

# The Fruit Fly Lure CeraTrap: An Effective Tool for the Study of the Arboreal Ant Fauna (Hymenoptera: Formicidae)

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## Abstract

Despite preliminary reports of ants trapped in food-baited fruit fly traps, little is known regarding the identity of the myrmecofauna that can be sampled using this technique. This study aimed to examine the inventory completeness, activity and species occurrence of canopy ant assemblages collected in baited traps used for monitoring fruit flies in different fruit orchards in central Veracruz, Mexico. The trap models used in the sampling were Multilure, McPhail glass, and 500 ml blue polyethylene bottles. Three commercial fruit fly food attractants (CeraTrap, Captor + Borax, and BioLure) and two grape juice products (Jumex grape juice and Tang) were used as baits for sampling. In total 3,626 ant workers belonging to 54 species, 19 genera, 10 tribes, and 5 subfamilies were collected. Among the five food attractants used in this study, CeraTrap recorded a markedly higher inventory completeness, ant activity and species occurrence per trap. This study reports for the first time the use of CeraTrap, as a promising and effective food attractant for collecting the foraging ants in the canopy of agroecosystems, which may be applicable to other habitats such as natural forests, mangroves, or agricultural settings such as coffee plantations.

**Key words:** agroecology, biodiversity, myrmecofauna, ant sampling, canopy ant

Ants (Hymenoptera: Formicidae) are one of the most abundant and diverse insect groups in most terrestrial ecosystems (Hölldobler and Wilson 1990), inhabiting or foraging in a great number of microhabitats from the subsoil to the arboreal canopy (Rojas 2001). Canopy ant assemblages contribute to around half of the overall myrmecofauna diversity in tropical ecosystems (Floren et al. 2014).

Ecological studies on arboreal ant diversity are commonly based on canopy fogging systems (Castaño-Meneses 2014), pitfall traps baited with proteins (sardine or tuna), nitrogen-rich (diluted urine), or sugar-rich (diluted honey) baits (Oliveira-Santos et al. 2009, Powell et al. 2011), and direct visual search and hand collection (Zina et al. 2017). Although these techniques are commonly used and capture various species, some of them requires a lot of work and may result in incomplete or biased inventories (Yanoviak et al. 2003). As diversity comparisons are only ecologically appropriate for communities with a similar level of inventory completeness, the use of a single or insecticide-based technique can hinder myrmecological studies (Ribeiro and Espírito-Santo 2007, Hsieh et al. 2016). For this reason, it is important to identify an efficient and environmentally friendly sampling technique for the study of arboreal canopy ant communities.

In tropical agroecosystems, Hymenoptera, particularly ants, have been reported as the main nontarget order of insects caught in traps baited with food attractants used for monitoring and mass trapping of the Mexican fruit fly, *Anastrepha ludens* Loew (Diptera: Tephritidae) (Herrera et al. 2016, Velázquez et al. 2018). Despite preliminary reports of ants trapped in food-baited fruit fly traps, little is known regarding the identity of the myrmecofauna that can be sampled using this technique. Thus, this study aimed to examine the inventory completeness, activity (the total number of ant workers) and species occurrence (the number of times that a given species was collected) of the assemblages of canopy ants collected in baited traps used for monitoring fruit flies in grapefruit (*Citrus paradisi* Macfad.), mango (*Mangifera indica* L.), sapodilla (*Manilkara zapota* L.), and guava (*Psidium guava* L.) orchards in central Veracruz, Mexico.

## Materials and Methods

The findings reported for the first time in this article come from a series of experiments developed to test different lures for monitoring *A. ludens* in different fruit orchards in the central Veracruz, Mexico (Table 1).

A temperate, humid climate prevails throughout most of the catchment area. Mean annual precipitation and temperature range from 1,500 mm and 25°C at sea level to 2,000 mm and 12°C at 2,500 m a.s.l. (Muñoz-Villiers and López-Blanco 2008). Seven fruit orchards located between 21 and 1,183 m a.s.l. were selected. Orchards were located from 3 to 90 km apart, their size ranged from 2.5 to 10 ha. Producers did not apply pesticides or chemical fertilizers to their orchards.

