs, and ants on 10 stems and 10 branches of *T. dactyloides* and *A. pennatula* respectively in burned (in June 2012) and unburned sites at locations in Jalisco, Mexico at the end of the wet season in September 2012.

Site	Ant/Leafhopper interaction on *Tripsacum dactyloides*				Ant/*Acacia* interaction		
	Dalbulus quinquenotatus		Ants	Ant species	*A. pennatula* EFNs	Ants	Ant Species
	Nymphs	Adults					
Both resources burned							
1. Zapopan	0.9 ± 0.7	0.5 ± 0.2	1.1 ± 0.5	*A.gracilipes*	5.5 ± 0.1	0.9 ± 0.2	*A. gracilipes* *Camponotus* sp.
2. Los Chorros	9.5.0 ±2.1	1.0 ± 0.2	10.0 ± 1.3	*B. obscurior*	5.0 ± 0.2	0	-
Both resources unburned							
3. San Isidro	15.1± 5.4	1.5± 0.5	14.3± 6.1	*B. obscurior*	5.6 ± 0.1	0	-
4. San Agustín	8.9± 1.3	1.9± 0.3	1.4± 0.4	*B. obscurior*	5.4± 0.1	0	-
5. La Mimila	1.2± 0.3	0.9± 0.3	1.4± 0.3	*A. gracilipes*	4.4 ± 0.2	0	-
6. El Arenal	6.9± 2.8	3.9± 1.3	3.9± 1.4	*A. gracilipes*	4.9 ± 0.1	2.8 ± 0.6	*A. gracilipes*

hoppers produce honeydew at a consistent rate (Larsen et al.,1992), whereas EFNs are highly variable in nectar production over the course of a day, resulting in a less predictable resource for the ants. For example, nectar production is highly variable in the plant *Macaranga tanarious* (L.) Muell. Arg. (Heil et al., 2000). Second, *D. quinquenotatus* is sedentary and gregarious (Heady & Nault 1985), resulting in a higher density of both nymphs and adult leafhoppers on the basal leaves of *T. dactyloides*. At higher leafhopper densities, more honeydew is produced in a concentrated area allowing easy collection by the ants. Third, *D. quinquenotatus* responds to the stroking of their abdomen by antennae of tending ants by excreting and holding honeydew droplets until droplets are removed by ants (Larsen et al., 1992). Ant-tended *Dalbulus quinquenotatus* leafhoppers secrete three to six times the volume of honeydew compared with other species of non-myrmecophilous *Dalbulus* leafhoppers (Larsen et al., 1992), increasing the availability of honeydew for tending ants. In contrast, EFNs of *A. pennatula* do not respond to antennation by ants by increasing extrafloral nectar secretions. However, this is not universal as *Inga* plants have been shown to increase nectar production in response to tending ants (Bixenmann et al., 2011). Fourth, *D. quinquenotatus* leafhoppers and their tending ants often live together in mud shelters made by tending ants on the basal leaves of the gamagrass. Within these shelters, high densities of ants and leafhoppers occur and parasitism is reduced (Moya-Raygoza & Larsen, 2008). These shelters help to increase the quantity of honeydew for tending ants by concentrating the leafhoppers, whereas *A. pennatula* does not provide shelters for ants in the form of big thorns as is found on other *Acacia* species. Providing shelter for members of the mutualism is important in establishing obligatory relationships (Speight et al., 1999). Fifth, the honeydew of myrmecophilous hemipterans contains melezitose that provide nitrogen and is a higher quality nectar than nectar from EFNs (Cook & Davidson, 2006). Sixth, excess *D. quinquenotatus* leafhoppers are sometimes eaten by tending ants (Moya-Raygoza & Nault, 2000), making the leafhoppers a high quality source of protein. Ant colony growth and reproduction requires substantial quantities of protein (Davidson et al., 2003).

