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Cotton Fleahopper[1] Biology and Ecology Relevant to Development of Insect Resistance Management Strategies

Kristin Hamons[2], Tyler Raszick[2], Lindsey Perkin[3], Gregory Sword[2], and Charles Suh[3]*

Abstract. Since the success of the U.S. Boll Weevil Eradication Program and widespread adoption of genetically modified crops, the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), has reemerged as a significant cotton pest. Current management strategies for the pest are based primarily on foliar applications of insecticides. A line of cotton that produces modified *Bt* Cry51Aa2 protein was developed for *Lygus spp.*, but the protein has also shown activity against the cotton fleahopper. Consequently, before this new *Bt* line can be released commercially, refuge requirements and other Insect Resistance Management (IRM) strategies mandated by the Environmental Protection Agency need to be established to prevent or delay development of resistance by cotton fleahopper to the new toxin. In response, we provide and discuss relevant information on the biology and ecology of the cotton fleahopper, including life history, host preference, dispersal, and population genetic structure, that may be useful for developing IRM strategies for the pest.

Introduction

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), was initially described as *Psallus seriatus* in 1842. However, the first report of the insect damaging cotton was not recorded until the end of the 19th Century (Howard 1898). The cotton fleahopper is believed to be native to Texas, but it has been reported throughout the southern United States (U.S.) and northern Mexico (Hixson 1940, Little and Martin 1942). Although the cotton fleahopper prefers wild weed hosts (Reinhard 1926, Holtzer and Sterling 1980, Barman 2011), adults move to and reproduce on cotton as spring and early-summer weed hosts mature and become less attractive (Reinhard 1926, Gaines 1933, Almand et al. 1976). Adults and nymphs damage cotton plants by sucking sap from young squares and terminal growth, which can result in excessive fruit loss, abnormal plant growth, and delayed plant maturity (Reinhard 1926, Almand et al. 1976, Ring et al. 1993).

During the early 1900s, the cotton fleahopper was considered to cause as much damage as the boll weevil, *Anthonomus grandis grandis* Boheman (Reinhard 1926). However, as boll weevils spread throughout Texas and eventually throughout the entire U.S. Cotton Belt, the economic significance of the cotton fleahopper was largely overshadowed by the boll weevil and eventually the heliothine complex (cotton

[1]*Pseudatomoscelis seriatus* (Reuter) (Hemipera: Miridae)

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bollworm and tobacco budworm). Since the success of the U.S. Boll Weevil Eradication Program and wide-spread adoption of genetically modified crops in the mid-to-late 1990s, the cotton fleahopper reemerged as a significant cotton pest. In 1999, the cotton fleahopper was considered the most economically damaging insect pest of U.S. cotton, costing producers an estimated $196 million in control and yield losses (Williams 2000). The following year, the cotton fleahopper was ranked the ninth-most damaging insect pest of cotton, infesting approximately 42% of U.S. cotton acreage (Williams 2001). In 2007, the cotton fleahopper was considered the fourth-most damaging insect pest of cotton and was deemed responsible for loss of 120,000 bales (Williams 2008). During the past decade, the cotton fleahopper has consistently ranked between the fourth- and sixth-most damaging insect pest of U.S. cotton, with most damage reported in Texas and Oklahoma, and occasionally in Arizona, Arkansas, Louisiana, Mississippi, and New Mexico (Williams 2008-2017).

Currently, cotton producers rely exclusively on foliar applications of insecticides to manage the cotton fleahopper, typically administering one to four applications during the initial 3 or 4 weeks of squaring when plants are most susceptible to damage by cotton fleahoppers (Parker et al. 2008, Vyavhare and Reed 2020). Acephate has historically been the standard choice of insecticide against the cotton fleahopper, but neonicotinoid insecticides (e.g., thiomethoxam) are now preferred and recommended (Parker 2012, Vyavhare and Kerns 2017). Treatment thresholds in Texas are based on the combined number of adults and nymphs on plants, but action levels vary among production regions. For example, the action level for the Blackland production region is 10-15 cotton fleahoppers per 100 terminals, whereas the recommended level in the Rolling Plains and Panhandle production regions is 25-30 cotton fleahoppers per 100 terminals. In addition to cotton fleahopper densities, action levels in some production regions (e.g., South and Rolling Plains, Panhandle, Permian Basin, Trans Pecos) also consider the amount of square set relative to the week of squaring. Once plants begin to bloom, insecticide is not needed for cotton fleahoppers, even though cotton fleahoppers may continue to be present in fields throughout the entire growing season. Insecticidal application to manage the cotton fleahopper is often followed by an outbreak of secondary pests because beneficial insects are eliminated (Almand et al. 1976). To prevent outbreaks of secondary pests, new management strategies for the cotton fleahopper are needed that reduce the need for insecticide and preserve beneficial insects.

The Monsanto Company (now Bayer Crop Science) developed a line of cotton (MON88702) that produces a modified *Bt* Cry51Aa2 protein selective against a number of hemipteran and thysanopteran pest species (Bachman et al. 2017). Although development of the new *Bt* cotton line was intended for the tarnished plant bug, *Lygus lineolaris* (Palisot de Bauvois), and western tarnished plant bug, *L. hesperus* (Knight), the protein also was active against the cotton fleahopper. As is the case for other insect pests on *Bt* crops, development of resistance to the toxin is a major concern. Consequently, before the new *Bt* line can be released commercially, refuge requirements and other Insect Resistance Management (IRM) strategies mandated by the Environmental Protection Agency (EPA) must be established to prevent or at least delay development of cotton fleahopper resistance to the new toxin. In response, the objective of this paper is to provide a synthesis of cotton fleahopper literature that may be useful for development of an IRM strategy for this cotton pest. Information on biology and ecology of the cotton fleahopper with emphasis on its life history, seasonal utilization of weed hosts, dispersal capabilities, and genetic diversity and gene flow patterns are presented and discussed.

Life History

The life cycle of the cotton fleahopper consists of three main stages: 1) egg; 2) five nymphal instars; and 3) adult. Gaylor and Sterling (1975) estimated a development threshold of 7.0°C and provided the average development time for each life stage at various constant temperatures (Table 1). In general, non-diapausing eggs hatch in about 10 days, and nymphs need 2 to 3 days to complete each of five instars. Thus, it takes 20 to 30 days for the cotton fleahopper to complete a life cycle (egg to adult), or about 570 to 600°C-days for the total life cycle (egg to egg). There usually are six to eight generations in a year, most of which are spent on weed hosts. However, as many as three generations may occur in cotton (Bohmfalk et al. 2005).

Cotton Fleahopper Eggs. Eggs are elongate (~0.8 to 0.9 mm) and about 0.2 mm in diameter around the widest part (Fig. 1). Eggs are typically oviposited under the bark of host plants where they are protected from predators and environmental elements (Fig. 1A, B). Initially, eggs are creamy white, but become yellowish as they age (Fig. 1C). The red eyes of developing embryos become visible before nymph emergence.

Eggs also serve as the overwintering stage for the cotton fleahopper by entering a state of diapause in late summer/early fall (Reinhard 1928, Gaylor and Sterling 1977). Oviposition of diapausing eggs typically occurs at the beginning of September in Central Texas, and the incidence of diapausing eggs increases throughout the month. Almost all eggs laid after the first week of October are in diapause (Gaylor and Sterling 1977). Diapause is induced by decreasing photophase, which must be experienced earlier than the third instar of the parental generation to cause eggs to diapause. The authors also reported that about 50% of diapausing eggs collected from the field were in an "intense" form of diapause and subsequently required exposure to temperatures cooler than 10°C before diapause could be terminated. The other 50% of eggs seemed to be in a less intense form of diapause. In either case, diapausing eggs require exposure to humidity (e.g., rainfall) and warm temperatures to terminate diapause. Following adequate exposure to moisture and warm temperatures in spring, diapause is terminated and most eggs in the field begin to hatch in March and April (Reinhard 1928).

Diapausing eggs are distinguished from non-diapausing eggs by their appearance after submersion in water (Gaylor and Sterling 1977). Diapausing eggs remain opaque and yellowish upon submersion, while non-diapausing eggs begin to

Table 1. Mean Number of Days Spent in Respective Life Stages of the Cotton Fleahopper at Various Temperatures

		Nymphal instars						
Temp (C°)	Egg	1st	2nd	3rd	4th	5th	Total (1st-5th)	Adult
40.6	-	1.0	1.2	3.1	2.3	2.0	8.6	-
35.0	9.5	2.0	1.2	1.7	1.7	2.3	8.9	11.6
29.4	9.4	2.3	2.0	1.9	2.0	2.7	10.9	14.8
26.7	11.0	3.4	2.4	2.3	2.6	3.2	13.9	22.1
23.9	11.8	5.3	3.2	2.8	3.3	4.2	18.8	21.3
15.0	-	14.4	10.7	11.8	11.9	15.5	63.8	-
12.8	-	18.8	11.8	23.7	4.0	-	-	-

Table modified from Gaylor and Sterling (1975).

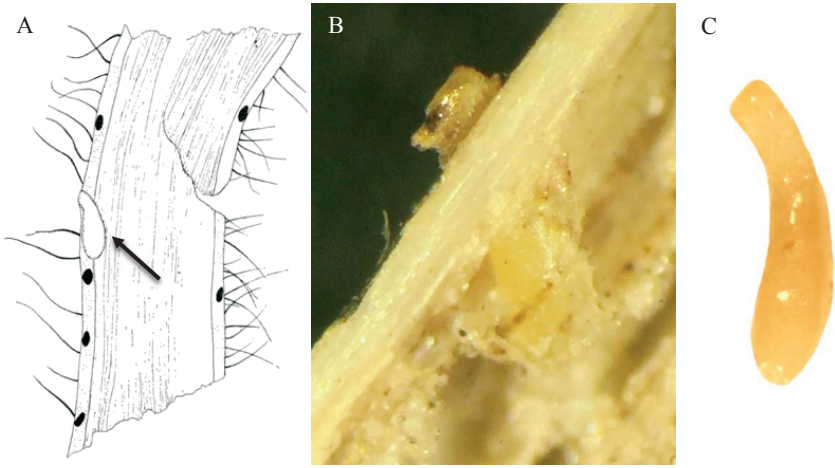

Fig. 1. A) Section of cotton stalk showing position in which the egg was laid beneath the bark (arrow). Figure from Reinhard (1926), B) cotton fleahopper egg in cross-section of stem of woolly croton, C) cotton fleahopper egg dissected from croton stem.

clear, allowing visualization of the yolk or developing embryo. Breene et al. (1989) provided methods to terminate diapause and induce hatching of eggs in laboratory conditions and provided rearing methods for subsequent nymphs. The approach is especially useful for generating and maintaining large numbers of nymphs for experiments. Croton (*Croton* spp. L.) stems containing diapausing eggs are submerged in water every other day for 15 minutes to simulate spring rain. The stems are allowed to dry for approximately 30 minutes after each soaking before being put in an environmental chamber (30°C, 14:10 light:dark hour photoperiod). Eggs begin to hatch in 14 days, and subsequent nymphs can be reared to adulthood on green beans (*Phaseolus* spp.) and sliced potatoes (*Solanum tuberosum* L.). However, nymphs and adults can be reared entirely on green beans without any adverse effects on survival or longevity of adults (Suh unpublished data).

Cotton Fleahopper Nymphs. Reinhard (1926) provided guidelines for distinguishing five nymphal instars of the cotton fleahopper based on the shape and relative size of wing pads and average width of the head capsule. However, inexperienced observers typically have difficulty discerning wing pads in early instars. Suh (2007) observed the average and range of head-capsule widths for each instar depended on the host on which nymphs were reared, and ranges among later instars slightly overlapped. Consequently, the author suggested using the width of head capsules to distinguish the first three instars (Table 2) and wing-pad characteristics to distinguish fourth and fifth instars (Fig. 2).

Cotton Fleahopper Adults. The adult cotton fleahopper is approximately 3 mm long, yellow-green, with reddish eyes (Fig. 3). Four dark spots on the cuneus of the cotton fleahopper hemelytra (Fig. 3A) help distinguish adults from similar-looking insects. Other unique features include dark striations or spots on the legs and antennae, and spines on the legs (Fig. 3B, Reinhard 1928).

Table 2. Average and Range of Head-Capsule Widths for the Five Nymphal Instars of the Cotton Fleahopper Reared on Green Beans or Collected from Horsemint and Cotton Plants in the Field.

	Mean (mm)			Range (mm)		
Instar	Green bean	Horsemint	Cotton	Green bean	Horsemint	Cotton
1	0.263	0.257	0.211	0.225-0.280	0.200-0.275	0.225-0.275
2	0.340	0.347	0.333	0.315-0.350	0.313-0.375	0.313-0.356
3	0.414	0.436	0.415	0.388-0.438	0.406-0.463	0.381-0.438
4	0.500	0.530	0.505	0.450-0.538	0.488-0.569	0.475-0.538
5	0.589	0.626	0.590	0.550-0.625	0.588-0.669	0.556-0.625

Table modified from Suh (2007).

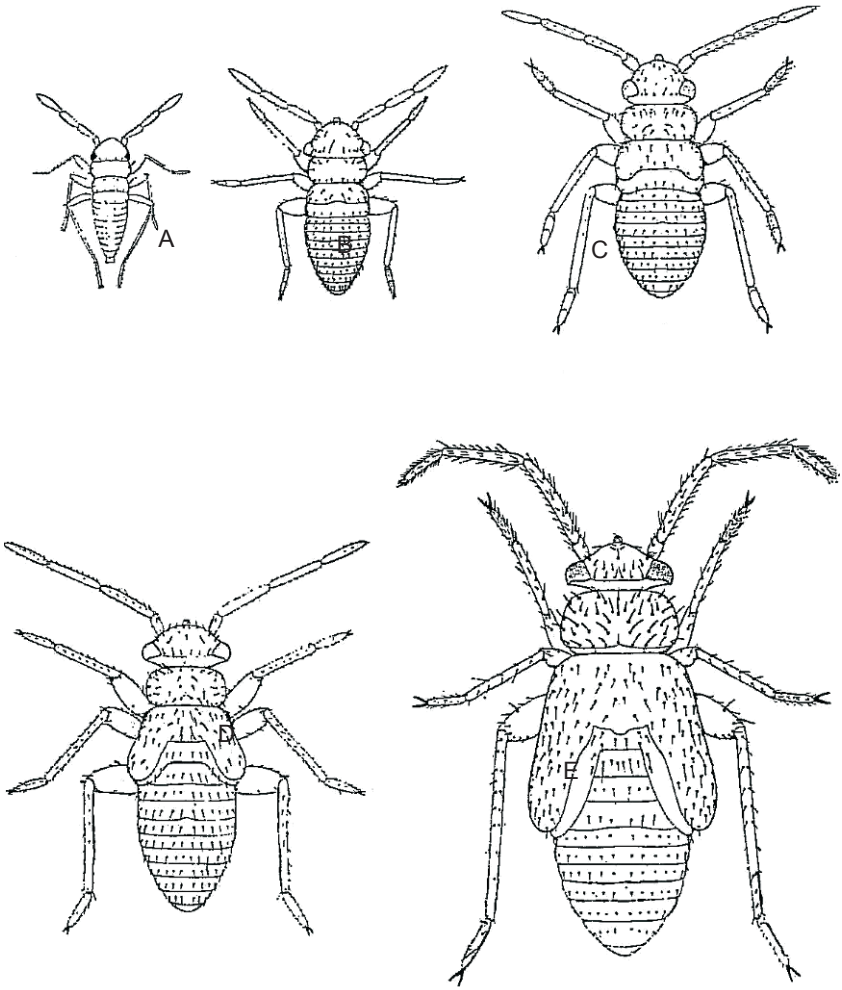

Fig. 2. Wing-pad development on five nymphal instars of the cotton fleahopper: A) first, B) second, C) third, D) fourth, and E) fifth. Figure from Reinhard (1928).

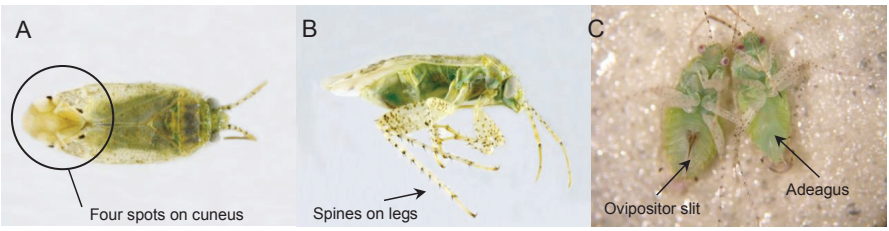

Fig. 3. A) Adult cotton fleahopper dorsal view with four distinguishing spots on the cuneus, B) adult cotton fleahopper lateral view showing dark striations on legs and antennae and spines on legs, C) ventral view of adult cotton fleahopper female and male, arrows point to the ovipositor slit (female) and adeagus (male).

Unfortunately, the intensity of the striations tends to fade in dead specimens, particularly on those captured on sticky traps or stored in alcohol (Suh unpublished data). Males and females are dimorphic as adults; females easily can be distinguished from males based on the presence of a ventral abdominal slit that houses the ovipositor (Fig. 3C). The outline of the slit is translucent in newly enclosed females, but the outer portion darkens with age as it becomes sclerotized. Likewise, the outline of the aedeagus within the abdomen of adult males becomes more visible as it becomes sclerotized with age.

Host Preference and Seasonal Utilization of Hosts

The cotton fleahopper was reported on more than 160 species in 35 families in North America. Esquivel and Esquivel (2009) provided a compendium of host plants reported in the literature from 1898 to 2008. Thus, we did not discuss the species in the survey, but outlined the main seasonal hosts in Central Texas that are generally recognized to play a major role in cotton fleahopper development and life cycle. The hosts include horsemint (spring), cotton (summer), and croton (late summer and winter) (Fig. 4).

Croton spp. (commonly referred to as woolly croton, goat weed, dove weed, sage weed) play a key role as a late-season and overwintering host for the cotton fleahopper. Diapausing eggs are laid in terminal ends of croton plants as early as September but most are oviposited in October. Upon terminating diapause in the spring, emerging nymphs feed on tender vegetative growth within 30 m of their emergence site to complete development to adulthood (Hixson 1941). Adults from diapausing eggs seek preferred host plants such as horsemint, *Monarda* spp., where they continue to reproduce and accumulate en masse. Cotton fleahopper adults may move to cotton as preferred spring-time weed hosts begin to mature and become less attractive (Almand 1976). Adults move into cotton as early as April, but most dispersal occurs from mid-May to the first part of June (Gaines 1933), which corresponds with the initial onset of pre-floral buds (squares) on cotton plants in Central Texas (Parker et al. 2008). Although cotton is not a preferred host, the cotton fleahopper may remain in cotton for several generations until preferred host plants are available or cotton is harvested. By the end of July, croton is readily available and cotton fleahoppers begin to accumulate en masse on the host. The cotton fleahopper may complete one or more generations on croton before using the plants as an overwintering host (Hixson 1941).

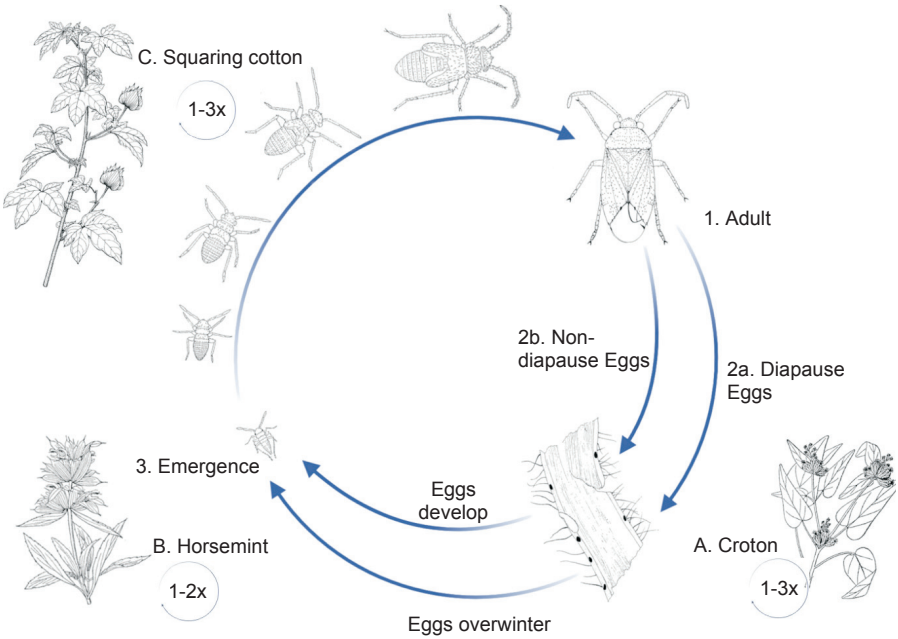

Fig. 4. Seasonal sequence of plant hosts used by the cotton fleahopper in Central Texas. 1) Adult cotton fleahoppers seek out seasonal host plants to feed and reproduce, including croton, horsemint, and cotton (A, B, C). Eggs are laid on croton (A) where they overwinter in a state of diapause under the bark (2a) or continue to develop (2b). 3) Nymphs emerge from eggs and develop on seasonal hosts, such as horsemint (B) near their emergence site for one to two generations. After seasonal hosts mature, cotton fleahoppers can infest cotton (C) for up to 3 generations. Lastly, cotton fleahopper adults seek croton when it becomes available again in mid- to late-summer and the cycle repeats.

Dispersal and Distribution Within Fields

Adult cotton fleahoppers disperse from native weed host plants and into cotton during the spring and early summer. Cotton fleahoppers, because of their small body size, originally were assumed to move only from local or adjacent fields into cotton. Thus, a county-wide (Calhoun Co., TX) effort was made in 1933 to eliminate all croton plants, the main overwintering host plant containing cotton fleahopper eggs, within a 40-km area surrounding cotton fields to reduce numbers of cotton fleahoppers in cotton the following spring. However, cotton fields from those areas still became heavily infested by cotton fleahoppers the following spring (Gaines and Ewing 1938). In fact, the distance between *Croton* spp. fields and cotton did not influence quantities of cotton fleahoppers infesting cotton; fields adjacent to croton had similar numbers of cotton fleahoppers compared to fields a distance away (at least 32 km; Gaines 1933, Gaines and Ewing 1938). The findings led the authors to conclude that cotton fleahoppers were capable of moving long distances despite having a small body. Results of the studies also led the authors to speculate that cotton fleahoppers might use wind currents to travel long distances. Balloon release and recovery were studied

in 17 south-central counties in Texas during late spring and summer when cotton fleahoppers move from weed hosts to cotton. Approximately 10% of the released balloons were recovered, with an average drift distance of 69 km and a drift range of 39 to 604 km. The prevailing wind was generally in a north-northeast direction from areas with light soils where *Croton* spp. grow abundantly in the fall, to heavy soils where cotton was cultivated (Gaines and Ewing 1938). Thus, it seems possible that cotton fleahoppers use wind-assisted dispersal to move to cotton at similar distances. Also, Tanglefoot® traps, extended up to 8 m into the air, and netting attached to airplanes were used to determine what height cotton fleahoppers reached during peak dispersal periods. Cotton fleahoppers were caught on Tanglefoot® traps in greater numbers and more frequently at 6-8 m than at 1-3 m in early spring. Based on aerial surveys using netting attached to airplanes, cotton fleahopper adults and nymphs were caught up to 609 m above the ground. The studies suggested that wind currents play a major role in dispersal, allowing cotton fleahoppers to travel long distances and at high altitudes to infest cotton many kilometers away. Mass movements of adult cotton fleahoppers into cotton have been observed immediately following passage of major northern frontal systems (Suh and Spurgeon 2004).

Other abiotic factors that influence the seasonal abundance of cotton fleahoppers in cotton from year to year are rainfall (timing and amounts) and land usage. Rainfall events that occur in the early fall promote growth of host plants, mainly *Croton* spp., thus supporting more cotton fleahoppers and diapausing eggs that emerge the following spring (Gaines 1933). Disturbance of the soil can also result in large quantities of croton (Fletcher 1940). For example, one grazing field in the Brazos River Bottom increased from 45 plants per hectare to more than 8,094 plants per hectare after it was terraced. The plants in newly disturbed soil also tended to be taller and have more seed capsules per plant. Fields fallow for a year had more croton with taller and more branched plants allowing cotton fleahoppers to flourish. However, if the field was fallow for 2 or more years, croton populations were substantially reduced by competition with annual weeds that became more abundant with time. Thus, a combination of seasonably early fall rains and land management practices can have a profound effect on abundance of cotton fleahoppers emerging from *Croton* spp. and subsequent numbers eventually dispersing to cotton in the spring.

Several degree-day models were developed to determine factors that might influence timing of spring emergence and ultimately infestation of cotton by cotton fleahoppers. Sterling and Hartstack (1979) used cotton fleahopper emergence threshold with a thermal accretion model to forecast spring emergence trends. Additionally, development of cotton fleahopper was determined not only to be affected by temperature, but also the host plant on which it fed (Gaylor and Sterling 1976). Almand (1976) documented seasonal dispersal of the cotton fleahopper across a variety of host plants, but the information has yet to be incorporated into a model. Current and ongoing research is revisiting these studies to build upon and develop a new, more realistic simulation model that incorporates development and seasonal host preferences of cotton fleahoppers. The model would allow manipulation in many aspects such as extending or reducing the availability of host plants to observe seasonal distribution of the cotton fleahopper. Preliminary results showed that increasing the amount and duration of horsemint availability might reduce infestation by cotton fleahopper in cotton (unpublished data). This suggests that when spring weather conditions are favorable for horsemint, delaying senescence, it might retain cotton fleahoppers that would otherwise infest cotton. Alternatively, it might be possible to use horsemint as a wild refuge or trap crop planted near a cotton field if

wild populations of horsemint are insufficient. A simulation model might enable further understanding of the complex relationship between pest and hosts, and thus aid in Insect Resistance Management strategies in cotton.

Once in cotton, cotton fleahoppers tend to prefer the upper portion of plants. Within-plant distribution averaged 65, 24, and 11% of the total abundance of cotton fleahoppers in the upper, middle, and lower one-third of the plant, respectively (Parajulee et al. 2006). A later study by Suh and Westbrook (2010) came to a similar conclusion and found 64% of adults and 78% of nymphs on cotton terminals, regardless of the time of the day plants were sampled. Cotton fleahopper nymphs also tend to be more aggregated than adults on a plant, which probably is because of their inability to move readily, causing them to stay mostly near the site where they were oviposited (Hixson 1941). Conversely, adults have the ability to fly and are not found as often in clusters as are nymphs (Riley and Sterling 1983).

Genetic Diversity and Gene Flow

Despite the depth of study into patterns of plant use and dispersal by the cotton fleahopper, only a few studies explored evolutionary impacts of those behaviors on the cotton fleahopper genome at the population level. As a result, little is known of the population genetic dynamics within and among cotton fleahopper populations. There also is lack of understanding on how dispersal might influence gene flow, which has important implications for management, especially in regard to evolution of resistance to insecticides and transgenic crops over time (Georghiou and Taylor 1977, Guse et al. 2002, Onstad et al. 2002). Gene flow between pest populations feeding on transgenic crops and those feeding on refuge plants has played an important role in managing development of insect resistance to *Bt* crops (Caprio 2001, Caprio et al. 2004, Carrière et al. 2004). A robust understanding of the population genetic structure of the cotton fleahopper is critical for implementation of an effective IRM plan with regard to the MON88072 line. The few studies that explored population genetics in cotton fleahopper (Barman et al. 2012, 2013; Antwi et al. 2015; Raszick et al. 2020) have had some notable limitations. First, only one study broadly described cotton fleahopper genetic diversity on a large geographical scale (Barman et al. 2013). Barman et al. (2012) and Antwi et al. (2015) narrowly focused on genetic diversity within Texas and studied host-race formation via host-associated differentiation. Both host-associated differentiation studies were limited by weaknesses in their designs including use of Amplified Fragment Length Polymorphism (AFLP) genetic markers and mobile adults instead of nymphs. Finally, Raszick et al. (2020) assessed the population genetic structure of nymphs and adults on mid-season and overwintering host plants using single nucleotide polymorphic (SNP) markers but did not sample early spring hosts (e.g., *Monarda* spp.). Additionally, Raszick et al. (2020) revealed a pattern of temporal instability in the population genetic structure from year to year, further complicating interpretation of the three previous studies.

Geographical Structure of Cotton Fleahopper Populations. The only broad survey of genetic diversity across a large portion of cotton fleahopper native range was by Barman et al. (2013). The study sampled 172 individuals collected exclusively from cultivated cotton across almost the entirety of its distribution (11 sampling localities across cotton-growing regions of the US, and a single sampling locality at Tamaulipas, Mexico). The authors found three regional population groups: western (AZ), central (TX, OK, AR, MS, LA, AL, and Mexico), and eastern (FL, GA, SC, and NC). Gene flow within regions was relatively high, but gene flow among regions was

low, similar to regional population genetic patterns observed in other species with limited dispersal (Meng et al. 2008, Elameen et al. 2016, Wang et al. 2017). This finding was further supported by a pattern of isolation-by-distance, wherein significant and positive correlation was detected between geographical regions and genetic distance of cotton fleahopper populations. Within regions, genetic diversity of the western and eastern regions was low compared with the central region that had more diversity within populations. The pattern is consistent with the idea that cotton fleahopper individuals are non-migratory and weak fliers that do not regularly travel long distances during their lifetime.

However, because Barman et al. (2013) specifically aimed to document genotypic variation across cotton-associated populations in the U.S., the study did not consider genetic diversity potentially present in non-cotton populations. Because the cotton fleahopper has been reported to use a broad diversity of host plants across many families, there probably is much genetic diversity in wild populations that was not investigated by the study. Indeed, Barman et al. (2012) identified a role of host-associated differentiation in driving evolutionarily significant diversification in the species, in which populations associated with different host plants display distinct genotypes. The pattern is mediated to some degree by geography, but nonetheless illustrates that sampling solely from cotton might bias data toward under representation of true genetic diversity within and among locations.

Host-Associated Differentiation. Barman et al. (2012) was the first study to explicitly investigate cotton fleahopper population genetics in the context of host-associated differentiation. In the study, cotton fleahoppers were sampled from three important host plants (horsemint, croton, and cultivated cotton) at peak flowering times in five cotton-growing regions of Texas (Lubbock, San Angelo, College Station, Weslaco, and Corpus Christi). The authors presented evidence supporting the existence of at least one distinct host-race associated with horsemint and a second host-race that may be associated with cotton and croton (Fig. 5). However, the pattern was influenced by geography wherein host-associated differentiation was observed at some sampling localities but not at others. Genotypes observed in all three host

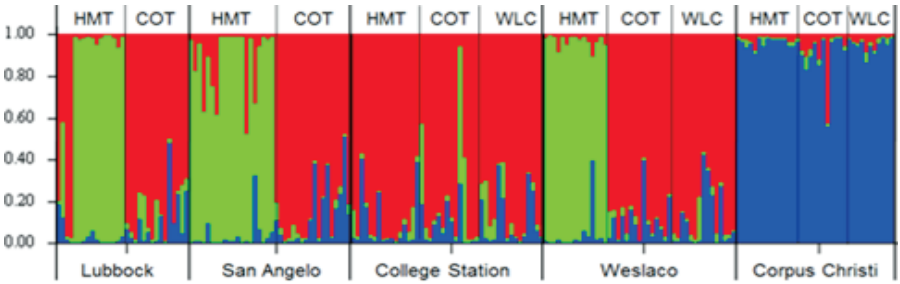

Fig. 5. STRUCTURE analysis of cotton fleahopper populations inferred from AFLP markers. Individuals are organized according to location and within each location, by each host plant (HMT, horsemint; COT, cotton; WLC, croton). Three inferred genetic clusters (K = 3) are represented by green, red, and blue. Individuals are represented by a narrow vertical column divided into segments in proportion to inferred membership of each of the three clusters. Figure adapted from Barman et al. (2012).

plants at Corpus Christi were unique to that locality, adding support for a strong influence of geography. Corpus Christi is located centrally among the sampled populations along the latitudinal gradient and upwind of the prevailing wind current direction. Consequently, observed distributions of genotypes are inconsistent with expected patterns of dispersal. Ultimately, the limited range of host plants in the study makes it impossible to draw conclusions about whether these apparent host-races are uniquely associated with a single host plant or possibly others. The fact that the same genotypes were present on multiple hosts in some localities suggests the latter.

Antwi et al. (2015) expanded on the work of Barman et al. (2012) by including 10 additional host plant species and a finer scale of geographical sampling in Texas. In the study only one genetic population was exclusively associated with a single host plant, and a second genetic population closely associated with horsemint also was found on other host plants, suggesting the host-race identified by Barman et al. (2012) was not exclusively associated with horsemint. A pattern of host-associated differentiation was found only when examining genotypes of nymphs. This is consistent with knowledge that individuals opportunistically disperse to alternative host plants from their natal host plants during adult life, but it questions the notion that specific genotypes might be exclusively associated with one or more host plants.

Barman et al. (2012) and Antwi et al. (2015) identified host-associated differentiation in the cotton fleahopper, but with limited and varying amounts of support. Although host-associated differentiation seems to play a role in cotton fleahopper population genetic dynamics, clearly geography also plays a role, but neither mechanism provides an entirely satisfying explanation for observed patterns of differentiation among and within cotton fleahopper populations. Raszick et al. (2020) sampled cotton fleahopper individuals from cotton fields multiple times across one growing season and found each population dominated by a different genotype over time. However, the pattern was not observed in the 2nd year of the study. Temporal variation in genotypes on cotton might be because of colonization from different genetic populations dispersing from other host plants. It is well known that differences in host plant phenology can play a role in differentiation, even in conjunction with host-associated differentiation, as described in *Rhagoletis* spp. (Feder et al. 1990), yet the temporal component of cotton fleahopper population dynamics has received little attention thus far. Nonetheless, Raszick et al. (2020) did support the status of croton as a natural refuge in the Brazos Valley growing region of Texas. However, a comprehensive IRM plan should consider volatility of plant communities and differences among regions where host-associated differentiation might be more prevalent.

In conclusion, for many years, the cotton fleahopper has consistently ranked as a top pest of cotton in the U.S. The cotton fleahopper is currently managed with foliar insecticide application which can result in outbreaks of secondary pests. The new cotton line (MON88702) that produces modified *Bt* Cry51Aa2 protein might provide a management alternative that is robust to variability in timing of infestation by cotton fleahopper. The *Bt* line could reduce the need for insecticide applications which would subsequently reduce the potential of secondary pest outbreaks. However, before the *Bt* line of cotton can be released commercially, refuge requirements and other IRM strategies need to be established to prevent or delay development of cotton fleahopper resistance to the new toxin. Based on genetic diversity of the cotton fleahopper within and among regions, cotton fleahopper adults do not seem to regularly travel long distances during their lifetime, although there is some evidence of mass dispersal by wind (Suh and Spurgeon 2004, Meng et al. 2008, Elameen et al.

2016, Wang et al. 2017). Therefore, IRM planning also should consider regional and yearly variation in local availability of preferred host plants which can substantially influence the intensity and timing of cotton fleahopper dispersal into cotton.

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References Cited

Almand, L. K., W. L. Sterling, and C. L. Green. 1976. Seasonal abundance and dispersal of the cotton fleahopper as related to host plant phenology. Texas Agricultural Experiment Station Bulletin 1170.

Antwi, J. B., G. A. Sword, and R. F. Medina. 2015. Host-associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. Ecology and Evolution 5: 2533-2543.

Bachman, P. M., A. Ahmad, J. E. Ahrens, W. Akbar, J. A. Baum, S. Brown, T. L. Clark, J. M. Fridley, A. Gowda, J. T. Greenplate, P. D. Jensen, G. M. Mueller, M. L. Odegaard, J. Tan, J. P. Uffman, and S. L. Levine. 2017. Characterization of the activity spectrum of MON88702 and plant-incorporated protectant Cry51Aa2.834_16. PLoS ONE. 12: e0169409.

Barman, A. K. 2011. Interactive Effects of Geography and Host Plant Species on Genetic and Phenotypic Variations of Cotton Fleahopper Populations. Ph.D. dissertation, Texas A&M University, College Station.

Barman, A. K., M. N. Parajulee, C. G. Sansone, C. P.-C. Suh, and R. F. Medina. 2012. Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus*. Entomol. Exp. Appl. 143: 31-41.

Barman, A. K., C. G. Sansone, M. N. Parajulee, and R. F. Medina. 2013. Population genetic structure of *Pseudatomoscelis seriatus* (Hemiptera: Miridae) in the cotton-growing regions of the United States. J. Econ. Entomol. 106: 2225-2233.

Bohmfalk, G. T., R. E. Frisbie, W. L. Sterling, R. B. Metzer, and A. E. Knutson. 2005. Identification, biology and sampling of cotton insects. Texas Cooperative Extension Texas A&M University. http://www. soilcropandmore.info/crops/ CottonInformation/insect/B-933/b-933.htm

Breene, R. G., W. R. Martin Jr., D. A. Dean, and W. L. Sterling. 1989. Rearing methods for the cotton fleahopper. Southwest. Entomol. 14: 249-253.

