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First report about ants associated with *Diaphorina citri* Kuwayama in Mexican lime (*Citrus aurantiifolia* Swingle) in the Apatzingán Valley, Michoacán, México

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ABSTRACT

Objective: To carry out taxonomic studies that determine which formicine species are associated with the cultivation of Mexican lime.

Design/Methodology/Approach: The study was conducted in eight municipalities and 59 localities of the Apatzingán Valley region, Michoacán, Mexico, where Mexican lime (*Citrus aurantiifolia* Swingle) is produced. A randomized complete blocks design was used in the experiment, where each municipality represented a block. The repetitions were the number of collections, while the experimental unit was a lime sprout infested with *Diaphorina citri* and ants. The collections were direct and were made with an entomological aspirator. The values obtained were analyzed with the SAS University Edition software (2018).

Results: The studied ants belong to the Dolichoderinae, Formicinae, Myrmicinae, Pseudomyrmecinae, and Ponerinae subfamilies, which represent 15 genera and the same number of species. The subfamilies with the greatest presence in the eight municipalities were: Myrmicinae, Dolichoderinae, and Formicinae. The species with the highest impact were *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*, while *Cardiocondyla minutior* and *Odontomachus* sp. had a lower impact.

Study limitations/implications: Social insecurity in the eight municipalities and the new form of coexistence.

Findings/Conclusions: The studied species prefer dry, warm, and disturbed sites.

Keyword: Myrmicinae, Dolichoderinae, *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*.

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INTRODUCTION

Ants are the most successful group in terrestrial environments; its almost 13,000 species present great diversity, both taxonomically and functionally. In Mexico, 93 genera of ants, divided into 927 species, have been reported (Vásquez-Bolaños, 2015); however, they are a little explored group, despite their great success and diversity. They perform important predatory, herbivore, or detritivore functions and take part in the physicochemical processes of the soil, as well as in the decomposition and recycling of nutrients (Rojas, 2001). However, despite their importance in ecosystems, some species are considered economically important, since they can act as agricultural pests or be associated with other pests (Della, 2003). The *Solenopsis xyloni*, *Linepithema humile*, *Formica aerata*, and *Tapinoma sessile* ants impact the natural enemies of hemiptera (DeBach *et al*., 1951; Martínez-Ferrer *et al*., 2003; Bradford and Silverman, 2010). Another case is the Asian Citrus Psyllid *Diaphorina citri* Kirkaldi, which is associated with various species of ants such as *Pheidole megacephala*, *Brachymyrmex patagonicus*, *Solenopsis invicta*, and *Brachymyrmex obscurior* (Navarrete *et al*., 2013). This association poses a serious threat to citrus farming, not only because the psyllid extracts the sap of the citrus plant, distorts the leaf, and causes sooty mold, but also because it transmits the *Candidatus liberibacter* bacterium, the causative agent of the worst disease that affects citrus in the world: Huanglongbing (HLB) or Citrus Greening (Halbert and Manjunath, 2004). The ecological interaction between *Diaphorina citri* and ants is important, because they protect the hemiptera from their natural enemies and quickly eliminate honeydew excretions, reducing bacterial and fungal infections; in return, ants obtain the honeydew from the plant that serves as an important food resource (Stachowicz, 2001).

Few current works refer to the psyllid-ants interaction. Navarrete *et al*. (2013) carried out one of the few of such studies in the state of Florida, USA. They mention that both in *Murraya paniculata* and in *Citrus latifolia*, parasitism of *Diaphorina citri* by *Tamarixa radiata* increased by 20.36% when ants were absent, compared with 0.39% parasitism when ants were present. Despite their importance, no studies about the ants associated with *Diaphorina citri* have been carried out in Mexico. The symbiosis between *Diaphorina citri* and ants poses as threat to the important production of citrus in the state of Michoacán. Consequently, taxonomic studies were carried out to find out which ant species are associated with *Diaphorina citri* in the Mexican lime cultivation in the Apatzingán Valley, Michoacán, Mexico.

MATERIALS AND METHODS

This study was carried out in the Apatzingán Valley region, which is located in the southwestern part of the state of Michoacán, Mexico, and has a total area of 951,769 hectares (19° 05’ 03” N and 102° 21’ 15” W) (Google-Earth, 2022).