Ants were collected from traps in seven different experiments developed between April and October 2014 (Table 1). Each orchard was divided into six blocks, where monitoring traps were hung at the upper one-third of the total tree height inside the tree canopy at a central point of lateral branches and on trees separated at least by 50 m. The number of traps per sampling unit varied from two to four traps, depending on the number of baits tested within each orchard. To hang traps, we used a 10-cm-long suspension hook made of galvanized iron wire, of 3 mm in diameter for glass traps, and of 2 mm in diameter for the plastic traps. Depending on the fruit orchard, there were one to six sampling events in which traps were monitored at weekly intervals and captured ants were collected. The installation of traps in different trees was randomized first, but traps were rotated clockwise each week to avoid the effect of the position of each tree. Due to the nature of the mentioned experiments, we were

not interested in comparing ant samplings among the different fruit orchards. However, this work was only focused in the comparison of inventory completeness, activity, and species occurrence of ant assemblages among the different baits assessed at orchard level.

The trap models (Fig. 1) used in the sampling were 1) Multilure traps (Better World Manufacturing, Inc., Fresno, CA), which are invaginated plastic traps with 12.8 cm diameter, 15 cm height, and a 5.8-cm diameter entrance hole (Martinez et al. 2007); 2) McPhail glass traps with 17 cm diameter, 16 cm height, and a 7-cm diameter entrance hole; and 3) 500 ml blue polyethylene bottles of 21 cm in height (Tecni Plastic Containers S.A. de C. V., Martinez de la Torre, Mexico) that were modified by drilling three 10-mm-diameter holes, 5 cm apart, at two-thirds of the way up the side of the bottle (Lasa and Cruz 2014).

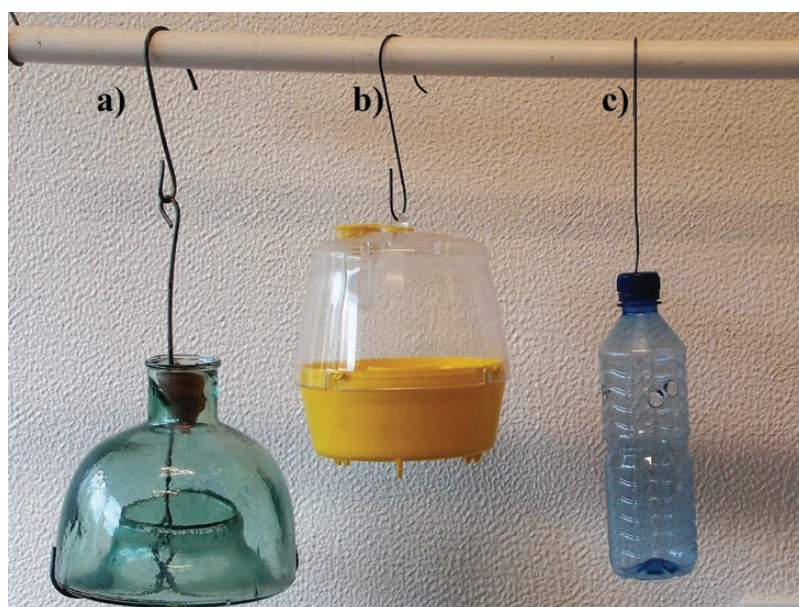
The commercial fruit fly food attractants, used as baits for sampling were: 1) CeraTrap (Bioibérica, Barcelona, Spain), a liquid lure consisting of enzymatically hydrolyzed proteins of animal origin that have been proven to attract several *Anastrepha* species (Lasa and Cruz 2014, Lasa et al. 2015, Rodríguez et al. 2015); 2) Captor + Borax, a chemical hydrolyzed protein combination that was prepared with 10 ml of Captor 300 (Promotora Agropecuaria Universal, Mexico City, Mexico), 5 g of borax (J. T. Baker, Mexico City) and