Caprio, M. A. 2001. Source-sink dynamics between transgenic and non-transgenic habitats and their role in the evolution of resistance. J. Econ. Entomol. 94: 698-705.

Caprio, M. A., M. K. Faver, and G. Hankins. 2004. Evaluating the impacts of refuge width on source-sink dynamics between transgenic and non-transgenic cotton. J. Insect Sci. 4: 3.

Carrière, Y., P. Dutilleul, C. Ellers-Kirk, B. Pederson, S. Haller, L. Antilla, and B. E. Tabashnik. 2004. Sources, sinks, and the zone of influence of refuges for managing insect resistance to *Bt* crops. Ecol. Applic. 14: 1615-1623.

Elameen, A., H. G. Eiken, and G. K. Knudsen. 2016. Genetic diversity in apple fruit moth indicate different clusters in the two most important apple growing regions of Norway. Diversity 8: 10.

Esquivel, J. F., and S. V. Esquivel. 2009. Identification of cotton fleahopper (Hemiptera: Miridae) host plants in Central Texas and compendium of reported hosts in the United States. Environ. Entomol. 38: 766-780.

Feder, J., C. Chilcote, and G. Bush. 1990. The geographic pattern of genetic differentiation between host associated populations of *Rhagoletis pomonella* (Diptera: Tephritidae) in the Eastern United States and Canada. Evolution 44: 570-594.

Fletcher, R. K. 1940. Certain host plants of the cotton flea hopper. J. Econ. Entomol. 33: 456-459.

Gaines, J. C. 1933. A study of the cotton flea hopper with special reference to the spring emergence, dispersal, and population. J. Econ. Entomol. 26: 963-971.

Gaines, J. C., and K. P. Ewing. 1938. The relation of wind currents, as indicated by balloon drifts, to cotton flea hopper dispersal. J. Econ. Entomol. 31: 674-677.

Gaylor, M. J. and W. L. Sterling. 1975. Effects of temperature on the development, egg production, and survival of the cotton fleahopper, *Pseudatomoscelis seriatus*. Environ. Entomol. 4: 487-490.

Gaylor, M. J., and W. L. Sterling. 1976. Effects of temperature and host plants on population dynamics of the cotton fleahopper, *Pseudatomoscelis seriatus.* Texas A&M University, Texas Agricultural Experiment Station B-1161.

Gaylor, M. J., and W. L. Sterling. 1977. Photoperiodic induction and seasonal incidence of embryonic diapause in the cotton fleahopper, *Pseudatomoscelis seriatus.* Ann. Entomol. Soc. Am. 70: 893-897.

Georghiou, G. P., and C. E. Taylor. 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 319-323.

Guse, C. A., D. W. Onstad, L. L. Buschman, P. Porter, R. A. Higgins, P. E. Sloderbeck, G. B. Cronholm, and F. B. Peairs. 2002. Modeling the development of resistance by stalk-boring Lepidoptera (Crambidae) in areas with irrigated transgenic corn. Environ. Entomol. 31: 676-685.

Hixson, E. 1940. The host relation of the cotton flea hopper. Iowa State College J. Sci. 16: 66-68.

Hixson, E. 1941. The host relation of the cotton flea hopper. Iowa State College J. Sci. 16: 66-68.

Holtzer, T. O., and W. L. Sterling. 1980. Ovipositional preference of the cotton fleahopper, *Pseudatomoscelis seriatus,* and distribution of eggs among host plant species. Environ. Entomol. 9: 236-240.

Howard, L. O. 1898. Cotton field insects, pp. 85-88. *In* Some miscellaneous results of the work of the division of entomology III. U.S. Department of Agriculture Division of Entomology Bulletin 18 (new series), USDA, Washington, DC.

Little, V. A., and D. F. Martin. 1942. Cotton Insects of the United States. Burgess, Minneapolis, MN.

Meng, X.-F., M. Shi, and X.-X. Chen. 2008. Population genetic structure of *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae): strong subdivision in China inferred from microsatellite markers and mtDNA gene sequences. Mol. Ecol. 17: 2880-2897.

Onstad, D. W., C. A. Guse, P. Porter, L. L. Buschman, R. A. Higgins, P. E. Sloderbeck, F. B. Peairs, and G. B. Cronholm. 2002. Modeling the development of

resistance by stalk-Boring Lepidopteran insects (Crambidae) in areas with transgenic corn and frequent insecticide use. J. Econ. Entomol. 95: 1033-1043.
Parajulee, M. N., R. B. Shrestha, and J. F. Leser. 2006. Influence of tillage, planting date and *Bt* cultivar on seasonal abundance and within-plant distribution patters of thrips and cotton fleahoppers in cotton. Int. J. Pest Manage. 52: 249-260.
Parker, R. D. 2012. Result of insect control evaluations on corn, sorghum, cotton, sunflower, pasture, and stored grain in Texas. https://agrilife.org/coastalbend/files/2011/09/2012-Research-Report1.pdf
Parker, R. D., M. J. Jungman, S. P. Biles, and D. L. Kerns. 2008. Managing cotton insects in the Southern, Eastern, and Blackland areas of Texas. Texas A&M AgriLife Extension Service Bulletin E-5: 17.
Raszick, T. J., C. P.-C. Suh, C. M. Dickens, and G. A. Sword. 2020. Genome-wide markers reveal temporal instability of local population genetic structure in cotton fleahopper, *Pseudatomoscelis seriatus* (Hemiptera: Miridae). Pest Manage. Sci. 76: 324-332.
Reinhard, H. J. 1926. The cotton fleahopper. Texas Agricultural Experiment Station Bulletin 39.
Reinhard, H. J. 1928. Hibernation of the cotton fleahopper. Texas A&M AgriLife Extension Service Bulletin 356: 32.
Riley, J. J., and W. Sterling. 1983. Dispersion patterns of the red imported fire ant (Hymenoptera: Formicidae), aphids, and some predaceous insects in East Texas cotton fields. Environ. Entomol. 12: 380-385.
Ring, D. R., J. H. Benedict, M. L. Walmsley, and M. F. Treacy. 1993. Cotton yield response to cotton fleahopper (Hemiptera: Miridae) infestations on the Lower Gulf Coast of Texas. J. Econ. Entomol. 86: 1811-1819.
Sterling, W. L., and A. W. Hartstack. 1979. Emergence threshold with validations for forecasting the spring emergence of cotton fleahoppers. Environ. Entomol. 8: 649-654.
Suh, C. P.-C. 2007. Head capsule widths of nymphal instars of the cotton fleahopper. Southwest. Entomol. 32: 127-130.
Suh, C. P.-C., and D. W. Spurgeon. 2004. Detecting cotton fleahopper movement into fields with sticky traps. (CD-ROM). *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, San Antonio, TX.
Suh, C. P.-C., and J. K. Westbrook. 2010. Relationship between population estimates of cotton fleahoppers (Hemiptera: Miridae) obtained by terminal and whole plant examinations. J. Entomol. Sci. 45: 204-210.
Vyavhare, S. S., and D. Kerns. 2017. Cotton Fleahoppers. Texas A&M AgriLife Extension Service Bulletin ENTO-037.
Vyavhare, S. S., and B. Reed. 2020. Control of cotton fleahopper in cotton using foliar applied insecticides, 2019. Arthropod Manage. Tests 45: tsaa090.
Wang, X.-Y., W.-M. Yang, B. Lu, L.-H. Zhou, and K.-M. Wu. 2017. Genetic variation and phylogeographic structure of the cotton aphid, *Aphis gossypii,* based on mitochondrial DNA and microsatellite markers. Sci. Rep. 7: 1920.
Williams, M. R. 2000. Cotton insect losses – 1999. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 887-913.
Williams, M. R. 2001. Cotton insect loss estimates – 2000. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 774-777.

Williams, M. R. 2008. Cotton insect losses – 2007. *In* S. Boyd, M. Huffman, D. Richter and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 887-913.

Williams, M. R. 2009. Cotton insect losses – 2008. S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 941-951.

Williams, M. R. 2010. Cotton insect losses – 2009. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 1099-1110.

Williams, M. R. 2011. Cotton insect losses – 2010. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 941-954.

Williams, M. R. 2012. Cotton insect losses – 2011. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 1013-1057.

Williams, M. R. 2013. Cotton insect losses – 2012. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, San Antonio, TX. 546-586.

Williams, M. R. 2014. Cotton insect losses – 2013. *In* S. Boyd, M. Huffman, and B. Robertson [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 798-812.

Williams, M. R. 2015. Cotton insect loss estimates – 2014. *In* S. Boyd, and M. Huffman [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 494-496.

Williams, M. R. 2016. Cotton insect losses – 2015. *In* S. Boyd and M. Huffman [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 507-525.

Williams, M. R. 2017. Cotton insect losses – 2016. *In* S. Boyd, M. Huffman, and A. Sarkissian [eds.], Proceedings Beltwide Cotton Conference, National Cotton Council, Memphis, TN. 710-754.

Geographic Variation of Bacterial Communities Associated with Cotton Fleahopper, *Pseudatomoscelis seriatus*[1]

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Abstract. Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is native to the southern United States and widespread throughout regions where cotton (*Gossypium hirsutum* L.) is commercially grown. It is considered a major cotton pest only in certain cotton-growing regions, while in other areas it rarely reaches pest status. Bacterial symbionts associated with sucking insects have been linked to their pest status on their insect hosts. We tested whether variation in bacterial communities within *P. seriatus* correlated with pest status of the insect on cotton. We used 454 pyrosequencing of the bacterial 16S ribosomal RNA (rRNA) gene to characterize the bacterial microbiota associated with *P. seriatus* from eight cotton-growing regions in the United States. We found 65 bacterial taxa in the dataset, and three phyla, Proteobacteria, Firmicutes, and Bacteroidota, accounted for 78.5, 16.3, and 4.6% of the bacterial community. Overall bacterial composition and diversity did not correlate with *P. seriatus* pest status or geographical origin. However, abundances of three taxa, *Spiroplasma* sp., *Swaminathania,* sp., and *Aureimonas* sp., were significantly different between regions in which *P. seriatus* occurs as major versus occasional pest. We found *Pseudomonas* spp. in all samples, with different strains/species in different samples, suggesting variant *Pseudomonas* could be a primary endosymbiont of *P. seriatus*. Characterization of the *P. seriatus* bacterial microbiota showed it was distinct from other well-characterized hemipteran herbivores. Whether variation in microbiota corresponds with variation in traits relevant to pest control warrants further investigation.

Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), is an herbivorous insect native to the southern United States and north-central Mexico (Knutson et al. 2002). It is currently widespread throughout regions where cotton (*Gossypium hirsutum* L.) is commercially grown in the United States (i.e., Cotton Belt) — from California to North Carolina (Henry 1991, Greenberg et al. 2012). Cotton fleahopper was first reported as a cotton pest in the United States during the 1920s (Reinhard 1926). Early in the growing season, numbers of cotton fleahoppers increase in cotton fields where adults and immatures feed on pinhead-sized floral buds (called "squares"

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Reinhard 1926). Damage inflicted by cotton fleahoppers, especially at such a critical stage of plant growth, makes the cotton fleahopper an important early season pest of the crop. Insecticide effectively controls cotton fleahoppers when numbers reach economic threshold (Drees 1985, Robinson and Stewart 1987). Abundance of cotton fleahopper was kept in check especially when frequent insecticide applications were used to control other insect pests (Greene et al. 2006).

Cotton fleahopper has reemerged as a major pest in some cotton-growing regions of the United States (Esquivel and Esquivel 2009). This change in pest status can be explained by improvement in management of other cotton pests such as the boll weevil, *Anthonomus grandis grandis* (Boheman), and cotton bollworm, *Helicoverpa zea* (Boddie) (Greene et al. 2006). Success of the Boll Weevil Eradication Program across most of the United States resulted in tremendous decrease in broad-spectrum insecticides in cotton fields (Moss 1914, Duffy and Hishamunda 2001). Additionally, since the 1990s, cotton growers have used transgenic *Bt* cotton that directly targets lepidopteran pests such as tobacco budworm (*Heliothis virescens* Fabricius) and cotton bollworm, further reducing insecticide inputs. Widespread reduction in insecticide led to a new suite of sucking hemipteran pests, including cotton fleahopper previously managed indirectly as part of intensive boll weevil and caterpillar control (Sansone et al. 2009, Greenberg et al. 2012).

Although cotton fleahopper is widely distributed throughout the Cotton Belt, it is considered a major pest only in some areas of Texas (e.g., East Texas, Coastal Bend, Upper Gulf Coast, and Lower Rio Grande Valley of South Texas), Oklahoma, and Louisiana (Parker et al. 2004, Sansone et al. 2009, Suh and Westbrook 2010). In West Texas (e.g., Texas High Plains and Rolling Plains), Arizona, Georgia, North Carolina, and South Carolina, cotton fleahopper is considered only an occasional pest (Sterling and Dean 1977, Sansone et al. 2009). Currently, uncertainty exists about why the pest status of cotton fleahopper is not consistent across cotton-growing regions of the United States. This could result from interlinked effects of multiple factors, e.g., annual precipitation, abundance and diversity of wild host plants (Barman et al. 2012, Antwi et al. 2015), within and across population variation of cotton fleahoppers (Raszick et al. 2020), and distinct geographic genotypes of cotton fleahopper (Barman et al. 2013). Annual precipitation and correspondingly large diversity and abundance of wild hosts in East compared to West Texas correlates with more abundant cotton fleahoppers in East Texas (Esquivel and Esquivel 2009). Also, cotton fleahoppers associated with one of its most abundant non-crop hosts, horsemint (*Monarda punctata*), are genetically distinct from cotton fleahoppers on cotton in West but not East Texas (Barman et al. 2012). While there are distinct geographic genotypes of cotton fleahopper populations across the Cotton Belt (Barman et al. 2012, 2013; Antwi et al. 2015), geographic variation in cotton fleahopper microbiota has not been examined.

Many hemipteran insects have established symbiotic relationships with bacteria that can be categorized as obligatory or primary and facultative or secondary. Obligatory bacterial symbionts are essential for survival and development of the insect hosts by providing essential nutrients (Douglas 1998, 2003; Hosokawa et al. 2010, Tada et al. 2011), such as *Buchnera aphidicola* within aphids, *Portiera aleyrodidarum* within whiteflies, and *Carsonella ruddii* within psyllids (Thao et al. 2000). Certain obligatory endosymbionts play causal roles in the pest status of some hemipterans. For example, obligatory *Candidatus Ishikawaella capsulata* determines whether sister species, *Megacopta punctatissima* and *M. cribraria*, can hatch and develop normally on legume crops (Hosokawa et al. 2007). Facultative symbionts

are not required for survival of the host, although they might provide significant fitness benefits, e.g., confer resistance to pathogenic microorganisms, protect against natural enemies, and confer resistance to insecticide (Scarborough et al. 2005, Oliver et al. 2010, Kikuchi et al. 2012, Kikuchi and Yumoto 2013). Certain facultative symbionts might promote adaptation of insects to their host plants (Tsuchida et al. 2004; Ferrari et al. 2007, 2012; Medina et al. 2011) as in aphids (Leonardo and Muiru 2003, Tsuchida et al. 2004, Brady and White 2013). There is notable individual and geographic variation in patterns of infection by endosymbionts (Tsuchida et al. 2002, Jones et al. 2011, Pan et al. 2012, Brady and White 2013, Apprill et al. 2013, Malacrinò et al. 2018). For example, pea aphids harbor multiple facultative symbionts yet under variable environmental conditions (e.g., temperature, precipitation, and host plant), certain symbionts might or might not be in every individual (Chen et al. 2000, Tsuchida et al. 2002, Simon et al. 2003). If the cotton fleahopper microbiota has any influence on cotton fleahopper host range or feeding ecology, variation in microbiota composition might help explain geographic variation in the pest status of the insect across its geographic distribution.

We examined whether cotton fleahopper pest status could be linked to variation in the microbial community harbored by the insect. Specific objectives were to: (1) characterize the microbiota of cotton fleahopper, (2) test whether microbiota diversity correlates with cotton fleahopper pest status across the United States Cotton Belt, and (3) determine the presence of any primary symbionts associated with cotton fleahopper.

Materials and Methods

Cotton fleahoppers were sampled from five states east to west across the United States Cotton Belt (Fig. 1): North Carolina (NC), South Carolina (SC), Georgia (GA), Texas and Arizona (AZ). Samples were from one location per state, except for Texas, where samples were taken from four locations (i.e., College Station (Cs), Corpus Christi (Cc), Weslaco (Ws), and Lubbock (Lb)). Based on geographical variation in cotton fleahopper pest status, we refer to Cs, Ws, and Cc as “major pest” locations, and AZ, Lb, GA, SC, and NC as “occasional pest” locations. At all locations, insects were collected from cotton fields at Agricultural Research and Extension centers. Standard sweep nets and a motorized blower (‘keep-it-simple’) sampler (Beerwinkle and Marshall 1999) were used to collect insects during summers of 2009 and 2012. Twelve adults were collected at each location. Samples were stored in 85% ethanol or at -80°C before DNA extraction.

To avoid contamination by external microbes, insects were washed with 70% ethanol for 1 minute, 10% sodium hypochlorite for 5 minutes, and rinsed five times with sterile water (Meyer and Hoy 2008). DNA was extracted from whole insects using a QIAGEN Blood & Tissue extraction kit (Valencia, CA) following the protocol of the manufacturer. DNA concentration was quantified using a NanoDrop spectrophotometer (Nyxor Biotech, Paris, France), after which concentrations were standardized to ~100ng/ul. One-microliter aliquots of DNA from each individual at each sampling location were pooled. Bacterial DNA were categorized using tag-encoded FLX 454 pyrosequencing (bTEFAP) at the Research and Testing Laboratory (Lubbock, TX). During library preparation, PCR was done in triplicate for each sample to minimize random amplicon biases often detected in sequences from single PCR amplicons (Engelbrektson et al. 2010, Haas et al. 2011). The 16S primers, 28F ‘GAGTTTGATCNTGGCTCAG’ and 519R ‘GTNTTACNGCGGCKGCTG’, spanning

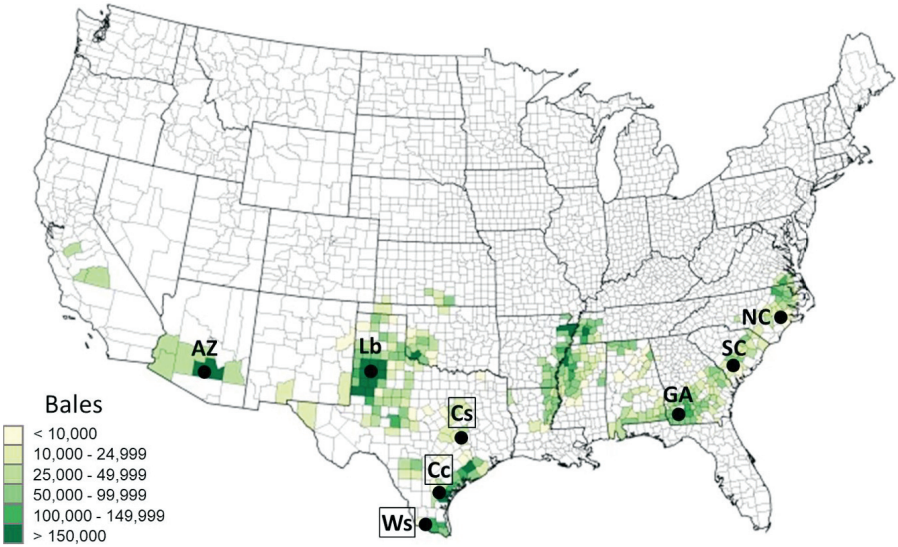

Fig. 1. Bales of cotton harvested (by county) in the US Cotton Belt in 2019 (USDA, National Agricultural Statistics Services). Black dots indicate where eight cotton fleahopper samples were collected in three major growing regions. East: North Carolina (NC), South Carolina (SC), Georgia (GA); Central: College Station (Cs), Corpus Christi (Cc), Weslaco (Ws); West: Lubbock (Lb) and Arizona (AZ). Samples labeled in black boxes indicate regions where cotton fleahoppers are considered major pests.

the variable regions V1-V3 of 16S bacterial rRNA gene were used for PCR. Equal volumes of each of the three PCR amplicons were pooled for emulsion PCR and bTEFAP using previously described methods (Dowd et al. 2008).

Pyrosequencing data in sff format were converted to fastq format using a script developed by Indraniel Das (https://github.com/indraniel/sff2fastq). Fastq reads were trimmed using Trimmomatic with “single-end” parameters (v0.38; Bolger et al. 2014), and FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess quality of the trimmed fastq sequences. All sequences were trimmed to 240 bp because at this length more than 85% of the sequences were retained across all samples. The R package DADA2 (Callahan et al. 2017) was used for analyses including error rate learning, de-replication, removal of chimeric sequences, sequence table construction, and taxonomy assignment. DADA2 infers sequences by resolving sequence differences to single nucleotides preferred for microbiome analysis rather than consolidating sequences at a fixed threshold (often 3%, Callahan et al. 2017, Prodan et al. 2020). Taxonomic assignment of unique sequences was based on the Silva database (release 138; Quast et al. 2013). Mitochondrial sequences were filtered because they probably were contamination sequences and bacterial taxa not characterized to Class. Taxa with low sequence abundances (i.e., taxa with mean relative abundance across eight samples <0.1%) were excluded from downstream analysis.

Downstream analysis involved five steps. All analyses used the R package phyloseq (McMurdie and Holmes 2013), vegan (Oksanen et al. 2007), and DESeq2 (Love et al. 2014). Plots were made using the R package ggplot2.

First, basic measurements commonly used to describe microbial community data, such as number of taxa, relative abundance of each taxa, Shannon, and Simpson indices (alpha diversity) were estimated. Second, a Bray-Curtis dissimilarity matrix was constructed from a rarefied count table, and Principal Coordinate Analysis (PCoA) was done based on the distance matrix. Third, a heatmap was generated using count data normalized to median sequencing depth across eight samples. Fourth, differences in microbial communities among geographic groups and samples classified as differing in pest status were evaluated using permutational multivariate ANOVA (PERMANOVA). For PERMANOVA, the adonis function in the vegan package (Oksanen et al. 2007) was used based on the Bray-Curtis dissimilarity matrix calculated aforenoted. Finally, R package DESeq2 (Love et al. 2014) was used to examine whether there were taxa whose abundances differed between samples that belonged to different pest status groups. Because there were only two such samples in the western geographic group (AZ and Lb), the analysis was limited to groups based on pest status only. Because DESeq2 accounted for library size differences, the raw count table without rarefaction or normalization was used in analysis.

Results

In total, 96 cotton fleahoppers (12 individuals per location) from cotton fields in the U.S. Cotton Belt were analyzed (Fig. 1). Pyrosequencing yielded 98,635 sequences. After quality trimming, 61,610 sequences remained. Chimeric sequences typically formed through fusion of phylogenetically distinct sequences either during PCR or sequencing could lead to overestimation of taxonomic units in pyrosequencing (Quince et al. 2009, Huse et al. 2010). Thus, an average of 16.45% of the sequences was removed in this step using the DADA2 pipeline. DADA2 outputs amplicon sequence variants (ASV) equivalent to the traditional OTU used in earlier programs that handle amplicon data, i.e., QIIME and Mothur (Prodan et al. 2020). The final DADA2 output records the frequency at which each ASV was observed in each sample. There were 333 ASV in the initial dataset across eight samples. However, many ASV had low abundance and were in only one sample. After removing ASV assigned as mitochondrial sequences and bacterial taxa not characterized to Class and above, 65 bacterial taxa were retained in the final dataset. Because most ASV were not assigned to species, the 65 taxa henceforth were referred to as ASV.

Sequencing depth varied among samples, with least for NC and Ws (2,108 and 2,311 reads after abundance and contamination filtering. Rarefaction curves that determined how close sampling effort came to fully describe the bacterial community tended toward saturation after sequencing depth reached 1,000. This indicated that despite the low sequencing depth of a few samples, the bacterial communities of cotton fleahoppers were fully represented.

Alpha diversity measured by Simpson and Shannon indices of AZ was substantially less than other samples (Fig. 2). The number of taxa of AZ was least among all eight samples. Nonetheless, high sequencing depth did not correlate with high alpha diversity. The sequencing depth of sample Cc was second greatest, but the alpha diversity and number of taxa was second lowest.

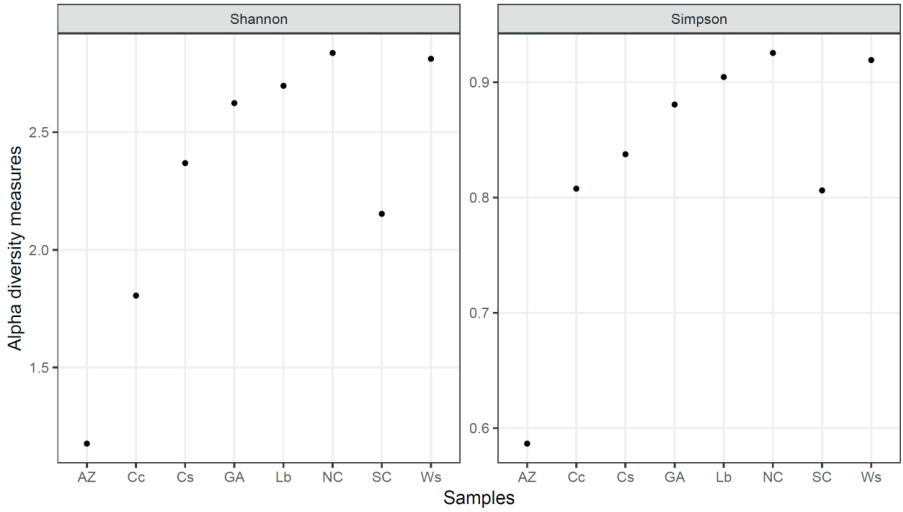

Fig. 2. Alpha diversity indices for cotton fleahopper samples.

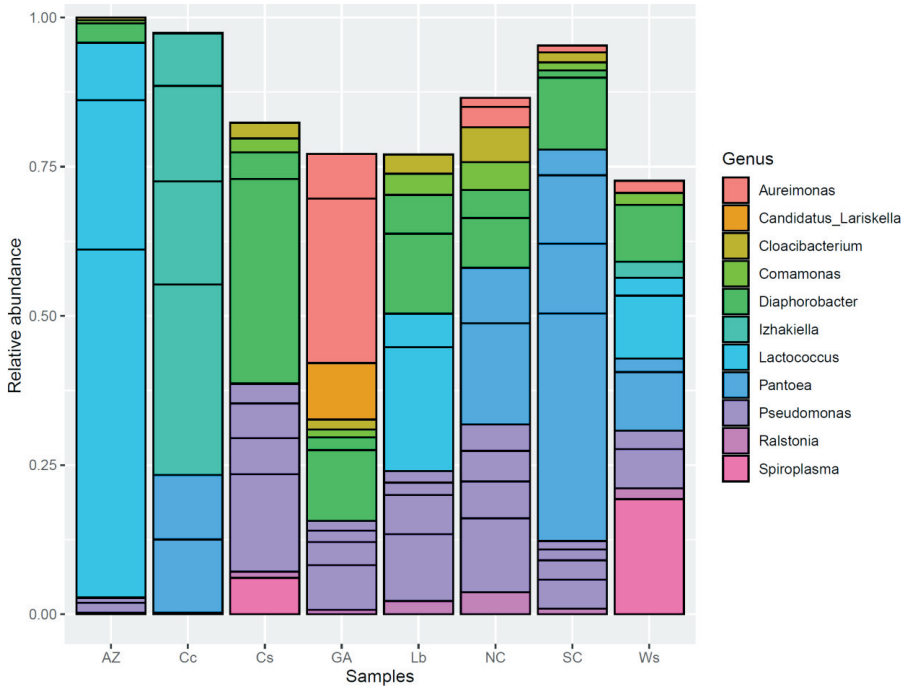

Fig. 3. Relative abundance of top 25 amplicon sequence variants agglomerated to genus. Different ASVs were separated by solid black lines within a sample.

At the phylum level, bacterial communities were dominated by three main taxa: Proteobacteria, Firmicutes, and Bacteroidota, accounting for 78.5, 16.3, and 4.6%, respectively, of all ASV when the 65 taxa were agglomerated into the phylum level. At the phylum level, the composition of AZ was different from the other seven samples. In particular, relative abundance of Firmicutes was much greater than the counterpart in the other seven samples.

Because only three ASV were characterized to species, we focused on reporting the microbial community at the genus level. The five most-abundant genera in descending order were *Diaphorobacter*, *Lactococcus*, *Pseudomonas*, *Pantoea*, and *Izhakiella*. Collectively, the five genera made up more than 75% of the microbial community across the eight locations sampled.

Only genus *Pseudomonas* was in all eight samples. Six ASVs were characterized as *Pseudomonas*. Although 5/6 ASVs could not be characterized into lower taxonomic levels (one ASV was *Pseudomonas aeruginosa*), clearly different *Pseudomonas* sequences were harbored by cotton fleahoppers at different locations.

Microbial composition differed greatly among samples when the top 25 ASV were visualized (Fig. 3). For example, *Lactococcus* spp. were abundant in AZ, scarce

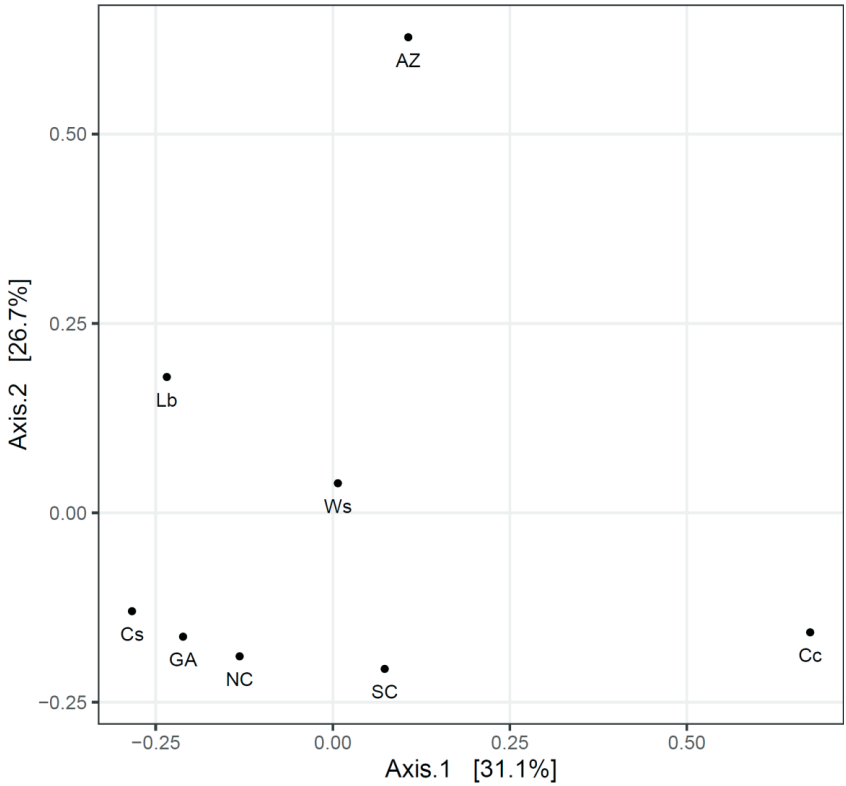

Fig. 4. Principal Coordinate Analysis (PCoA) of bacterial communities based on Bray-Curtis dissimilarity distance matrix was built from rarified count table.

at Cs, Lb, and Ws, and absent at Cc, GA, NC, and SC. *Izhakiella* was only at Cc and in low abundance at Ws. *Aureimonas* was very abundant in GA, but absent in AZ, Cs, and Lb. It also was common to have multiple taxa of the same genus at one location, e.g., three taxa of *Lactococcus* in AZ, four of *Izhakiella* at Cc, and four taxa of *Pantoea* in SC. Despite the low resolution of the sequences to characterize the taxa to species (we truncated the reads to 240 bp), this indicated the microbial community within cotton fleahopper is complex, with evidence of different microbial strains or species coexisting within cotton fleahoppers at each location.

To clearly visualize overall variation in bacterial composition among samples, we generated an ordination plot based on Bray-Curtis dissimilarity matrix (Fig. 4). The microbial communities within cotton fleahoppers from AZ and CC were different from the other six samples. Cotton fleahopper bacterial communities from Cs, GA, and NC clustered together, indicating similarities in microbial composition. Bacterial communities from Lb, Ws, and SC cotton fleahoppers were similar to communities characterized at Cs, GA, and NC cotton fleahoppers, but distant from AZ and Cc. Additionally, a heatmap was made based on normalized count data of all 65 ASVs in the final dataset (Fig. 5). This recapitulated the findings from other analyses based on different algorithms. AZ and Cc had different profiles from other samples, e.g., absence of many taxa present in other samples. NC, SC, and GA had similar profiles.

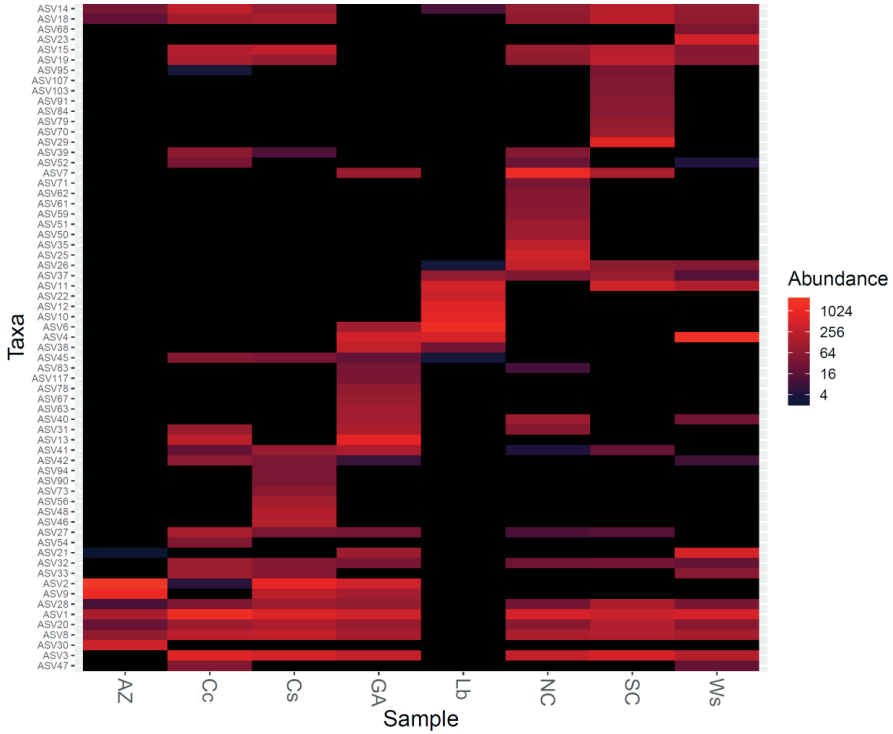

Fig. 5. Heatmap of all 65 amplicon sequence variants based on a count table normalized to the median sequencing depth.

Table 1. Bacterial ASVs Showing Statistically Significant Differences in Abundance between Cotton Fleahopper Considered a Major Pest and as Occasional Pests

	ASV13	ASV26	ASV38
Phylum	Firmicutes	Proteobacteria	Proteobacteria
Class	Bacilli	Alphaproteobacteria	Alphaproteobacteria
Order	Entomoplasmatales	Rhizobiales	Acetobacterales
Family	Spiroplasmataceae	Rhizobiaceae	Acetobacteraceae
Genus	Spiroplasma	Aureimonas	Swaminathania
log2FoldChange	-8.37	4.92	-6.16
Adjust *p*-value	3.12E-05	0.05	0.0019
[a]**Count data:**			
*Cc	0	5 (0.08%)	48 (0.75%)
*Cs	576 (6.1%)	0	0
*Ws	446 (19.3%)	0	164 (7.1%)
AZ	0	0	0
GA	0	371 (7.5%)	0
Lb	0	0	0
NC	0	32 (1.52%)	0
SC	0	53 (1.14%)	0

[a]raw reads and relative abundance shown in the count table; abundance waws compared because sequencing depth varied between samples.
*samples collected from areas in which cotton fleahopper is considered a major pest.

PERMANOVA was used to test whether microbial composition differed among geographic clusters and samples that differed in pest status. There was no evidence of association between bacterial community composition and geography or pest status (p = 0.196 for geographic cluster; p = 0.587 for pest status). Abundances of three ASVs (A, B, and C) differed significantly between major and occasional pest groups (Table 1). Although the genus *Pseudomonas* was in all cotton fleahopper samples, no single ASV was consistently present or absent in either group. *Spiroplasma* sp. and *Swaminathania* sp. ASVs were in 2/3 samples from major pest areas and absent from occasional pest areas. *Aureimonas* sp. was in 3/5 samples from the occasional pest area but only in low abundance at Cc (major pest area).