In the described region, a randomized complete block design was established, which enabled a comparison of the diversity of ants collected in each locality and municipality. Two formicines samplings were carried out. The first sampling was made from August 18 to 20, 2014, and 36 collections were obtained. The second sampling took place from March 16 to 24, 2015, with the same number of collections. A total of 72 collections were therefore gathered in eight municipalities: Aguililla, Apatzingán, Buena Vista, Gabriel Zamora, La Huacana, Francisco J. Múgica, Parácuaro, and Tepalcatepec. A population of 59 localities where Mexican lime (*Citrus aurantiifolia* Swingle) is produced was chosen from these municipalities.

In the orchards, the collection of ants was random. An entomological aspirator was used to isolate most of the specimens that were found in at least five sprouts of Mexican lime trees infested by formicines and by *D. citri* (Table 1).

The collected ants were deposited in Eppendorf® tubes containing 70% alcohol. Subsequently, they were classified by morphospecies and placed in glass jars with rubber seal lids. Some samples were mounted on No. 2 entomological pins and small ants were glued to cardboard triangles with Resistol 850® glue. Once the insects were labeled, a database was developed with Windows® Excel. The study was carried out at the Laboratorio de Invertebrados of the Facultad de Biología of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán. A LEICA® stereoscopic microscope, the dichotomous keys developed by Mackay and Mackay (1989), and the descriptions proposed by Bolton (1994) were used to determine the genus of the ants. Finally, the specialist Mr. Miguel Bolaños Vásquez (MSc), of the Universidad Autónoma de Guadalajara, was consulted to determine the species of some ants. The SAS University Edition 2018 software was used to analyze the data on the number of ants associated with *Diaphorina citri*, with a Tukey test at 0.05 (Castillo, 2003).

RESULTS AND DISCUSSION

Field work

We collected 2,218 ants associated with *Diaphorina citri* in 59 locations belonging to eight municipalities in the state of Michoacán, Mexico. The number of specimens collected ranged from 2 to 289 ants. Based on the highest to lowest number of ants collected, the municipalities where most ants were collected were: Buenavista, Apatzingán, F. J. Múgica, La Huacana, Parácuaro, Tepalcatepec, Aguililla, and Gabriel Zamora (Table 1).

The foregoing assumes that the density of ants is influenced by the surface of cultivated Mexican lime. Although this fruit is grown in 27 municipalities, most of the surface is concentrated in Buenavista (33%) and Apatzingán (32.5%); in the rest of municipalities the cultivated area is smaller, ranging from 3,500 and 160 to 1.6 hectares (SAGARPA, 2014).

Table 1 shows a contrast in the number of ants collected in the adjoining localities of Ampliación Presa del Rosario and Ejido Presa del Rosario, in the municipality of Apatzingán. Hypothetically, this is the result of the phytosanitary management that is given to the crop and the use of the land, since the lime trees in Ampliación Presa del Rosario are found in backyards and have little commercial area. In general, based on the ants-D. *citri* association, ants obtain sugary substances through associations with Hemiptera: Sternorrhyncha insects (Ramos and Serna, 2004). This relationship is called trophobiosis and is understood as the symbiotic association between organisms (Hölldobler and Wilson, 1990).

In Mexico, research has already been carried out about the relationships between ants, insects of the Hemiptera order, and plants (Ibarra and Dirzo, 1990; Rico *et al.*, 1998;

Table 1. Municipalities and localities of the Apatzingán Valley, Michoacán where ants associated with *Diaphorina citri* were collected (2014-2015).

Municipality	Locality	Number of ants collected (n)
Aguililla	El Aguaje	147
Apatzingán	Altamira	15
	Amp. Presa del Rosario	6
	Chiquihuitillo	18
	Ejido Apatzingán	39
	Ejido Presa del Rosario	97
	El Guayabo	9
	EL Mirador	45
	El Morado	6
	El Pino	22
	El Recreo	91
	La Nopalera	6
	Las Colonias	13
	Las Tinajas	10
	Mata de plátano	4
	Puerta de Alambre	12
	San Antonio la Labor	11
	San Fernando	18
	San Juan de los Plátanos	19
Buena Vista	18 de Marzo	109
	Catalinas	50
	Doroteo Arango	9
	El Porvenir	15
	El Recreo	31
	El Terreno	34
	Emiliano Zapata	145
	Felipe Carrillo Puerto	129
	Punta de Agua	56
	San José Piedras Blancas	2
	Santa Ana Amatlán	8
Gabriel Zamora	El Capire	140
La Huacana	Cupuán del Río	34
	Cupuancillo	24
	El Chauz	65
	La Peña	12
	Los Olivos	9
	Mártires de la parota	109
	Zicuirán	10
Francisco J. Múgica	El Ceñidor	289
	Nueva Italia	3

Table 1. Continues.