**Table 1.** Sampling methods of myrmecofauna in seven orchards monitored in central Veracruz, Mexico

Characteristics	Grapefruit	Mango	Sapodilla 1	Sapodilla 2	Guava 1	Guava 2	Guava 3
Latitude (N)	20° 11'05"	19° 19'47"	19° 19'35"	19° 20'16"	19° 25'08"	19° 21'45"	19° 26'50"
Longitude (W)	96° 56'06"	96° 45'18"	96° 43'22"	96° 43'53"	96° 58'30"	96° 40'12"	96° 24'08"
Elevation (m)	21	336	317	337	1,183	440	26
Municipality	Martínez de la Torre	Apazapan	Apazapan	Apazapan	Xico	Emiliano Zapata	Zempoala
Area (ha)	3	6	10	2.5	1	1	1
Collection date in 2014	April 11–May 2	June 2–30	April 14–May 26	June 2–July 7	Sept. 2–23	Sept. 15–Oct. 13	Sept. 15
Tree canopy height (m)	3.5	4.5	4.5	4	3	3	3
No. sampling events	4	4	7	6	4	4	1
Trap type	Multilure	Multilure	McPhail	Multilure	PET bottle	PET bottle	PET bottle
Lure tested	CB, CT, JX, TG	CB, CT, JX, TG	CB, CT	CB, CT, BL	CB, CT	CB, CT	CB, CT

PET, polyethylene.

Fruit fly attractants were CeraTrap (CT), Captor + Borax (CB), Jumex grape juice (JX), Tang grape powder drink (TG) and BioLure (BL).



**Fig. 1.** Trap models used in the sampling: (a) McPhail glass trap; (b) Multilure trap; and (c) blue polyethylene bottle.

235 ml of water; 3) BioLure (Suterra Inc., Bend, OR), a dry lure containing ammonium acetate and putrescine in sachets in which insects were trapped in a mixture of water and 10% propylene glycol (Heath et al. 1997). The following grape juice products were also tested: 1) Grape juice (Grupo Jumex, Mexico), 2) Tang, a grape-flavored powdered drink mix (Kraft Foods de Mexico, Mexico City), that was prepared with water as recommended on the packet (15 g powder + 2 liters water).

All collected ants were preserved in 70% ethanol, and one to three of the collected specimens per sample that differed morphologically were dry-mounted. The key of Mackay and Mackay (1989) was used to identify ant genera, along with several additional keys for species identification, depending on the genus involved. All ants were deposited in the entomological collection of the Instituto de Ecología A.C. in Xalapa, Veracruz, Mexico (IEXA; Reg. SEMARNAT: Ver. IN.048.0198).

The inventory completeness for each orchard was calculated using the sample coverage estimator ( $\hat{C}_n$ ), which is a less biased estimator of inventory completeness than nonparametric methods. This estimator indicates the proportion of the “total community” represented by the trapped species and when  $\hat{C}_n \approx 100\%$ , sampling is complete given the effort and capture technique used (Chao and Jost 2012). Values of  $\hat{C}_n$  were calculated using iNEXT package for R (Hsieh et al. 2016). We then fitted generalized linear models (GLMs), assuming a Poisson error distribution and a log link function, to test for differences in activity and species occurrence of ants per trap and post hoc contrast tests to identify differences among bait types using the *gmodels* package for R (Warnes et al. 2015). Finally, ant species were catalogued as arboreal, generalist or ground-dwelling according to their nesting habits available in AntWeb v7.34.4 (AntWeb 2018) and then the proportion of each nesting habit in each orchard was compared using G tests with Williams’s correction.

## Results

In total 3,626 ant workers belonging to 54 species, 18 genera, 10 tribes, and 5 subfamilies were collected (Table 2). The subfamily Myrmicinae had the highest number of tribes, genera, and species. The genus *Camponotus* had the highest number of species (14 spp.), followed by *Pseudomyrmex* (6 spp.), *Pheidole* (5 spp.), *Azteca* and *Crematogaster* (4 spp.), *Forelius* (3 spp.) and finally *Brachymyrmex*, *Cephalotes*, *Monomorium*, *Nesomyrmex*, *Solenopsis*, and *Tetramorium* (2 spp. each). The six remaining genera were represented by one species each.