Discussion

Amplicon sequencing was used to characterize cotton fleahopper bacterial microbiota and test whether bacterial community composition might correlate with cotton fleahopper pest status. Despite overall bacterial composition and diversity not correlating with cotton fleahopper pest status (Figs. 4-5), the abundances of three ASV, *Spiroplasma* sp., *Swaminathania,* sp., and *Aureimonas* sp., were significantly different between locations in which cotton fleahopper is considered a major versus an occasional pest. Bacterial diversity and composition varied greatly among locations, suggesting different endosymbiont communities associated with cotton fleahopper across its broad geographic distribution. Nevertheless, our sequence libraries were dominated by phyla Proteobacteria, Firmicutes, and Bacteroidota, comprising more than 99% of the microbial community across eight relatively distant locations. The five most abundant genera were *Diaphorobacter*, *Lactococcus*, *Pseudomonas*, *Pantoea*, and *Izhakiella*, constituting 75% of the microbial community

across eight locations. Other obligatory endosymbionts such as *Buchnera aphidicola*, *Portiera aleyrodidarum*, and *Carsonella ruddii* (Douglas 1998, 2003; Thao et al. 2000; Hosokawa et al. 2010, Tada et al. 2011) commonly associated with other hemipteran herbivores were not detected. Because our sampling effort came close to fully capturing the community structure of bacteria within cotton fleahoppers, it is unlikely we missed any common taxa. Finding variants of *Pseudomonas* in all tested samples suggests a potential endosymbiotic role that warrants further study.

Microbial communities within insects can greatly affect host phenotypes. Because phytophagous insects are ubiquitously associated with bacterial symbionts, some of which improve insect nutrition and confer resistance to environmental stressors (Douglas 1998, Brownlie and Johnson 2009, Gross et al. 2009), it seemed reasonable to suspect some symbionts could predict variation in pest status in cotton fleahopper. For insects in the family Plataspidae (e.g., *M. punctatissima* and *M. cribraria*), the bacterial symbiont *Candidatus* Ishikawaella capsulata makes them pestiferous when associated with leguminous plants (Hosokawa et al. 2007, Brown et al. 2014). Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, harbors gut microbiota that improve tolerance to anti-herbivory defenses in soybean leaves (Chu et al. 2013). Yet, strict association was not apparent for cotton fleahoppers on cotton plants. The only marginal association between pest status and microbes was the abundances of ASVs *Spiroplasma* sp., *Swaminathania* sp., and *Aureimonas* sp. (Table 1). *Spiroplasma* sp. is found in multiple hemipteran insects (Ammar et al. 2011, Watanabe et al. 2014). In contrast, only one study indicated *Aureimonas* sp. within hemipteran insects (Kang et al. 2019). To date, no other study indicated association of *Swaminathania* sp. with insects; nonetheless, the bacterium was associated with plants (Loganathan and Nair 2004).

Among the five most abundant genera in our study, *Pseudomonas* and *Pantoea* both contain plant pathogens that can be vectored by cotton fleahopper. In addition to transmitting bacterial pathogens, e.g., *Pseudomonas* and *Pantoea*, cotton fleahopper also can transmit fungal pathogens (e.g., *Fusarium smitectum* Berk., *F. equiseti* (Corda) Sacc and *Alternaria*) to cotton while feeding (Martin et al. 1987). Infected plants typically have excessive abscission of new growth, including squares because the pathogens induce production and greater amounts of ethylene, a phytohormone that regulates fruit abscission in cotton (Grisham et al. 1987). *Pantoea*-infected cotton plants, after infestation by cotton fleahoppers, also experience flower bud necrosis that results in abscission of flower buds and young fruit (bolls) (Bell et al. 2007). Thus, one could think that although pestiferous and non-pestiferous cotton fleahoppers do not differ greatly in overall bacteria composition, perhaps key pathogenic bacteria like *Pseudomonas* or *Pantoea* could explain cotton fleahopper pest status at some locations. To address this, it is critical to accurately characterize the pathogen status of the taxa by using a combination of PCR, sequencing, carbohydrate utilization, and microscopy. The genus *Pseudomonas* was consistently present across all eight samples. Six ASVs were designated as *Pseudomonas* spp., with only one characterized as *Pseudomonas aeruginosa* that infects both plants and animals (Wu and Li 2015). Considering there was no further characterization of the other five *Pseudomonas* ASVs, it is unclear whether the bacteria are plant pathogens or insect pathogens, or commensal or beneficial symbionts. Another possibility is that several ASVs within the genus *Pseudomonas* might be obligatory endosymbionts in cotton fleahoppers. In a study on hemlock woolly adelgids (Hemiptera: Adelgidae), *Pseudomonas adelgestsugas* was detected in all samples by PCR, suggesting its obligate role (Mech et al. 2019).

Clarification on the potential role of *Pseudomonas* ASVs warrants further study. ASVs within *Lactococcus, Diaphorobacter,* and *Izhakiella* are associated with hemipteran insects (Thaochan et al. 2010, Salem et al. 2013, Aizenberg-Gershtein et al. 2016, Gordon et al. 2016, Hong et al. 2017). *Lactococcus* spp. is capable of regulating growth of other microbes (Pérez et al. 2010). Species of *Lactococcus* in the gut of the western corn rootworm inhibit growth of bacteria suspected to increase resistance to plant defenses (Chu et al. 2013).

Bacterial microbiota varies substantially across sampling locations, e.g., AZ sample had least alpha diversity and its community composition differed from other samples. Several factors might have contributed to variation in cotton fleahopper microbiota. Cotton-growing areas in AZ experience less annual precipitation (<50% compared with the next most Western location, Lb) and warmer temperatures (Gleason et al. 2008). Conditions change eastward from AZ and Lb to central (i.e., Ws, Cc, and Cs) and eastern (i.e., GA, SC, and NC) locations with more annual precipitation and cooler temperatures). Composition of bacterial microbiota of other insects varies geographically depending on climatic conditions. For example, Toju and Fukatsu (2011) reported differences in infection frequencies by *Wolbachia* and *Rickettsia* in chestnut weevil, *Curculio sikkimensis*, at localities with different atmospheric temperatures. Scarce precipitation also inhibits growth of pathogens such as *Pseudomonas* within plants (Wright and Beattie 2004). Variation in climatic conditions can alter amounts of infection of bacterial pathogens or even trigger growth of otherwise nonpathogenic bacteria to a pathogenic level (Hube 2004, Apprill et al. 2013).

To our knowledge, this is the first study to provide a comprehensive description of cotton fleahopper microbiota. Martin et al. (1987) attempted to quantify cotton fleahopper microbes but their culture-dependent study limited cotton fleahopper microbes to only a few taxa. While our work is informative, we need to be cautious in interpreting the data. We did not include controls in the study considering it is debatable what samples should be used as meaningful positive and negative controls in sequencing-based microbiome studies. Multiple procedures probably introduce bias to sequencing-based microbiome studies, including but not limited to DNA extraction, PCR amplification bias and error, and clustering, filtering, and taxonomic assignment in bioinformatics processing (Hornung et al. 2019). Considering such complexity, in combination with the exploratory and descriptive nature of current microbiome research, future research is needed to determine functional roles, if any, of currently unknown taxa in cotton fleahoppers. Our study indicated the overall composition of microbiota did not predict cotton fleahopper pest status. However, abundance of three bacterial sequences differed significantly between groups with different pest status. Specific bacterial taxa could be better predictors of pest status as in other sucking insects (Hosokawa et al. 2007, Kikuchi et al. 2012, Brown et al. 2014). Although several bacteria associated with cotton fleahoppers might be commensals, others might function within cotton fleahoppers and such roles might differ depending on environmental conditions. Rapid accumulation of microbiome data might yet reveal patterns worthy of further ecological and evolutionary research.

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References Cited

Aizenberg-Gershtein, Y., S. Laviad, M. Samuni-Blank, and M. Halpern. 2016. *Izhakiella capsodis* gen. nov., sp. nov., in the family *Enterobacteriaceae*, isolated from the mirid bug *Capsodes infuscatus*. Int. J. Syst. Evol. Microbiol. 66: 1364-1370.

Ammar, E.-D., G. E. Gasparich, D. G. Hall, and S. A. Hogenhout. 2011. Spiroplasma-like organisms closely associated with the gut in five leafhopper species (Hemiptera: Cicadellidae). Arch. Microbiol. 193: 35-44.

Antwi, J. B., G. A. Sword, and R. F. Medina. 2015. Host-associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. Ecol. Evol. 5: 2533-2543.

Apprill, A., K. Hughen, and T. Mincer. 2013. Major similarities in the bacterial communities associated with lesioned and healthy Fungiidae corals. Environmental Microbiology 15: 2063-2072.

Barman, A. K., M. N. Parajulee, C. G. Sansone, C. P. C. Suh, and R. F. Medina. 2012. Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus*. Entomol. Exp. Appl. 143: 31-41.

Barman, A. K., C. G. Sansone, M. N. Parajulee, and R. F. Medina. 2013. Population genetic structure of *Pseudatomoscelis seriatus* (Hemiptera: Miridae) in the cotton-growing regions of the United States. J. Econ. Entomol. 106: 2225-2233.

Beerwinkle, K. R., and H. F. Marshall. 1999. Cotton fleahopper (Heteroptera: Miridae) responses to volatiles from selected host plants. Journal of Cotton Science 3: 153-159.

Bell, A. A., E. G. Medrano, J. D. Lopez, and R. K. Luff. 2007. Transmission and importance of *Pantoea ananatis* during feeding on cotton buds (*Gossypium hirsutum* L.) by cotton fleahoppers (*Pseudatomoscelis seriatus* Reuter). World Cotton Research Conference, Lubbock, TX, 10-14 September 2007.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-2120.

Brady, C. M., and J. A. White. 2013. Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. Ecol. Entomol. 38: 433-437.

Brown, A. M. V., L. Y. Huynh, C. M. Bolender, K. G. Nelson, and J. P. McCutcheon. 2014. Population genomics of a symbiont in the early stages of a pest invasion. Mol. Ecol. 23: 1516-1530.

Brownlie, J. C., and K. N. Johnson. 2009. Symbiont-mediated protection in insect hosts. Trends. Microbiol. 17: 348-354.

Callahan, B. J., P. J. McMurdie, and S. P. Holmes. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11: 2639-2643.

Chen, D., C. Montllor, and A. Purcell. 2000. Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. Entomol. Exp. Appl. 95: 315-323.

Chu, C.-C., J. L. Spencer, M. J. Curzi, J. A. Zavala, and M. J. Seufferheld. 2013. Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm. P. Natl. Acad. Sci. USA. 110: 11917-11922.

Douglas, A. E. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annu. Rev. Entomol. 43: 17-37.

Douglas, A. E. 2003. *Buchnera* bacteria and other symbionts of aphids, pp. 23-38. *In* T. A. Miller and K. Bourtzis [eds.], Insect Symbiosis. CRC Press, Boca Raton, FL.

Dowd, S. E., T. R. Callaway, R. D. Wolcott, Y. Sun, T. McKeehan, R. G. Hagevoort, and T. S. Edrington. 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 8: 1-8.

Drees, B. M. 1985. Management of cotton insects in south and east Texas counties. Texas Agric. Ext. Ser. Bull. B-1204

Duffy, P. A., and N. Hishamunda. 2001. Economic impacts of boll weevil eradication, pp. 157-174. *In* W. A. Dickerson, A. L. Brashear, J. T. Brumley, F. L. Carter, W. J. Grefenstette, and F. A. Harris [eds.], Boll Weevil Eradication in the United States through 1999. Cotton Foundation Reference Book Series Number 6. Memphis, TN.

Engelbrektson, A., V. Kunin, K. C. Wrighton, N. Zvenigorodsky, F. Chen, H. Ochman, and P. Hugenholtz. 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J. 4: 642-647.

Esquivel, J. F., and S. V. Esquivel. 2009. Identification of cotton fleahopper (Hemiptera: Miridae) host plants in Central Texas and compendium of reported hosts in the United States. Environ. Entomol. 38: 766-780.

Ferrari, J., C. L. Scarborough, and H. C. J. Godfray. 2007. Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. Oecologia 153: 323-329.

Ferrari, J., J. A. West, S. Via, and H. C. J. Godfray. 2012. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. Evolution 66: 375-390.

Gleason, K. L., J. H. Lawrimore, D. H. Levinson, T. R. Karl, and D. J. Karoly. 2008. A revised US climate extremes index. J. Climate 21: 2124-2137.

Gordon, E. R. L., Q. McFrederick, and C. Weirauch. 2016. Phylogenetic evidence for ancient and persistent environmental symbiont reacquisition in Largidae (Hemiptera: Heteroptera). Appl. Environ. Microb. 82: 7123-7133.

Greenberg, S. M., J. J. Adamczyk, and J. S. Armstrong. 2012. Principles and practices on integrated pest management on cotton in the Lower Rio Grande Valley of Texas, p. 246. *In* S. Soloneski and M. L. Larramendy [eds.], Integrated Pest Management and Pest Control-current and Future Tactics. IntechOpen, London, UK.

Greene, J. K., C. S. Bundy, P. M. Roberts, and B. R. Leonard. 2006. Identification and management of common boll feeding bugs in cotton. Clemson University, Coop. Ext. Serv. Bull. EB 158.

Grisham, M. P., W. L. Sterling, R. D. Powell, and P. W. Morgan. 1987. Characterization of the induction of stress ethylene synthesis in cotton caused by the cotton fleahopper (Hemiptera, Miridae) and its microorganisms. Ann. Entomol. Soc. Am. 80: 411-416.

Gross, R., F. Vavre, A. Heddi, G. D. D. Hurst, E. Zchori-Fein, and K. Bourtzis. 2009. Immunity and symbiosis. Mol. Microbiol. 73: 751-759.

Haas, B. J., D. Gevers, A. M. Earl, M. Feldgarden, D. V. Ward, G. Giannoukos, D. Ciulla, D. Tabbaa, S. K. Highlander, E. Sodergren, B. Methe, T. Z. DeSantis, J. F. Petrosino, R. Knight, B. W. Birren, and H. M. Consortium. 2011. Chimeric

16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res. 21: 494-504.
Henry, T. J. 1991. Revision of Keltonia and the cotton fleahopper genus *Pseudatomoscelis*, with the description of a new genus and an analysis of their relationships (Heteroptera, Miridae, Phylinae). J. New York Entomol. Soc. 99: 351-404.
Hong, Y., T. Yi, X. Tan, J. Su, and F. Ge. 2017. Microbes affected the TYLCCNV transmission rate by the Q biotype whitefly under high O_3. Sci Rep. 7: 14412.
Hornung, B. V. H., R. D. Zwittink, and E. J. Kuijper. 2019. Issues and current standards of controls in microbiome research. FEMS Microbiol. Ecol. 95: fiz045
Hosokawa, T., Y. Kikuchi, M. Shimada, and T. Fukatsu. 2007. Obligate symbiont involved in pest status of host insect. Proc. Biol. Sci. 274: 1979-1984.
Hosokawa, T., R. Koga, Y. Kikuchi, X.-Y. Meng, and T. Fukatsu. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. U.S.A. 107: 769-774.
Hube, B. 2004. From commensal to pathogen: stage- and tissue-specific gene expression of *Candida albicans*. Curr. Opin. Microbiol. 7: 336-341.
Huse, S. M., D. M. Welch, H. G. Morrison, and M. L. Sogin. 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ. Microbiol. 12: 1889-1898.
Jones, R. T., A. Bressan, A. M. Greenwell, and N. Fierer. 2011. Bacterial communities of two parthenogenetic aphid species cocolonizing two host plants across the Hawaiian Islands. Appl. Environ. Microbiol. 77: 8345-8349.
Kang, J.-Y., Y.-S. Kwon, G. Jeong, I. An, and S. Park. 2019. Characteristics of microbial communities of *Pachygrontha antennata* (Hemiptera: Pachygronthidae) in relation to habitat variables. Int. J. Environ. Res. Public Health 16: 4668
Kikuchi, Y., and I. Yumoto. 2013. Efficient colonization of the bean bug *Riptortus pedestris* by an environmentally transmitted *Burkholderia* symbiont. Appl. Environ. Microbiol. 79: 2088-2091.
Kikuchi, Y., M. Hayatsu, T. Hosokawa, A. Nagayama, K. Tago, and T. Fukatsu. 2012. Symbiont-mediated insecticide resistance. Proc. Natl. Acad. Sci. U.S.A. 109: 8618-8622.
Knutson, A., J. Bernal, and A. Gonzalez-Hernandez. 2002. Parasites of the cotton fleahopper, *Pseudatomoscelis seriatus*: searching in Mexico. Proc. Beltwide Cotton Conferences Natl. Cotton Counc. Am., Memphis, TN.
Leonardo, T. E., and G. T. Muiru. 2003. Facultative symbionts are associated with host plant specialization in pea aphid populations. Proc. Biol. Sci. 270 Suppl 2: S209-212.
Loganathan, P., and S. Nair. 2004. *Swaminathania salitolerans* gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (*Porteresia coarctata* Tateoka). Int. J. Syst. Evol. Microbiol. 54: 1185-1190.
Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15: 550.
Malacrinò, A., O. Campolo, R. F. Medina, and V. Palmeri. 2018. Instar- and host-associated differentiation of bacterial communities in the Mediterranean fruit fly *Ceratitis capitata*. PLoS ONE 13: e0194131.

Martin Jr., W. R., M. P. Grisham, C. M. Kenerley, W. L. Sterling, and P. W. Morgan. 1987. Microorganisms associated with cotton fleahopper, *Pseudatomoscelis seriatus* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 80: 251-255.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8: e61217.

Mech, A. M., S. J. Harper, N. P. Havill, C. D. von Dohlen, and G. R. Burke. 2019. Ecological factors influencing the beneficial endosymbionts of the hemlock woolly adelgid (Hemiptera: Adelgidae). Insect Sci. 26: 97-107.

Medina, R. F., P. Nachappa, and C. Tamborindeguy. 2011. Differences in bacterial diversity of host-associated populations of *Phylloxera notabilis* Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory. J. Evolution Biol. 24: 761-771.

Meyer, J. M., and M. A. Hoy. 2008. Molecular survey of endosymbionts in Florida populations of *Diaphorina citri* (Hemiptera: Psyllidae) and its parasitoids *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae). Fla. Entomol. 91: 294-304.

Moss, B. L. 1914. The boll weevil problem: an analysis of its significance to the southern farmer and business man. Progressive Farmer.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, M. H. H. Stevens, M. J. Oksanen, and M. Suggests. 2007. The vegan package. Community ecology package 10: 719.

Oliver, K. M., P. H. Degnan, G. R. Burke, and N. A. Moran. 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu. Rev. Entomol. 55: 247-266.

Pan, H., X. Li, D. Ge, S. Wang, Q. Wu, W. Xie, X. Jiao, D. Chu, B. Liu, and B. Xu. 2012. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci*. PloS ONE 7: e30760.

Parker, R. D., D. D. Fromme, A. E. Knutson, and M. Jungman. 2004. Managing cotton insects in the southern, eastern, and blackland areas of Texas, Texas Cooperative Extension, Texas A&M University System.

Pérez, T., J. L. Balcázar, I. Ruiz-Zarzuela, N. Halaihel, D. Vendrell, I. de Blas, and J. L. Múzquiz. 2010. Host-microbiota interactions within the fish intestinal ecosystem. Mucosal Immunol. 3: 355-360.

Prodan, A., V. Tremaroli, H. Brolin, A. H. Zwinderman, M. Nieuwdorp, and E. Levin. 2020. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. PLoS ONE 15: e0227434.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41: D590-596.

Quince, C., A. Lanzén, T. P. Curtis, R. J. Davenport, N. Hall, I. M. Head, L. F. Read, and W. T. Sloan. 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. Nat. Methods 6: 639-641.

Raszick, T. J., C. P.-C. Suh, C. M. Dickens, and G. A. Sword. 2020. Genome-wide markers reveal temporal instability of local population genetic structure in the cotton fleahopper, *Pseudatomoscelis seriatus* (Hemiptera: Miridae). Pest Manag. Sci. 76: 324-332.

Reinhard, H. J. 1926. Control of cotton fleahopper in Texas, pp. 7-34. *In* Texas Agric. Exp. Stn. Bull., Texas A&M University, College Station, TX.

Robinson, J. V., and J. W. Stewart. 1987. Management of cotton insects in the southern, eastern and Blackland areas of Texas. Texas Agric. Exp. Bull. B-1204.

Salem, H., E. Kreutzer, S. Sudakaran, and M. Kaltenpoth. 2013. Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). Environ. Microbiol. 15: 1956-1968.

Sansone, C. G., M. N. Parajulee, R. R. Minsenmayer, C. Suh, A. K. Barman, and R. F. Medina. 2009. Investigations into the timing and frequency of insecticide applications for cotton fleahopper, pp. 1133-1138. National Cotton Council of America, San Antonio, TX.

Scarborough, C. L., J. Ferrari, and H. C. J. Godfray. 2005. Aphid protected from pathogen by endosymbiont. Science 310: 1781-1781.

Simon, J.-C., S. Carre, M. Boutin, N. Prunier-Leterme, B. Sabater-Muñoz, A. Latorre, and R. Bournoville. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. P. Roy. Soc. Lond. B. Bio. 270: 1703-1712.

Sterling, L. W., and A. D. Dean. 1977. A bibliography of the cotton fleahopper *Pseudatomoscelis seriatus* (Reuter). Texas Agricultural Experiment Station.

Suh, C.-C., and J. K. Westbrook. 2010. Relationship between population estimates of cotton fleahoppers (Hemiptera: Miridae) obtained by terminal and whole plant examinations. J. Entomol. Sci. 45: 204-210.

Tada, A., Y. Kikuchi, T. Hosokawa, D. L. Musolin, K. Fujisaki, and T. Fukatsu. 2011. Obligate association with gut bacterial symbiont in Japanese populations of the southern green stinkbug *Nezara viridula* (Heteroptera: Pentatomidae). Appl. Entomol. Zoo. 46: 483.

Thao, M. L., N. A. Moran, P. Abbot, E. B. Brennan, D. H. Burckhardt, and P. Baumann. 2000. Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl. Environ. Microbiol. 66: 2898-2905.

Thaochan, N., R. A. I. Drew, J. M. Hughes, S. Vijaysegaran, and A. Chinajariyawong. 2010. Alimentary tract bacteria isolated and identified with API-20E and molecular cloning techniques from Australian tropical fruit flies, *Bactrocera cacuminata* and *B. tryoni*. J. Insect Sci. 10: 131.

Toju, H., and T. Fukatsu. 2011. Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants. Mol. Ecol. 20: 853-868.

Tsuchida, T., R. Koga, H. Shibao, T. Matsumoto, and T. Fukatsu. 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum*. Mol. Ecol. 11: 2123-2135.

Tsuchida, T., R. Koga, and T. Fukatsu. 2004. Host plant specialization governed by facultative symbiont. Science 303: 1989-1989.

Watanabe, M., F. Yukuhiro, T. Maeda, K. Miura, and D. Kageyama. 2014. Novel strain of *Spiroplasma* found in flower bugs of the genus *Orius* (Hemiptera: Anthocoridae): transovarial transmission, coexistence with *Wolbachia* and varied population density. Microb. Ecol. 67: 219-228.

Wright, C. A., and G. A. Beattie. 2004. *Pseudomonas syringae* pv. tomato cells encounter inhibitory levels of water stress during the hypersensitive response of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U.S.A. 101: 3269-3274.

Wu, M., and X. Li. 2015. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, pp. 1547-1564. *In* Y. Tang, D. Liu, J. Schwartzman, M. Sussman, and I. Poxton [eds.], Molecular Medical Microbiology. Academic Press, London, UK.

Field Efficacy of Synthetic and Botanical-derived Insecticides against *Melanaphis sacchari*[1], and Non-target and Beneficial Species Associated with Cultivated Sorghum

Efectividad de Campo de Insecticidas Sintéticos y Botánicos contra *Melanaphis sacchari*[1], y Especies Benéficas y No-objetivo Asociadas al Cultivo de Sorgo

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Abstract. The purpose of the study was to investigate the field efficacy of commercial insecticides against *Melanaphis sacchari* (Zehntner) and determine the diversity of non-target species associated before and after treatment. Treatments were applied at 35 and 50 days after germination, and associated insects were sampled with yellow traps 48 hours after application of each product. Insect abundance differed significantly from the control that had 1,006 and 3,750 aphids on the first and second days of evaluation, with fewer aphids at the first sampling (35 days). The most effective products were flupyradifurone (Sivanto®), imidacloprid (Uniprid®), acephate (Unifate®), and *Chenopodium ambrosioides* botanical extract (Requiem®), with efficacies of 99.95% (0.5 aphids/plant), 99.05 (9.5 aphids/plant), 99.41% (6.0 aphids/plant), and 76.78% (233.5 aphids/plant) ($F_{n,3}$ = 19.78; df = 5,12; p < 0.001, r^2 = 0.8918), respectively. At the second sampling (50 days), efficacy of flupyradifurone was 100%, and *Ch. ambrosioides* plant-derived product and spinetoram (Palgus®) were opposite ($F_{n,3}$ = 43.14; df = 5,12; p < 0.001, r^2 = 0.9473). We collected 980 insects associated with sorghum, in five orders, 26 families, and 53 species of Coleoptera, Diptera, Hemiptera, Hymenoptera, and Neuroptera. Species richness increased as abundance decreased. Species richness and abundance were greatest at the first assessment. The Shannon-Wiener diversity index was between 1.2 and 1.6 for the first assessment and 1.8 and 2.4 for the second evaluation. Flupyradifurone, imidacloprid and acephate should be included in integrated management of *M. sacchari,* considering adequate use of the product.

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Resumen. El objetivo de este estudio fue investigar la eficacia de los insecticidas comerciales en el campo contra *Melanaphis sacchari*, y determinar la diversidad de especies no objetivo asociadas antes y después de la aplicación del tratamiento. Aplicamos los tratamientos a los 35 y 50 días después de la germinación y realizamos el muestreo de insectos asociados con trampas amarillas 48 horas después de las aplicaciones de cada producto. Se observaron diferencias significativas entre los datos de densidad de insectos, y se comparó con el grupo control que durante el primer y segundo día de evaluación registró 1,005.66 y 3,750 áfidos, se produjo una reducción de la población de áfidos en el primer muestreo (35 días); los productos más representativos fueron flupiradifurone (Sivanto®), imidacloprid (Uniprid®), acefate (Unifate®) y extracto botánico de C*henopodium ambrosioides* (Requiem®), con eficacia del 99.95% (0.5 áfidos/planta), 99.05 (9.5 áfidos/planta), 99.41% (6.0 áfidos/planta), y 76.78% (233.5 áfidos/planta) ($F_{n,3}$ = 19.78; df = 5,12; $p < 0.001$, $r^2 = 0.8918$), respectivamente, mientras que para el segundo muestreo (50 días) la flupiradifurone registró una eficiencia del 100%, y el producto derivado de la planta *Ch. ambrosioides* y el Spinetoram (Palgus®) registraron lo contrario ($F_{n,3}$ = 43.14; df = 5,12; $p < 0.001$, $r^2 = 0.9473$). Se recolectaron 980 insectos asociados con cultivo de sorgo, determinando cinco órdenes, 26 familias, y 53 especies, distribuidas en Coleoptera, Diptera, Hemiptera, Hymenoptera, y Neuroptera. La riqueza de las especies aumentó a medida que la abundancia disminuyó. La primera evaluación mostró la mayor abundancia y riqueza de especies. Se registró un índice de diversidad Shannon-Wiener entre 1.2 y 1.6 para la primera evaluación, y para la segunda evaluación se observó un incremento con valores entre 1.8 y 2.4. Los resultados sugieren que el flupiradifurone, imidacloprid y acefate pueden incluirse en el manejo integrado de *M. sacchari*, considerando un uso adecuado del producto.

Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehnther) Hemiptera: Aphididae, is native to Asia and/or African regions but has cosmopolitan distribution (Singh et al. 2004, Peña-Martínez et al. 2016). The insect in the United States of America is in Florida, Hawaii, Louisiana, and Texas; in Mexico, it is at alarming levels in several states such as Tamaulipas, Nuevo Leon, San Luis Potosi, Veracruz, and Michoacan (Hernandez et al. 2014, SENASICA 2014, 2015).

The insect is a danger to all sorghum-producing areas and affects crop developmental stages. Aphids feeding on sap cause plant foliage to be yellowish and then brown, excrete honeydew that encourages growth of sooty mold fungi affecting photosynthesis, delays vegetative growth, and depending on the infestation decreases the yield of the crop 77 to 100% (Singh et al. 2004, Hernández et al. 2014, Villanueva et al. 2014, Bowling et al. 2016). The aphid transmits viruses such as sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (ScYLV) to sorghum (El-Sayed 2013).

Strategies to control the aphid are based on application of synthetic organic insecticides such as flupyradifurone, sulfoxaflor, spirotetramat, imidacloprid, pymetrozine, and afidopyropen that keep pest abundance below the economic threshold (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Tejeda-Reyes et al. 2017). However, effects of synthetic insecticides have unfortunate consequences for many non-target organisms, especially beneficial species of natural enemies and pollinators (Rodríguez 1980, Ivés and Settle 1996, Banken and

Stark 1998, MEA 2005, El-Heneidy et al. 2015, Adly 2016, Garibaldi et al. 2016, Goda et al. 2016, Rader et al. 2016). The study aimed to determine toxicity of biorational and synthetic insecticides on *Melanaphis sacchari* and determine diversity of non-target and beneficial species associated with sorghum.

Materials and Methods

The research was done in fields of 'Fortuna' sorghum at the experimental station of the Facultad de Ciencias Agropecuarias (FCA), belonging to the Universidad Michoacana de San Nicolas de Hidalgo, at Apatzingan, Michoacán (19°04'58.57" N, 102°22'19.55" O), Mexico. Crop cultural practices were homogeneous following technical recommendations of the Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias (INIFAP) of Mexico for the region (González et al. 2009). Experiments were initiated when aphid abundance was the threshold of 50 per leaf (Rodríguez-del-Bosque 2015, Bowling et al. 2016).

The experimental design was a randomized complete block with six treatments and three replications. Each experimental unit was two 12.75-m-long rows 0.8-m apart and separated from the next experimental unit by 0.5-m.

Chemical and biorational insecticides tested were: 1) Requiem® (*Chenopodium ambrosioides* botanical extract 16.75% CE) 1.0 L ha^{-1}, 2) Palgus® DowAgrosciences (spinetoram 5.87% SC) 100 ml ha^{-1}, 3) Uniprid® AgroMéxico 350 SC (imidacloprid 30.20%) 1.0 L ha^{-1}, 4) Sivanto® Prime 20 SL (flupyradifurone 20%) 1.0 L ha^{-1}, 5), Unifate® Ultra AgroMéxico (acephate 97%) Pellet 0.55 kg ha^{-1}, and 6) absolute control (tap water) without insecticide. A motorized backpack sprayer with a two-point flat fan nozzle was used to apply solutions as a fog at 80 psi pressure to the drip-point. Outputs of 400 and 500 liters/ha were used for the first and second applications, respectively. Spraying was approximately 40-cm from the foliage, for complete coverage. Before application, the pH of the water was adjusted to 5.5 with acidifier, and 1 ml per liter of the adjuvant Inex-A® (Cosmocel S.A.) was added. Doses recommended by the manufacturer were applied at 35 and 50 days after germination.

Insects were counted 2 days after application of insecticide. Two plants from each row per experimental unit were selected at random (12 plants). On each plant, one leaf from the lower and one from the upper part of the plant were selected at random and the total number of aphids (sum of winged and nonwinged) was counted with the help of a 10x magnifying glass.

For sampling, yellow plastic plates 20-cm in diameter containing 250 ml of 5% soapy water (yellow traps) were used. One trap was put on the ground in each replication. Preliminary sampling at 48 hours assessed insect abundance before insecticides were applied to the crop. Insects in each trap were collected 2 days later and preserved in ethyl alcohol (70%) for taxonomic identification. Beneficial insects associated with the crop were identified by using taxonomic keys of Roberts (1972), Michener et al. (1994), Ibañez-Bernal et al. (2004), Valencia et al. (2006), Cadena et al. (2007), Choate (2010), and Ascher and Pickering (2017).

The numbers of insects at each sampling were subjected to analysis of variance and a multiple comparison test (Dunnett, $\alpha = 0.05$) using SAS v8.0 statistical package. Diversity and richness of species were determined according to the formula by Shannon Wiener (1949), equity rates, and abundance with Pisces Conservation 2007 software (Pielou 1975).

Results and Discussion

Insect abundance differed significantly between the first and second field assessment and compared with the control. There were 1,005.7 and 3,750.0 aphids/plant on the first and second evaluation days, with fewest aphids at the first sampling (35 days). The most effective products were flupyradifurone, imidacloprid, acephate, and *Ch. ambrosioides* botanical extract, with efficacies of 99.95% (0.5 aphids/plant), 99.05 (9.5 aphids/plant), 99.41% (6.0 aphids/plant), and 76.78% (233.5 aphids/plant) ($F_{n,3}$ = 19.78, *df* = 5, 12, $p < 0.001$, $r^2 = 0.8918$), respectively. At the second sampling (50 days), efficacy of flupyradifurone was 100%, but *Ch. ambrosioides* plant-derived product and spinetoram were opposite ($F_{n,3}$ = 43.14; df = 5,12; $p < 0.001$, $r^2 = 0.9473$) (Table 1).

Table 1. Aphid Abundance and Efficacy (%) 48 Hours after Treatment of Sorghum
Cuadro 1. Densidad de Áfidos y Eficacia (%) 48 Horas Después de la Aplicación de Tratamientos en el Sorgo

	Number of aphids			
	1st evaluation		2nd evaluation	
Treatment	35 days	Efficacy (%)	50 days	Efficacy (%)
Flupyradifurone	0.50 ± 0.50b	99.95	0.00 ± 0.00b	100.00
Imidacloprid	9.50 ± 2.50b	99.05	7.50 ± 2.50b	99.80
Acephate	6.00 ± 1.00b	99.41	50.00 ± 10.00b	98.94
Ch. ambrosioides botanical extract	233.50 ± 26.50b	76.78	4500.00 ± 500.00a	0.00
Spinetoram	1112.5 ± 62.50a	0.00	4750.00 ± 750.00a	0.00
Control	1005.66 ± 495.0a	0.00	3750.00 ± 1250.00a	0.00

Means followed by different letters in a column are significantly different, $P \leq 0.05$.

The effect of insecticides depends on several factors such as insect species, developmental stage, and exposure time to the products. For example, Roubos et al. (2014) commented that spinetoram had no significant in lethal effect on *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). With broad-spectrum insecticides such as phosmet, zeta-cypermethrin, esfenvalerate, methomyl, and azinphos-methyl, mortal effects on *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) decreased until the 3rd day after product application, comparable to organic products such as novaluron, *Bacillus thuringiensis* subsp. *kurstaki*, methoxyfenozide, and chlorantraniliprole. The insecticide has a lethal effect on *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) comparable to broad-spectrum insecticides. Likewise, imidacloprid killed *Aphidius colemani* (Dalman) (Hymenoptera: Braconidae) and *O. insidiosus* similar to organic products, and had a toxic effect on *H. convergens* and *C. rufilabris* similar to broad-spectrum insecticides. Al-Kherb (2011) described low toxicity of imidacloprid on *Orius* sp., *Paederus alfierii* Koch (Coleoptera: Staphylinidae), *Coccinella undicempunctata* (Reiche), and *C. carnea*, while Sohrabi et al. (2013) reported the product was very toxic to *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Barbosa et al. (2017) reported susceptibility of adults of *C. carnea* to flupyradifurone and ovicidal and great mortality

of nymphs of *O. insidiosus.* However, they considered it relatively innocuous and compatible with integrated pest management.

In contrast, other authors mentioned 100% efficacy of flupyradifurone on *O. insidiosus* and 90% larvicidal activity and 80% toxic effect on adults of *H. convergens* (Colares et al. 2017, Cloyd and Herrick 2018). Bayoun et al. (1995) described low toxicity of acephate on parasitoid and predatory insects, and toxicity to *Diuraphis noxia* Kurdjumov (Hemiptera: Aphididae), while Carmo et al. (2010) reported toxicity of the product to the parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae). Several authors reported lethal effects on beneficial insects when botanical extracts and essential oils were used; for example, *Azadirachta indica* A. Juss (Meliaceae), *Citronella* sp. D. Don (Cardiopteridaceae), *Eucalyptus* sp. L'Hér. (Myrtaceae), *Allium sativum* L. (Amaryllidaceae), and *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. (Asteraceae) (Swaminathan et al. 2010; Bonsignore and Vacante 2012; Tunca et al. 2012, 2014; Xavier et al. 2015).

A total of 980 specimens was collected in five orders, 26 families, and 53 species, categorized as predators, parasitoids, and pollinators. The order Hymenoptera had 13 families with 29 species (n = 204 insects), Diptera had five families with 15 species (n = 723), Coleoptera had four families with five species (n = 23), Hemiptera had two families with three species (n = 7), and Neuroptera had one species in one family (n = 23). Species richness increased as abundance decreased, and the number of species was similar in most treatments. Species abundance and richness were most at the first evaluation. The Shannon-Wiener diversity index was between 1.2 and 1.6 for the first assessment and 1.8 to 2.4 for the second evaluation. The amount of species is not proportional to community richness, because it involves abundance and distribution, i.e., to higher equity, greater diversification, so it can have the same measure but different rates of dominance (Table 2).