Municipality	Locality	Number of ants collected (n)
Parácuaro	Antúnez	58
	Buenos Aires	38
	Cancita	14
	Ciudad Morelos	12
	El Carrizo	14
	El Junco	17
	El Varal	8
	Úspero	16
Tepalcatepec	Catarino Torres	7
	Colomotitán	4
	El Montoso	4
	La Bocanada	83
	La Ordeñita	14
	La Romera	29
	Las Primaveras	3
	Los Huiranches	7
	Los Tambores	5
	Nuevo Corongos	6
	Pancha López	8

Torres *et al.*, 2000). The following ant-plant relationships stand out: myrmecophile, which only refers to those beneficial to the plant (Bentley, 1976); myrmecotrophy, in which the plant produces nutritive particles to feed the ants; myrmecophyte plants are those that have myrmecodomatia, morphological structures produced by the plant in which ants live (Gaume *et al.*, 2005); myrmecochory is the mechanism by means of which ants disperse seeds; and myrmecophily is the pollination carried out by ants (Hölldobler and Wilson, 1990). Nectaries are glands that produce sugary exudates which attract ants. They can be found in various parts of the plant, such as stems, leaves, and flowers. The appearance of nectaries could have been the result of a process of co-evolution between different species of plants and ants (Delabie *et al.*, 2003). Bentley (1976) and others indicate that there is a positive correlation between the frequency of plants with extra-floral nectaries and the abundance of ants in natural forests.

Taxonomic determination of the studied ants

The collected ants belong to five subfamilies, 15 genera, and the same number of species (Table 2). Vásquez-Bolaños (2015) mentions that 11 subfamilies, 93 genera, and 927 species are known in Mexico, which is relevant because, in this work, five subfamilies were recorded in a relatively small region of the national surface. According to SIAP (2016), this fruit tree is grown in 24 states with an approximate area of 81,221.90 hectares. Therefore,

Table 2. Taxonomic grouping of ants associated with *D. citri* in Mexican lime cultivation, in the Apatzingán Valley, Michoacán, Mexico (2014-2015).

Locality	Taxonomic categories		
	Subfamily	**Genus**	**Species**
Aguililla	Dolichoderinae	*Dorymyrmex*	*flavus*[‡]
	Dolichoderinae	*Forelius*	*damiani*[†]
	Myrmicinae	*Monomorium*	*minimum*[†]
	Myrmicinae	*Novomessor*	*ensifer*[†]
	Formicinae	*Paratrechina*	*longicornis*[†]
Apatzingán	Myrmicinae	*Pheidole*	sp. [‡]
	Myrmicinae	*Atta*	*mexicana*[‡]
	Myrmicinae	*Solenopsis*	*geminata*[‡]
	Myrmicinae	*Crematogaster*	*crinosa*[‡]
	Dolichoderinae	*Forelius*	*damiani*[‡]
	Dolichoderinae	*Dorymyrmex*	*flavus*[‡]
	Formicinae	*Paratechina*	*longicornis*[†]
Buena Vista	Myrmicinae	*Crematogaster*	sp. [‡]
	Myrmicinae	*Monomorium*	*minimum*[†]
	Dolichoderinae	*Forelius*	*pruinosus*[†]
	Formicinae	*Paratrechina*	*longicornis*[†]
	Formicinae	*Nylanderia*	*terricola*[†]
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*[†]
Tepalcatepec	Myrmicinae	*Cardiocondyla*	*minutior*[‡]
	Myrmicinae	*Atta*	*mexicana*[‡]
	Myrmicinae	*Novomessor*	*ensifer*[†]
	Myrmicinae	*Monomorium*	*minimum*[†]
	Myrmicinae	*Crematogaster*	sp. [†]
	Dolichoderinae	*Dorymyrmex*	*flavus*[‡]
	Dolichoderinae	*Forelius*	*mccooki*[†]
	Formicinae	*Nylanderia*	*terricola*[‡]
	Formicinae	*Brachymyrmex*	*obcurior*[†]
	Formicinae	*Paratrechina*	*longicornis*[†]
	Pseudomyrmecinae	*Pseudomyrmex*	*elongatus*[†]
Gabriel Zamora	Myrmicinae	*Atta*	*mexicana*[†]
	Myrmicinae	*Solenopsis*	sp. [†]
	Dolichoderinae	*Forelius*	*mccooki*[†]
	Dolichoderinae	*Forelius*	*pruinosus*[†]
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*[†]
	Formicinae	*Paratrechina*	*longicornis*[†]