The inventory completeness recorded by CeraTrap-baited traps was significantly higher than grapefruit orchard, sapodilla orchard #2, and guava orchards #1, 2, and 3 (Fig. 2). In all orchards, inventory completeness recorded in CeraTrap-baited traps (85–96%) was not significantly different from that recorded by all food attractants grouped together. The Captor + Borax-baited traps had an intermediate inventory completeness in the grapefruit and mango orchards, similar to the inventory completeness values of traps baited with Jumex grape juice or, in the sapodilla orchard #1 with BioLure-baited traps. Traps baited with Tang grape drink had the lowest inventory completeness in the orchards in which this bait was tested.

GLM analyses indicated that ant activity was significantly affected by the factor bait type in sapodilla orchard #1 ( $F_{1,8} = 32.63$ ;  $P < 0.001$ ), sapodilla orchard #2 ( $F_{2,12} = 6.57$ ;  $P = 0.01$ ), grapefruit orchard ( $F_{3,16} = 64.11$ ;  $P < 0.001$ ), guava orchard #2 ( $F_{1,8} = 35.46$ ;  $P < 0.001$ ), guava orchard #3 ( $F_{1,8} = 120.2$ ;  $P < 0.001$ ), and mango orchard ( $F_{3,16} = 16.61$ ;  $P < 0.001$ ). Pairwise Tukey tests indicated the

traps baited with CeraTrap recorded the highest capture of ants in all these orchards (Fig. 2). The species occurrence per trap was significantly influenced by bait type in the orchards planted with sapodilla #1 ( $F_{1,8} = 17.31$ ;  $P = 0.003$ ), sapodilla #2 ( $F_{2,12} = 11.38$ ;  $P = 0.001$ ), grapefruit ( $F_{3,16} = 29.89$ ;  $P < 0.001$ ), guava #1 ( $F_{1,8} = 8.33$ ;  $P = 0.02$ ), guava #2 ( $F_{1,8} = 94.04$ ;  $P < 0.001$ ), guava #3 ( $F_{1,8} = 12.07$ ;  $P = 0.008$ ), and mango ( $F_{3,16} = 9.77$ ;  $P < 0.001$ ). Pairwise Tukey tests indicated that in all these orchards the traps baited with CeraTrap recorded the highest species occurrence per trap (Fig. 2).

The most common nesting habit, of the total sampled myrmecofauna, was arboreal (69%), followed by generalist (19%) and ground-dwelling (13%). At orchard level, the number of arboreal ants only was significantly higher in sapodilla orchard #1 ( $G_{2,6} = 17.12$ ;  $P < 0.001$ ), sapodilla orchard #2 ( $G_{2,8} = 18.59$ ;  $P < 0.001$ ), grapefruit orchard ( $G_{2,8} = 9.6$ ;  $P = 0.008$ ), and mango orchard ( $G_{2,8} = 25.2$ ;  $P < 0.001$ ).

## Discussion

The ant fauna collected in this study represented ~18% of the total myrmecofauna registered for the state of Veracruz (Vásquez-Bolaños 2015). The species *Camponotus conspicuus* Emery, *Camponotus curviscapus* Emery, *Camponotus textor* Forel, and *Pseudomyrmex viduus* (Smith) are new records for the state of Veracruz (Vásquez-Bolaños 2015). Almost all the revised specimens were ant workers, except for four captured queens of *Camponotus formiciformis* Forel.

Among the five food attractants used in this study, CeraTrap recorded a markedly higher inventory completeness and ant activity and species occurrence per trap (Fig. 2; Table 2). One of the characteristics that may have contributed to the efficacy of the CeraTrap bait seems to be the higher volatile emission of attractants and that the workers, trying to feed on it, were rapidly trapped by the low surface tension of CeraTrap (Lasa and Williams 2017). These physicochemical characteristics allowed long periods of sampling without the investigator’s interventions, which is commonly required in other bait samplings (Oliveira-Santos et al. 2009, Powell et al. 2011).