Hymenoptera was the most representative order with several species and families; pollinators, and parasitoids; solitary, semi-social and eusocial species were registered; some of them with colonies on tree trunks, stems, and substrates (Michener 2000). Most-represented were *Tiphia popilliavora* Rohwer (Thripidae) reported as a potential alternative in biological control because they attack ground beetle larvae; *Scolia dubia* (Say) (Hymenoptera: Scoliidae) has been used to manage *Popillia japonica* (Coleoptera: Scarabaeidae) in the field (Grissell 2007, Van Driesche et al. 2007, Obeysekara et al. 2014, Alcock 2016, Manju et al. 2018), and *Polistes pallipes* Lepeletier (Hymenoptera: Vespidae) used to regulate lepidopteran pests of avocado (*Persea americana* Mill.) and cabbage (*Brassica oleracea* L.) crops (West-Eberhard 2006, Carpenter et al. 2012). Among the most abundant pollinators were the wasp *Neozeloboria* sp. Turner (Hymenoptera: Vespidae), native honey bee *Melissodes* sp., and *Apis mellifera* L. (Hymenoptera: Apidae) (Garibaldi et al. 2013, Cunningham 2017).

Diptera was the order with the greatest number of specimens collected when the product was first evaluated. We observed many *Condylostylus melampus* (Loew) (Diptera: Dolichopodidae), but abundance of the predator decreased to zero at the second assessment when acephate was used. The most abundant genera of Diptera were *Condylostylus*, *Dexia* Meigen, *Aplomyiopsis* Villeneuve, *Archytas* Fabricius (Tachinidae), and *Allograpta* Say (Syrphidae).Species of the genus *Condylostylus* are cosmopolitan, with *C. melampus* and *C. occidentalis* common in agroecosystems (Bickel 1994, Yang and Saigusa 2005). Pollet and Brooks (2008) affirmed several *Condylostylus* species were predators of soft-bodied insects, such as mites, Collembola, aphids, and thrips. We found *Dexia* sp., *D. ventralis* Aldrich, and a

Table 2. Richness and Species Composition of Non-target Insects Associated with Sorghum Crop
Cuadro 2. Riqueza y Composición de Especies de Insectos No Objetivos Asociados al Cultivo de Sorgo

			Pre	1st evaluation (35 days)						2nd evaluation (50 days)					
Order	Family	Species	-	Cr	Ext	Spin	Imid	Flup	Ace	Cr	Ext	Spin	Imid	Flup	Ace
Coleoptera	Cantharidae	*Chauliognathus pennsylvanicus* (De Geer)	1	0	1	0	0	0	2	1	0	0	0	0	0
	Melyridae	*Collops* sp.	0	0	0	0	1	0	0	0	0	0	0	0	0
	Coccinellidae	*Cycloneda sanguinea* (L.)	0	5	1	0	2	0	0	3	0	0	1	0	0
		Harmonia axydiris (Pallas)	0	0	0	0	0	0	0	1	0	0	0	1	0
	Staphylinidae	Lathrobium sp.	3	0	0	0	0	0	0	0	0	0	0	0	0
Diptera	Asilidae	*Promachus vertebratus* (Say)	0	0	0	0	0	2	0	0	0	0	0	0	0
	Dolichopodidae	*Condylostylus melampus* (Loew)	180	62	54	58	62	55	41	13	4	10	9	4	0
		Condylostylus occidentalis (Bigot)	0	2	1	0	0	2	1	1	0	2	0	0	4
		Dolichopus sp.	0	5	2	0	0	0	0	0	0	0	0	0	0
	Stratiomyidae	*Hermetia illuceus* (Loew)	0	0	0	0	0	0	0	0	0	0	1	0	0
		Stratiomys sp.	0	1	0	0	0	0	0	0	0	0	0	0	0
	Syrphidae	*Allograpta* sp.	4	0	0	1	1	0	0	3	1	0	0	0	0
		Eristalis sp.	0	0	0	0	0	0	0	0	0	2	0	0	0
		Eristalis tenax (L.)	0	0	0	0	0	0	0	0	0	0	1	0	0
		Microdon sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
		Syrphus sp.	8	0	0	0	0	0	0	0	0	0	0	0	0
	Tachinidae	*Aplomyiopsis epilachnae* (Aldrich)	0	1	0	1	2	1	0	11	3	0	0	3	0
		Archytas sp.	4	2	0	2	3	1	4	13	22	8	10	5	3
		Dexilla sp.	0	2	3	1	0	0	1	0	2	3	5	0	0
		Dexilla ventralis (Aldrich)	0	0	0	2	0	5	2	0	0	0	0	0	0
Hemiptera	Reduviidae	*Zelus* sp.	0	1	1	0	1	0	0	0	0	2	0	0	0
		Zelus tetracanthus (Stål)	0	0	0	0	0	0	0	1	0	0	0	0	0
	Ampulicidae	*Ampulex* sp.	0	0	0	0	1	0	0	0	0	0	0	0	0
Hymenoptera	Anthophoridae	*Anthophora occidentalis* Cresson	0	0	0	0	0	0	0	0	0	0	1	0	2
		Melissodes sp.	0	0	2	0	0	0	0	0	1	0	0	2	2
	Apidae	*Apis melifera* L.	0	5	0	10	0	0	0	0	0	0	0	0	0

		Bombus sp.	0	0	0	0	0	2	0	0	0	0	0	0	0
	Cabronidae	*Crabro* sp.	0	0	0	0	0	0	3	0	0	0	0	0	0
	Chalcididae	*Spilachalcis* sp.	0	0	0	1	0	0	0	0	0	0	0	0	0
	Halictidae	*Agapostemon virescens* Fabricius	0	0	0	1	0	0	0	3	0	0	0	0	0
		Sphecodes sp	0	1	0	2	0	0	0	0	0	0	1	0	0
	Ichneumonidae	*Casinaria texana* (Ashmead)	0	0	0	0	0	0	0	0	0	0	1	0	0
		Megarhyssa sp.	1	0	0	0	0	0	0	0	0	2	0	0	0
	Mutillidae	*Dasymutilla* sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
	Reduviidae	*Narvesus* sp.	0	0	0	0	0	0	0	0	0	0	1	2	0
	Scoliidae	*Scolia dubia* Say	0	0	0	0	0	1	0	3	2	1	0	1	0
	Sphecidae	*Astata* sp.	0	1	1	0	0	0	3	0	0	2	9	0	3
		Ammophila nigricans Dahlbom	0	0	0	0	0	0	1	0	0	0	0	0	0
		Ammophila sp.	0	0	0	0	0	0	0	0	0	2	0	0	0
		Chlorion sp.	0	0	0	0	0	0	0	0	0	0	0	0	2
		Gorytes sp.	0	0	0	0	0	0	0	0	0	1	1	0	0
		Myzinum sp.	0	0	0	0	0	0	0	0	0	3	0	0	0
		Pemphredon sp.	2	0	0	0	1	0	0	0	0	0	1	0	0
		Sceliphron sp.	0	0	0	0	0	0	0	0	0	2	0	0	0
		Sphecius sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
	Tiphiidae	*Neozeloboria proximus* Turner	0	1	1	0	1	0	0	2	7	0	1	0	0
		Tiphia popilliavora Rohwer	0	10	4	15	2	8	3	0	0	4	3	6	4
	Vespidae	*Eumenes fraternus* (Say)	0	1	0	0	1	1	0	0	0	0	3	0	0
		Polistes pallipes Lepeletier	0	0	0	5	1	0	0	2	0	3	6	5	0
		Polistes sp.	0	0	1	0	0	0	1	0	8	0	0	0	0
		Rygchium dorsale Fabricius	0	0	0	0	1	0	0	0	0	0	0	0	0
	Vespula	*Vespula maculata* (Lin)	0	0	0	2	0	0	0	0	0	0	0	0	0
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)	2	0	0	2	4	1	0	2	3	1	3	5	0
Abundance			205	100	72	103	84	79	62	62	53	48	58	34	20
Number of species			9	15	12	14	15	11	11	17	10	16	18	10	7
Shannon Wiener Diversity Index			0.60	1.53	1.24	1.59	1.23	1.21	1.38	2.35	1.84	2.52	2.49	2.16	1.90
Pielou Equity Index			0.27	0.57	0.45	0.60	0.45	0.51	0.57	0.82	0.79	0.90	0.86	0.93	0.97

parasitoid of some Coleoptera such as *Maladera castanea* (Arrow), *Phyllophaga* spp. Harris, *P. japonica*, and *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) most abundant (Arnaud 1978, Vallejo and Carrillo-Sanchez 1987). We found *Aplomyiopsis epilachnae* (Aldrich) (Diptera: Tachinidae) parasitoids of *E. varivestis* (Borror and DeLong 1970), several species in the genus *Archytas* sp. that parasitize Lepidoptera larvae such as *Acrobasis nuxvorella* Neunzig (Pyralidae), and *Spodoptera frugiperda* Walker and *Trichoplusia ni* (Hubner) (Noctuidae), and immature *Allograpta* sp. prey on aphids while adults pollinate (Pérez et al. 2009, Stireman et al. 2009, Ríos-Velasco et al. 2011, Torres-Delgado et al. 2018).

Species of Coleoptera were dominant for biological control, including *Harmonia axyridis* Pallas that is an effective polyphagous predator (Almeida and Silva 2002, Koch 2003), *Cycloneda sanguinea* L. (Coccinellidae) that is a predator in both larval and adult stages (Miranda et al. 1998, Almeida et al. 2001, Faria et al. 2006, Evans 2008, Casari and Ide 2012), *Chauliognathus pennsylvanicus* (De Geer) (Cantharidae) that in larval stages prey on aphids while adults are pollinators (Baskin et al. 2000 Mahr 2009), and *Collops* sp. (Melyridae) used for integrated pest management of alfalfa (*Medicago sativa* L.) and cotton (*Gossypium* sp. L.) crops because it eats eggs, larvae, pupae, and nymphs of aphids, whitefly, and bugs such as *Bagrada hilaris* Burmeister (Hemiptera: Pentatomidae) (Ellsworth et al. 2011, Grasswitz 2016).

The most representative species of Hemiptera were *Zelus* sp. Fabricius (Reduviidae) predators that feed of a broad variety of insects such as *Aphis gossypii* Glover (Aphididae), eggs and larvae of Lepidoptera, larvae and adults of *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae), and also feed on such valuable species as coccinellids and big-eyed bug geocorids (Cogni et al. 2002, Weirauch et al. 2012). We also found *Aphytis* spp. (Hymenoptera: Aphelinidae) (Heimpel et al. 1997, Drees and Jackman 1999) and *Chrysoperla carnea* Stephen (Neuroptera: Chrysopidae) (Hodge 1999) applied for biological control of thrips and *Myzus persicae* (Sulzer), *Aphis pomi* (Degeer) (Hemiptera: Aphididae), and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Weirauch et al. 2012, Rodrigues et al. 2014). Monserrat and Marín (1994) mentioned that *C. carnea* has ecological versatility that facilitates colonization of multiple habitats.

Marrugan (1988) commented Shannon-Wiener index less than 1.5 is low diversity, between 1.6 and 3.0 is medium diversity, and values equal to or greater than 3.1 indicate much diversity. In this work, non-treatment resulted in great diversity of species because of side effects of the insecticides applied.

Chemical insecticides like imidacloprid, flupyradifurone, and acephate reduced abundance of *M. sacchari* below the economic threshold, unlike botanical extract of *Ch. ambrosioides* and spinetoram 50 days after application. Diversity of beneficial species was low during pre-sampling and the first evaluation. However, after the second application, most plots had medium diversity because abundance decreased in all treatments. Flupyradifurone, imidacloprid, and acephate best controlled pests.

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References Cited

Alcock, J. 2016. The Scramble Competition Mating System of *Scolia dubia* (Hymenoptera: Scoliidae). J. Kansas Entomol. Soc. 89: 168-173.

Al-Kherb, W. A. 2011. Field efficacy of some neonicotinoid insecticides on whitefly *Bemicia tabaci* (Homoptera: Aleyrodidae) and its natural enemies in cucumber and tomato plants in Al-qassim Region, KSA. J. Entomol. 8: 429-439.

Almeida, L. M, and V. B: Silva. 2002. Primeiro registro de *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae): um coccinelídeo originário da região Paleártica. Rev. Bras. Zool. 19: 941-944.

Almeida, S. G., P. Petersen, and A. Cordeiro. 2001. Crise socioambiental e conversao ecologica da agricultura brasileira: subsidios a formulacao de diretrizes ambientais para o desenvolvimento agricola. AS-PTA, Rio de Janeiro, Brazil.

Arnaud, P. H. 1978. A Host-parasite Catalog of North American Tachinidae (Diptera). United States Department of Agriculture.

Ascher, J. S., and J. Pickering. 2017. Discover Life Bee Species Guide and World Checklist (Hymenoptera: Apoidea: Anthophila). https://bugguide.net/node/view/468583 Accessed 26 October 2019.

Banken, J., and J. Stark. 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: a study of neem and the seven spotted lady beetle (Coleoptera: Coccinellidae). J. Econ. Entomol. 91: 1-6.

Barbosa, P. R., J. P. Michaud, C. L. Bain, and J. B. Torres. 2017. Toxicity of three aphicides to the generalist predators *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Orius insidiosus* (Hemiptera: Anthocoridae). Ecotoxicol. 26: 589-599.

Baskin, J. M., J. L. Walck, C. C. Baskin, and D. E. Buchele. 2000. Ecology and conservation biology of the endangered plant species *Solidago shortii* (Asteraceae). Native Plants Journal 1: 35-41.

Bayoun, I. M., F. W. Plapp Jr., F. E. Gilstrap, and G. J. Michels Jr. 1995. Toxicity of selected insecticides to *Diuraphis noxia* (Homoptera: Aphididae) and its natural enemies. J. Econ. Entomol. 88: 1177-1185.

Bickel, D. J. 1994. The Australian Sciapodinae (Diptera: Dolichopodidae), with a review of the Oriental and Australasian faunas, and a world conspectus of the subfamily. Rec. Aust. Mus. 21: 1-394.

Bonsignore, C., and V. Vacante. 2012. Influences of botanical pesticides and biological agents on *Orius laevigatus - Frankliniella occidentalis* dynamics under greenhouse conditions. J. Plant Prot. Res. 52: 15-23.

Borror, D. J., and D. M. DeLong. 1970. Introduction to the Study of Insects. 3rd ed. Holt, Rinehart, and Winston, New York.

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliott, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. J. Integr. Pest Manag. 7: 1-13.

Cadena, P., F. Ángel, L. A. Gómez, and R. González. 2007. Diferenciación morfológica y molecular de especies de crisópidos (Neuroptera: Chrysopidae). Rev. Colomb. Entomol. 33: 171-177.

Carmo, E. D., A. Bueno, and R. C. Bueno. 2010. Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. BioControl 55: 455-464.

Carpenter, J. M., B. R. Garcete-Barrett, and J. A. F. López. 2012. Las Vespidae (Hymenoptera: Vespidae) de Guatemala. Avispas de Guatemala. Biodivers. 2: 1-13.

Casari, S. A., and S. Ide. 2012. Coleptera, pp. 453-536. *In* J. A. Rafael, G. A. Melo, C. J. Carvalho, S. A. Casari, and R. Constantino [eds.], Insetos do Brasil: Diversidade e Taxonomia. Holos editora, Ribeirão Preto, Sao Paulo, Brazil.

Choate, P. M. 2010. Introduction to the Identification of Insects and Related Arthropods. 1-14.

Cloyd, R. A., and N. J. Herrick. 2018. Effects of pesticides on the survival of rove beetle (Coleoptera: Staphylinidae) and insidious flower bug (Hemiptera: Anthocoridae) adults. J. Econ. Entomol. 111: 78-88.

Cogni, R., A. V. Freitas, and B. F. Filho. 2002. Influence of prey size on predation success by *Zelus longipes* L. (Het., Reduviidae). J. Appl. Entomol. 126: 74-78.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2017. Relative toxicity of two aphicides to *Hippodamia convergens* (Coleoptera: Coccinellidae): implications for integrated management of sugarcane aphid, *Melanaphis sacchari* (Hemiptera: Aphididae). J. Econ. Entomol. 110: 52-58.

Cunningham, S. A. 2017. Human welfare and its connection to nature: What have we learned from crop pollination studies? Austral. Ecol. 42: 2-8.

Drees, B. M., and J. Jackman. 1999. Field Guide to Common Texas Insects. Gulf Publishing, Houston, TX.

Ellsworth, P. A., A. Mostafa, and L. Brown. 2011. *Collops* beetle: natural predator in field crops. Western Farm Press. University of Arizona; Steven Naranjo, USDA-ARS Accessed on 29 September 2019.

El-Sayed, A. 2013. Maize (*Zea mays* L.) constitutes a novel host to sugarcane yellow leaf virus. Can. J. Plant Pathol. 35: 68-74.

Evans, E. W. 2008. Multitrophic interactions among plants, aphids, alternate prey, and shared natural enemies-a review. Eur. J. Entomol. 105: 369-380.

Faria, M. R., J. G. Lundgren, E. M. G. Fontes, O. A. Fernandes, F. Schmidt, N. V. Tuat, and D. A. Andow. 2006. Assessing the effects of Bt cotton on generalist arthropod predators, pp. 175-199. *In* A. Hilbeck, D. A. Andow, and E. M. G. Fontes [eds.], Environmental Risk Assessment of Genetically Modified Organisms: Methodologies for Assessing Bt Cotton in Brazil. CABI, Cambridge.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, and S. A., et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Sci. 339: 1608-1611.

Garibaldi, L. A., L. G. Carvalheiro, B. E. Vaissière, B. Gemmill-Herren, J. Hipólito, B. M. Freitas, and J. An. 2016. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. Sci. 351: 388-391.

González, J. A., A. E. Aguirre, and A. J. A. Hernandez. 2009. Sorgo fortuna, una alternativa para producir forraje en la planicie Huasteca. Instituto Nacional de Investigaciones Forestales, Agrícolas INIFAP, Tamaulipas, Mexico.

Grasswitz, T. R. 2016. Laboratory evaluation of predation rates of two native natural enemies on the exotic pest *Bagrada hilaris*. J. Appl. Entomol. 140: 1-13.

Grissell, E. 2007. Scoliid Wasps of Florida, Campsomeris, *Scolia* and *Trielis* spp. (Insecta: Hymenoptera: Scoliidae). University of Florida IFAS Extension (OnLine). Accessed October 2019.

Heimpel, G. E., J. A. Rosenheim, and M. Mangel. 1997. Predation on adult *Aphytis* parasitoids in the field. Oecologia 110: 346-352.

Ibañez-Bernal, S., O. V. Hernández, and C. L. Miranda. 2004. Dolichopodidae (Diptera), pp. 759-765. *In* B. J. Llorente, J. J. Morrone, O. O. Yáñez, and F. I. Vargas [eds.], Biodiversidad, Taxonomia y Biogeografia de Artropodos de México. Hacia una síntesis de su conocimiento. 4th ed. Facultad de Ciencias, UNAM, DF México.

Ivés, A., and W. Settle. 1996. The failure of a parasitoid to persist with a super-abundant host: the importance of the numerical response. Oikos 75: 269-278.

Koch, R. L. 2003. The multicolored Asian lady beetle *Harmonia axyridis*: a review of this biology, uses in biological control and non-targets impacts. J. Insect Sci. 32: 1-16.

Mahr, S. 2009. Goldenrod Soldier Beetle, *Chauliognathus pennsylvanicus*. University of Wisconsin, Madison (online). Accessed on 29 September 2019.

Manju, P., P. M. Sureshan, and P. Girish. 2018. New record of two species of scoliid wasps (Hymenoptera: Scoliidae) from Kerala. J. Entomol. Res. 42: 103-106.

Marrugan, A. E. 1988. Ecological Diversity and Its Measurement. Croom Helm, London.

Michener, C. D. 2000. The Bees of the World. Johns Hopkins University Press, Baltimore, MD. 913 + xiv.

Michener, C. D., R. J. McGlinley, and B. Danforth. 1994. The bee genera of North and Central America (Hymenoptera: Apoidea). Smithsonian Institution Press, Washington, DC.

Millennium Ecosystem Assessment (MEA). 2005. Ecosystems and Human Well-being Synthesis. World Resources Institute, Island Press, Washington, DC.

Miranda, M. M. M., M. Picanço, A. L. Matioli, and A. Pallini-Filho. 1998. Distribuição na planta e controle biológico de pulgões (Homoptera: Aphididae) em tomateiros. Rev. Bras. Entomol. 42: 13-16.

Monserrat, V. J., and F. Marín. 1994. Plant substrate specificity of Iberian Chrysopidae (Insecta: Neuroptera). Acta Oecol. 15: 119-131.

Obeysekara, P. T., A. Legrand, and G. Lavigne. 2014. Use of herbivore-induced plant volatiles as search cues by *Tiphia vernalis* and *Tiphia popilliavora* to locate their below-ground scarabaeid hosts. Entomol. Exp. Appl. 150: 74-85.

Peña-Martínez, R., A. L. Muñoz-Viveros, R. Bujanos-Muñiz, J. Luévano-Borroel, F. Tamayo-Mejia, and E. Cortez-Mondaca. 2016. Formas sexuales del complejo pulgón amarillo del sorgo, *Melanaphis sacchari/sorghi* en México. Southwest. Entomol. 41: 127-132.

Pérez, T. B. C., A. Aragón-García, N. Bautista-Martínez, A. M. Tapia-Rojas, López- and J. F. Olguín. 2009. Entomofauna asociada al cultivo de Jamaica (*Hibiscus sabdariffa* L.) en el municipio de Chiautla de Tapia, Puebla. Acta Zool. Mex. 25: 239-247.

Pielou, E. C. 1975. Ecological Diversity. John Wiley Press, New York.

Pollet, M. A. A., and S. E. Brooks. 2008. Long-legged flies (Diptera: Dolichopodidae). *In* J. L. Capinera [ed.], Encyclopedia of Entomology. 2nd ed. Vol. 4, Springer, Dordrecht.

Rader, R., I. Bartomeus, L. A. Garibaldi, M. P. Garratt, B. G. Howlett, R. Winfree, and R. Bommarco. 2016. Non-bee insects are important contributors to global crop pollination. Proc. Natl. Acad. Sci. USA. 113: 146-151.

Ríos-Velasco, C., G. Gallegos-Morales, M. C. Rincón-Castro, K. A. Ulloa-Rubio, J. Cambero-Campos, and R. D. Valenzuela-García. 2011. Primer registro de *Archytas marmoratus* y *Voria ruralis* (Diptera: Tachinidae) y sus niveles de

parasitoidismo en dos lepidópteros plaga en Coahuila, México. Acta Zool. Mex. 27: 577-582.
Roberts, R. B. 1972. Revision of the bee genus *Agapostemon* (Hymenoptera: Halictidae). Univ. Kans. Sci. Bull. 49: 437-590.
Rodrigues, C. A., A. P. Battel, N. M. Martinelli, R. D. A. Moral, R. K. Sercundes, and W. A. C. Godoy. 2014. Dynamics and predation efficiency of *Chrysoperla externa* (Neuroptera: Chrysopidae) on *Enneothrips flavens* (Thysanoptera: Thripidae). Fla. Entomol. 97: 653-659.
Rodríguez-del-Bosque, L. A., and A. Terán. 2015. *Melanaphis sacchari* (Hemiptera: Aphididae) a new sorghum insect pest in México. Southwest Entomol. 40: 433-434.
Rodríguez, M. 1980. Manejo y Control de Plagas en Plantas y Animales. 3rd ed. Limusa, México.
Roubos, C. R., C. Rodriguez-Saona, R. Holdcraft, K. S. Mason, and R. Isaacs. 2014. Relative toxicity and residual activity of insecticides used in blueberry pest management: mortality of natural enemies. J. Econ. Entomol. 107: 277-285.
SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria). 2014. Pulgón amarillo (*Melanaphis sacchari*) (Zehntner). SAGARPA. Ficha Técnica 43, Mexico.
SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria). 2015. Acciones contra el pulgón amarillo del sorgo (*Melanaphis sacchari*) en México. Circular 101, 17 Agosto 2015. México.
Shannon, C., and W. Weaver. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, IL.
Singh, B. U., P. G. Padmaja, and N. Seetharama. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. J. Crop Protec. 23: 739-755.
Sohrabi, F., P. Shishehbor, M. Saber, and M. S. Mosaddegh. 2013. Lethal and sublethal effects of imidacloprid and buprofezin on the sweet potato whitefly parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae). Crop Protec. 45: 98-103.
Stireman, J. O., H. F. Greeney, and L. A. Dyer. 2009. Species richness and host associations of Lepidoptera-attacking Tachinidae in the northeast Ecuadorian Andes. J. Insect Sci. 9: 1-19.
Swaminathan, R., J. Hansa, and T. Hussain. 2010. Side effects of a few botanicals on the aphidophagous coccinellids. J. Biopestic. 3: 81-84.
Tejeda-Reyes, M. A., J. Díaz-Najera, J. Rodriguez-Maciel, M. Vargas-Hernández, J. F. Solís-Aguílar, S. Ayvar-Serna, and J. A. Flores-Yáñez. 2017. Evaluación en campo de insecticidas sobre *Melanaphis sacchari* (Zehntner) en sorgo. Southwest. Entomol. 42: 545-550.
Torres-Delgado, M. G., E. Ruiz-Cancino, V. Ávila-Rodríguez, U. Nava-Camberos, J. M. Coronado-Blanco, O. G. Alvarado-Gómez, and E. M. Olais. 2018. Parasitoides del gusano barrenador de la nuez, *Acrobasis nuxvorella* Neunzig en La Comarca Lagunera, México. Southwest. Entomol. 43: 175-182.
Tunca, H., N. Kilinçer, and C. Özkan. 2012. Side-effects of some botanical insecticides and extracts on the parasitoid, *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae). Turk. J. Entomol. 36: 205-214.
Tunca, H., N. Kilinçer, and C. Özkan. 2014. Toxicity and repellent effects of some botanical insecticides on the egg-larval parasitoid *Chelonus oculator* Panzer (Hymenoptera: Braconidae). Sci. Res. Essays 9: 106-113.

Valencia, L. L. A., N. J. Romero, C. J. Valdez, S. J. L. Carrillo, and M. V. López. 2006. Taxonomía y registros de Chrysopidae (Insecta: Neuroptera) en el estado de Morelos, México. Acta Zool. Mex. 22: 17-61.

Vallejo, T., and J. L. Carrillo-Sanchez. 1987. Parasitismo de *Aplomyiopsis epilachnae* (Diptera: Tachinidae) sobre *Epilachna varivestis* (Coleoptera: Coccinellidae) en Chapingo, Mexico. Folia Entomol. Mex. 141-156.

Van Driesche, R., M. Hoddle, T. D. Center, C. E. Ruvz, B. J. Coronado, and A. J. Manuel. 2007. Control de Plagas y Malezas por Enemigos Naturales (No. 632.96 V33). US Department of Agriculture, US Forest Service, Forest Health Technology Enterprise Team.

Villanueva, R. T., M. Brewer, M. O. Way, S. Biles, D. Sekula, E. Bynum, J. Swart, C. Crumley, A. Knutson, P. Porter, R. Parker, G. Odvody, C. Allen, D. Ragsdale, W. Rooney, G. Peterson, D. Kerns, T. Royer, and S. Armstrong. 2014. Sugarcane aphid: a new pest of sorghum. Texas A&M Agrilife Extension Ento-035. Weslaco, TX.

Weirauch, C., C. Alvarez, and G. Zhang. 2012. *Zelus renardii* and *Z. tetracanthus* (Hemiptera: Reduviidae): biological attributes and the potential for dispersal in two assassin bug species. Fla. Entomol. 95: 641-649.

West-Eberhard, M. J., J. M. Carpenter, and P. E. Hanson. 2006. Familia Vespidae, pp. 617-644. *In* P. E. Hanson and I. D. Gauld [eds.], Hymenoptera de la Region Tropical. Vol. 77. Memoirs of the American Entomological Institute, Gainesville.

Xavier, V. M., M. C. Picanço, M. Chediak, P. A. S. Júnior, R. S. Ramos, and J. C. Martins. 2015. Acute toxicity and sublethal effects of botanical insecticides to honey bees. J. Insect Sci. 15: 137.

Yang, D., and T. Saigusa. 2005. Diptera: Dolichopodidae, pp. 740-765. *In* X. K. Yang [ed.], Insect Fauna of Middle-West Qinling Range and South Mountains of Gansu Province. Science Press, Beijing.

Relationships among *Melanaphis sacchari*[1], its Predator Insect Communities, and Weather Conditions in Sorghum Crops at Colima, Mexico

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Abstract. Principal component analysis was used to search for relationships among communities of predators (Coccinellidae, Syrphidae, and Chrysopidae), aphids, and environmental conditions in crops of sorghum (*Sorghum bicolor* (L.) Moench) in 2016, 2017, and 2018. The aphid species collected were *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch), and *Melanaphis sacchari* (Zehntner). *M. sacchari* was most abundant, with abrupt decrease in abundance over the years. Twelve species of Coccinellidae predators, two Chrysopidae, and 13 of Syrphidae were identified. Principal component analysis revealed a stronger relationship between *M. sacchari* and predators than weather. The relationship among *Hippodamia convergens* Guérin-Méneville, *Scymnus* (*Pullus*) *dozieri* Gordon, and *M. sacchari* was excellent.

Resumen. Se realizó un análisis de componentes principales con la finalidad de buscar relaciones entre las comunidades de insectos depredadores (Coccinellidae, Syrphidae, y Chrysopidae), áfidos y condiciones ambientales en cultivos de sorgo. Las recolectas se llevaron a cabo en tres periodos de muestreo, durante los años 2016, 2017, y 2018. Tres especies de áfidos fueron colectadas *Schizaphis graminum* (Rondani), *Rhopalosiphum maidis* (Fitch), y *Melanaphis sacchari* (Zehntner), siendo esta última la más abundante, con un notable decremento a través de los años; asimismo, se identificaron 12 especies de depredadores de la familia Coccinellidae, dos de Chrysopidae, y 13 especies de Syrphidae. El análisis de componentes principales reveló mayor relación entre las especies de depredadores y *M. sacchari*, que con las condiciones ambientales. Se destaca la relación entre *Hippodamia convergens* Guérin-Méneville, *Scymnus* (*Pullus*) *dozieri* Gordon, y *M. sacchari*.

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Introduction

Since arrival of the sugarcane aphid, *Melanaphis sacchari* (Zehntner), to Mexico in 2013 (INIFAP 2015), several studies provided information and tools for its control. However, few publications dealt with relationships among predator insects, *M. sacchari* (or other aphid species), and weather variables in crops of sorghum (*Sorghum bicolor* (L.) Moench). Vázquez-Navarro et al. (2016) studied predators on a sorghum fodder crop infested with the pest, identifying the coccinellid *Hippodamia convergens* Guérin-Méneville as the dominant predator species. In the United States of America, Colares et al. (2015b), evaluated using native predators against *M. sacchari* and *Schizaphis graminum* (Rondani), an established insect pest species. They concluded that in most cereal crops, aphidophagous insects were pre-adapted to respond to, and feed on any aphid, whether newly arrived or already established. Aphidophagous insects fed on *M. sacchari* the same as on *S. graminum*. For that reason, Colares et al. suggested that aphidophagous insects, despite often being underestimated, could control invasive aphids. This would explain why effective biological control of aphids typically evolves over time, even when classical programs are unable to achieve the same amount of control.

Melanaphis sacchari is capable of rapidly increasing in abundance in sorghum (Colares et al. 2015a). For example, in a nonmanaged crop, 50 initial individuals can increase to more than 10,000 aphids per leaf (Bowling et al. 2016). A severe infestation is devastating and substantially reduces sorghum yield and grain quality, causing chlorosis, necrosis, and even plant death (Bayoumy et al. 2015, Bowling et al. 2016). Losses in nontreated fields can be 100% when infested during early plant development. Insecticide usually is applied to suppress aphids in sorghum, but it is expensive (Elliott et al. 2017) and has severe environmental implications as well as aphids developing resistance.

Non-chemical management strategies are needed to reduce management challenges and costs while limiting resistance to insecticide. Using natural enemies in integrated pest management (IPM) has potential to reduce use of insecticide and costs of suppressing aphids (Zhang and Swinton 2012). Effect of biological control on *M. sacchari* in sorghum fields has not been quantified and might be underestimated (Colares et al. 2015a). Singh et al. (2004) reported 47 species of pathogens, parasitoids, and predators of *M. sacchari* in different countries. Among natural enemies, coccinellids, chrysopids, and syrphids were observed most often and probably kill many *M. sacchari*. The objective of this study was to analyze the relationship of crop phenology, environmental factors, and *M. sacchari* and other aphids with Coccinellidae, Chrysopidae, and Syrphidae aphid predators in sorghum crops as a basis for implementation of biological control in integrated pest management.

Materials and Methods

The study was in sorghum crops at two locations of the municipality of Tecoman in the State of Colima, Mexico. No insecticide was applied, with the intention of not interfering with insects. The town of Tecuanillo at 18°51'31" N, 103°52'55" W, with an elevation of 17 m above sea level had 4 ha of *S. bicolor* (SG Pichon 70, fodder variety) planted manually at a depth of 1.5 cm, with 20 cm between plants and 90 cm between rows. The experimental field of the Faculty of Biological and Agricultural Sciences, at the University of Colima, Campus Tecoman was 40 km

from the Colima-Manzanillo highway, at 18° 57' N, 103° 53' W and an elevation of 50 m above sea level. The experimental area was 3,052 m² planted the same as at Tecuanillo.

Each of three sampling periods was a complete sorghum crop cycle. In each period, samples were collected each week with a sweep net covering 180 linear meters per collection. The first sampling period was at Tecoman with 21 collections from 18 February to 6 July 2016. The second period was at the experimental field with 15 collections from 28 September 2017 to 4 January 2018. The third period was at the experimental field with 17 collections from 15 March to 13 July 2018.

Insects collected were killed and preserved in 70% alcohol. Coccinellidae, Syrphidae, and Chrysopidae individuals were mounted on entomological pins and labelled. Aphids were stored in 70% alcohol in 10-ml glass containers labelled using the corresponding data.

Coccinellids were identified based on literature by Gordon (1985, 2000), Romero-Nápoles (1988), and Vandenberg (1992). Chrysopidae specimens were identified based on Brooks (1994) and De Freitas et al. (2009), while Syrphidae were identified using keys by Miranda et al. (2013). Aphididae species were identified using Remaudière and Remaudière (1997) and Peña-Martínez et al. (2016). All specimens were deposited at the Colección de Insectos Entomófagos del Centro Nacional de Referencia de Control Biológico (CIE-CNRCB) del Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA); (Entomophagous Insect Collection of the National Reference Center for Biological Control of the National Service for Agrifood Health, Safety and Quality) at Tecoman, Colima, Mexico.

Correlation coefficient analysis helped elucidate relationships between predators and aphid species. In addition, two matrices of factors were constructed for multivariate analysis. The first contained abundant data of aphid abundance, phenological state of the crop each week, and weekly average of environmental conditions (14 factors total) (Table 1). The second matrix contained data of predator abundance (sampled weekly) (27 factors total). Both matrices had 53 samples, each corresponding to a week.

Detrended correspondence analysis was used to learn the length of gradient. Principal component analysis was used to estimate relationship among environmental conditions (from the National Institute for Forestry, Agricultural and Livestock Research) (INIFAP 2020) and abundance of predators and aphids (weekly). PC-ORD V 5.31 was used for both analyses (McCune and Mefford 2006).

Results

In total, 30,526 specimens of the family Aphididae were collected during the three sampling periods (Table 2). Three aphid species were *Rhopalosiphum maidis* (Fitch), *M. sacchari*, and *S. graminum*. *M. sacchari* was most abundant (98% of individuals). Predator insects were 2,088 specimens -- 1,342 Coccinellidae adults of 12 species, 622 individuals in two species of Chrysopidae, and 124 specimens of 13 species of Syrphidae.

During the first sampling period, 26 predatory insect species were identified of the 1,573 specimens collected; 11 species were identified from the 212 specimens in the second period, and 11 species were identified of the 303 specimens in the third period (Table 2). Greater abundance in the third compared to the second period was mainly because of increased abundance of *Chrysoperla externa* (Hagen). In the first period, 27,254 individuals of *M. sacchari* and 650 of *S. graminum* were collected,

while in the second period, 2,122 specimens were collected, all of them *M. sacchari*. During the third period, 198 *M. sacchari* and 102 *R. maidis* were collected.

Correlation coefficient analysis showed significant relationship among *M. sacchari* and mostly *Cycloneda sanguinea sanguinea* L., *H. convergens*, and *Sycmnus* (*Pullus*) *dozieri* Gordo predators (Table 3). Positive relationship was observed among predators *C. sanguinea sanguinea* with *H. convergens*, *S*. (*P*.) *dozieri* with *H. convergens*, *Diomus roseicollis* (Mulsant) with *Coleomegilla maculata lengi* Timberlake, *S*. (*P*.) *dozieri* with *D*. *roseicollis*, *Ceraeochrysa valida* (Banks) with *Sthetorus* sp., *C*. *externa* with *C*. *valida*, and *Sthetorus* sp. with *S*. (*P*.) *dozieri*.