Table 2. Continues.

Locality	Taxonomic categories		
	Subfamily	Genus	Species
La Huacana	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Novomessor*	*ensifer*†
	Myrmicinae	*Monomorium*	*minimum*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†
Francisco J. Múgica	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Solenopsis*	sp. †
	Myrmicinae	*Crematogaster*	sp. †
	Dolichoderinae	*Azteca*	sp. †
	Dolichoderinae	*Forelius*	*mccooki*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†
	Formicinae	*Brachymyrmex*	*obcurior*†
	Ponerinae	*Odontomachus*	sp.
Parácuaro	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Solenopsis*	sp.†
	Myrmicinae	*Crematogaster*	sp.†
	Dolichoderinae	*Forelius*	*mccooki*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Dolichoderinae	*Azteca*	sp. †
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†

‡=taxonimical determination of the ants carried out by Miguel Vásquez-Bolaños.
†=taxonimical determination of the ants carried out by Ana Leticia Escalante Jiménez.

the presence of formicines can be considered as a bioindicator for the phytosanitary management of different citrus agroecosystems since some are more disturbed than others.

The subfamilies with the greatest presence in the eight municipalities were: Myrmicinae, Dolichoderinae, and Formicinae. These results match the findings of Bolton (1995), who points out that only six of the 16 registered subfamilies of the ant fauna in question are distributed in all of the eight zoogeographical regions: Cerapachyinae, Dolichoderinae, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae.

Khachonpisitsa *et al.* (2020) and other authors confirm that the most abundant subfamilies are Mirmicinae which comprises 36.70% of all genera and 40.83% of all species, followed by Formicinae (17.43% and 10.59%), Dorylinae (11.01% and 9.83%), and Dolichoderinae (6.42% and 6.62%).

In the Apatzingán Valley, the subfamily with the least presence was Ponerinae, which was only collected in the municipality of F.J. Mugica. For their part, two to five species of the Myrmicinae subfamily, two to three Dolichoderinae species, and one to three Formicinae species were found in the eight municipalities (Figure 1). Rojas (2011) mentions that the ant fauna of the soil comprises 407 species: Myrmicinae is the most diverse subfamily, it includes 53% of the total number of species, and it dominates the tropical areas of Mexico, while Formicinae has been collected in temperate zones. The Dolichoderina subfamily has a cosmopolitan distribution and can be found everywhere, except for the polar areas (Ward *et al.*, 2010). It comprises approximately 900 described species (Bolton *et al.*, 2007), including some of the world's most successful invaders, such as the Argentine ant (*Linepithema humile*), the ghost ant (*Tapinoma melanocephalum*) and the white-footed ant (*Technomyrmex* spp.) (Williams 1994; Holway *et al.*, 2002). The adaptation and success of the ants is caused by the presence of the metapleural gland, whose phenylacetic acid secretion differentially inhibits the growth of microorganisms within the nests (Maschwitz *et al.*, 1970).

Regarding the distribution of ant genera and species by municipality, the subfamily Formicinae: *Paratrechina longicornis* was collected in all the study sites, followed by Myrmicinae: *Atta mexicana* (6 municipalities). In contrast, *Solenopsis geminata*, *Cardiocondyla minutior*, *Pheidole* sp., and *Crematogaster crinosa* were only recorded in one municipality. For its part, Pseudomyrmecinae: *Pseudomyrmex simplex* was found in five municipalities; meanwhile, two species from this subfamily (*Pseudomyrmex elongatus* and Ponerinae: *Odontomachus* sp.) were collected only in one municipality. (Table 3).