The fruit fly lure CeraTrap is a liquid food attractant consisting of a mixture of enzymatic hydrolyzed proteins of animal origin (extracted from mammal intestinal mucosa) that release a series of volatile compounds, mostly amines and organic acids that are highly attractive to fruit flies (Lasa and Cruz 2014). In contrast, other widely used food attractants used to monitor fruit flies are derived from protein sources, such as Torula yeast, produced by the ascomycete *Candida utilis* (Henneberg) (Saccharomycetales: Saccharomycetidae), whereas Captor 300 and other commercial chemical hydrolyzed proteins are plant-derived products and BioLure, which is a synthetic attractant that includes ammonium acetate and putrescine (Lasa et al. 2014). A previous study revealed that those fruit fly attractants, mainly CeraTrap, may capture several orders of insects like Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Orthoptera (Herrera et al. 2016). However, this is the first report that has identified ants collected by CeraTrap in agricultural ecosystems.

An additional advantage of CeraTrap is that, due its high stability, the release of attractant volatiles is homogeneous over time and the lure remains attractive during periods exceeding 6 weeks (Lasa et al. 2015). The product stability appears to be related with the presence of preservatives in the formulation, which prevent microbial growth and limit product degradation over time (Lasa and Williams 2017). This also favors the preservation of captured ants for later species identification (Table 2). We believe that this product