Detrended correspondence analysis showed gradient length was 2.15, so principal component analysis was justified. The first axis of the principal component analysis explained 25.83% of total variation, the second explained 12.60%, and the third 8.53%. Total accumulated variation explained by the three axes was 46.97% (Table 4).

The species correlated with the three components (bold) were *C. sanguinea sanguinea*, *H. convergens*, *S.* (*P.*) *dozieri, C. valida*, and *Allograpta exotica.*

Table 1. Matrix Factors Studied for Predators and Aphids in Sorghum at Colima, Mexico

First matrix		Second matrix	
Acronym		Acronym	
Melsac	*Melanaphis sacchari*	Cycsan	*Cycloneda sanguinea sanguinea*
Shigra	*Schizaphis graminum*	Hipcon	*Hippodamia convergens*
Rhomai	*Rhopalosiphum maidis*	Colmac	*Coleomegilla maculata lengi*
Prec	Precipitation (mm)	Ollvni	*Olla v-nigrum*
MaxT	Maximum temperature (°C)	Dioroc	*Diomus roseicollis*
MinT	Minimum temperature (°C)	Exochi	*Exochomus childreni guexi*
AvT	Average temperature (°C)	Scydoc	*Scymnus* (*Pullus*) *dozieri*
MaxWS	Maximum wind speed (km/h)	Nepsp1	*Nephus* sp. 1
WS	Wind speed (km/h)	Nepsp2	*Nephus* sp. 2
GRad	Global radiation (w/m2)	Stesp	*Sthetorus* sp.
RH	Relative humidity (%)	Hypsp	*Hyperaspis* sp.
ET	Reference evapotranspiration (mm)	Scyloe	*Scymnus* (*Pullus*) *loewii*
PE	Potential evaporation (mm)	Cerval	*Ceraeochrysa valida*
PC	Phenology of crop	Chrext	*Chrysoperla externa*
		Allexo	*Allograpta exotica*
		Allobl	*Allograpta obliqua*
		Ocyant	*Ocyptamus antiphates*
		Ocydim	*Ocyptamus dimidiatus*
		Ocygas	*Ocyptamus gastrostactus*
		Psecla	*Pseudodorus clavatus*
		Toxdis	*Toxomerus dispar*
		Toxmac	*Toxomerus maculatus* Macquart
		Toxmar	*Toxomerus marginatus*(Say)
		Toxpol	*Toxomerus politus*
		Toxpue	*Toxomerus puellus* (Hull)
		Toxpul	*Toxomerus pulchellus* (Macquart)
		Toxwat	*Toxomerus watsoni* (Curran)

Table 2. Abundance of Species of Aphids and Predators Sampled in 2016, 2017, and 2018 in Sorghum at Colima, Mexico

Species	Order and Family	2016	2017	2018
Melanaphis sacchari	Hemiptera: Aphididae	27,454	2,122	198
Rhopalosiphum maidis	Hemiptera: Aphididae	0	0	102
Schizaphis graminum	Hemiptera: Aphididae	650	0	0
Coleomegilla maculata lengi	Coleoptera: Coccinellidae	41	41	44
Cycloneda sanguinea sanguinea	Coleoptera: Coccinellidae	342	52	4
Diomus roseicollis	Coleoptera: Coccinellidae	132	39	27
Exochomus childreni guexi	Coleoptera: Coccinellidae	4	0	0
Hyperaspis sp.	Coleoptera: Coccinellidae	2	0	0
Hippodamia convergens	Coleoptera: Coccinellidae	159	22	11
Nephus sp. 1	Coleoptera: Coccinellidae	1	0	0
Nephus sp. 2	Coleoptera: Coccinellidae	1	0	0
Olla v-nigrum	Coleoptera: Coccinellidae	2	0	0
Scymnus (*Pullus*) *dozieri*	Coleoptera: Coccinellidae	353	28	8
Scymnus (*Pullus*) *loewii*	Coleoptera: Coccinellidae	4	0	0
Stethorus sp.	Coleoptera: Coccinellidae	17	7	1
Ceraeochrysa valida	Neuroptera: Chrysopidae	80	0	0
Chrysoperla externa	Neuroptera: Chrysopidae	338	12	192
Allograpta exotica	Diptera: Syrphidae	51	0	1
Allograpta obliqua	Diptera: Syrphidae	3	0	0
Ocyptamus antiphates	Diptera: Syrphidae	1	0	0
Ocyptamus dimidiatus	Diptera: Syrphidae	3	0	0
Ocyptamus gastrostactus	Diptera: Syrphidae	1	0	0
Pseudodorus clavatus	Diptera: Syrphidae	9	0	0
Toxomerus dispar	Diptera: Syrphidae	18	0	6
Toxomerus maculatus	Diptera: Syrphidae	3	1	0
Toxomerus marginatus	Diptera: Syrphidae	1	0	0
Toxomerus politus	Diptera: Syrphidae	0	6	8
Toxomerus puellus	Diptera: Syrphidae	1	0	0
Toxomerus pulchellus	Diptera: Syrphidae	4	1	0
Toxomerus watsoni	Diptera: Syrphidae	2	3	1

Table 3. Correlation Coefficient Values between the Most Abundant Predator Species and *Melanaphis sacchari*

	Cycsan	Hipcon	Colmac	Dioroc	Scydoc	Stesp	Cerval	Chrext	Allexo	Melsac
Cycsan	1.000									
Hipcon	**0.868**	1.000								
Colmac	0.429	0.417	1.000							
Dioroc	0.437	0.219	**0.527**	1.000						
Scydoc	**0.745**	**0.906**	0.331	**0.592**	1.000					
Stesp	0.339	0.141	0.168	0.469	**0.884**	1.000				
Cerval	**0.645**	**0.450**	0.158	**0.663**	0.301	**0.545**	1.000			
Chrext	0.432	0.316	0.326	0.372	0.222	0.412	**0.542**	1.000		
Allexo	**0.609**	0.430	0.245	**0.684**	0.293	0.392	**0.715**	**0.549**	1.000	
Melsac	**0.871**	**0.790**	0.450	0.150	**0.719**	0.144	0.386	0.348	0.353	1.000

Abbreviations in Table defined in Table 1.

Table 4. Principal Component Analysis. Percentage of accumulated explained variance and correlation coefficients of species and environmental variables with the components.

	Component 1	Component 2	Component 3
% Variance	25.83	12.60	8.53
% Accumulated variance	25.83	38.43	46.97
	Correlation	Correlation	Correlation
Cycsan	**0.893**	-0.131	0.161
Cheext	**0.611**	0.266	-0.058
Scyloe	**0.587**	-0.305	0.210
Stesp	0.451	0.285	0.087
Hipcon	**0.825**	-0.447	0.007
Ocygas	0.373	**-0.511**	-0.159
Toxdis	**0.547**	0.447	-0.230
Psecla	0.424	**-0.565**	-0.134
Dioroc	**0.557**	**0.557**	-0.083
Cerval	**0.791**	0.454	0.045
Allexo	**0.788**	0.377	-0.295
Allobl	0.421	**0.514**	0.265
Exochi	**0.560**	**-0.586**	0.332
Scydoc	**0.709**	**-0.630**	0.028
Colmac	0.370	-0.162	0.496
Hypsp	0.448	0.337	**0.668**
Ocyant	**0.630**	0.043	-0.391
Ocydim	0.575	-0.113	-0.482
Ollvni	0.333	0.346	**0.592**
ET	0.409	0.096	-0.029
Grad	0.342	0.171	-0.083
Melsac	**0.683**	-0.356	-0.007
MinT	-0.221	-0.309	0.083
Prec	-0.326	0.013	0.030
MaxT	0.007	-0.060	0.017
AvT	-0.133	-0.297	0.089
MaxWS	0.006	0.001	0.005
WS	0.002	0.053	-0.036
RH	-0.192	-0.131	-0.049
PE	0.287	0.197	-0.049
Shigra	-0.109	0.010	-0.026
Rhomai	-0.129	0.078	-0.040
PC	0.090	-0.225	0.040

Statistically significant are in bold. Acronyms of factors were defined in Table 1.

(Widemann) (for the first component); *S.* (*P.*) *dozieri* and *Exochomus childreni guexi* LeConte with the second component; and *Olla v-nigrum* (Mulsant) and *Hyperaspis* sp. correlated to the third component (Table 4). Most weather conditions except global radiation and reference evapotranspiration were not significantly correlated with the components. Abundance of *M. sacchari* was the factor from the first matrix with the greatest weighting on the positive side of Component 1 (Fig. 1). Principal component analysis formed three groups of predators. Group 1 had *H. convergens*,

S. (*P*.) *dozieri*, *Sycmnus* (*Pullus*) *loewii* Mulsant, *Ocyptamus dimidiatus* (Fabricius), *Pseudodorus clavatus* (Fabricius), *E. childreni guexi*, and *Toxomerus marginatus* (Say) which are related to *M. sacchari*. Group 2 formed by *Toxomerus politus* (Say), *C. sanguinea sanguinea*, and *Ocyptamus antiphates* (Walker) was related to reference evapotranspiration, and Group 3 with *C. externa*, *D. roseicollis*, *Sthetorus* sp., *Toxomerus dispar* (Fabricius), *Nephus* sp., *C. valida*, *A. exotica*, *Allograpta obliqua* (Say) *Hyperaspis* sp., and *O. v-nigrum* was related to global radiation.

All predator species except *Nephus* sp. 1 and *T. politus* were in the positive portion of Axis 1 (Fig. 1). In contrast, *H. convergens*, *C. sanguinea sanguinea, S.* (*P*.) *loewii*, and *S.* (*P.*) *dozieri* were predator species with greatest relationship to *M. sacchari*, as shown by close proximity.

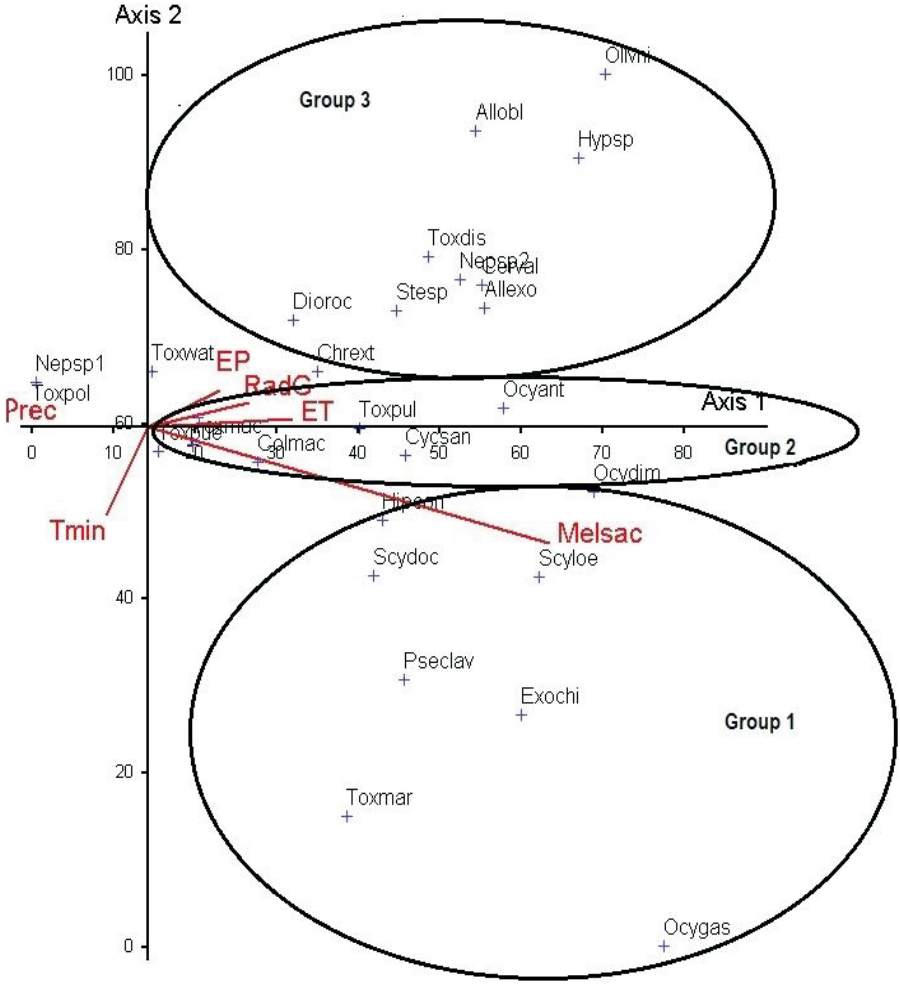

Fig. 1. Principal component analysis diagram with predator species (+) and environmental variables (-).

Discussion

There is information on natural enemies that attack *M. sacchari* in different parts of the world (Singh et al. 2004; Colares et al. 2015a,b) including Mexico (Cortez-Mondaca et al. 2016; Rodríguez-Vélez 2016, 2019; Rodríguez-del-Bosque et al. 2018; Villa-Ayala et al. 2020). According to correlation coefficient analysis, *C. sanguinea sanguinea*, *H. convergens*, and *S.* (*P.*) *dozieri* were species most correlated with *M. sacchari* in sorghum crops at Colima, Mexico (Table 1). The same results were found with the first principal component; the chrysopids *C. valida* and *C. externa* and the syrphid *A. exotica* also were moderately correlated (Table 4).

Coccinellid adults killed most *M. sacchari* in a sorghum monoculture with closed canopy at Kansas State Agricultural Research Station-Hays (Colares et al. 2015b). Syrphid larvae killed most in open habitats with adjacent trees and flowers. Chrysopids and aphelinids were secondary in killing *M. sacchari* at both habitats. Percentages of Coccinellidae, Chrysopidae, and Syrphidae were similar.

Only Syrphidae adults were collected, in contrast to the study by Colares et al. (2015b). Percentages of adults collected by Colares et al. (7.0%) and in this study (5.9%) were similar and the least of the three families. However, predatory activity of the syrphids might be underestimated by the Colima study where only adults were analyzed; based on the Kansas study, adults collected in the field compared with immature stages of Syrphidae were only 0.27% of the total syrphids collected. Larvae were 40% of the total number of flower flies, an important percentage considering larvae of predaceous syrphids feed on aphids and other soft-bodied insects and are important in suppressing phytophagous insects (Bugg et al. 2008).

Of the adult insects collected in both studies, Coccinellidae was most abundant [63.1% (Colares et al. 2015b) and 64.2% (this study)]. *C. sanguinea sanguinea, H. convergens*, and *S.* (*P.*) *dozieri* were important in the State of Colima. *C. sanguinea sanguinea* and *H. convergens* already were reported feeding on the sugarcane aphid in sorghum crops in Dutch Guiana, Puerto Rico, and USA (Florida) (Singh et al. 2004). In Mexico, *C. sanguinea sanguinea* preyed on the sugarcane aphid at Colima, Michoacán, Nuevo León (Rodríguez-Vélez et al. 2019), and Tamaulipas (Rodríguez-Vélez et al. 2016, 2019). *C. sanguinea* without mentioning the subspecies was recorded at Chiapas (Salas-Marina et al. 2017), Guanajuato (Marín-Jarillo 2017), and Nayarit (Rodríguez-Palomera et al. 2016).

H. convergens, the species second most related to sugarcane aphid in this study (Table 3), killed most in a sorghum monoculture with closed canopy at Kansas and preyed on *M. sacchari* in the USA (Singh 2004, Colares et al. 2015b). In Mexico, *H. convergens* preyed on the sugarcane aphid at Chiapas (Salas-Marina et al. 2017), Guanajuato (Marín-Jarillo 2017), Nayarit (Rodríguez-Palomera et al. 2016), Durango (Vázquez-Navarro et al. 2016), Colima, Michoacán, and Nuevo León (Rodríguez-Vélez et al. 2019), and Tamaulipas (Rodríguez-Vélez et al. 2016, 2019). Delgado-Ramírez et al. (2019) successfully evaluated predation by *H. convergens* feeding on *M. sacchari*, supporting results of this research suggesting *H. convergens* was a dominant predator species associated with *M. sacchari* (Colares et al. 2015a,b).

Scymnus (*Pullus*) *dozieri*, the species third most closely related to the sugarcane aphid, preyed on *M. sacchari* at Guanajuato (Marín-Jarillo 2017) and Colima (Rodríguez-Vélez et al. 2019). Although the authors did not do correlation analysis among *M. sacchari* and coccinellids collected, the predator-prey relationship among the species was registered in sorghum crops, thus supporting results of the importance of Coccinellidae as a predator of *M. sacchari*.

Adult Chrysopidae, the second most abundant family, were 29.7% of total abundance (of the three families) in the Kansas study (Colares et al. 2015b); the same percentage was at Colima. At Kansas, chrysopids and aphelinids killed the second most amount of *M. sacchari.* Based on results of the first principal component at Colima, *C. valida* (0.791), *C. externa* (0.611), and *M. sacchari* (0.683) were moderately correlated. In Mexico, eight species of chrysopids were recorded in sorghum crops; at Tamaulipas, *C. carnea s. l*. (Rodríguez-del-Bosque et al. 2018), with *C. valida* and *C. externa* at Colima. At Sinaloa, eight species were recorded at 10 locations (Cortez-Mondaca et al. 2016); four to seven species were recorded at each of the locations. At Kansas, only *C. carnea* was recorded feeding on *M. sacchari*; the percentage of its abundance was equal to the abundance of *C. valida* and *C. externa* at Colima, Mexico. The information supports results of research by Colares et al. (2015b) and this study in which chrysopids were important natural enemies, but did not kill the most sugarcane aphids.

Although predator species are correlated with *M. sacchari* as a main food source, others are only moderately correlated. All form an important guild of predators that control sugarcane aphids. Regardless of species at each locality, Coccinellidae, Chrysopidae, and Syrphidae were most correlated with *M. sacchari* in sorghum crops.

At Colima, abundance of *M. sacchari* in the third period was less than 1% compared with the first period (Table 4). Colares et al. (2015a) described how infestations by exotic aphids changed through three stages: 1) epidemic stage, in which large numbers of aphids devastate a wide geographical region of agricultural production; 2) attenuation stage, in which infestations gradually decrease to more limited periodic and intermittent outbreaks in a geographical range; and 3) endemic stage, in which control by a complex of resident natural enemies is widely established and infestation by the pest insect that caused economic loss becomes rare and generally limited to situations where natural enemies are interrupted by weather or cultural practices.

Despite decreases of *M. sacchari* in recent years (as shown by the present study), it does not necessarily mean the sugarcane aphid reached the endemic stage at Colima. However, conservation biological control should be a priority in integrated pest management to control sugarcane aphids on sorghum in Mexico.

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References Cited

Bayoumy, M. H., R. Perumal, and J. P. Michaud. 2015. Comparative life histories of greenbugs and sugarcane aphids (Hemiptera: Aphididae) coinfesting susceptible and resistant sorghums. J. Econ. Entomol. 109: 385-391.

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliott, D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane aphid

(Hemiptera: Aphididae): a new pest on sorghum in North America. J. Integr. Pest. Manag. 7: 1-13

Brooks, S. J. 1994. A taxonomic review of the common green lacewing genus *Chrysoperla* (Neuroptera: Chrysopidae). Bull. Br. Nat. Hist. 6: 137-210.

Bugg, R. L., R. G. Colfer, W. E. Chaney, E. A. Smith, and J. Cannon. 2008. Flower flies (Syrphidae) and other biological control agents for aphids in vegetable crops. UCANR publication 8285.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015a. Indigenous aphid predators show high levels of preadaptation to a novel prey, *Melanaphis sacchari* (Hemiptera: Aphididae). J. Econ. Entomol. 108: 2546-2555.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015b. Recruitment of aphidophagous arthropods to sorghum plants infested with *Melanaphis sacchari* and *Schizaphis graminum* (Hemiptera: Aphididae). Biol. Control 90: 16-24.

Cortez-Mondaca, E., M. López-Buitimea, J. López-Arroyo, F. J. Orduño-Cota, and G. Herrera-Rodríguez. 2016. Especies de Chrysopidae asociadas al pulgón del sorgo en el Norte de Sinaloa, México. Southwest. Entomol. 41: 541-546.

De Freitas, S., N. D. Penny, and P. A. Adams. 2009. A revision of the New World genus *Ceraeochrysa* (Neuroptera: Chrysopidae). Proc. Calif. Acad. Sci. 60: 503-610.

Delgado-Ramírez, C. S., M. D. Salas-Araiza, O. A. Martínez-Jaime, R. Guzmán-Mendoza, and S. Flores-Mejia. 2019. Predation capability of *Hippodamia convergens* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) feeding of *Melanaphis sacchari* (Hemiptera: Aphididae). Fla. Entomol. 102: 24-28.

Elliott, N., M. Brewer, N. Seiter, T. Royer, R. Bowling, G. Backoulou, J. Gordy, K. Giles, J. Lindenmayer, B. McCornack, and D. Kernsz. 2017. Sugarcane aphid spatial distribution in grain sorghum fields. Southwest. Entomol. 42: 27-36.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. N. Y. Entomol. Soc. 93: 1-912.

Gordon, R. D. 2000. South American Coccinellidae (Coleoptera) Part VII: A systematic revision of South American *Scymnus* (*Pullus*) Mulsant. (Scymninae: Scymnini). Frustula. Entomol. 23: 56-108.

INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias). 2015. Pulgón amarillo: nueva plaga de sorgo en México. http://www.inifapcirne.gobmx/Eventos/2015/BE%20Pulgon%20amarillo,nueva%20plaga%20en%20Mexico.pdf Accessed 19 May 2020.

INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias). 2020. Red de Estaciones de INIFAP. https://clima.inifap.gob.mx/lnmysr/Estaciones/ConsultaDiarios15Min?Estado=6&Estacion=3634 19 May 2020.

Marín-Jarillo, A. 2017**.** Enemigos naturales asociados al pulgón amarillo del sorgo *Melanaphis sacchari* (Zehntner) en la región del Bajío. http://www.pulgonamarillogto.com/exteduc/capacitacion/universidades/2017/4_Enemigos_naturales_PAS.pdf Accessed 11 September 2020.

McCune, B., and M. J. Mefford. 2006. PC-Ord. Multivariate analysis of ecological data. Version 5.31. MjM Software, Gleneden Beach, OR.

Miranda, G. F. G., A. D. Young, M. M. Locke, S. A. Marshall, J. H. Skevington, and F. C. Thompson. 2013. Key to the genera of Nearctic Syrphidae. Can. J. Arthropod Identif. 23**:** 1-351.

Peña-Martínez, R., A. L. Muñoz-Viveros, R. Bujanos-Muñiz, J. Luévano-Borroel, F. Tamayo-Mejía, and E. Cortez-Mondaca. 2016. Formas sexuales del complejo pulgón amarillo del sorgo, *Melanaphis sacchari*/*sorghi* en México. Southwest. Entomol. 41: 127-132.

Remaudière, G., and M. Remaudière. 1997. Catalogue des Aphididae du Monde (Homoptera: Aphidoidea). Institut National de la Recherche Agronomique, Paris, France.

Romero-Nápoles, J. 1988. Características morfológicas útiles para diferenciar *Hippodamia convergens* Guerin de *H. koebelei* Timberlake (Coleoptera: Coccinellidae) en el área de Chapingo, México. Folia Entomol. Mex. 76:45-54.

Rodríguez-del-Bosque, L. A., B. Rodríguez-Vélez, M. A. Sarmiento-Cordero, and H. C. Arredondo-Bernal. 2018. Natural enemies of *Melanaphis sacchari* on grain sorghum in northeastern Mexico. Southwest. Entomol. 43: 277-279.

Rodríguez-Palomera, M., J. Cambero-Campos, G. Luna-Esquivel, O. Estrada-Virgen, N. De Dios Ávila, and A. C. Cambero. 2016. Coccinélidos depredadores del pulgón amarillo del sorgo *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) en Nayarit, México. Entomol. Mex. 3: 361-364.

Rodríguez-Vélez, J. M., B. Rodríguez-Vélez, M. A. Sarmiento-Cordero, M. Palomares-Pérez, and H. C. Arredondo-Bernal. 2016. Species of Coccinellidae (Coleoptera: Cucujoidea) associated with *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) in Tamaulipas, Mexico. Entomol. News 126: 97-105.

Rodríguez-Vélez, J. M., A. Gallou, M. A. Najar-Pacheco, C. A. Uribe-Mú, F. M. Huerta-Martínez, A. Contreras-Ramos, and H. C. Arredondo-Bernal. 2019. Morphological and COI identification of predatory Coccinellidae (Coleoptera) of *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) of Mexico. Coleopt. Bull. 73: 1-9.

Salas-Marina, M. A., V. Hernández-García, C. Ríos-Velasco, N. Lule-Chávez, J. A. Mauricio-Castillo, and M. H. Chávez-Hernández. 2017. *Melanaphis sacchari*, a new pest of *Sorghum halepense* at Chiapas, México, and its predators. Southwest. Entomol. 42: 401-404.

Singh, B. U., P. G. Padmaja, and N. Seetharama. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop. Prot. 23: 739-755.

Vandenberg, N. J. 1992. Revision of the New World lady beetles of the genus *Olla* and description of a new allied genus (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 85: 370-392.

Vázquez-Navarro, J. M., J. C. Carrillo-Aguilera, and B. A. Cisneros-Flores. 2016. Estudio poblacional en un cultivar de sorgo forrajero infestado con pulgón amarillo del sorgo *Melanaphis sacchari* (Zehntner, 1897) (Hemiptera: Aphididae) en la comarca lagunera. Entomología Mexicana 3: 395-400.

Villa-Ayala, P., G. Sánchez-Rivera, B. Rodríguez-Vélez, and F. Castrejón-Ayala. 2020. Parasitoides e hiperparasitoides asociados al pulgón amarillo del sorgo, *Melanaphis sacchari* (Zehntner), en el Estado de Morelos, México. Southwest. Entomol. 45: 563-566.

Zhang, W., and S. M. Swinton. 2012. Optimal control of soybean aphid in the presence of natural enemies and the implied value of their ecosystem services. J. Environ. Manage. 96: 7-16.

Increase in Importance of *Aphelinus nigritus* (Howard) on Winter Crops in Oklahoma Coincides with Invasion of Sugarcane Aphid on Sorghum in Oklahoma

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Abstract. Aphid parasitoids on the Southern Great Plains provide important pest suppression services in summer and winter crops. The primary parasitoids *Lysiphlebus testaceipes* (Cresson), *Aphelinus nigritus* (Howard), and *Diaretiella rapae* (McIntosh) use multiple aphid host species on multiple crops in agricultural landscapes and historically, *L. testaceipes* has been the most abundant and arguably most important. However, after the sugarcane aphid, *Melanaphis sacchari* (Zehntner), infested sorghum (*Sorghum bicolor* (L.) Moench) on the Southern Plains, *A. nigritus* increased in abundance and impact on this summer crop. We hypothesized that *A. nigritus* also would be more abundant in fields of winter wheat (*Triticum aestivum* L.) and winter canola (*Brassica napus* L.) in the region following the sugarcane aphid. From 2016-2019, we documented aphid parasitoid abundance and percentage of parasitism in Oklahoma winter crops during late fall and early spring using a standardized sentinel aphid deployment retrieval and rearing approach. Unexpectedly, *A. nigritus* was relatively abundant during Fall 2016, parasitizing as many as 5.4% of sentinel aphids in winter wheat fields which was more than the typically dominant parasitoid *L. testaceipes.* However, percentage of parasitism by *A. nigritus* decreased during the study. Our results suggested that abundance of *A. nigritus* mirrored those of sugarcane aphids in the region, and the temporary presence of the parasitoid in winter crops reflected dynamics at a landscape level.

Introduction

Winter wheat (*Triticum aestivum* L.) and winter canola (*Brassica napus* L.) have been grown as rotational crops in Oklahoma since 2001, primarily to manage grassy weeds through selective herbicide use (DeVuyst et al. 2009, Boyles et al. 2012, Bushong et al. 2012, Vitale et al. 2014, Royer and Giles 2017a). Wheat and canola comprise a large percentage of the actively growing winter vegetation during autumn through spring, and fields of both crops are sporadically infested with aphid herbivores that can reduce plant growth and yield. The primary aphid pests on winter wheat are greenbug, *Schizaphis graminum* (Rondani), and birdcherry-oat aphid, *Rhopalosiphum padi* L., whereas canola fields are regularly colonized by turnip aphid,

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Lipaphis pseudobrassicae Davis, and less commonly colonized by green peach aphid, *Myzus persicae* Sulzer, and cabbage aphid, *Brevicoryne brassicae* L. (Jessie et al. 2015; Royer and Giles 2015, 2017b; Giles et al. 2017).

Aphid management programs on the Southern Plains have been developed for both crops, and conservation of parasitoids and judicious use of insecticide are foundations of the programs (Giles et al. 2003, 2008, 2017; Royer et al. 2005, 2015ab; Hunger et al. 2017, Royer and Giles 2017a, Vitale et al. 2020). On winter wheat, *Lysiphlebus testaceipes* (Cresson) is the dominant aphid parasitoid on the Southern Plains, representing >90% of all primary parasitoids documented. *Diaeretiella rapae* (McIntosh) is regularly observed at low levels in winter wheat fields, while *Aphelinus nigritus* (Howard) is rarely found during the fall and early spring months (Kring and Gilstrap 1983, Giles et al. 2003, Jessie 2017a, Elliott et al. 2019). Only a few studies documented parasitism in winter canola fields on the Southern Plains, and the studies demonstrated that *D. rapae* was the only parasitoid to emerge from winter canola aphids (Elliott et al. 2014, Jessie 2017b).

Aphid parasitoids and parasitism rates are affected by temporal and spatial landscape diversity in agricultural landscapes (Theis et al. 2005, Jonsson et al. 2015, Haan et al. 2020), and both abundance of and parasitism rates by *L. testaceipes* have been enhanced on winter wheat by increased summer crops in the landscape (Elliott et al. 2018). In this study, sorghum was hypothesized to act as a green bridge or relay crop for dispersing *L. testaceipes* on the Southern Plains. Indeed, Gilstrap et al. (1984) showed sorghum supported *L. testaceipes* and *A. nigritus* during the summer. Relatively recent research by Elliott et al. (2014, 2018) was done before invasion by the sugarcane aphid (*Melanaphis sacchari* Zehntner) onto the Southern Plains (Bowling et al. 2016), and the subsequent unexpected increase in abundance and impact of *A. nigritus* on sorghum (*Sorghum bicolor* (L.) Moench) (Maxon et al. 2019).

Based on large recent populations of *A. nigritus* on sorghum (Maxon et al. 2019, N.C. Elliott unpublished data), documented landscape linkage between aphid parasitism on sorghum and winter crops (Elliott et al. 2018), and broad host range of Aphelinidae parasitoids (Brewer et al. 2008), we hypothesized the abundance and impact of *A. nigritus* should be greater in winter crops than previously reported (Kring and Gilstrap 1983, Giles et al. 2003, Jessie 2017a, Elliott et al. 2018) as a result of spillover from sorghum and perhaps non-cultivated Johnsongrass (*Sorghum halepense* (L.) Per.), that is also a host of sugarcane aphids throughout much of the Southern Plains.

Our objective was to document the aphid parasitoid assemblage and quantify aphid parasitism during fall and spring in winter wheat and winter canola fields during a 3-year period from 2016-2019. To ensure accurate representation of parasitism of aphids with varying abundance among fields, we used sentinel plants with aphids on both crops. The study is the first to quantify aphid parasitism on both winter crops produced on the Southern Plains since invasion by sugarcane aphids on sorghum.

Materials and Methods

Using a standardized sentinel aphid deployment, retrieval, and rearing approach (Brewer et al. 2008; Elliott et al. 2018, 2019), we documented the aphid parasitoid assemblage and quantified aphid parasitism in winter wheat and winter canola fields during the fall and spring from 2016 through 2019. In total, 23 wheat and 23 canola fields in northcentral Oklahoma (approximately 35.884 to 36.637°N,

97.588 to 98.281°W) were sampled during the 3-year period. Fields within a growing season (fall-to-spring) were the same, but new fields were selected for sampling each fall.

Using 15-cm-diameter pots with a 2:1 mixture of peat moss and fritted clay, wheat plants (10 seedlings, cv. 'Jagger') or canola plants (one rosette stag plant, cv. 'Wichita') were infested with birdcherry-oat aphids or turnip aphids, respectively; both aphids are common in Oklahoma (Royer and Giles 2015, 2017a). Individual pots with plants and parasitoid-free aphids were covered with a 14-cm diameter by 35-cm tall circular clear vented plastic cage and maintained in a greenhouse until aphid numbers reached about 388 per pot.

During late-October/early-November and mid-late March when parasitoids are most active (Giles et al. 2003), nine aphid-infested pots were placed 25 m apart in a T-shaped pattern in respective commercial wheat or canola fields for a period of 3 days. For each time-period deployment event, five to seven check sentinel pots for each crop were retained in the greenhouse to ensure aphid colonies were parasitoid free. Sentinel pots were caged as they were retrieved from fields, maintained for 7 days at 21 ± 2°C and 16:8 light:dark hours, then plants from each pot were cut and placed in a labeled emergence canister and maintained for another 7 days to allow emergence of adult parasitoids. Parasitoid adults from each emergence canister were counted and identified to species. The 3-day deployment (nine canisters per field) allowed for calculation of a functional measure of field parasitism during fall and spring (Elliott et al. 2018). In addition, although we did not quantify aphid abundance for the study, visual observations during deployment and retrieval of sentinel aphids indicated each field harbored aphid numbers well below economic thresholds which is consistent with the low aphid-parasitoid dynamics typically observed on the Southern Plains (Giles et al. 2003, Royer et al. 2015ab). We summarized simple descriptive statistics for the study including 1) total number of parasitoid adults of each species per field and season and 2) mean ± SE percentage of parasitism per field (based on approximately 388 aphids per canister) for each species of parasitoid for each field and season. Check plants were processed similarly to sentinel plants, and no adult parasitoids were observed in emergence canisters during the 3-year study.

Results and Discussion

Overall parasitoid numbers and percentage parasitism levels were relatively low in most fields and at levels among fields similar to previous winter parasitism studies (Kring and Gilstrap 1983; Rice and Wilde 1988; Giles et al. 2003; Elliott et al. 2014, 2019; Jessie 2017ab). No hyperparasitoids were detected, which is consistent with previous studies in Oklahoma (Giles et al 2003, Elliott et al. 2018). Although we hypothesized that *A. nigritus* migrate from sugarcane aphid-infested sorghum fields and Johnsongrass to wheat during the fall, we were surprised the species was the dominant parasitoid during fall 2016 and 2017 in wheat fields (Fig. 1). Percentage of parasitism by *A. nigritus* was relatively low, exceeding 5% in only one field during fall 2016, was much less during fall 2017, and absent after fall 2018 (Tables 1-3). The presence of *A. nigritus* on canola during fall 2016 (Fig. 2) was unexpected but not surprising considering its increased abundance in the region (Maxon et al. 2019) and the relatively broad host range of Aphelinidae (Brewer et al. 2008). Interestingly, colonization of winter crop fields by *A. nigritus* during the fall did not translate into greater numbers during spring 2017 and 2018 suggesting the species is not as well

adapted to numerically respond at winter temperatures as is *L. testaceipes* (Figs. 1, 2). Indeed, several studies demonstrated that *L. testaceipes* was well adapted to finding, attacking, and reproducing in aphids on winter crops (Jones et al. 2003, 2007; Hughes et al. 2010, 2011) and might be a superior competitor during cooler environmental conditions especially in winter wheat habitats (Jessie 2017a).

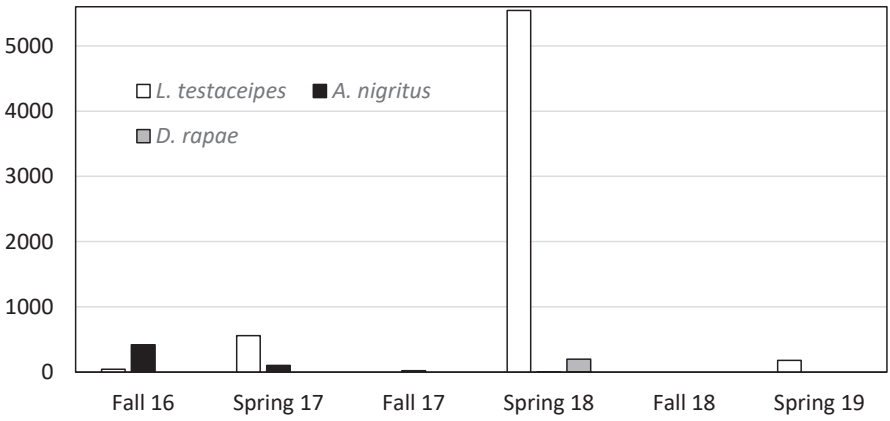

Fig. 1. Total adult parasitoids by species collected from sentinel aphids put in winter wheat fields in north-central Oklahoma from 2016-2019.