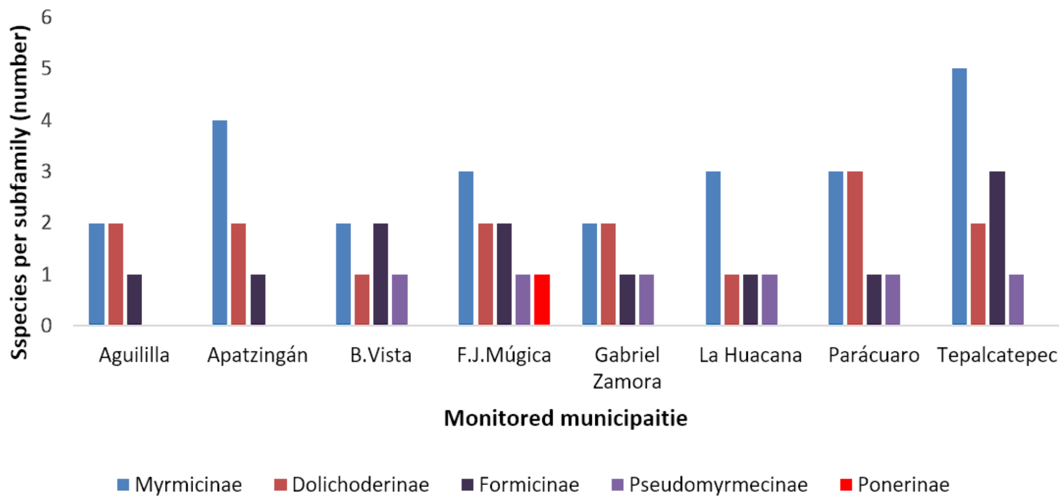

Figure 1. Ant subfamilies collected in eight Apatzingán Valley municipalities, Michoacán, Mexico (2014-2015).

Table 3. Distribution of ants associated with *Diaphorina citri* collected in Mexican lime trees, in eight municipalities of the state of Michoacán, Mexico (2014 and 2015).

Species \ Municipality	Aguililla	Apatzingán	Buena Vista	Francisco J. Múgica	Gabriel Zamora	La Huacana	Tepalcatepec	Parácuaro
Atta mexicana	Presente	Ausente	Ausente	Presente	Presente	Presente	Presente	Presente
Azteca sp.	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Ausente
Brachymyrmex obcurior	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Presente	Ausente
Cardiocondyla minutior	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Crematogaster crinosa	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Crematogaster sp.	Ausente	Ausente	Presente	Presente	Ausente	Ausente	Presente	Presente
Dorymyrmex flavus	Presente	Presente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Forelius damiani	Presente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Forelius mccooki	Ausente	Ausente	Ausente	Presente	Presente	Ausente	Presente	Presente
Forelius pruinosus	Ausente	Ausente	Presente	Ausente	Presente	Presente	Ausente	Presente
Monomorium minimum	Presente	Ausente	Presente	Ausente	Ausente	Presente	Presente	Ausente
Novomessor ensifer	Presente	Ausente	Ausente	Ausente	Ausente	Presente	Presente	Ausente
Nylanderia terricola	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Presente	Ausente
Odontomachus sp.	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Ausente
Paratrechina longicornis	Presente	Presente	Presente	Presente	Presente	Presente	Presente	Presente
Pheidole sp.	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Pseudomyrmex simplex	Ausente	Ausente	Presente	Presente	Presente	Presente	Ausente	Presente
Pseudomyrmex elongatus	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Solenopsis geminata	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Solenopsis sp.	Ausente	Ausente	Ausente	Presente	Presente	Ausente	Ausente	Presente

(green) = Ausente. (red) = Presente.

Formicinae: *Paratrechina longicornis* is present in all municipalities (Table 3). This species is defined as polygynous and polycalic; it dominates in highly stressed or disturbed environments, and limits the existence of other species (Smith, 1965). It should be monitored, given its omnivorous, opportunistic, predatory, and scavenger status, and its adaptability to both dry and humid environments. It is ecologically important —because it can displace other native ant or invertebrate species—, economically important —as an agricultural pest—, and socially important —because it causes damage to domestic spaces.