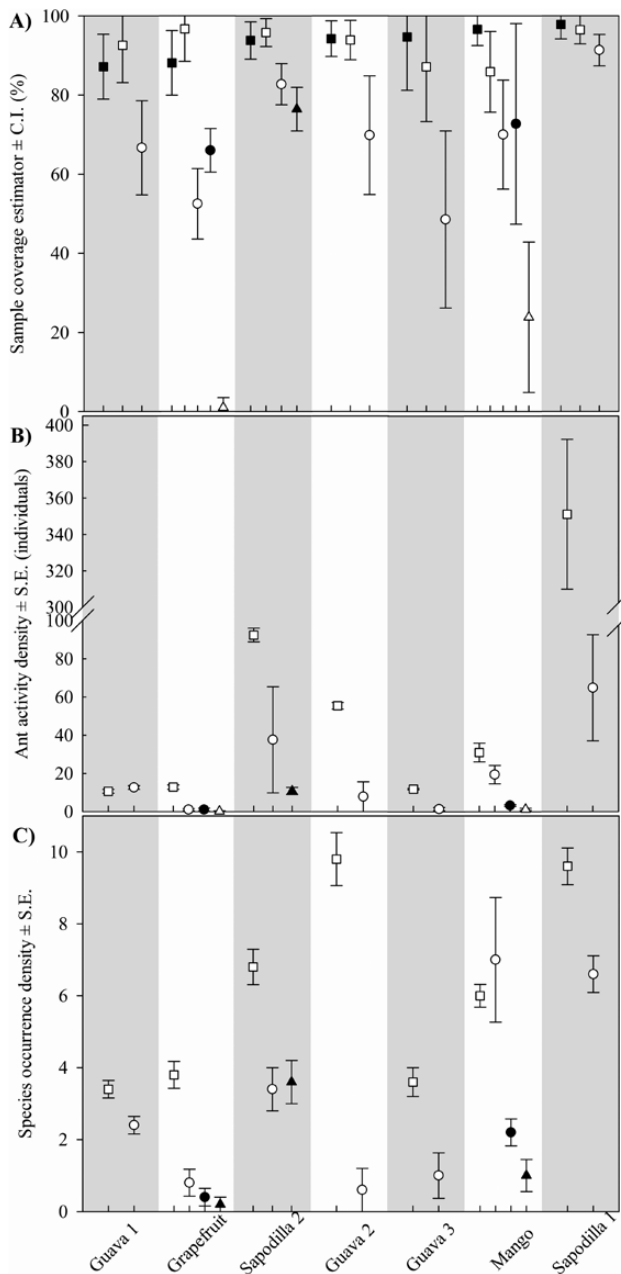


Table 2. Continued

Parameters and ant species	Sapodilla 1			Sapodilla 2			Grapefruit			Guava 1			Guava 2			Guava 3			Mango		
	CT	PH	BL	CT	PH	BL	CT	PH	TG	CT	PH	CT	PH	CT	PH	CT	PH	CT	PH	TG	
<i>B. muscillus</i> Forel <sup>§</sup>							4/28														
<b>Myrmicinae</b>																					
<b>Attini</b>																					
<i>Cephalotes minutus</i> (Fabricius) <sup>a</sup>	1/1	5/5	1/1	1/2														1/1			1/1
<i>C. scutellatus</i> (Smith) <sup>a</sup>				1/1																	
<i>Pheidole punctatissima</i> Mayr <sup>§</sup>																					
<i>Pheidole</i> sp. 1 <sup>a</sup>																					
<i>Pheidole</i> sp. 2 <sup>a</sup>																					
<i>Pheidole</i> sp. 3 <sup>§</sup>																					
<i>Pheidole</i> sp. 4 <sup>§</sup>																					
<b>Crematogastrini</b>																					
<i>Crematogaster crinosa</i> Mayr <sup>a</sup>	7/10	4/15	1/1	1/1																	
<i>C. curvispinosa</i> Mayr <sup>a</sup>																					
<i>C. obscurata</i> Emery <sup>a</sup>																					
<i>C. torosa</i> Mayr <sup>a</sup>																					
<i>Nesomyrmex echinatoidis</i> (Forel) <sup>a</sup>																					
<i>N. wilda</i> (Smith) <sup>a</sup>																					
<i>Tetramorium lanuginosum</i> Mayr <sup>§</sup>																					
<i>T. spinosum</i> (Pergande) <sup>§</sup>	1/1			1/1																	
<b>Solenopsidini</b>																					
<i>Monomorium ebeninum</i> Forel <sup>§</sup>																					
<i>M. floricola</i> (Jerdon) <sup>§</sup>																					
<i>Solenopsis geminata</i> (Fabricius) <sup>§</sup>																					
<i>S. picea</i> Emery <sup>a</sup>																					
<b>Pseudomyrmecinae</b>																					
<b>Pseudomyrmecini</b>																					
<i>Pseudomyrmex elongatus</i> (Mayr) <sup>a</sup>	3/3																				
<i>P. gracilis</i> (Fabricius) <sup>a</sup>	1/1	1/1																			
<i>P. elongatulus</i> Dalla Torre <sup>a</sup>																					
<i>P. pallidus</i> (Smith) <sup>a</sup>																					
<i>P. simplex</i> (Smith) <sup>a</sup>																					
<i>P. viduus</i> (Smith) <sup>a,*</sup>																					
<b>B) Diversity measures</b>																					
Observed species richness	14	9	6	13	7	6	9	2	3	1	9	6	12	2	10	4	12	5	18	2	
Species occurrence per trap	48	33	18	34	17	18	19	2	4	1	17	12	49	3	18	5	30	11	35	5	
Total individuals captured	1,738	332	54	462	188	54	64	5	5	1	53	63	277	39	59	7	155	16	97	6	
Captured arboreal species	12	8	6	11	7	6	7	1	2	1	5	3	5	2	5	3	12	4	17	2	
Captured generalist species	0	0	0	2	0	0	2	1	1	0	3	3	3	0	3	0	0	1	1	0	
Captured ground-dwelling species	2	1	0	0	0	0	0	0	0	0	1	0	4	0	2	1	0	0	0	0	

Numbers listed indicate the observed species occurrences (values above diagonal) and number of individuals captured (values below diagonal) in each orchard. All species are sorted by subfamily and tribe. Fruit fly attractants were Cera Trap (CT), Captor + Borax (CB), Jumex grape juice (JX), Tang grape powder drink (TG), and BioLure (BL). The nesting habits are arboreal (<sup>a</sup>), generalist (<sup>g</sup>), and ground-dwelling (<sup>gd</sup>), and an \* indicates the species that are new records for the state of Veracruz.