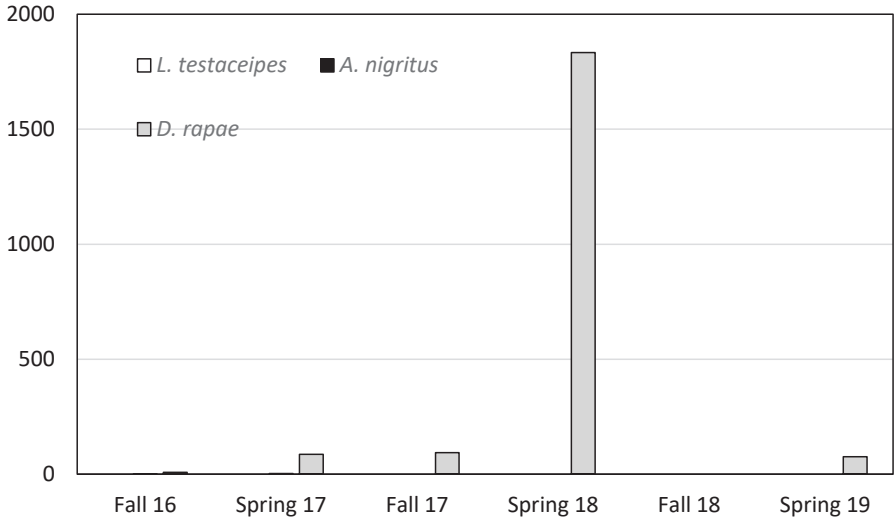

Fig. 2. Total adult parasitoids by species collected from sentinel aphids placed in winter canola fields in north central Oklahoma from 2016-2019.

Lysiphlebus testaceipes at low levels was in wheat fields each fall of the study, but emerged as expected (Giles et al. 2003) each spring as the dominant primary cereal aphid parasitoid collected from sentinel aphids (Fig. 1, Tables 1-3). Parasitism by *L. testaceipes* was relatively consistent among wheat fields each spring and reached as much as ~40%, and observed parasitism levels on low aphid densities regularly exceeded natural enemy thresholds used on the Southern Plains (Patrick and Boring 1990, Royer et al. 2015b). *A. nigritus* on wheat during fall 2016 and 2017 were very abundant but seemed to have little impact on spring populations and parasitism by *L. testaceipes* (Fig. 1, Tables 1, 2). As expected and observed in other studies, *L. testaceipes* was not found in turnip aphids placed in canola fields although adults often are observed in very large numbers in canola fields (Elliott et al. 2014, Jessie 2017a).

Diaeretiella rapae successfully attacked aphids on winter wheat and canola on the Southern Plains (Jessie 2017b) and emerged from sentinel aphids in both crops during the study (Fig. 2, Tables 1-3). Although it was outcompeted by *L. testaceipes* on wheat (Jessie 2017b), *D. rapae* parasitized brassica specialist aphids on winter canola and as observed in previous studies (Elliott et al. 2014, Jessie 2017a) was the dominant primary parasitoid on the crop (Fig. 2). *Diaeretiella rapae* at low levels was in canola fields each fall and emerged as expected each spring as the dominant primary aphid parasitoid from sentinel turnip aphids (Fig. 2, Tables 1-3). Parasitism by *D. rapae* was also relatively consistent among canola fields each spring, and reached as much as ~16%. The presence of *A. nigritus* on canola in 2016 and 2017 was minimal and seemed to have little impact on the presence and effectiveness of *D. rapae* (Fig. 2).

Table 1. Summary Statistics (Mean ± SE) for Percentage Parasitism by *Lysiphlebus testaceipes, Aphelinus nigritus,* and *Diaeretiella rapae* on Sentinel Aphids Put in Winter Wheat and Winter Canola Fields in North-central Oklahoma for the 2016-2017 Field Season

Wheat	Fall 2016			Spring 2017		
field	*L. testaceipes*	*A. nigritus*	*D. rapae*	*L. testaceipes*	*A. nigritus*	*D. rapae*
1	0.23 ± 0.23	1.03 ± 0.34	0	0	0	0
2	0.92 ± 0.63	5.41 ± 1.80	0	0.03 ± 0.03	0	0
3	0.03 ± 0.03	1.80 ± 0.60	0	0	0	0
4	0.03 ± 0.03	2.72 ± 0.91	0	0	0	0
5	0	0.06 ± 0.02	0	10.17 ± 4.63	0.09 ± 0.06	0
6	0.03 ± 0.03	0	0	4.30 ± 2.82	2.86 ± 2.62	0
7	0.06 ± 0.06	1.00 ± 0.33	0	1.49 ± 0.97	0	0
Canola field						
1	-	-	-	0	0	0.03 ± 0.03
2	-	-	-	0	0	0.30 ± 0.19
3	0	0	0	0	0	0
4	0	0	0	0	0	0.89 ± 0.43
5	0	0	0	0	0.13 ± 0.07	1.55 ± 0.84
6	0	0	0.20 ± 0.20	0	0	0.26 ± 0.23
7	0	0.03 ± 0.03	0.03 ± 0.03	0	0	0

Table 2. Summary Statistics (Mean ± SE) for Percentage of Parasitism by *Lysiphlebus testaceipes, Aphelinus nigritus,* and *Diaeretiella rapae* on Sentinel Aphids Put in Winter Wheat and Winter Canola Fields in North-central Oklahoma for the 2017-2018 Field Season

Wheat	Fall 2017			Spring 2018		
field	*L. testaceipes*	*A. nigritus*	*D. rapae*	*L. testaceipes*	*A. nigritus*	*D. rapae*
1	0	0.14 ± 0.11	0	21.48 ± 10.57	0.03 ± 0.03	1.29 ± 0.98
2	0	0.09 ± 0.09	0	26.69 ± 5.51	0	3.44 ± 2.49
3	0	0.03 ± 0.03	0	24.34 ± 5.19	0	0.12 ± 0.11
4	0	0	0	14.55 ± 4.19	0	0.14 ± 0.11
5	0	0.29 ± 0.21	0	39.58 ± 9.17	0	0
6	0	0	0	13.32 ± 3.08	0	0
7	0	0	0	7.93 ± 2.26	0	0
8	0	0.06 ± 0.06	0	10.91 ± 3.52	0	0.73 ± 0.44
Canola field						
1	0	0	1.69 ± 0.83	0	0	0
2	0	0	0.40 ± 0.32	0	0	6.33 ± 2.53
3	0	0	0	0	0	5.64 ± 2.90
4	0	0	0	0	0	1.80 ± 0.66
5	0	0	0.60 ± 0.60	0	0	16.38 ± 2.56
6	0	0	0	0	0	2.41 ± 2.18
7	0	0	0	0	0	12.97 ± 2.77
8	0	0	0	0	0	6.96 ± 1.23

Table 3. Summary Statistics (Mean ± SE) for Percentage Parasitism by *Lysiphlebus testaceipes, Aphelinus nigritus,* and *Diaeretiella rapae* on Sentinel Aphids Put in Winter Wheat and Winter Canola Fields in North-central Oklahoma for the 2018-2019 Field Season

Wheat	Fall 2018			Spring 2019		
field	*L. testaceipes*	*A. nigritus*	*D. rapae*	*L. testaceipes*	*A. nigritus*	*D. rapae*
1	0	0	0	1.55 ± 1.55	0	0
2	0	0	0	0.86 ± 0.86	0	0
3	0	0	0	2.15 ± 2.15	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0.66 ± 0.66	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
Canola field						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	2.18 ± 2.18
8	0	0	0	0	0	0

No studies to date documented *A. nigritus* as the dominant primary aphid parasitoid on winter crops on the Southern Plains, and we speculate that large numbers of the parasitoid moved from nearby sorghum fields and possibly Johnsongrass into wheat fields during fall 2016 and 2017. During our study, sorghum fields in Oklahoma were infested with sugarcane aphids during the summer and early fall, but not surprisingly, regional outbreak of sugarcane aphids on sorghum was also decreasing from 2016-2019 (Bowling et al. 2016, Elliott et al. 2017, Maxon et al. 2019, Lindenmayer et al. 2020, N.C. Elliott unpublished data) as were the number of hectares planted to the crop (USDA NASS 2017, 2018, 2019). The regional decrease in infestation by sugarcane aphids also coincided with a 2016-2019 decrease of *A. nigritus* on winter crops in Oklahoma (Figs. 1, 2).

The opportunistic appearance of *A. nigritus* on winter crops during 2016 and 2017 does however highlight the need to further investigate ecological interactions among aphids and parasitoids in agricultural landscapes of the US Southern Plains. The impact of the invasive sugarcane aphid on sorghum to temporarily shift parasitoid assemblages on winter crops was not anticipated. This temporary shift might have been caused by host suitability issues; *A. nigritus* is thought to have a broader host range and is consistently found emerging from sugarcane aphids on sorghum on the Southern Plains, whereas *L. testaceipes* is most often in other cereal aphids in Oklahoma (N.C. Elliott unpublished data). Because of their mobility and ability to use a range of aphid herbivores, more generalist natural enemies such as *A. nigritus* are more likely to survive and reproduce in diverse cropping systems that have a range of spatial and temporal resources (Brewer and Elliott 2004, Bianchi et al. 2006, Schellhorn et al. 2015, Tscharntke et al. 2016, Haan et al. 2020). Perhaps our data revealed that *A. nigritus* is a superior competitor when aphids are abundant during warm summer and early fall months common on the Southern Plains. Future landscape-level studies integrating data from summer and winter crops are needed, as well as studies addressing host suitability and competition among aphid parasitoids.

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References Cited

Bianchi, F. J. J. A., C. J. H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proc. Royal Soc. B: Biol. Sci. 273: 1715-1727.

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. C. Elliott, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane

aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. J. Integrated Pest Management 7: 12. doi:10.1093/jipm/pmw011

Boyles, M., J. Bushong, H. Sanders, and M. Stamm. 2012. Great Plains canola production handbook. KSU Agricultural Experiment Station and Cooperative Extension Service. MF-2734.

Brewer, M. J., and N. C. Elliott. 2004. Biological control of cereal aphids in North America and mediating effects of host plant and habitat manipulations. Annu. Rev. Entomol. 49: 219-242.

Brewer, M. J., T. Noma, N. C. Elliott, A. N. Kravchenko, and A. L. Hild. 2008. A landscape view of cereal aphid parasitoid dynamics reveals sensitivity to farm- and region-scale vegetation structure. European J. Entomol. 105: 503-511.

Bushong, J. A., A. P. Griffith, T. F. Peeper, and F. M. Epplin. 2012. Continuous winter wheat versus a winter canola–winter wheat rotation. Agron. J. 104: 324-330.

DeVuyst, E. A., F. Epplin, T. F. Peeper, and M. C. Boyles. 2009. Oklahoma canola systems vs. continuous wheat budget comparison. Oklahoma Cooperative Extension Service Fact Sheet and excel spreadsheet. AGEC-257, http://canola.okstate.edu/ocesfactsheets.

Elliott, N. C., G. F. Backoulou, K. L. Giles, and T. A. Royer. 2014. Aphids and parasitoids in wheat and nearby canola fields in central Oklahoma. Southwest. Entomol. 39: 23-28.

Elliott, N. C., M. Brewer, N. Seiter, T. Royer, R. Bowling, G. Backoulou, J. Gordy, K. Giles, J. Lindenmayer, B. McCornack, and D. Kerns. 2017. Sugarcane aphid spatial distribution in grain sorghum fields. Southwest. Entomol. 42: 27-35.

Elliott, N. C., M. J. Brewer, and K. L. Giles. 2018. Landscape context affects aphid parasitism by *Lysiphlebus testaceipes* in wheat fields. Environ. Entomol. 47: 803-811.

Elliott, N. C., K. L. Giles, M. J. Brewer, and C. N. Jessie. 2019. Early season parasitism of cereal aphids in wheat fields and field borders. Southwest. Entomol. 44: 11-19.

Giles, K. L., D. B. Jones, T. A. Royer, N. C. Elliott, and S. D. Kindler. 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. J. Econ. Entomol. 96: 975-982.

Giles, K. L., G. Hein, and F. Peairs. 2008. Areawide pest management of cereal aphids in dryland wheat systems of the Great Plains, pp. 441-466. *In* O. Koul, G. W. Cuperus, and N. Elliot [eds.], Areawide Pest Management: Theory and Implementation. CABI, UK.

Giles, K. L., B. P. McCornack, T. A. Royer, and N. C. Elliott. 2017. Incorporating biological control into IPM decision making. Current Opinion Insect Science 20: 84-89.

Gilstrap, F. E., T. J. Kring, and G. W. Brooks. 1984. Parasitism of aphids (Homoptera: Aphididae) associated with Texas sorghum. Environ. Entomol. 13: 1613-1617.

Haan, N. L., Y. Zhang, and D. A. Landis. 2020. Predicting landscape configuration effects on agricultural pest suppression. Trends in Ecol. & Evol. 35: 175-186.

Hughes, G. E., E. Owen, G. Sterk, and J. S. Bale. 2010. Thermal activity thresholds of the parasitic wasp *Lysiphlebus testaceipes* and its aphid prey: implications for the efficacy of biological control. Physiol. Entomol. 35: 373-378.

Hughes, G. E., G. Sterk, and J. S. Bale. 2011. Thermal biology and establishment potential in temperate climates of the aphid parasitoid, *Lysiphlebus testaceipes*. BioControl 56: 19-33.

Hunger, B., A. de Oliveira Silva, T. Royer, and K. Giles. 2017. Effect of planting date and seed treatment on diseases and insect pests of wheat. Oklahoma Cooperative Extension Service Fact Sheet: CR-7088.

Jessie, C. N. 2017a. Diversity, Relative Abundance, Movement, and Fitness of Insect Predators in Winter Canola Agricultural Landscapes in the United States Southern Plains. Ph.D. dissertation. Oklahoma State University, Stillwater, OK.

Jessie, W. P. 2017b. Effects of Interspecific Competition and Narrow-spectrum Insecticides on the Survival and Development of *Diaeretiella rapae* in Oklahoma Winter Canola. Ph.D. dissertation. Oklahoma State University, Stillwater, OK.

Jessie, W. P., K. L. Giles, E. J. Rebek, M. E. Payton, C. N. Jessie, and B. P. McCornack. 2015. Preference and performance of *Hippodamia convergens* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) on *Brevicoryne brassicae*, *Lipaphis erysimi*, and *Myzus persicae* (Hemiptera: Aphididae) from winter-adapted canola. Environ. Entomol. 44: 880-889.

Jones, D. B., K. L. Giles, R. C. Berberet, T. A. Royer, N. C. Elliott, and M. E. Payton. 2003. Functional responses of an introduced parasitoid and an indigenous parasitoid on the greenbug at four temperatures. Environ. Entomol. 32: 425-432.

Jones, D. B., K. L. Giles, T. A. Royer, N. C. Elliott, and M. E. Payton. 2007. Parasitism of greenbug, *Schizaphis graminum,* by the parasitoid *Lysiphlebus testaceipes,* at winter temperatures. Environ. Entomol. 36: 1-8.

Jonsson, M., C. S. Straub, R. K. Didham, H. L. Buckley, B. S. Case, R. J. Hale, C. Gratton, and S. D. Wratten. 2015. Experimental evidence that the effectiveness of conservation biological control depends on landscape complexity. J. Applied Ecol. 52: 1274-1282.

Kring, T. J., and F. E. Gilstrap. 1983. Within-field distribution of greenbug (Homoptera: Aphididae) and its parasitoids in Texas winter wheat. J. Econ. Entomol. 76: 57-62.

Lindenmayer, J. C., K. L. Giles, N. C. Elliott, A. E. Knutson, R. Bowling, M. J. Brewer, N. J. Seiter, B. McCornack, S. A. Brown, A. L. Catchot and T. A. Royer. 2020. Development of binomial sequential sampling plans for sugarcane aphid *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) in commercial grain sorghum. J. Econ. Entomol. https://doi.org/10.1093/jee/ toaa064

Maxson, E. L., M. J. Brewer, W. L. Rooney, and J. B. Woolley. 2019. Species composition and abundance of the natural enemies of sugarcane aphid, *Melanaphis sacchari* (Zehnter) (Hemiptera: Aphididae), on sorghum in Texas. Proc. Entomol. Soc. WA 121: 657-680.

Patrick, C. D., and E. P Boring, III. 1990. Managing Insect and Mite Pests of Texas Small Grains. TA&M Extension Pub # B-1251.

Rice, M. E., and G. E. Wilde. 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: aphididae) in sorghum and wheat. Environ. Entomol. 17: 836-841.

Royer, T. A., and K. L. Giles. 2015. Management of Insect and Mite Pests in Small Grains. Oklahoma Cooperative Extension Service CR-7194.

Royer, T. A., and K. L. Giles. 2017a. The OKANOLA project: challenges in managing insect pests of canola in the Southern Plains, pp. 147-156. *In* G. V. P. Reddy [ed.], Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops. CAB International, Boston, MA.

Royer, T. A., K. L. Giles. 2017b. Aphids in Winter Canola and Their Management. http://factsheets.okstate.edu/documents/epp-7097-aphids-in-winter-canola - and-their-management/

Royer, T. A., K. L. Giles, T. Nyamanzi, R. M. Hunger, E. G. Krenzer, N. C. Elliott, S. D. Kindler, and M. Payton. 2005. Economic evaluation of the effects of planting date \ and application rate of imidacloprid for management of cereal aphids and barley yellow dwarf in winter wheat. J. Econ. Entomol. 98: 95-102.

Royer, T. A, B. Pendleton, N. C. Elliott, and K. L. Giles. 2015a. Greenbug (Hemiptera: Aphididae) biology, ecology and management in wheat and sorghum. J. Integrated Pest Management. http://dx.doi.org/10.1093/jipm/pmv018

Royer, T. A., K. L. Giles, N. C. Elliott, and S. D. Kindler. 2015b. The cereal aphid Expert system and Glance ’n Go sampling for greenbugs: questions and answers. Oklahoma Cooperative Extension Service CR-7191.

Schellhorn, N. A., F. Bianchi, and C. L. Hsu. 2014. Movement of entomophagous arthropods in agricultural landscapes: links to pest suppression. Annu. Rev. Entomol. 59: 559-581.

Thies, C., I. Roschewitz, and T. Tscharntke. 2005. The landscape context of cereal aphid-parasitoid interactions. Proc. Biol. Sci. 272: 203-210.

Tschamtke, T., D. S. Karp, R. Chaplin-Kramer, P. Batary, F. DeClerck, C. Gratton, L. Hunt, A. Ives, M. Jonsson, A. Larsen, E. A. Martin, A. Martinez-Salinas, T. D. Meehan, M. O’Rourke, K. Poveda, J. A. Rosenheim, A. Rusch, N. Schellhorn, T. C. Wanger, S. Wratten, and W. Zhang. 2016. When natural habitat fails to enhance biological pest control – five hypotheses. Biol. Conservation 204: 449-458.

USDA, NASS (National Agricultural Statistics Survey). 2017. http://www.nass.usda.gov/.

USDA, NASS (National Agricultural Statistics Survey). 2018. http://www.nass.usda.gov/.

USDA, NASS (National Agricultural Statistics Survey). 2019. http://www.nass.usda.gov/.

Vitale, P. P., F. M. Epplin, K. L. Giles, N. C. Elliott, P. A. Burgener, and S. P. Keenan. 2014. Crop diversity on traditional Great Plains wheat farms. J. Am. Soc. Farm Managers Rural Appraisers 77: 145-159.

Vitale, J. D., P. P. Vitale, F. M. Epplin, K. L. Giles, N. C. Elliott, F. B. Peairs, G. J. Michels Jr., G. L. Hein, P. A. Burgener, and S. P. Keenan. 2020. Farm management practices used by wheat producers in the western Great Plains: estimating their productivity and profitability. J. Applied Farm Econ. https://docs.lib.purdue.edu/jafe/vol3/iss1/4/

Field Parasitism of the Neotropical Cornstalk Borer, *Diatraea lineolata* (Walker)[1], by *Apanteles diatraeae* Muesebeck[2]

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Abstract. The Neotropical cornstalk borer, *Diatraea lineolata* (Walker), is a pest of economic importance in maize, *Zea mays* L., in Mexico. The objective was to evaluate natural parasitism by *Apanteles diatraeae* Muesebeck on *D. lineolata* larvae during four 'Mejen' maize-growing seasons at Tabasco, Mexico. Parasitism by the braconid *A. diatraeae* on *D. lineolata* larvae was relatively low, from 0.29 to 2.8%. Of a total of 15 parasitized larvae, 46.6% were 3rd instar, 26.7% 4th instar, and 26.7% 5th-instar larvae. *A. diatraeae* is a gregarious endoparasitoid, with an average of 57.1 wasps emerging per parasitized larva and a sex ratio of 1:0.44 (female:male). The braconid should be studied in greater detail to know more about its interrelationship with its host during phenological stages of the crop, before physiological maturity of maize plants.

Introduction

The Neotropical cornstalk borer, *Diatraea lineolata* (Walker) (Lepidoptera: Crambidae), was first described in Venezuela (Walker 1856). Its distribution includes Mexico, Central America, from Venezuela to Colombia, Ecuador, and the Caribbean Islands (Dyar and Heinrich 1927, Bleszynski 1969). In Mexico, *D. lineolata* significantly damages maize, especially in the states of Tamaulipas (Rodríguez-del-Bosque et al. 1988), Veracruz (Aguirre 2007), Jalisco (Pérez-Domínguez and Ireta-Moreno 2017), and Tabasco (Osorio-Osorio et al. 2015). In this last State, damage reached 62.4% bored stalks in native Mejen maize.

A challenge facing crop protection in Mexican agriculture is availability of alternatives or technological packages based on biological control (Williams et al. 2013, Rodríguez del Bosque et al. 2015), which is a safe and sustainable approach that takes advantage of natural enemies such as predators, parasitoids, or pathogens to control agricultural pests (Wang et al. 2019). Parasitoids are the most common natural enemies in biological pest control programs (Carballo 2002). One of the most important groups of parasitoid wasps in terms of species richness and economic importance is the subfamily Microgastrinae (Hymenoptera: Braconidae) (Rodríguez

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et al. 2013, Whitefield 2017, Whitfield et al. 2018) that comprises 2,999 described species with many yet to be described (Fernández-Triana et al. 2020).

Microgastrinae wasps are characterized by 18 antennal segments (although these might appear subdivided when placode sensilla are evenly arranged in two ranks per flagellomere). The spiracles of the first metasomal segment are on the lateral tergites, rather than on the median tergite, and they tend to have apically reduced wing venation (Whitfield 2017). Currently, *Apanteles* Foerster is the largest genus of Microgastrinae, with approximately 1,000 described species from all biogeographical regions (Yu et al. 2012, Fernandez-Triana et al. 2020). Many families of Lepidoptera have been recorded as hosts for *Apanteles*, but many records probably are incorrect and/or need further verification (Fernandez-Triana et al. 2020). In Costa Rica most known hosts belong to three families: Crambidae, Depresariidae, and Hesperiidae (Fernandez-Triana et al. 2014). Austin and Dangerfield (1989) reviewed the taxonomy of species in the braconid subfamily Microgastrinae that parasitize larvae of *Diatraea* in the New World.

The parasitoid *Apanteles diatraeae* Muesebeck was described in Cuba as a parasitoid of larvae of *Diatraea saccharalis* Fabricius (Muesebeck 1921). However, hosts of the parasitoid also include *D. lineolata* (Muesebeck 1957) and *Diatraea grandiosella* Dyar (Davis et al. 1933, Davis 1944). Worldwide, *A. diatraeae* was reported in Arizona and Florida (USA), Colombia, Cuba, the Dominican Republic, Grenada, Guatemala, Haiti, Honduras, Jamaica, Mexico, Nicaragua, Panama, Puerto Rico, Trinidad and Tobago, and Venezuela (Fernandez-Triana et al. 2020). In Mexico, the species was a stalk-borer parasitoid in maize crops in the states of Nuevo León, Morelos, Tabasco, and Tamaulipas, although only in the State of Morelos was natural parasitism evaluated (Van Zwaluwenburg 1926, Rodríguez-del-Bosque and Smith 1995).

During development of a study to evaluate damage by cornstalk borers in maize at Macuspana, Tabasco, Mexico (Osorio-Osorio et al. 2015), an endoparasitoid species that attacks *D. lineolata* larvae was detected. The objective was to evaluate natural parasitism by the parasitoid on *D. lineolata* larvae in four maize crop-sowing seasons in the region.

Materials and Methods

The study was at the Chinalito ejido, municipality of Macuspana, Tabasco, Mexico (17º 45´12´´ N, 92º15´47´´ W, 11 m above sea level). Under rainfed agricultural conditions in the study area, 45,000 native Mejen maize were planted in a 1-ha area during the seasons: autumn-winter 2016, spring-summer 2017, summer-autumn 2018, and winter-spring 2019. In each planting season, sampling was done during maximum outbreak of *D. lineolata* (Osorio-Osorio et al. 2015), which coincided with physiological maturity of the maize plants (unpublished data). Four sample periods were 85 to 95 days after maize was planted (21 January 2016, 26 August 2017, 1 November 2018, and 8 April 2019). On each sampling date, 500 maize plants were selected randomly and cut at the base. The stalks were opened longitudinally to extract *D. lineolata* larvae and pupae that were put individually into plastic containers. For larvae to continue developing, they were given diet proposed by Badilla et al. (1994) and kept at 28 ± 5°C, 70 ± 5% relative humidity, and a photoperiod of 12:12 light:dark hours in a brood chamber until borer adults or the parasitoid emerged. In addition to extraction of *D. lineolata* larvae from stalks, cocoon masses of the parasitoid were found inside bored stalk galleries with signs *D. lineolata* larvae

were attacked. The percentage of parasitism was estimated from the number of parasitized larvae among the total number of larvae collected from *D. lineolata*. The identity of the Neotropical cornstalk borer was confirmed by comparing genitalia of emerged adult males with taxonomic keys by Dyar and Heinrich (1927) and Bleszynski (1969). The parasitoid was identified through taxonomic works of Muesebeck (1921, 1957), Austin and Dangerfield (1989), Whitfield (2017), and Fernandez-Triana et al. (2014, 2020). Hyperparasitoids that emerged from cocoon masses in the field were identified with the work of Schauff et al. (1997).

Results and Discussion

In total, 814 larvae of *D. lineolata* were collected -- 253 on 22 January 2016 (autumn-winter 2016), 101 on 26 August 2017 (spring-summer 2017), 365 on 1 November 2018 (summer-autumn 2018), and 95 on 8 April 2019 (winter-spring 2019). During the planting seasons, seven larvae were parasitized in the first season by *A. diatraea* (Fig. 1), five in the second, one in the third, and two in the fourth. Parasitism percentages of *A. diatraea* by sampling date were 2.8, 4.9, 0.29, and 2.1%, respectively.

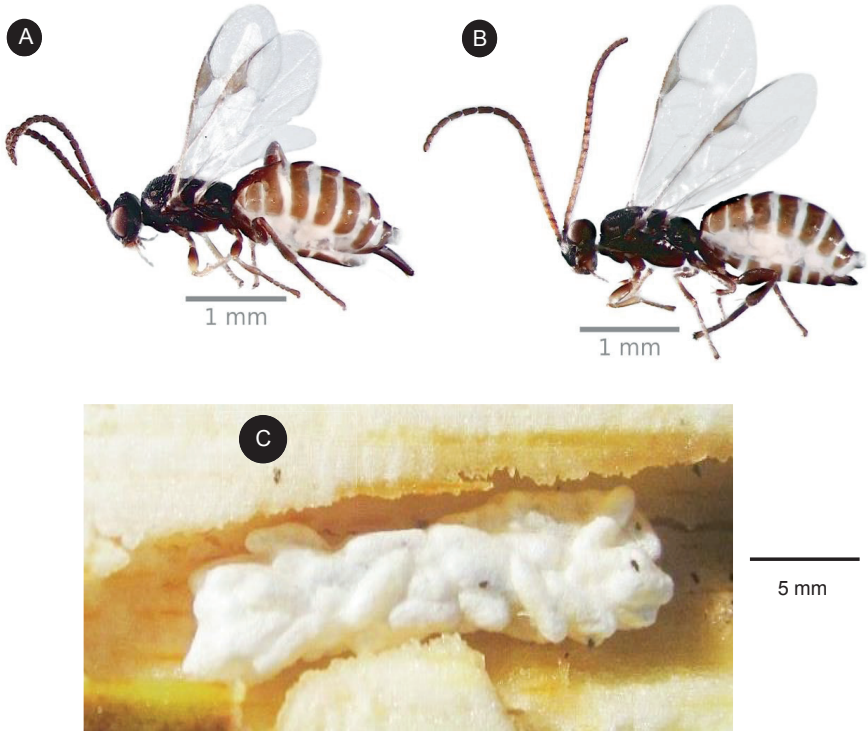

Fig. 1. *Apanteles diatraeae*: female (A), male (B), and cocoons covering a Neotropical cornstalk borer larva (C).

Of the parasitized larvae, 46.6% were found in the 3rd instar, 26.7% in the 4th, and 26.7% in the 5th. Individual confinement of each larva collected from *D. lineolata* allowed confirming that *A. diatraeae* is a gregarious endoparasitoid. An average of 57.1 wasps emerged per larva, and the sex ratio (female:male) of *A. diatraeae* was 1:0.44. Likewise, in 14 of the 15 parasitized larvae, more females emerged than males. In addition to *D. lineolata* larvae collected, on 21 January 2016, three cocoon masses of *A. diatraeae* were found, from which specimens of *Horismenus* sp. emerged. On 26 August 2017, two cocoon masses attacked by the same hyperparasitoid also were found, thereby confirming the previous finding that the hyperparasitoid *Horismenus* sp. attacked *A. diatraeae* in the State of Tabasco, Mexico (Rodriguez-del-Bosque and Smith 1995).

The biology of *A. diatraeae* was studied on southwestern corn borer *D. grandiosella* for 4 years (1931-1934) at Tucson, AZ (Davis 1944). Davis (1944) observed during the spring season at the beginning of each year, the percentage of parasitism on wintering larvae of *D. grandiosella* was low, but throughout the year and as the next three generations of the pest developed, parasitism increased significantly. On average, parasitism was 1.4% for wintering larvae, 3.6% for 1st generation, 32.4% for 2nd generation, and 78.9% for 3rd generation *D. grandiosella* (Davis 1944). In a similar study on the Caribbean island of Trinidad (from 1941-1942), parasitism of *A. diatraeae* on *Diatraea* spp. in maize did not exceed 1% (Hynes 1944). However, in maize in the State of Morelos, Mexico, as many as 50% of *Diatraea* spp. larvae were parasitized by the parasitoid (Van Zwaluwenburg 1926). While searching for natural enemies of grass stem-borers in various regions of Mexico, Rodríguez-del-Bosque and Smith (1995) reported that *A. diatraeae* parasitized *Diatraea* spp. larvae in maize at Nuevo León, Tamaulipas, and Tabasco, but the amounts of parasitism were not indicated.

In conclusion, parasitism by *A. diatraeae* on *D. lineolata* larvae in maize at Tabasco, Mexico was relatively low (0.29 to 2.8%). However, the parasitoid should be studied in greater detail before physiological maturity of maize plants, because it might help mitigate abundance of the Neotropical cornstalk borer during crop phenological stages of flowering and grain formation.

Acknowledgment

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References Cited

Aguirre H., A. 2007. Pérdida del Rendimiento de Maíz por Daño del Barrenador Neotropical *Diatraea lineolata* (Walker) y *Fusarium* spp., en el Trópico Húmedo. Tesis de Licenciatura, Universidad Autónoma Agraria "Antonio Narro", Saltillo, Coahuila, México.

Austin, A. D., and P. C. Dangerfield. 1989. The taxonomy of new world microgastrine braconids (Hymenoptera) parasitic on *Diatraea* spp. (Lepidoptera: Pyralidae). Bull. Entomol. Res. 79: 131-144.

Badilla, F. F., S. A. I. Solis, y S. D. Alfaro. 1994. Manual de Producción del Parasitoide *Cotesia flavipes* para el Control Biológico de los Taladradores de

la Caña de Azúcar *Diatraea* spp. en Costa Rica. Dirección de Investigación y Extensión de la Caña de Azúcar (DIECA), Ed. Conmemorativa del 10 Aniversario del Programa de Entomología.

Bleszynski, S. 1969. The taxonomy of the Crambinae moth borers of sugar cane, pp. 11-41. *In* J. R. Williams, J. R. Metcalfe, R. W. Montgomery, and R. Mathes [eds.], Pest of Sugar Cane. Elsevier Publishing, Amsterdam.

Carballo, M. 2002. Manejo de insectos mediante parasitoides. Manejo Integrado de Plagas y Agroecología (Costa Rica) 66: 118-122.

Davis, E. G. 1944. *Apanteles diatraeae*, a Braconid Parasite of the Southwestern Corn Borer. Tech. Bull. 871. U.S. Department of Agriculture, Washington, DC.

Davis, E. G., J. R. Horton, C. H. Gable, E. V. Walter, and R. A. Blanchard. 1933. The Southwestern Corn Borer. Tech. Bull. 388. U.S. Department of Agriculture. Washington, DC.

Dyar, H. G., and C. Heinrich. 1927. The American moths of the genus *Diatraea* and allies. Proceedings U.S. National Museum 71: 1-48.

Fernandez-Triana, J. L., J. B. Whitfield, J. J. Rodriguez, M. A. Smith, D. H. Janzen, W. D. Hallwachs, M. Hajibabaei, J. M. Burns, M. A Solis, J. Brown, S. Cardinal, H. Goulet, and P. D. N. Hebert. 2014. Review of *Apanteles sensu stricto* (Hymenoptera, Braconidae, Microgastrinae) from area de conservation Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. ZooKeys 383: 1-565.

Fernandez-Triana, J., M. R. Shaw, C. Boudreault, M. Beaudin, and G. R. Broad. 2020. Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). ZooKeys 920: 1-1089.

Hynes, H. B. N. 1942. Lepidopterous pests of maize in Trinidad. Trop. Agric. 19: 194-202.

Muesebeck, C. F. W. 1921. A revision of the North American species of Ichneumon-flies belonging to the genus *Apanteles*. Proceedings U.S. National Museum 58: 483-576.

Muesebeck, C. F. W. 1957. New World *Apanteles* parasitic on *Diatraea* (Hymenoptera: Braconidae). Entomological News 17: 19-27.

Osorio-Osorio, R., V. Hernández-García, K. M. Harris, L. U. Hernández-Hernández, E. de la Cruz-Lázaro, C. Márquez-Quiroz, D. Mota-Sánchez, and L. A. Aguirre-Uribe. 2015. Species of stalk borers (Lepidoptera: Crambidae) and damage to maize in southeastern México. Southwest. Entomol. 40: 831-835.

Pérez-Domínguez, J. F., y J. Ireta-Moreno. 2017. Daños causados por barrenadores del tallo y pudrición de tallo en maíz de la región Ciénega de Chapala, Jalisco. Entomología Agrícola 5: 428-434.

Rodriguez, J. J., J. L. Fernández-Triana, M. A. Smith, D. H. Janzen, W. Hallwachs, T. L. Erwin, and J. B. Whitfield. 2013. Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). Insect Conservation and Diversity 6: 530-536.

Rodríguez-del-Bosque, L. A., y J. W. Smith, Jr. 1995. Casos de hiperparasitismo en barrenadores del tallo, *Diatraea* spp. (Lepidoptera: Pyralidae), en México. Vedalia 2: 41-42.

Rodríguez-del-Bosque, L. A., J. W. Smith Jr., and H. W. Browning. 1988. Damage by stalkborers (Lepidoptera: Pyralidae) to corn in Northeastern Mexico. J. Econ. Entomol. 81: 1775-1780.

Rodríguez-del-Bosque, L. A., H. C. Arredondo, T. Williams, y J. F. Barrera. 2015. Pasado, presente y pespectivas del control biológico en México, pp. 17-28. *In* H. C. Arredondo B. y L. A. Rodríguez-del-Bosque [eds.], Casos de Control Biológico en México, Vol. 2. Colegio de Posgraduados, Chapingo, México.

Schauff, M. E., J. LaSalle, and L. D. Coote. 1997. Eulophidae, pp. 327-429. Chapter 10. *In* G. A. P. Gibson, J. T. Huber, and J. B. Woolley [eds.], Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). Research Press, National Research Council of Canada, Ottawa, Ontario.

Van Zwaluwenburg, R. H. 1926. Insect enemies of sugarcane in western Mexico. J. Econ. Entomol. 19: 664-669.

Walker, F. 1856. List of the Specimens of Lepidopterous Insects in the Collection of the British Museum. Part IX. Noctuidae. London.

Wang Z., Y. Liu, M. Shi, J. Huang, and X. Chen. 2019. Parasitoid wasps as effective biological control agents. Journal of Integrative Agriculture 18: 705-715.

Whitfield, J. B. 2017. Subfamily Microgastrinae, pp. 9941-11071. *In* R. A. Wharton, P. M. Marsh, and M. J. Sharkey [eds.], Manual to the New World Genera of the Family Braconidae (Hymenoptera). 2nd ed. (Kindle version). The International Society of Hymenopterists, Washington, DC.

Whitfield, J. B., A. D. Austin, and J. L. Fernandez-Triana. 2018. Systematics, biology, and evolution of Microgastrine parasitoid wasps. Annu. Rev. Entomol. 63: 389-406.