Vásquez-Bolaños (2015) reports that *P. longicornis* is present in various states of the Mexican Republic, but not in Michoacán; therefore, this is the first time that this species has been reported in the state.

Its dispersion is favored by trade and, in general, by human transport. It currently is one of the wandering ant species with the widest geographic distribution (Wetterer *et al.*, 1999). Regarding the species Myrmicinae: *Atta Mexicana*, Rojas (1989) reports its presence in several Mexican states, but not in Michoacán. This species is cataloged as a pest of economic importance, because it causes considerable losses when it defoliates timber trees and agricultural crops (Serratos *et al.*, 2017). Infante-Rodríguez *et al.* (2020) point out that the leaves that are not attacked have alkaloids, phenols, flavonoids, and tannins, as well as high concentrations of chlorogenic acid, (-)-epicatechin, quercetin-3, 4-di-O-glucoside, shikimic acid, ellagic acid, and scopoletin. These compounds have been linked to antifungal effects and deter leafcutter ants.

Despite the chemical defense strategies of the plants —which include the production of specific secondary metabolites, which are toxic to leafcutter ants (Cherrett 1972; Boulogne *et al.*, 2012; van Bael *et al.*, 2011), or which also inhibit the growth and development of their symbiotic fungus, *Leucoagaricus gongylophorus* (Möller) (Miyashira *et al.*, 2012; Lobo-Echeverri *et al.*, 2016)—, it has been reported that the symbiotic fungus of some *Atta* species can detoxify certain phenolic compounds present in the leaves of forage plants (Cherret 1980; Powell and Stradling, 1986; Nichols-Orians, 1991). In addition, the worker ant obtains certain enzymes (laccases or polyphenol oxidases) from the fungus. These fungal enzymes pass through the ant gut without becoming denatured (De Fine Licht *et al.*, 2013), are added by the ants as fecal droplets to processed plant material (Rockwood 1976), and help to detoxify some compounds present in the leaves (Aylward *et al.*, 2013). Consequently, the range of plants that leafcutter ants can harvest seems to be broadened through fungal-derived enzymatic detoxification techniques. The presence of this ant in the eight municipalities studied is worrisome, because in view of the lack of a comprehensive lime crop management, various cultural tasks —such as gravity irrigations, pruning, and nitrogen-based fertilization— induce the permanent presence of sprouts, propitiating the ideal environment for colonization by ants of the genus *Atta mexicana*.

As for *Monomorium minimum*, the little black ant is an occasional invader of human dwellings (Smith 1965). They are diurnal and consume immobilized arthropods, as well as sternorrhyncha honeydew (Stein and Thorvilson, 1989). *M. minimum* was found in soybean aphid (*Aphis glycinas*) populations, harassing or killing *Orius insidiosus* and *Harmonia axyridis*, which resulted in reduced predation and an up to 10-fold increase in aphid numbers when ants were present. Ants were not observed directly interfering with the *Aphidius colemani* parasitoid, although the number of parasitized aphids was higher in aphid colonies left unattended by ants (Sharaf *et al.*, 2015).

According to its biology and behavior when it becomes associated with *D. citri*, this situation results from the fact that this is where *M. minimum* finds its ideal food diet (meat=proteins and sugars=energy source). Therefore, the contribution of this work was the accurate identification of the formicines, which enabled an easier and more effective ant control. An erroneous identification can lead to inappropriate and expensive (time and money) control tactics and lead to unnecessary risks to people or the environment.

For its part, the genus *Crematogaster* is a widespread and distinctive lineage of myrmicine ants (Hölldobler and Wilson 1990). In most species, colonies can be huge (covering the canopy of trees) or tiny (contained within a single dead twig). Huge colonies are often polydomous, aggressive, and territorial. *Crematogaster* appear to be very generalized collectors and omnivores, with most of its species nesting on dead wood and in stem, branch, or trunk hollows (Longino, 2003).

The genus *Crematogaster* is clearly monophyletic, with a unique and apomorphic arrangement of postpetiole and gaster. The teardrop-shaped gaster points backwards and the postpetiole is attached to the dorsal surface of the fourth abdominal tergite. Consequently, the gaster is suspended below the postpetiole, rather than being clearly behind it. The petiole has no dorsal node and, when the gaster is elevated, the petiole fits level with the propodeum. This combination of characteristics is probably related to the defensive or offensive behavior during which the workers wave their gasters in the air, exuding a drop of venom on the spatula of the stinger (Buren, 1959).