Moreover, this *D. quinquenotatus* leafhopper-ant association is an obligate and highly specialized mutualism as compared with the more general and facultative ant-*A. pennatula* mutualism. Moya-Raygoza (2005) found that the ant *B. obscurior* visits active EFNs of *A. pennatula* but does not protect this species of *Acacia* from herbivores. Lack of protection by ants against herbivores is common among plants with EFNs (Buckley, 1983; Heads, 1986; Oliveira et al., 1999; Ruhren, 2003). In contrast, both Moya-Raygoza and Nault (2000) and Larsen et al. (2001) have shown that tending ants protect both nymph and adult *D. quinquenotatus* from predators. Thus, this mutualism between *D. quinquenotatus* and ants is obligatory, as these leafhoppers apparently cannot live without tending ants.

Post-fire response

Both *T. dactyloides* and *A. pennatula* respond quickly to a fire event with new growth, producing young leaves ready to be colonized by herbivorous insects. Previous studies conducted in the tropics have found that some species of plants respond to fire with vigorous growth, which can be colonized rapidly by herbivores (Prada et al., 1995; Vieira et al., 1996). We found that ants are adapted to colonize plants quickly after fire, taking advantage of new resources such as honeydew offered by *D. quinquenotatus* feeding on *T. dactyloides* and extrafloral nectar produced by EFNs of *A. pennatula*, resulting in the reestablishment of these mutualistic interactions only a few days after fire.

We found more ants tending leafhoppers than visiting EFNs at burned sites where both *T. dactyloides* and *A. pennatula* were found. Fire does not kill *T. dactyloides*, but instead stimulates the growth of new stems from *T. dactyloides* rhizomes. These new stems are the first food resources that appear within the community and are quickly recolonized by *D. quinquenotatus*. These leafhoppers may come from contiguous unburned sites. These immigrant leafhoppers start to feed and produce large quantities of honeydew that attract large numbers of ants. The numbers of ants revealed this fast recolonization by leafhoppers and ants at sites where fire occurred in either May 2007 or June 2012. In contrast, in June 2012 no leafhoppers or ants were found on *T. dactyloides* leaves at unburned sites because those leaves were dried out. Although EFNs at unburned sites were actively producing extrafloral nectar at that time, few ants were present.

No previous studies have compared the ant abundance at leafhoppers and EFNs on fire-disturbed habitats when both resources are available at the same time. Schowalter (2006), reported that ants and sap-sucking insects such as leafhoppers dominate early-successional tropical forests as they contain an abundance of young, succulent leaf tissue that favor sap-sucking hemipterans and tending ants. In North American grasslands, populations of some leafhopper species are significantly greater following fire due to immigration from unburned areas into rapidly growing burned areas (Warren et al., 1987). Previously, Moya-Raygoza (1995) found that *D. quinquenotatus* leafhoppers were found in larger numbers and tended by a greater number of ants in burned than unburned *T. dactyloides* colonies, because recently burned plants produce new young leaves with higher concentrations of nitrogen.

Similar results have been found in the interaction between ants and EFN-bearing plants in other systems after disturbance. For example, pruned plants (*Conocarpus erectus* L.) grew faster and produced higher numbers of extrafloral nectaries and attracted a higher density of ants (Piovia-Scott, 2011). Leaf damage also increases the production of extrafloral nectar in different plants (Heil et al., 2001). In another case, higher abundance of ants was found in the shrub *B. campestris* after fire because of a high concentration of extrafloral nectar (Alves-Silva & Del-Claro, 2013). Similarly Alves-Silva (2011) and Koptur et al. (2010) found a more diverse ant fauna guarding plants from herbivory after fire occurred due to the high production of extrafloral nectar. This is not surprising as ants are attracted to high quality sugar resources produced by plants with EFNs (Heil & McKey, 2003).

Therefore, the availability of honeydew and extrafloral nectar to ants after fire is important because it can regulate ecological dominance, affecting the ant trophobiont and plant communities. Greater numbers of ants tending leafhoppers may result in better protection of these honeydew producers by ants compared with the ant protection of plants with EFNs that can also occur in these fire-prone sites. Moreover, colonization by ants after fire is important to initiate these mutualisms with both hemipterans and EFNs. Our results highlight the importance of investigating mutualisms not only in paired species, but also among multiple mutualisms involving ants when a system is disturbed.

Acknowledgments

We are grateful to Miguel Vasquez Bolaños for the identification of some of the ant taxa. We also appreciate the comments and suggestions of two anonymous reviewers.

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