Williams, T., H. C. Arredondo-Bernal, and L. A. Rodríguez-del-Bosque. 2013. Biological pest control in Mexico. Annu. Rev. Entomol. 58: 119-140.

Yu, D. S. K., C. van Achterberg, and K. Horstmann. 2012. Taxapad 2012, Ichneumonoidea 2011. Database on flash-drive. www.taxapad.com, Ottawa, Ontario, Canada.

A New Ant Species of the Genus *Pheidole* Westwood, 1839[1] from Miocene Mexican Amber

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Abstract. A fossil species of *Pheidole* Westwood, 1839 (Formicidae, Myrmicinae) was described based on an amber inclusion found in the Miocene strata at Chiapas, southwestern Mexico. It was named *Pheidole pauchil* sp. nov., which is the first described species of the genus in Mexican amber. This broadens our understanding of the diversity of *Pheidole* in the Miocene in the southernmost part of North America.

Resumen. Se describe una especie fósil de *Pheidole* Westwood, 1839 (Formicidae, Myrmicinae) proveniente de una inclusión de ámbar encontrada en el estrato del Mioceno de Chiapas, Sureste de México. El espécimen se nombró *Pheidole pauchil* sp. nov., el cual representa la primera especie del género en ámbar de México. Esto amplía nuestro conocimiento sobre la diversidad de *Pheidole* en la parte más austral de Norteamérica en el Mioceno.

Introduction

The ant genus *Pheidole* (Fomicidae, Myrmicinae), with 1, 047 extant species according to Bolton (2020) and continuously increasing description of new members (Longino 2009, 2019), is widely distributed throughout the world. This hyperdiverse and cosmopolitan taxon is not well represented and has few specimens in the fossil record. The genus *Pheidole* currently is known in the Cenozoic strata of North and Central America. The fossil species *Pheidole tertiaria* Carpenter, 1930, is known from Eocene rocks of Colorado **(**Perkovsky 2016, Casadei-Ferreira et al. 2019). Other fossil species described from Miocene amber of the Dominican Republic include *Pheidole tethepa* Wilson, 1985; *Pheidole primigenia* Baroni Urbani, 1995; and *Pheidole anticua* Casadei-Ferreira, Chaul & Feitosa, 2019. *Pheidole* also was preliminarily recorded in Miocene amber of southern Mexico (Solórzano-Kraemer 2007), but no species were described.

Pheidole cordata Holl, 1829*,* is another specimen described in Eocene Baltic amber, but provenance of the specimen was questioned by Wilson (2003) who suggested *P. cordata* probably was a specimen embedded in copal (recent plant resin) or fake amber. A second specimen known as *Pheidole rasnitsyni* Dubovikoff, 2011 also was originally interpreted as amber inclusion from Eocene Baltic sediments (Dubovikoff 2011), but recently was considered a copal inclusion (Perkovsky 2016,

[1]Formicidae, Myrmicinae

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Cassadei-Ferreira et al. 2019: Table 1, p. 123). We describe a fossil ant from Miocene Mexican amber that best matches the genus *Pheidole* Westwood, 1839. It was erected as *Pheidole pauchil* sp. nov. Diagnosis and illustration are presented.

Amber-bearing beds of Simojovel are widely accepted as part of Mazantic shale and Balumtum sandstone strata dated as early to the middle Miocene (Poinar 1992, Perrilliat et al. 2010, Durán-Ruiz et al. 2013, Riquelme et al. 2013). The botanical source of Chiapas amber is attributed to an extinct legume tree species of the genus *Hymenaea* Linné (sensu Langenheim 1966). Chiapas amber has chemical signatures in common with plant resins of living *Hymenaea courbaril* Linné and *Hymenaea verrucosa* Gaertner that now are distributed in the tropics (Langenheim 2003, Riquelme et al. 2014a). The sedimentary record and associated paleobiota suggest resin-producing trees of *Hymenaea* were distributed in a lowland-fluvial environment close to a coastal plain (Graham 1999, Langenheim 2003, Durán-Ruiz et al. 2013, Riquelme et al. 2014b).

Materials and Methods

The fossil specimen treated in this study is embedded in golden amber with translucent to cloudy glossiness. It comes from the La Pimienta site at the town of Simojovel, Chiapas, southwestern México. It was designated Holotype CPAL.401 and deposited in the Colección de Paleontología, Universidad Autonoma del Estado de Morelos, Mexico (CPAL-UAEM).

Anatomical data were collected using high-resolution microscopy and multiple-image stacking for three-dimensional focus expansion as presented by Riquelme et al. (2014b) (Fig. 1). Schematic drawings were hand traced by electronic pen using a stereomicroscope and photomicrographs, and Corel Draw X7® was used for graphic editing (Fig. 2). Anatomical measurements are in millimeters, which were collected using an Olympus AZ binocular dissecting microscope and a U-OCM10/100 1 mm micrometer with 0.1- mm intervals. To help identify the fossil, the free version of Lucid software v.3.3 was used for the New World *Pheidole* data. Abbreviations and indices follow Longino (2019).

The electronic version of the article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version were effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains were registered in ZooBank, the online registration system for the ICZN. The ZooBank Life Science Identifiers (LSID) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is 0101.

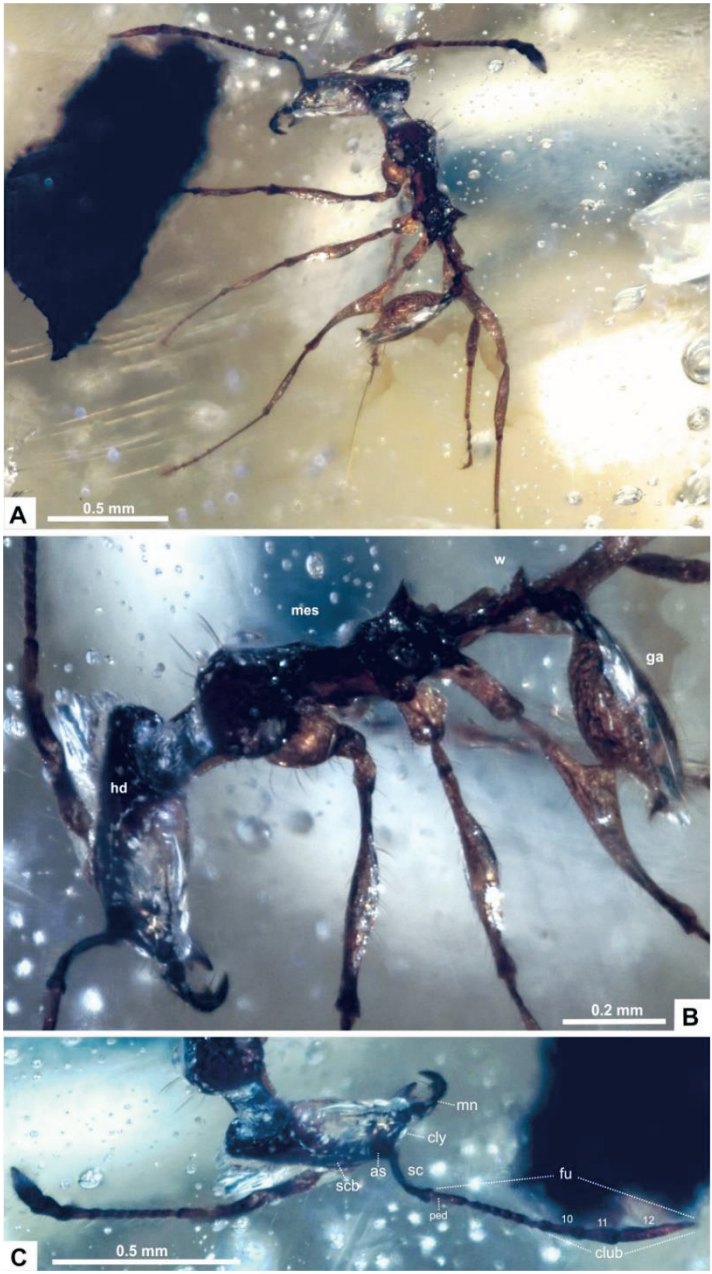

Fig. 1. *Pheidole pauchil* sp. nov. A. Holotype CPAL.401, amber inclusion, worker. B. Lateral view of the head (hd), mesosoma (mes), waist (w), and gaster (ga). C. Closer view of antenna. Abbreviations: (as) antennal socket, (cly) clypeus, (fu) funiculus, (mn) mandible, (ped) pedicel, (sc) scape, (scb) antennal scrobe.

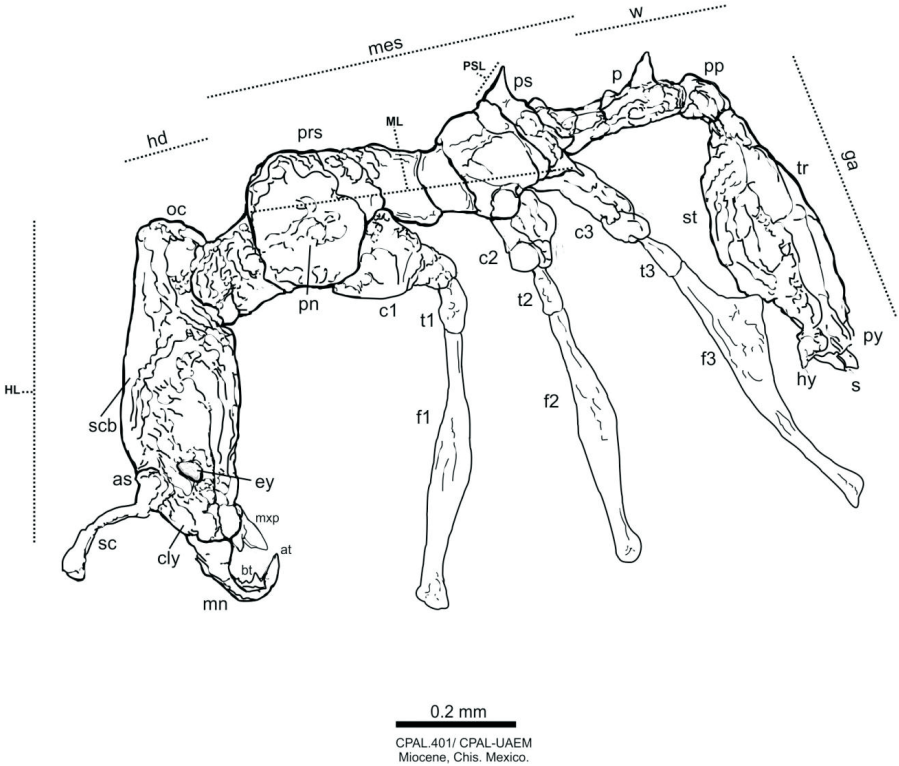

Fig. 2. *Pheidole pauchil* sp. nov. Holotype CPAL.401, line drawing of the head, mesosoma, petiole, and gaster in lateral view, based on photomicrographs and stereomicroscope observations. Abbreviations: (as) antennal socket, (at) apical tooth of mandible, (bt) basal tooth of mandible, (cly) clypeus, (c) coxa, (f) femur, (ga) gaster, (hd) head, (HL) head length, (hy) hypopygium, (mn) mandible, (mes) mesosoma, (ML) mesosoma length, (oc) occipital region, (p) petiole, (pp) post-petiole, (pn) pronotum, (prs) promesonotum, (ps) propodeal spine, (PSL) propodeal spine length, (py) pygidium, (sc) scape, (scb) antennal scrobe, (s) sting, (st) sternites, (t) trochanter, (tr) tergites, (w) waist.

HL: head length; in full-face view, maximum length of head, from line tangent to anterior-most projection of head capsule or clypeus to line tangent to posterior-most projection of vertex margin.
HW: head width; in full-face view, maximum width of head capsule not including eyes, if eyes protrude beyond margins of head, measured above or below eyes, depending on which is widest.
CI: cephalic index: 100*HW/HL.
SL: scape length; length of scape shaft from apex to basal flange, not including basal condyle and neck.
SI: scape index: 100*SL/HW.
ML: mesosoma length; in lateral view, distance from base of anterior face of pronotum, at inflection point between downward-sloping anterior face and flange-like

anteriormost projection of pronotum, to posteriormost extension of metapleural or propodeal lobes.
PSL: propodeal spine length; viewed laterally such that side of spine is roughly perpendicular to viewing angle, distance from inflection point between dorsal face of propodeum and base of spine to tip of spine.
SPLI: propodeal spine index: 100*PSL/HL.

Results

Systematic Paleontology.
Class Insecta Linnaeus, 1758
Order Hymenoptera Linnaeus, 1758
Family Formicidae Latreille, 1809
Subfamily Myrmicinae Lepeletier de Saint-Fargeau, 1835
Genus *Pheidole* Westwood, 1839
Pheidole pauchil sp. nov. Varela-Hernández & Riquelme. Type species by original designation.
ZooBank LSID: urn:lsid:zoobank.org:author:656B353A-952C-447A-A26D-0455E742C0AA

Diagnosis. With traits of the genus *Pheidole* and the following combination of diagnostic characters: tiny ant, 42 mm total length from head to posterior end of the abdomen. HL and HW equal in size, scape proportionally long. Antennal club 3-segmented, first funicular segment as long as the next three. Propodeal spines long and triangular, broad at the bases and pointed at the apex. Hind femur with a conspicuous lateral flange at about one third the distance to the apical end. Readily distinguished from *P. anticua* by the presence of long scapes, surpassing posterior end of the head; petiolar node sharp at the apex, with anterior and posterior sides meeting in an acute angle; extensor surface of posterior femur flattened and forming an acute angle approximately one third the distance to the apical end, while *P. anticua* has scapes short, not surpassing posterior end of the head; dorsal margin of the node meeting in a more or less sub-squared form; extensor surface of posterior femur not flattened and forming an acute angle.

Type Material. Holotype CPAL.401, amber inclusion, worker, and only specimen known (Fig. 1). Housed in the Colección de Paleontología, Universidad Autónoma del Estado de Morelos, Mexico (CPAL-UAEM).

Locality. La Pimienta site: 17°09´11´´N, 92°46´08´´W, Simojovel, Chiapas, México.

Horizon. Mazantic shale and Balumtum sandstone strata, Miocene, ca. 23 Ma (Poinar 1992, Perrilliat et al. 2010, Durán-Ruiz et al. 2013, Riquelme et al. 2013).

Etymology. The epithet "*pauchil*" means "*amber*" in the Tzeltal language, a modern version of Mayan language currently spoken at Simojovel, Chiapas, Mexico.

Description. Holotype CPAL.401, major worker; tiny sized ant, body length 42 mm, general measurements (mm): HL 0.35; HW 0.35, SL 0.4, CI 100; SI 114.28; ML 0.6, PSL 0.075; PSLI 21.42.

Head: in full face view, with lateral margins parallel; head compressed dorsoventrally, numerous erect hairs on dorsum of the head. Mandibles notably curved inward, two conspicuous apical and subapical teeth and four intercalary teeth in the masticatory margin. Hypostomal teeth not visible. Antenna 12-segmented, club 3-segmented. Club longer than rest of funiculus. Scapes long, surpassing vertex of head, abundant erect hairs all along the scape. First funicular segment very long, as much as the next three segments. Funiculus with numerous suberect hairs.

Mesosoma: in dorsal view, promesonotum with a well-developed neck, slightly longer than wide; pronotum longer than wide, wider anteriorly, lateral margins in dorsal view parallel from anterior side to half the length of pronotum, then stretching gradually and meeting posteriorly; long erect hairs on dorsum of pronotum. Mesonotum about as long as wide, triangular in dorsal view. Metanotum slender in dorsal view, depressed laterally between mesonotum and propodeum; mesonotal groove deep. Propodeum in lateral view, with long spine projections, sharp at the posterior edge, very wide at their base giving the impression of a well-defined triangle. Hind legs greatly expanded at the extensor surface of the femur about one third the distance to the apical end, forming a more or less right angle at the point of intersection of the sides that form the angle.

Petiole and Gaster: in profile view, petiolar peduncle long and slender; dorsal margin meeting anterior side of the node in a defined angle. Petiolar node in profile sharp, with anterior and posterior sides converging gradually at the apex of the node. In dorsal view, node markedly narrow, much broader than long. Gaster tergites, in profile view, relatively flattened with long fine erect hairs.

Remarks. Holotype CPAL.401 shows diagnostic characters that include the antennal club 3-segmented, which is not exclusive to all members of the genus, some species show antennal club 4-segmented or 5-segmented, but at least antennal club must be 3-segmented; propodeal spines also are present in CPAL.401, spines are variable in form and size in *Pheidole* but at least tiny denticles in the propodeum must be present. However, CPAL.401 is distinct from other congeners in having different specific proportions of the head, such as HL, HW, and SL, which are uncommon among the group. In extant species of *Pheidole*, the length of the head is typically longer than wide, whereas in CPAL.401, HL and HW are the same size, and the scape is also proportionally longer. Additionally, the size and shape of the head in CPAL.401 resembles a major worker.

Discussion

Pheidole is a successful group in evolutionary terms, with most taxa correlated to different biomes (Wilson 2003, Economo et al. 2015). Molecular phylogenetic analysis by Moreau (2008) suggests *Pheidole* emerged about 58.4-61.2 Ma. However, the oldest record of an undisputed fossil of *Pheidole* is *P. tertiaria* that dates from the late Eocene of North America, ca. 34 Ma (Carpenter 1930, Wilson 2003). However, a stem age of 59.8 Ma and crown age of 37.3 Ma were suggested for *Pheidole* (Economo et al. 2015). This radiation estimated in the Paleogene is consistent with the occurrence of *P. tertiaria.* New World origin and tropical distribution of *Pheidole* was discussed in Wilson (2003). Accordingly, the genus *Pheidole* was able to diversify widely in the Miocene tropics of North America, extending to the south of the continent, in response to intrinsic adaptability to a great range of environmental conditions, which is consistent with the occurrence of *Pheidole pauchil* sp. nov. in Miocene strata of southern Mexico, with an estimated age of 23 million years.

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References Cited

Bolton, B. 2020. An online catalog of the ants of the world https://antcat.org. Accessed on 06.05.2020.

Carpenter, F. M. 1930. The fossil ants of North America. Bulletin of the Museum of Comparative Zoology 70: 1-66.

Casadei-Ferreira, A. J., C. M. Chaul, and R. M. Feitosa. 2019. A new species of *Pheidole* (Formicidae, Myrmicinae) from Dominican amber with a review of the fossil records for the genus. ZooKeys 866: 117-125.

Dubovikoff, D. A. 2011. The first record of the genus *Pheidole* Westwood, 1839 (Hymenoptera: Formicidae) from the Baltic amber. Russian Entomological Journal 20: 255-257.

Durán-Ruíz, C., F. Riquelme, M. Coutiño-José, G. Carbot-Chanona, G. Castaño-Meneses, and M. Ramos-Arias. 2013. Ants from the Miocene Totolapa amber (Chiapas, Mexico), with the first record of the genus *Forelius* (Hymenoptera: Formicidae). Canadian Journal of Earth Sciences 50: 495-502.

Economo, E. P., P. Klimov, E. M. Sarnat, B. Guénard, M. D. Weiser, B. Lecroq, and L. L. Knowles. 2015. Global phylogenetic structure of the hyperdiverse ant genus *Pheidole* reveals the repeated evolution of macroecological patterns. Proceedings of the Royal Society B 282: 20141416.

Graham, A. 1999. Studies in Neotropical Paleobotany. XIII. An OligoMiocene palynoflora from Simojovel (Chiapas, Mexico). American Journal of Botany 86: 17-31.

Langenheim, J. H. 1966. Botanical source of amber from Chiapas, Mexico. Ciencia 24: 201-211.

Langenheim, J. H. 2003. Plant Resins: Chemistry, Evolution, Ecology and Ethnobotany. Timber Press, Oregon.

Longino, J. T. 2009. Additions to the taxonomy of New World *Pheidole* (Hymenoptera: Formicidae). Zootaxa 2181: 1-90.

Longino, J. T. 2019. *Pheidole* (Hymenoptera: Formicidae) of Middle American wet forest. Zootaxa 4599: 1-126.

Moreau, C. S. 2008. Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution 48: 224-239.

Perkovsky, E. E. 2016. Tropical and Holarctic ants in Late Eocene ambers. Vestnik Zoologii 50: 111-122.

Perrilliat, M. C., F. J. Vega, and M. A. Coutiño. 2010. Miocene mollusks from the Simojovel area in Chiapas, Southwestern Mexico. Journal of South American Earth Sciences 30: 111-119.

Poinar, G. O. 1992. Life in Amber. Stanford University Press, CA.

Riquelme, F., J. Alvarado-Ortega, M. Ramos-Arias, M. Hernández, I. Le Dez, T. A. Lee-Whiting, and J. L. Ruvalcaba-Sil. 2013. A fossil stemmiulid millipede (Diplopoda: Stemmiulida) from the Miocene amber of Simojovel, Chiapas, México. Historical Biology 26: 415-427.

Riquelme, F., P. Northrup, J. L. Ruvalcaba-Sil, V. Stojanoff, D. P. Siddons, and J. Alvarado-Ortega. 2014a. Insights into molecular chemistry of Chiapas amber

using infrared-light microscopy, PIXE/RBS, and sulfur K-edge XANES spectroscopy. Applied Physics A 116: 97-109.
Riquelme, F., M. Hernández-Patricio, A. Martínez-Dávalos, M. Rodríguez-Villafuerte, M. Montejo-Cruz, J. Alvarado-Ortega, J. L. Ruvalcaba-Sil and L. Zúñiga-Mijangos. 2014b. Two flat-backed polydesmidan illipedes from the Miocene Chiapas-amber Lagerstätte, Mexico. PLoS ONE 9: e105877.
Solórzano-Kraemer, M. M. 2007. Systematic, palaeontology and palaeobiogeography of the insect fauna from Mexican amber. Palaeontographica, Albteilung A 282: 1-133.
Wilson, E. O. 2003. *Pheidole* in the New World. A Dominant, Hyperdiverse Ant Genus. Harvard University Press, Cambridge, MA.

Effects of Concentrations of Azadirachtin Oil on Mortality and Post-Exposure Time of *Atta mexicana*[1] Leaf-Cutter Worker Ants

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Abstract. The leaf-cutting ant, *Atta mexicana* Smith, is a pest commonly managed by synthetic insecticides. Considering negative environmental and health impacts produced by insecticides, alternative strategies such as plant-derived insecticides are an option for controlling the ant. Effects of 20 serial dilutions of refined azadirachtin oil and three post-exposure times were assessed on *A. mexicana* forage workers in a laboratory. The dilutions were applied directly to ants. Azadirachtin concentration and post-exposure time significantly affected mortality of *A. mexicana*. The mean lethal concentration at 0 hours post-exposure was 2.26×10^{1} mg/ liter and at 50 and 100 hours was 1.67×10^{-6} mg/ liter and 1.65×10^{-14}mg/ liter, respectively. Findings on toxicity of azadirachtin to *A. mexicana* are of great importance for pest management strategies in agriculture. Insecticides based on azadirachtin oil should be considered among integrated pest management strategies recommended by the Plant Health Commission in the Americas.

Introduction

Leaf-cutter ants are an economically important pest in many crops in the Americas (Leal et al. 2014). They are a group of ants represented by the genera *Atta* and *Acromyrmex* in the Attini tribe (Ward et al. 2015). The ants cut fresh plant material to feed and grow symbiotic fungus inside the nest of the colony (Aragón-García et al. 2016). Because of their foraging activity, some *Atta* species are considered primary pests and defoliate cultivated plants, while others are secondary pests and defoliate shade and forage trees or cause serious damage to annual crops or fruit trees. Among crops most damaged by leaf-cutter ants are Prince-of-Wales feather (*Amaranthus hypochondriacus* L. [Caryophyllales: Amaranthaceae]), citrus trees (*Citrus* spp. [Sapindales: Citroideae]), cacao (*Theobroma cacao* L. [Malvales: Malvaceae]), coffee (*Coffea arabica* L. [Gentianales: Rubiaceae]), maize (*Zea mays* L. [Poales: Poaceae]), roselle (*Hibiscus sabdariffa* L. [Malvales: Malvaceae]), heliconias (*Heliconia* spp. [Zingiberales: Heliconiaceae]), and sugarcane (*Saccharum officinarum* L. [Poales: Poaceae]) (Valenzuela-González et al. 2008, Pérez-Torres et

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al. 2009, 2011, Aristizábal et al. 2013a, b, Landero-Torres et al. 2014, Murguía-González et al. 2018).

Leaf-cutter ants usually are controlled by broad-spectrum, very toxic chemical insecticides with low specificity (Santos et al. 2007). Use of such pesticides with lasting persistence in crops can generate long-term risks to human health (Stokstad 2013), specifically when consumers ingest fruits, vegetables, and products treated with agrochemicals. Pesticide use also can generate environmental and phytosanitary risks in agroecosystems, potentially resulting in elimination of beneficial organisms or natural enemies, development of populations of resistant insects, or contamination of soil and groundwater (Médiène et al. 2011).

Assessing eco-friendly strategies to control pests that threaten crops is necessary to reduce use of pesticides (Murguía-González et al. 2018). Less common cultural techniques for pest management include digging and removing nests (Oliveira et al. 2011). Among biological control methods used for leaf-cutter ants are application of entomo- and mycopathogenic fungi or plant extracts (Bueno and Bueno 2011, Oliveira et al. 2011, Aragón-García et al. 2016, Serratos et al. 2017).

Use of bioinsecticides especially is considered an environmentally friendly strategy because they do not damage human health or beneficial organisms or natural enemies of pests because of their specificity and short persistence (Oliveira et al. 2011). Bioinsecticides formulated from plant extracts are a biorational strategy to control pests such as leaf-cutter ants (Bueno and Bueno 2011). Chemical compounds, especially azadirachtin (Angulo-Escalante et al. 2004), from the neem plant, *Azadirachta indica* A. Juss (Sapindales: Meliaceae) (Benelli et al. 2017), are useful in preventing or repelling most insects including leaf-cutting ants (Santos-Oliveira et al. 2006).

Only a few studies in Brazil evaluated toxicity of products from *A. indica* on leaf-cutter ants of the genus *Atta*. Raw extracts of neem seed oil were very toxic to workers of *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) in a laboratory (Santos-Oliveira et al. 2006) and *Atta sexdens piriventris* Santschi (Hymenoptera: Formicidae) in the field (Boff et al. 2016). Raw solid-paste extracts of neem seeds were effective in the laboratory (Santos-Oliveira et al. 2006) and field (Gruber and Valdix 2003). Despite the promising relevance of neem derivatives, no assessment to date evaluated concentration and post-exposure time that cause efficacy of commercial neem products on ants of the genus *Atta* (Gahukar 2014).

In Mexico, previous studies indicated *A. mexicana* was one of the most abundant species of leaf-cutter ants and caused significant economic losses in different crops (Pérez-Torres et al. 2009, Perez et al. 2011, Vásquez-Bolaños 2015, Aragón-García et al. 2016, Serratos et al. 2017). Several producers expressed interest and need for agroecological alternatives to efficiently control the pest (Valenzuela-González et al. 2008, Soti et al. 2018). The purpose of this work was to assess factors that affect refined azadirachtin oil on mortality of forager workers of the leaf-cutter ant *A. mexicana* in the laboratory. We assessed whether azadirachtin concentration and post-exposure time were potential factors killing ants. Considering the growing demand for insecticide-free agricultural production, it is important to elucidate the efficacy of alternative or plant-derived insecticides for controlling leaf-cutter ants and other pests.

Materials and Methods

A colony of *Atta mexicana* was founded by fertilized queens collected in May 2010 from a coffee plantation at Coatepec, Veracruz, Mexico. The colony was reared at 25 ± 1°C and fed fresh leaves of *Mangifera indica* L. (Sapindales: Anacardiaceae), *Palicourea padifolia* Roem & Schult (Gentianales: Rubiaceae), *Rosa* spp. L. (Rosales: Rosaceae), *Styrax glabrescens* Benth (Ericales: Styracaceae), and *Trema micrantha* Roem & Schult (Rosales: Cannabaceae).

Four years after colony formation, ant workers of the soldier sub-caste were extracted, dry mounted, and identified morphologically. Keys by Mackay and Mackay (1989) were used to identify to genus and by Borgmeier (1959) to species. Specimens had smooth and shiny occipital lobes with no setae or pubescence. The first segment of the gaster had a shallow occipital groove with an opening of more than 90º and a head width greater than 4.6 mm. Specimens were corroborated by Formicidae curators (Luis N. Quiroz and Jorge E. Valenzuela) after deposited at the Entomological Collection of the Instituto de Ecología, A.C. in Xalapa, Veracruz, Mexico (IEXA; Reg. SEMARNAT: Ver. IN.048.0198). Ant workers from the same experimental colony were used in a previous study assessing leaf quality of different fern species (Mehltreter and Valenzuela 2012). All bioassays and maintenance of the *A. mexicana* colony were done at the Unidad de Manejo y Conservación de Recursos Genéticos of the Facultad de Ciencias Biológicas y Agropecuarias region Orizaba-Córdoba in Amatlán de Los Reyes, Veracruz, Mexico.

Commercial insecticide Azaním® was used for all bioassays (Biokrone 2015). It is a certified natural botanical insecticide formulated from 30 g/ liter or 30,000 mg/ liter of refined azadirachtin oil (Biokrone 2015).

The bioassays had a completely randomized design with bifactorial structure. Experimental factors were concentration of azadirachtin (10 serial dilutions) and post-exposure time (0, 50, and 100 hours). An experimental unit was a colorless, cylindrical, plastic container 7 cm tall and 8 cm in diameter containing 30 worker ants of the forager subcaste. Being a very polymorphic species, ants with similar body size (head width between 4.7 and 5.1 mm) were used throughout the experiment.

Thirty worker ants (experimental unit) were exposed by direct contact to a 2.5-ml solution of 20 different concentrations of azadirachtin: 3×10^4, 3×10^3, 3×10^2, 3×10^1, 3×10^0, 3×10^{-1}, 3×10^{-2}, 3×10^{-3}, 3×10^{-4}, 3×10^{-5}, 3×10^{-6}, 3×10^{-7}, 3×10^{-8}, 3×10^{-9}, 3×10^{-10}, 3×10^{-11}, 3×10^{-12}, 3×10^{-13}, 3×10^{-14}, or 3×10^{-15} mg/ liter. The solutions were made with sterile distilled water in sterile conditions, and no adjuvants or adherents were used. The solutions were applied using a cylindrical atomizer of Uline® brand, model S-20077 (Uline Shipping Supplies, S. de R. L. de C. V., Apodaca, Nuevo León, Mexico). Because the atomizer spray volume was 0.25 ml, 10 applications were made in the experimental unit to uniformly cover all 30 ants. At 24 hours after exposure, ants in the experimental units received water and *Rosa* sp. stems as food during the remaining experiment. The check was 2.5 ml of sterile distilled water. All experimental units were maintained at 25 ± 1°C.

The number of dead ants in each experimental unit was recorded at Time 0 immediately after spraying different concentrations of azadirachtin and at 50 and 100 hours. Because azadirachtin is hydro- and photosensitive, its insecticidal activity was deactivated 100 hours after application (Singh 2012). Therefore, the number of dead ants was recorded at 50 and 100 hours after exposure to the insecticide. Mortality was determined by quantifying the number of dead ants observed at each exposure time and visually corroborated by recording the number of live ants.

Based on the first bioassay to detect susceptibility of ants to azadirachtin, mean lethal concentration (LC_{50}) at 0, 50, and 100 hours was determined for 10 azadirachtin concentrations (3×10^4 to 3×10^{-15} mg/ liter) to obtain a range of concentrations that killed 10 to 90% of the ants.

The response variable in all experiments was the number of dead ants expressed as a percentage. Because mortality is a binomial variable dependent on counts, it does not meet normality assumptions. To avoid transforming it, means were analyzed by non-parametric statistical tests. Distributions of ants were examined using a Kaplan-Meier survival test in response to azadirachtin concentrations.

To determine the effect of the evaluated concentrations and exposure times on ants, Generalized Linear Models (GLMs) with a binomial distribution and *logit* link function were used. Evaluated concentrations and post-exposure times at which dead ants were counted were considered fixed factors. When significant effects were found for either evaluated factor, paired Tukey HSD comparisons were made between levels of each factor to determine significant differences between treatments.

LC_{50} of each exposure time (0, 50, and 100 hours) was estimated by regression analysis with a binomial distribution using *Probit* link function (Pacheco and Rebelo 2013). All analyses were done using statistical package R 3.5 (R Development Core Team 2016).

To classify toxicity of the concentrations, a scale proposed by the International Organization for Biological Control (IOBC) was used, which is defined by the magnitude of mortality reduction compared with the check. The scale ranged from 1 to 4: 1 was a harmless insecticide that killed <30%; 2 was slightly toxic, killing 30 to 79%; 3 was moderately toxic, killing 80 to 99%; and 4 was very toxic, killing >99% (Hassan et al. 1994).

Results

A Kaplan-Meier test indicated significant difference in mortality rate between exposure concentrations ($P < 0.05$), and a marked difference among large concentrations and the check ($P < 0.01$, Fig. 1). Azadirachtin concentration ($F_{1,9} = 53.83$, $P < 0.001$) and post-exposure time ($F_{1,9} = 256.35$, $P < 0.001$) independently and significantly affected mortality of *A. mexicana* (Fig. 2). Interaction between both factors resulted in significant variation in numbers of workers killed ($F_{1,9} = 15.30$, $P < 0.001$). Paired comparisons indicated that at 0 hours post-exposure, *A. mexicana* workers were very susceptible to concentrations of 3×10^4, 3×10^3, and 3×10^2 mg/ liter of azadirachtin, while concentrations of 3×10^{-15} to 3×10^{-1} mg/ liter did not have any effect on mortality (Tukey, $P < 0.001$, Fig. 2a). At 50 hours, ants exposed to concentrations of 3×10^4 to 3×10^1 mg/ liter were significantly more susceptible compared to those treated with concentrations less than 3×10^{-12} mg/ liter of azadirachtin ($t = 12.67$, $P < 0.001$, Fig. 2b). Finally, at 100 hours post-exposure, the ants were significantly susceptible to the 20 evaluated concentrations mostly between 3×10^4 and 3×10^{-6} mg/ liter (Tukey, $P < 0.01$, Fig. 2c).

According to IOBC classification, at 0 hours post-exposure, only the pure concentration (3×10^4 mg/ liter) was categorized as toxic, while concentrations of 3×10^3 and 3×10^2 mg/ liter were moderately toxic, and of 3×10^1 mg/ liter was slightly toxic. The remaining 16 concentrations were considered harmless. At 50 hours post-exposure, the toxic concentrations were 3×10^4, 3×10^3, 3×10^2, and 3×10^1 mg/

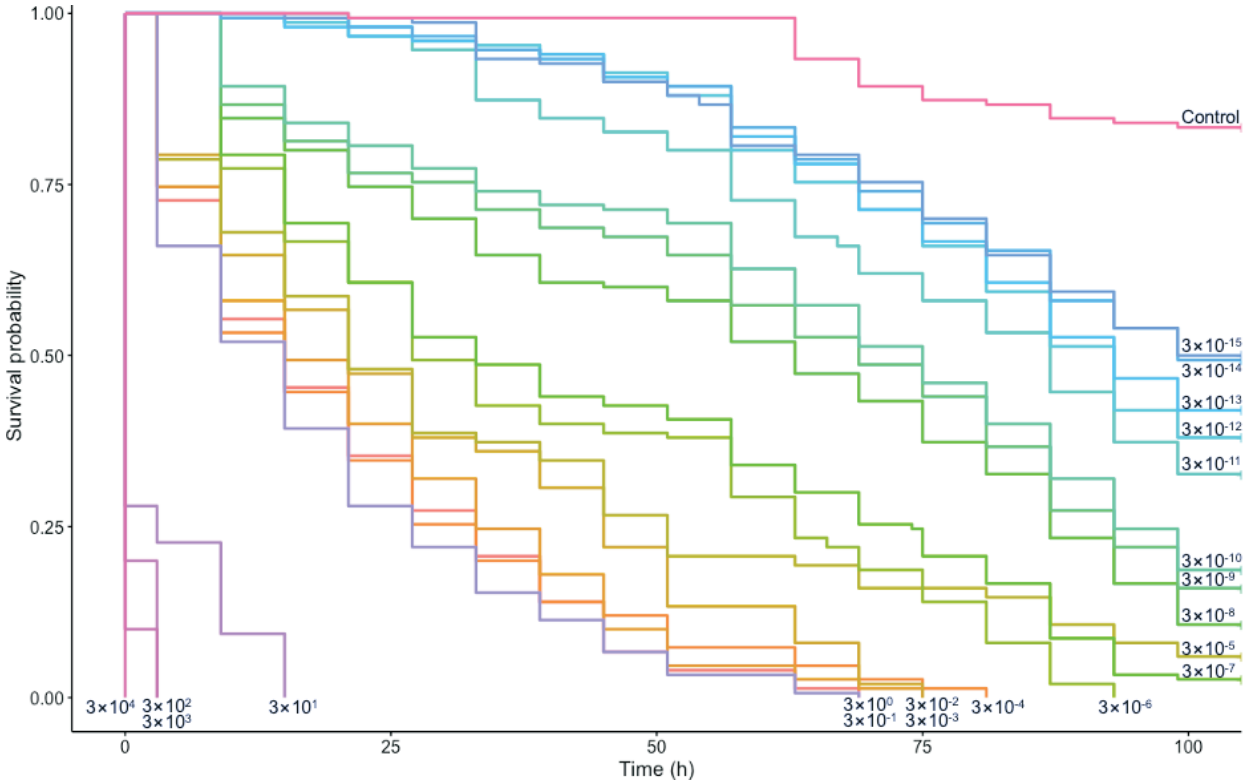

Fig. 1. Survival of *Atta mexicana* workers treated with 20 azadirachtin concentrations. Solid lines represent cumulative survival curves from Kaplan-Meier analysis. Pairwise comparisons with the Kaplan-Meier survival test indicated differences among concentrations ($P < 0.01$).