The antennae have 11 segments with the exception of a lineage of Asian and African species, which have antennae with 10 segments and a terminal bundle of 2-4 segments. The propodeum usually has a pair of dorsal spines (lack of spines is rare among New World species). Species differ in the petiole and postpetiole shape, general pilosity characteristics, and surface sculpture (Bolton, 1995).

In this sense, *Crematogaster crinosa* was reported in Costa Rica as a very widespread species associated with lime cultivation, that prefers isolated and seasonally dry habitats. Workers are omnivores, are attracted to protein and carbohydrate baits, feed on dead or injured insects, visit extrafloral nectaries, and take care of homoptera. When their nests are disturbed, they can be aggressive. *Crematogaster crinosa* is the only member of the group that regularly dominates mangrove habitats (Longino, 2003). The presence of this ant in the Apatzingán Valley, Michoacán, is a clear sign that insects maintain magnificent alarm sensors, which makes them excellent bioindicators of an ecosystem's environmental health status; being poikilotherms, they easily respond to any stimulus, through physiological or biochemical mechanisms. In other words, the presence of this species suggests that most of the lime orchards in the study sites are neglected and old.

The subfamily Pseudomyrmecinae is monophyletic; it arose during the Cretaceous and probably most of its diversification took place during the Tertiary (Wilson and Hölldobler, 2005). They are fast-moving ants, with large eyes and a well-developed stinger; they generally inhabit the tree layer (Ward, 1990). Larsen and Philpott (2010) mention that *Pseudomyrmex ejectus*, *Pseudomyrmex simplex*, and *Pseudomyrmex* PSW-53 nest in twigs and act as predators of the coffee berry borer. Their presence may not be as closely related to the intensity of shade management as has been suggested for other arthropod predators of the coffee berry borer. The *Pseudomyrme simplex* species has been registered in the state of Tamaulipas, Mexico (Coronado-Blanco, 2013).

Ants have a remarkable functional diversity, since they comprise a wide spectrum of trophic guilds. However, from the point of view of their diet, ants always select the most nutritious materials (Stradling, 1978). They take food from various trophic levels such as seeds, nectar, fungi, insect secretions, carcasses, faeces, live prey (various arthropods), or a combination of these elements (Bolton, 1994; Petal, 1978).

In addition, *Citrus aurantiifolia* is a citrus species that produces vegetative sprout throughout the year. Consequently, tender tissues are available that favor the presence of overlapping generations of *D. citri* and allows nymphs to feed on the sap of leaves and tender stems, during their five stages. The result of this food process is the secretion of large amounts of sugar that largely define the Citrus-*Diaphorina*-formicines association.

The genera *Azteca* and *Forelius* —part of the subfamilies and genera Myrmicinae: *Solenopsis* and *Dolichoderinae*— are commonly found in warm environments and arid zones; according to the climatic classification developed by García (1987), the Apatzingán Valley is a region with an arid climate and scarce rainfall.

Finally, the records presented by Soto-Cárdenas *et al.* (2019) also agree that the most abundant subfamily was Myrmicinae, followed by Dolichoderinae and Formicinae, while the least abundant was Ponerinae. The least abundant genera were: *Brachymyrmex*, *Odontomachus*, and *Cardiocondyla*.

Statistical analysis of ants by municipality, genera, and species

The information from 72 collections was statistically analyzed; the collections were separated in eight blocks —one block per each of the municipalities severely infested by *D. citri*. The results show that the experimental model was significant, given the value of F_{cal}=2.19 *vs.* Pr>F=0.0019. On the contrary, the blocks were not significant when F_{cal}=1.20 *vs.* Pr>F=0.3216, which indicates that the municipalities are physically similar (climate, altitude, variety of plantations, and agronomic management). Likewise, ant dispersion can be favored by the lack of phytosanitary control or good practices when the materials used during the sowing of the orchards and the harvesting of the limes are handled, as well as during the transportation of plants to other plots or the packaging of the fruits.