**Fig. 2.** Inventory completeness (A), ant activity (B), and species occurrence (C) of ants collected with attractants and traps used for fruit flies monitoring and mass trapping in fruit orchards in Veracruz, Mexico. (A) Comparison of inventory completeness (as measured by sample coverage estimator based on sample size) differences are statistically significant when 95% confidence intervals do not overlap, while with overlap no differences are assumed ( $\alpha = 0.05$ ). (B) Variation in the mean number of ant workers  $\pm$  SE captured by each bait per trap. (C) Variation in the mean number of species occurrences  $\pm$  SE recorded by each bait per trap. Baits were CeraTrap ( $\square$ ), Captor + Borax ( $\circ$ ), Jumex grape juice ( $\bullet$ ), Tang grape powder drink ( $\Delta$ ), and BioLure ( $\blacktriangle$ ). The overall inventory completeness ( $\blacksquare$ ) considering all food attractants grouped together is only indicated in (A).

could be effectively used to complement other sampling techniques used to study arboreal species, especially for studies that compare alpha and beta diversities of ant assemblages across multiple sites including sampling in the tree or crop canopy (Powell et al. 2011, Castaño-Meneses 2014, Vasconcelos et al. 2018).

Recently, some studies carried out in Brazil have highlighted the use of urine, a rich nitrogen bait, as an attractant in arboreal pitfall

traps (Powell et al. 2011, Camarota et al. 2016, Vasconcelos et al. 2018). However, the myrmecofauna captured with human urine usually is complemented with that collected in honey-baited traps and despite that, the inventory remains incomplete (Powell et al. 2011). Unfortunately, an empirical and preliminary assay developed to know the attractiveness of human urine for collecting ants in tropical forests of central Veracruz did not show the same effectiveness reported in central Brazilian forests (M. A. García-Martínez, unpublished data). The notably efficacy of human urine for collecting arboreal Brazilian ants may be due to a more limiting source of sodium for ant assemblages in the impressive tropical dry ecosystem of central Brazil than in tropical subhumid regions that are relatively near to the coastal plain, such as our study area (Clay et al. 2015, Vasconcelos et al. 2018).

Regarding the nesting habits observed in the sampled myrmecofauna, CeraTrap captured between 67% and 86% of the total arboreal species recorded in each orchard. This arboreal myrmecofauna was composed of various nectarivorous and predator ant species (AntWeb 2018), such as *Azteca* spp., *Camponotus* spp., *Cephalotes* spp., *Crematogaster* spp., *Nesomyrmex* spp., *Pseudomyrmex* spp., some species of *Solenopsis* and *Pheidole* (Table 2). In contrast, we also observed how the relative low proportion of collected ground-dwelling ants increased in low-canopy orchards (e.g., guava orchards) (Table 2). The ground-dwelling species attracted to the arboreal traps and in particular to CeraTrap bait belong to the genera *Forelius*, *Pheidole* and *Solenopsis*, *Monomorium*, and *Tetramorium* (Table 2). These results confirm the effectiveness of CeraTrap for capturing diverse arboreal ants in agricultural canopies and suggest a possible attractiveness to ground-dwelling ants.

The sampling program performed in this study demonstrated a remarkable diversity of foraging ants in the canopy of fruit orchards. This study also reports for the first time the use of CeraTrap, as a promising and effective food attractant for collecting the foraging ants in the canopy of agroecosystems, which may be applicable to other habitats such as natural forests, mangroves, or agricultural settings such as coffee plantations. The importance of using CeraTrap is that ant abundance impressively increases, which significantly improves the inventory completeness of assemblages (Fig. 2). A complete inventory has important consequences for studies that compare different sites at local scales, or studies that seek to understand differences or similarities among ant assemblages at different geographical locations. Given that the use of baited traps has been tested to monitor arboreal ants (Oliveira-Santos et al. 2009), future studies should focus on evaluating the efficacy of CeraTrap-baited traps across a range of situations and environments as a potentially important tool for the acquisition of complementary information on the myrmecofauna.

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