The result obtained from the analysis of the dependent ant variable was significant. This value (F_{cal}=3.34 *vs.* Pr>F=0.0006) means that there is a very close ants-*D. citri* relationship and that at least one genus of ants represented greater association. Therefore, according to the decision rule, the null hypothesis is rejected (Table 4). The determination coefficient (r^2) explains by 86.30% that the density of ants is closely related to *Diaphorina citri*. The result of the coefficient of variation (CV=28.45) suggests that the accuracy with which the treatments were compared is reliable and that the experiment is statistically acceptable (Table 4).

Table 4. Analysis of variance of the number of ants associated with *Diaphorina citri* in Mexican lime orchards, in the Apatzingán Valley, Michoacán (2014-2015).

Source of Variation	Degrees of Freedom	Sum of Squares	F_{cal}	Pr>F
Modelo	23	202221.73	2.69	0.0019
Error	48	156937.55	-0-	-0-
Total	71	359159.30	-0-	-0-
Blocks	7	25472.19	1.10	0.3706
Ants	14	174783.15	3.34	0.0006

R^2	Coefficient of variation
0.8630	28.4532

For the interpretation of Tukey's test (α=0.05), the assigned letters belonged to five groups. However, the most abundant genera and species in this research were *Paratrechina longicornis*, followed by *Forelius mccoki*, and *Atta Mexicana*: statistically, they presented the highest mean and, during the field practice, they showed greater density in the municipalities studied. These results reflect a close association with *D. citri* and, in the case of *Atta mexicana*, it is related to vegetative sprouting. The genera with the least impact were *Odontomachus* sp. and *Cardiocondyla minutior* (Table 5).

CONCLUSIONS

The municipalities where more ants were collected were Buenavista, Apatzingán, and F. J. Múgica. In the 59 localities, five subfamilies associated with lime cultivation and with *Diaphorina citri* were collected: Dolichoderinae, Myrmicinae, Formicinae, Pseudomyrmecinae, and Ponerinae. The dominant species were *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*. The least abundant species were *Solenopsis geminata*, *Crematogaster crinosa*, *Cardiocondyla minutior*, and *Odontomachus* sp. The genera Azteca, *Forelius*, *Dorimyrmex*, and *Paratrechina* were collected in the driest, warmest, and most disturbed municipalities. *Diaphorina citri* and the ants that cohabit in the Apatzingán

Table 5. Grouping of ants associated with *Diaphorina citri* in Mexican lime orchards, in the Apatzingán Valley, Michoacán (2014-2015).

Tukey group	Average	Ant
A	31.50	*Paratrechina longicornis* (Latreille, 1802).
A	30.50	*Forelius mccooki* (McCook 1880).
A	30.00	*Atta mexicana* (F. Smith, 1858).
A	30.00	*Forelius pruinosus* (Roger, 1863).
B	23.250	*Forelius damiani* Guerrero & Fernández, 2008
B	22.71	*Crematogaster crinosa* Mayr, 1862
B	22.50	*Crematogaster* sp. Lund 1831.
C	19.75	*Monomorium mínimum* (Buckley, 1867).
C	16.37	*Pseudomyrmex simplex* (Smith, 1877).
C	13.00	*Dorymyrmex flavus* (McCook, 1879).
C	11.25	*Solenopsis* sp. (Westwood, 1840).
D	7.50	*Pseudomyrmex elongatus* (Mayr, 1870).
D	7.50	*Azteca* sp. Forel, 1878.
D	7.00	*Brachymyrmex obscurior* (Forel, 1893).
D	7.00	*Nylanderia terrícola* (Buckley, 1866).
D	7.00	*Novomessor ensifer* (Forel, 1899).
D	6.75	*Pheidole* sp. (Westwood, 1839).
D	6.75	*Solenopsis geminata* (Fabricius, 1804).
E	1.00	*Cardiocondyla minutior* Forel, 1899.
E	1.00	*Odontomachus* sp. Latreille, 1804.

Means with the same letters are not significantly different. Means with different letters are statistically different.

Valley region are adapted to a warm climate without a defined winter. Lime plants grow sprouts on a permanent basis, a condition that favors the plant-insect relationship. The association between *Diaphorina citri* and ants from the following genera was confirmed: *Pheidole*, *Brachymyrmex*, and *Solenopsis*.

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