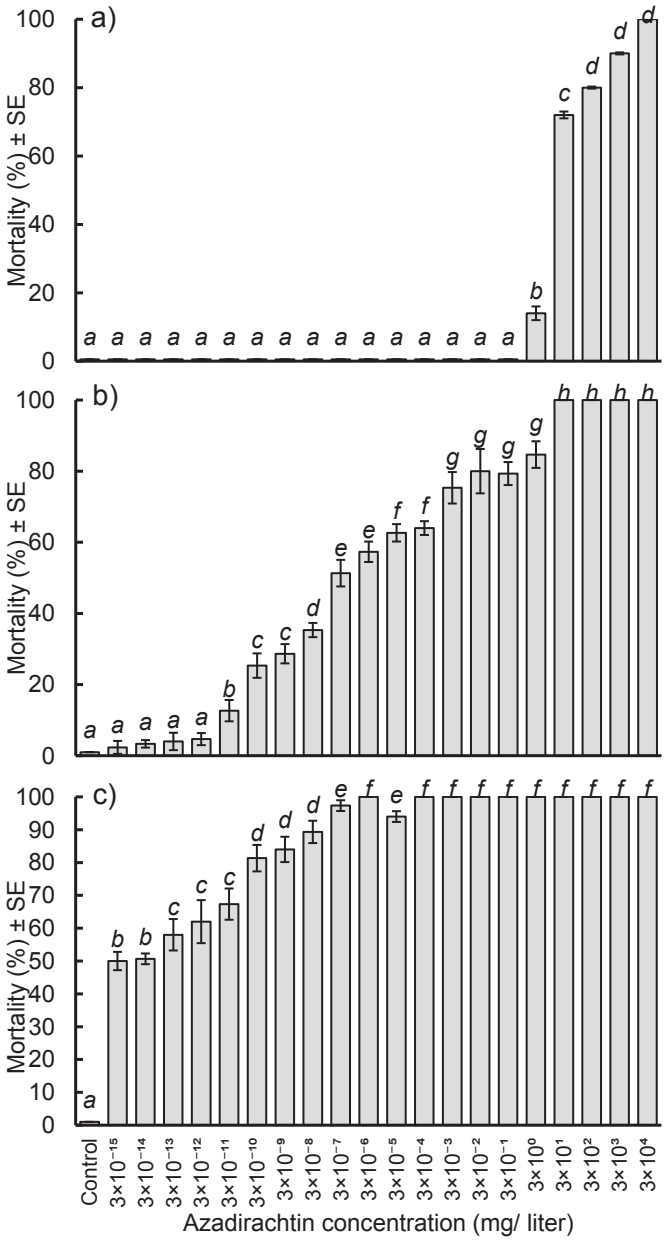

Fig. 2. Susceptibility of forager workers of *Atta mexicana* to different concentrations of azadirachtin at 0 (a), 50 (b), and 100 hours (c) post-exposure. Mortality of *A. mexicana* (percentage of dead ants) ± standard error (SE) was compared between different concentrations of azadirachtin and the check treatment; bars labeled with the same letter are not significantly different (HSD Tukey test, $P < 0.05$).

liter, while moderately toxic were 3×10^0 and 3×10^{-2} mg/ liter. The concentrations of 3×10^{-1}, 3×10^{-3}, 3×10^{-4}, 3×10^{-5}, 3×10^{-6}, 3×10^{-7}, and 3×10^{-8} mg/ liter were slightly toxic, and the remaining seven concentrations were harmless. At 100 hours post-exposure, the toxic concentrations were 3×10^4, 3×10^3, 3×10^2, 3×10^1, 3×10^{-1}, 3×10^{-3}, 3×10^{-4}, and 3×10^{-6} mg/ liter. Moderately toxic concentrations were 3×10^{-5}, 3×10^{-7}, 3×10^{-8}, 3×10^{-9}, and 3×10^{-10} mg/ liter, and slightly toxic were 3×10^{-11}, 3×10^{-12}, 3×10^{-13}, 3×10^{-14}, and 3×10^{-15} mg/ liter.

LC_{50} estimated by the *Probit* regression model varied significantly according to post-exposure time (Table 1). At 0 hours post-exposure, the estimated LC_{50} was between the concentrations 3×10^0 and 3×10^3 mg/ liter, at 50 hours between 3×10^{-11} and 3×10^0 mg/ liter, and at 100 hours between 3×10^{-15} and 3×10^{-8} mg/ liter (Table 1, Fig. 2).

Table 1. Comparison of Toxicity of Azadirachtin on Foraging Workers of *Atta mexicana* at Different Post-exposure Times

Post-exposure time (hours)	LC_{50} (mg/ liter)	Fiduciary limits 95% Lower	Upper	Heterogeneity X^2	d.f.	P
0	2.26×10^1	1.54×10^1	3.27×10^1	123.75	1	< 0.001
50	1.67×10^{-6}	7.59×10^{-7}	3.68×10^{-6}	101.63	1	< 0.001
100	1.65×10^{-14}	4×10^{-15}	6.86×10^{-14}	51.66	1	< 0.001

Discussion

Concentration and post-exposure time of refined azadirachtin oil shape mortality of the leaf-cutter ant, *Atta mexicana* (Figs. 1-2, Table 1). Worker ants were very susceptible to azadirachtin. Concentrations of 300 to 30,000 mg/ liter of azadirachtin were very toxic to *A. mexicana* worker ants, killing 100% following direct spray application. We decided to test a wide range of concentrations because there is not enough information on the toxic effect and azadirachtin concentration required to kill *A. mexicana* worker ants.

Azadirachtin was proposed as a very toxic organic insecticide for cutter ants (Santos et al. 2007). Numbers of treated ants increased significantly and gradually in response to increasing concentration of azadirachtin oil (Fig. 2). Along the evaluated gradient, it was possible to observe some indicators of symptomatology previously reported for azadirachtin intoxication (Singh 2012, Biokrone 2015). In general, in this study ants poisoned by azadirachtin began to move slower and be disoriented. The activity of workers (that is, the number of ants in motion) decreased significantly compared to the check group, for which no decrease was observed. Therefore, use of azadirachtin oil to control *A. mexicana* would directly impact and decrease foraging activity.

In the second bioassay, mean lethal concentration was determined at different post-exposure times. Only a very small quantity of azadirachtin was needed to kill 50% of the experimental ants (Table 1). Similar results were reported by Amaral et al. (2019), where 6.0 mg/ml of concentrated azadirachtin killed all of *A. sexdens* worker ants 72 hours post-exposure. The results differed greatly from previous studies in which much greater concentrations were required to kill 50% of different *Atta* spp. (Santos-Oliveira et al. 2006, Bueno and Bueno 2011). The differences

might be caused by use of a concentrated product versus extracted compounds or the application of vegetative parts of the plant in mixtures. Therefore, the botanical insecticide Azaním® formulated from refined azadirachtin oil can be used to effectively control *A. mexicana*. The method is an alternative agroecological management strategy with low cost and low environmental impact on agroecosystems.

Based on previously analyzed methods and techniques of applying agrochemicals in central Veracruz (Landero-Torres personal communication), the direct contact method was chosen to apply insecticide to the studied ants. The reliability of insecticide products was found to increase when applied directly (Boff et al. 2016). In addition, direct application by spray or drip seems a viable technique for applying azadirachtin to wild ant nests in the field (Bueno and Bueno 2011, Biokrone 2015). Use of sublethal doses of azadirachtin undetectable by exposed ants could encourage horizontal transmission of the toxic agent. The scenario would be feasible only if ants intoxicated or contaminated with azadirachtin were introduced to an anthill and then transmitted the toxic agent during contact or grooming with ants in the colony (Oliveira et al. 2011, Della Lucia et al. 2014).

The direct contact method contrasts with other methods of intoxication to control other species of *Atta* spp. reliant on indirect contact or ingestion of toxic substances (Gruber and Valdix 2003, Santos-Oliveira et al. 2006, Boff et al. 2016). We avoided using the methods because ants, especially those of the genus *Atta,* have great olfactory capacity and can detect toxic elements. In *A. mexicana*, the greatest olfactory capacity is in the forager worker sub-caste (López-Riquelme et al. 2006). Previously, neem extracts were reported as insect repellents (Santos-Oliveira et al. 2006, Singh 2012). Therefore, ingestion or application of azadirachtin oil by indirect contact could have low efficacy in *A. mexicana*.

In conclusion, toxicity of azadirachtin on the leaf-cutter ant *A. mexicana* is of great importance for agroecological pest management strategies in agriculture. Application of botanical insecticide such as Azaním®, instead of chemical insecticides would have a positive impact on biotic and abiotic resources of agroecosystems. Also, use of organic insecticides has many ecological and economic advantages for producers. For example, producers able to control pests in crops without using polluting products could obtain organic certification for their crops. Thus, disseminating findings of this work and other work evaluating organic insecticides is important for the agricultural sector and corresponding phytosanitary authorities to increase the likelihood the findings will be applied in the field.

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References Cited

Amaral, K. D., L. C. Gandra, M. A. de Oliveira, D. J. de Souza, and T. M. Della Lucia. 2019. Effect of azadirachtin on mortality and immune response of leaf-cutting ants. Ecotoxicology 28: 1190-1197.

Angulo-Escalante, M., A. Gardea-Béjar, R. R. Vélez, G. R. Estrada, A. Carrillo-Fasio, C. Cháidez-Quiroz, and J. Partida-López. 2004. Contenido de azadiractina A en semillas de nim (*Azadirachta indica* A. Juss) colectadas en Sinaloa, México. Rev. Fitotec. Mex. 27: 305-311.

Aragón-García, A., B. C. Pérez-Torres, G. Calderón-García, D. Castillo-Hernández, M. Aragón-Sánchez, and D. J. Ramón. 2016. Uso del complejo de hongos de la tortilla de maíz (*Zea mays* L.) para el combate de la hormiga arriera *Atta mexicana* (Smith 1858) (Hymenoptera: Formicidae). Entomol. Mex. 3: 153-158.

Aristizábal, L. F., L. V. Cardona, E. R. Henao, M. Salgado, and S. P. Arthurs. 2013a. Insects associated with tropical foliage produced in the coffee growing region of Colombia. Rev. Bras. Entomol. 57: 313-318.

Aristizábal, L. F., K. A. Ospina, U. A. Vallejo, E. R. Henao, M. Salgado, and S. P. Arthurs. 2013b. Entomofauna associated with *Heliconia* spp. (Zingiberales: Heliconiaceae) grown in the central area of Colombia. Fla. Entomol. 96: 112-119.

Benelli, G., A. Canale, C. Toniolo, A. Higuchi, K. Murugan, R. Pavela, and M. Nicoletti. 2017. Neem (*Azadirachta indica*): towards the ideal insecticide? Nat. Prod. Res. 31: 369-386.

Biokrone. 2015. Azaním. http://www.biokrone.com/azanim.php Accessed March 2019.

Boff, P., A. Giesel, and M. I. Carissimi Boff. 2016. No-residual baits and farmer perception to manage leaf-cutting ants. Agroecol. Sustain. Food Syst. 40: 451-465.

Borgmeier, T. 1959. Revision der gattung *Atta Fabricius* (Hymenoptera, Formicidae). Studia Ent. 2: 321-390.

Bueno, O. C., and F. C. Bueno. 2011. Plantas insecticidas: perspectivas de uso no controle de formigas-cortadeiras, pp. 359-372. *In* T. M. C. Della Lucia [ed.], Formigas-Cortadeiras: Da Bioecologia ao Manejo. Editora UFV, Viçosa, Brazil.

Della Lucia, T. M., L. C. Gandra, and R. N. Guedes. 2014. Managing leaf-cutting ants: peculiarities, trends and challenges. Pest Manag. Sci. 70: 14-23.

Gahukar, R. T. 2014. Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: a review. Crop Prot. 62: 93-99.

Gruber, A. K., and J. K. Valdix. 2003. Control de *Atta* spp. con prácticas agrícolas e insecticidas botánicos. Manejo Integr. Plagas y Agroecol. 87-90.

Hassan, S. A., F. Bigler, H. Bogenschütz, E. Boller, J. Brun, J. N. M. Calis, J. Coremans-Pelseneer, C. Duso, A. Grove, U. Heimbach, N. Helyer, H. Hokkanen, G. B. Lewis, F. Mansour, L. Moreth, L. Polgar, L. Samsøe-Petersen, B. Sauphanor, A. Stäubli, G. Sterk, A. Vainio, M. van de Veire, G. Viggiani, and H. Vogt. 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group «pesticides and beneficial organisms». Entomophaga 39: 107-119.

Landero-Torres, I., M. Á. García-Martínez, M. E. Galindo-Tovar, O. R. Leyva-Ovalle, H. E. Lee-Espinosa, J. Murguía-González, and J. Negrín-Ruiz. 2014. An ornamental heliconias crop as a reservoir of the native myrmecofauna: a case of tropical horticulture in central Veracruz, Mexico. Southwest. Entomol. 39: 135-147.

Leal, I. R., R. Wirth, and M. Tabarelli. 2014. The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. Biotropica 46: 516-528.

López-Riquelme, G. O., E. A. Malo, L. Cruz-López, and M. L. Fanjul-Moles. 2006. Antennal olfactory sensitivity in response to task-related odours of three castes

of the ant *Atta mexicana* (Hymenoptera: Formicidae). Physiol. Entomol. 31: 353-360.

Mackay, W. P., and E. E. Mackay. 1989. Clave de los géneros de hormigas en México (Hymenoptera: Formicidae), pp. 1-82. *In* L. N. Quiroz-Robledo, and L. M. P. Garduño-Hernández [eds.], Memorias del II Simposio Nacional de Insectos Sociales. SME-CIEAMAC. Oaxtepec, Morelos, México. https://www.researchgate.net/publication/255645332_CLAVE_DE_LOS_GENEROS_DE_HORMIGAS_EN_MEXICO_HYMENOPTERA_FORMICIDAE Accessed March 2019.

Médiène, S., M. Valantin-Morison, J. P. Sarthou, S. De Tourdonnet, M. Gosme, M. Bertrand, J. Roger-Estrade, J. N. Aubertot, A. Rusch, N. Motisi, C. Pelosi, and T. Doré. 2011. Agroecosystem management and biotic interactions: a review. Agron. Sustain. Dev. 31: 491-514.

Mehltreter, K., and J. Valenzuela. 2012. Leafcutter ants as test organisms for leaf quality of ferns. Indian Fern J. 29: 262-268.

Murguía-González, J., I. Landero-Torres, O. R. Leyva-Ovalle, M. E. Galindo-Tovar, R. C. Llarena-Hernández, E. Presa-Parra, and M. A. García-Martínez. 2018. Efficacy and cost of trap-bait combinations for capturing *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) in ornamental palm polycultures. Neotrop. Entomol. 47: 302-310.

Murguía-González, J., O. R. Leyva-Ovalle, M. E. Galindo-Tovar, I. Landero-Torres, R. C. Llarena-Hernández, and M. A. García-Martínez. 2018. New records of ants in Oaxaca, Mexico: their agricultural importance in sugarcane crops. Agrociencia 52: 379-391.

Oliveira, M. A., M. S. Araújo, C. G. S. Marinho, M. M. R. Ribeiro, and T. M. C. Della Lucia. 2011. Manejo de formigas-cortadeiras, pp. 400-419. *In* T. M. C. Della Lucia [ed.], Formigas-cortadeiras: Da Bioecologia ao Manejo. Editora UFV, Viçosa, Brazil.

Pacheco, A. G., M. de F. Rebelo. 2013. A simple R-based function to estimate lethal concentrations. Mar. Environ. Res. 91: 41-44

Pérez-Torres, B. C., A. Aragón-García, N. Bautista-Martínez, A. M. Tapia-Rojas, and J. F. López-Olguín. 2009. Entomofauna asociada al cultivo de jamaica (*Hibiscus sabdariffa* L.) en el municipio de Chiautla de Tapia, Puebla. Acta Zool. Mex. 25: 239-247.

Pérez-Torres, B. C., A. Aragón-García, R. Pérez Avilés, L. R. Hernández, and J. F. López-Olguín. 2011. Estudio entomofaunístico del cultivo de amaranto (*Amaranthus hypochondriacus* L.) en Puebla, México. Rev. Mex. Cienc. Agric. 2: 359-371.

R Development Core Team. 2016. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput. Vienna Austria.

Santos, A. V., B. L. De Oliveira, and R. I. Samuels. 2007. Selection of entomopathogenic fungi for use in combination with sub-lethal doses of imidacloprid: perspectives for the control of the leaf-cutting ant *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Mycopathologia 163: 233-240.

Santos-Oliveira, M. F. S., O. C. Bueno, T. Marini, I. C. Reiss, and F. C. Bueno. 2006. Toxicity of *Azadirachta indica* to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Sociobiology 47: 423-431.

Serratos, C., A. Aragón-García, B. C. Pérez-Torres, and F. López. 2017. Alternativa agroecológica para el manejo de *Atta mexicana* en Puebla, México. Southwest. Entomol. 42: 261-273.

Singh, D. K. 2012. Toxicology: Agriculture and Environment - Pesticide Chemistry and Toxicology. Bentham Science Publishers.

Soti, P., Q. Van Camp, and A. Racelis. 2018. Laboratory evaluation of efficacy of entomopathogenic nematodes on Texas leaf-cutting ants, *Atta texana*. Southwest. Entomol. 43: 635-637.

Stokstad, E. 2013. Pesticide planet. Science 341: 730-731.

Valenzuela-González, J., L. Quiroz-Robledo, and D. L. Martínez-Tlapa. 2008. Hormigas (Insecta: Hymenoptera: Formicidae), pp. 107-121. *In* R. H. Manson, V. Hernández-Ortiz, S. Gallina, K. Mehltreter [eds.], Agroecosistemas Cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología A.C. (INECOL) e Instituto Nacional de Ecología (INE-SEMARNAT), Xalapa, Mexico http://www2.inecc.gob.mx/publicaciones2/libros/542/cap8.pdf Accessed March 2019.

Vásquez-Bolaños, M. 2015. Taxonomía de formicidae (Hymenoptera) para México. Métodos en Ecol. y Sist. 10: 1-53.

Ward, P. S., S. G. Brady, B. L. Fisher, and T. R. Schultz. 2015. The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Syst. Entomol. 40: 61-81.

Effects of *Equisetum arvense* Ethanolic Extract on Biological Parameters of *Tetranychus merganser* Boudreaux[1]

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Abstract. The red spider mite, *Tetranychus merganser* Boudreaux (Acari: Tetranychidae), is an important agricultural pest in Australia, China, Thailand, the United States, and Mexico because feeding causes severe damage and defoliation of plants. The aim of the study was to evaluate effects of *Equisetum arvense* L. ethanolic extract on biological parameters of *T. merganser* in a laboratory. Different concentrations (10, 50, 100, 250, 500, and 1,000 µg/ml) of ethanolic extract were applied to *T. merganser* females. *E. arvense* extract did not kill *T. merganser*, but increased the number of eggs by 19.9, 22.5, and 21.2% compared with a check at 24, 48, and 72 hours, respectively. It also augmented the growth rate and finite rate of increase, and decreased doubling time of the red spider mite. The effects require further study because the plant extracts could have considerable implications for natural enemies and also might be suitable for mass production of red spider mites for other experiments in a laboratory.

Introduction

The red spider mite, *Tetranychus merganser* Boudreaux (Acari: Tetranychidae), is an important agricultural pest in Australia, China, Thailand, the United States, and Mexico (Ullah et al. 2011ab, Chacón-Hernández et al. 2020a, Montelongo-Ruíz et al. 2020). Feeding causes severe damage by destroying epidermal tissue, parenchyma, and chloroplasts of leaves and affecting growth, development, and production of host plants (López-Bautista 2014, López-Bautista et al. 2016). Control of *T. merganser* includes synthetic insecticides, biological control (predators) (Reyes-Pérez et al. 2015), or such cultural practices as crop rotation, fertilization, and irrigation management (López-Bautista 2014). However, red spider mites are persistent and return to crops, possibly because of elimination of natural predators (Reyes-Pérez et al. 2015) or development of resistance (Christal-Catalani et al. 2016) because of excess chemical control. Another explanation is the phenomenon of hormoligosis that involves increased reproductive rate, shorter

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development time, or change to early reproduction because of application of low concentrations of pesticides, antibiotics, hormones, temperature, radiation, or phytochemicals (Luckey 1968, Costa-Comelles et al. 1988, Cohen 2006, Guedes et al. 2009).

Use of plant extracts for biological control is an alternative to control mites. Horsetail, *Equisetum arvense* L. (Equisetaceae), is used for biological control of arthropod pests in the tea crop [*Camellia sinensis* (L.) O. Kuntze (Theaceae)] (Gurusubramanian et al. 2008, Roy et al. 2016) and has acaricidal effects against purple tea mite, *Calacarus carinatus* Green (Acari: Eriophyidae) (CABI 2019). It also is used in a smoker for fumigating honey bee colonies infested by *Varroa jacobsoni* (Mesostigmata: Varroidae) (FAO 1986). The plant is an important source of phytochemical compounds such as alkaloids, carbohydrates, phytosterols, saponins, sterols, ascorbic acid, silicic acid, phenol, tannin, flavonoids, triterpenoids, volatile oils, minerals, and other biologically active components (Mir et al. 2013, Al-Snafi 2017). Horsetail extracts are used mostly for medicine (Do Monte et al. 2004, Gallardo-Pérez et al. 2006, Bessa-Pereira et al. 2012, Al-Snafi 2017).

Few studies reported that plant extracts stimulate oviposition by mites such as ethanolic extract of *Chamaedorea radicalis* (Arecaceae) on *T. merganser* (Montelongo-Ruiz et al. 2020), and aqueous extract of *Polygonum hydropiper* L. (Polygonaceae) on *Oligonychus coffeae* Nietner (Tetranychidae: Acarina) (Roy et al. 2011). This study evaluated different concentrations of *E. arvense* ethanolic extract on biological parameters of *T. merganser*.

Materials and Methods

An initial colony of *Tetranychus merganser* was started with biological material collected from papaya (*Carica papaya* L., Caricaceae) plants at Victoria City (23° 44' 38.4'' N y 99° 9' 57.599 W, 329 m above sea level), Tamaulipas State, Mexico. The red spider mites were maintained on bean [*Phaseolus vulgaris* L. (Fabaceae)] plants at 29 ± 4°C and 60 ± 15% relative humidity in a greenhouse.

Dry *E. arvense* was purchased from Plantavit in Mexico City. Dry aerial parts of *E. arvense* were crushed in a mortar, sieved through a 2.8-mm mesh (No. 7), and subsequently dried for 72 hours) at 45°C in an oven (ON-12G, Lab Companion, Korea). Extraction was by mashing 100 g of dry plant material in 1,000 ml of 70% ethanol (CTR, Mexico) for 5 days at 25 to 37°C at room temperature in the dark. The ethanol was evaporated by rotary evaporation (RE100-Pro, DLAB Scientific, United States) and stored at 4°C until used (García et al. 2012). Four milligrams of solid ethanolic extract of *E. arvense* was weighed and diluted in 2 ml of ethanol to obtain a concentration of 2,000 µg/ml. Different concentrations (1,000, 500, 250, 100, 50, and 10 µg/ml) were prepared from the concentrated solution.

The experiment was done at 27 ± 1°C, 70-80% relative humidity, and photoperiod of 12:12 light:dark hours in a laboratory. A sand technique by Ahmadi (1983) was used with slight modification. A bean leaf disc 2.5-cm in diameter was put with the underside up on water-saturated cotton in a 5-cm-diameter Petri dish.

Ten adult females were put with five adult males of red spider mite to improve reproduction and oviposition in each experimental unit. After 24 hours, a brush was used to help remove males, leaving only females on leaf discs (Chacón-Hernández et al 2020b, Montelongo-Ruíz et al. 2020). Different concentrations of *E. arvense* extract (10, 50, 100, 250, 500, and 1,000 µg/ml) were applied.

A sample unit of mites on three replications (leaf discs) was counted for each treatment and check, analyzing 21 replications per date counted. The treatments and check were arranged in a completely randomized design. A manual sprayer (Model 14687, Truper®, Victoria City, Tamaulipas, Mexico) was used to spray each bean leaf disc with 1.4 ± 0.1 ml of extract concentration. The check was sprayed only with water. Mites were counted at 24, 48, and 72 hours after application of each treatment.

The Kramer and Mulla (1979) formula was used to determine "Oviposition Activity Percentage" (OAP) for each concentration:

$$OAP = [(N_T - N_C)/(N_T + N_C)] \times 100 \qquad (1)$$

where N_C is the number of eggs in the check and N_T is the number of eggs in the treated group. Percentages ranged from +100 to -100. Positive values indicated more oviposition in the treatment than the check (indicating the treatment stimulated oviposition), and conversely, more oviposition in the check than in the treatment resulted in a negative OAP (indicating the treatment inhibited oviposition).

Instantaneous rate of increase(r_i), finite rate of increase(λ), and population doubling time (D_t) were calculated to indicate population growth r_i calculated by:

$$r_i = \frac{ln(N_f/N_0)}{\Delta t} \qquad (2)$$

where N_0 is the initial number of spider mites (i.e., 10 adult females per replication), N_f is the end number of spider mites (i.e., number of adult females that survived and several eggs laid at the end of the bioassay, i.e., third day), and Δt is the number of days of the experiment (i.e., 3 days). Positive r_i values indicated a growing population; negative r_i values indicated a decrease in abundance, and $r_i = 0$ indicated a stable population (Stark and Banks 2003). The finite rate of increase, or number of times the population multiplied in a unit of time, was calculated as:

$$\lambda = antilog_e\, r_i \qquad (3)$$

Doubling time in which the population doubled (Birch 1948) was calculated by:

$$D_t = Ln(2)/r_i \qquad (4)$$

Eggs laid were counted daily using a dissecting microscope (UNICO Stereo & Zoom Microscopes ZM180, Dayton, NJ). The number of eggs laid and demographic parameters (r_i, λ, and D_t) were analyzed using analysis of variance (ANOVA) with mean comparison by Tukey's HSD test. The R-project program (R Core Team 2018) was used for all analyses.

Results

Different concentrations of *E. arvense* extract did not kill *T. merganser* females, but the number of eggs was significantly more at 24, 48, and 72 hours (F = 65.656; df = 6,14; P < 0.0001; F = 36.978; df = 6,14; P < 0.0001; F = 43.622; df = 6,14; P < 0.0001), respectively. With 100 µg/ml concentration, eggs significantly increased by 7.3, 16.7, and 13.0% compared with the check at 24, 48, and 72 hours, respectively (Table 1). A maximum of 22.5% increase was reached with the 1,000 µg/ml concentration over the check at 48 hours.

The instantaneous rate of increase, finite rate of growth, and doubling time of red spider mites were significantly different among the treatments (F = 52.132; df = 6,14; P < 0.0001; F = 50.114; df = 6,14; P < 0.0001; F = 56.358; df = 6,14; P < 0.0001), respectively (Fig. 1). Mean (± SE) instantaneous rate of increase was 0.91 ± 0.00 by the check, to 1.05 ± 0.01 at 1,000 µg/ml (Fig. 1A). The average number of mites added to the population per female per day was 2.47 ± 0.01 by the check, to 2.85 ± 0.03 at 1,000 µg/ml (Fig. 1B). The mean time in which the *T. merganser* population

Table 1. Effect of Different Concentrations of *Equisetum arvense* Ethanolic Extract on *Tetranychus merganser* Oviposition

Treatment	Average number of eggs	OAP (%)	Average number of eggs	OAP (%)	Average number of eggs	OAP (%)
µg/ml	24 hours		48 hours		72 hours	
Check	48.33±0.67e*		111.67±2.02b		151.67±1.20d	
10	53.33±1.15d	4.91±0.54	116.67±4.16b	2.20±0.27	154.33±5.78d	0.87±0.48
50	55.00±1.00d	6.44±1.26	119.00±8.73b	3.13±1.37	158.67±8.66d	2.19±1.86
100	56.00±0.67cd	7.34±1.57	156.33±3.78a	16.66±1.57	197.00±1.73c	12.98±1.28
250	60.67±0.88c	11.32±0.63	160.00±1.45a	17.78±0.84	208.00±1.76bc	15.66±0.62
500	66.00±1.00b	15.45±0.71	163.00±2.90a	18.55±1.81	224.00±4.16ab	19.17±2.16
1,000	72.33±1.45a	19.87±1.59	176.67±3.38a	22.53±1.65	233.67±7.62a	21.22±1.72

*Mean ± (SE). Different letters indicate significant differences ($P < 0.05$; ANOVA and Tukey's HSD test). OAP: percentage of oviposition activity.

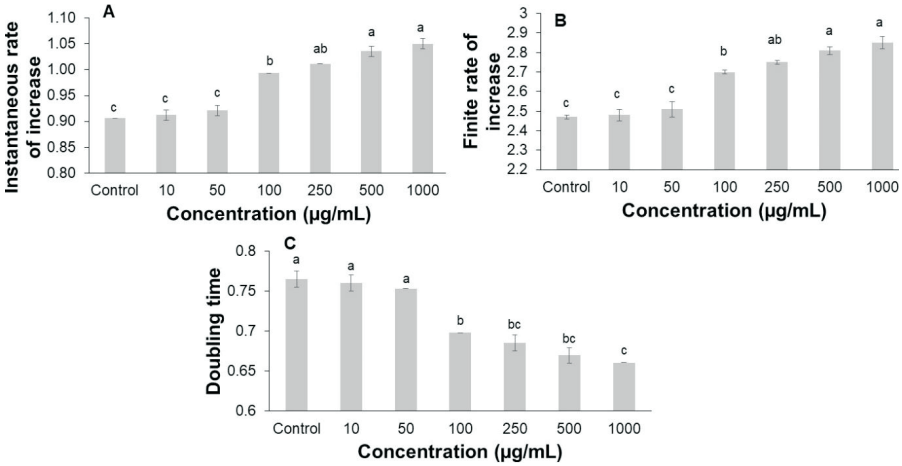

Fig. 1. Effect of *Equisetum arvense* ethanolic extract on (A) instantaneous rate of increase, (B) finite rate of increase, and (C) doubling time of *Tetranychus merganser.*

doubled decreased from 0.7648 ± 0.001 in the check, to 0.6602 ± 0.00 with 1,000 µg/ml (Fig. 1C). The increasing concentrations of the extract increased the population of red spider mites per female per day in less time than the check.

Discussion

The first stage of arthropod success in life is oviposition (Khaliq et al. 2015). Host plant phytochemical compounds such as carbohydrates, alkaloids, flavonoids, phenolics, and terpenoids increase eggs laid by several arthropods (Roy et al. 2011, Khaliq et al. 2015, Feng et al. 2017, Riddick et al. 2018, Montelongo-Ruíz et al. 2020). Montelongo-Ruíz et al. (2020) documented 2.7 to 11.1% increase in oviposition by *T.*

merganser with 5 and 1,000 µg/ml of ethanolic extract of *C. radicalis*, respectively, compared with the check. Roy et al. (2011) found that concentrations of 2, 4, and 6% of *P. hydropiper* aqueous extract stimulated oviposition in females of *O. coffeae*. Christal-Catalani et al. (2016) reported increase in eggs laid per female of *Tetranychus urticae* Koch from 24.4 in the check to 30.3 per female sprayed by potassium silicate at a dose of 4 liters ha^{-1}. Marazzi and Städler (2004) observed an increase in oviposition when 1 ml of methanolic extract of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) was applied to mature females of *Delia radicum* L. (Diptera: Anthomyiidae). Guedes and Cutler (2013) mentioned the insect could absorb an essential element that contained a stressor agent (e.g., bioinsecticide), in which the individual was deficient, favoring growth rate and possibly maximizing size, which increased reproduction and population growth rate.

No tests at cellular, biochemical, or physiological levels were done to obtain more information on oviposition stimulation effects on red spider mites. In several species of insects, cerebral neurosecretory cells produce a hormone that induces and enhances ovulation or oviposition (a myotropin) (Gillott 2018). Instantaneous growth of arthropods was favored by some factors and genes that play fundamental roles in reproduction, including data on hormonal balance associated with development of ovaries, egg yolk formation (vitellogenin), and formation and laying of eggs (Cohen 2006). Although further studies are required, results here document that concentrations of ethanolic extract of *E. arvense* stimulated *T. merganser* females by enhancing oviposition and increasing demographic parameters, which can increase the efficiency of red spider mites to develop a new system and adapt to a different environment.

In conclusion, ethanolic extract of *E. arvense* stimulated oviposition and increased instantaneous rate of increase (r_i) and finite rate of increase (λ), and decreased doubling time (D_t) of *T. merganser*. More studies are needed to evaluate other types of *E. arvense* extracts (aqueous, methanol, and acetone) with greater concentrations and assess their effect on natural enemies impacting *T. merganser*. The extract also might be suitable for mass production of red spider mites in other kinds of experiments in laboratory conditions.

References Cited

Ahmadi, A. 1983. Demographic toxicology as a method for studying the dicofol two-spotted spider mite (Acari: Tetranychidae) system. J. Econ. Entomol. 76: 239-242.

Al-Snafi, A. E. 2017. The pharmacology of *Equisetum arvense*- a review. IOSR J. Pharm. 7: 31-42.

Bessa-Pereira, C., P. S. Gomes, J. Costa-Rodrigues, R. Almeida-Palmas, L. Vieira, P. S. Ferraz, M. A. Lopes, and M. H. Fernandes. 2012. *Equisetum arvense* hydromethanolic extracts in bone tissue regeneration in vitro osteoblastic modulation and antibacterial activity. Cell Proliferat. 45: 386-396.

Birch, L. C. 1948. The intrinsic rate of increase of an insect population. J. Anim. Ecol. 17: 15-26.

CABI. 2019. *Calacarus carinatus* (purple tea mite). *In* Invasive Species Compendium. CAB International, Wallingford, UK. www.cabi.org/isc. Accessed 15 August 2020.

Chacón-Hernández, J. C., S. Ordaz-Silva, E. Mireles-Rodriguez, M. Rocandio-Rodríguez, I. V. López-Sánchez, R. T. Q. Heinz-Castro, F. Reyes-Zepeda, and

S. Castro-Nava. 2020a. Resistance of wild chili (*Capsicum annuum* L. var. *glabriusculum*) to *Tetranychus merganser* Boudreaux. Southwest. Entomol. 45: 89-98.

Chacón-Hernández, J. C., R. Arredondo-Valdés, J. C. Anguiano-Cabello, S. Ordaz-Silva, A. Hernández-Juárez, and F. Reyes-Zepeda. 2020b. Effect of *Magnolia tamaulipana* extract on egg laying and food intake of *Tetranychus urticae* (Acari: Tetranychidae). Intern. J. Acarol. 46: 108-110.

Christal-Catalani, G., M. R. Vieira, L. Santos-de-Camargos, N. C. Pires-Bomfim, and J. A. Agustini. 2016. Effects of potassium silicate application on papaya plants in the twospotted spider mite population. Rev. Bras. Frutic. 39: 1-9.

Cohen, E. 2006. Pesticide-mediated homeostatic modulation in arthropods. Pestic. Biochem. Physiol. 85: 21-27.

Costa-Comelles, J., F. García-Mari, F. Ferragut, R. Laborda, D. Roca, and C. Marzal. 1998. Influencia residual de los insecticidas butocarboxim, cipermetrina y metilazinfos en el potencial biótico de *Panonychus citri* (McGr.) (Acari: Tetranychidae). Bol. San. Veg. Plagas 14: 127-140.

Do Monte, F. H. M., J. G. dos-Santos, M. Russi, V. M. Lanziotti, L. K. Leal, and G. M. Cunha. 2004. Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L. in mice. Pharmacol. Res. 49: 239-243.

Feng, B., K. Qian, and Y.-J. Du. 2017. Floral volatiles from *Vigna unguiculata* are olfactory and gustatory stimulants for oviposition by the bean pod borer moth *Maruca vitrata*. Insects 8: 1-16.

FAO (Food and Agriculture Organization). 1986. Honeybee mites and their control: a selected annotated bibliography. FAO Agricultural Services Bulletin.

Gallardo-Pérez, J. C., M. L. Esparza-Aguilar, and A. Gómez-Campos. 2006. Importancia etnobotánica de una planta vascular sin semilla en México: *Equisetum*. Polibotánica 21: 61-74.

García, D., A. J. Ramos, V. Sanchis, and S. Marín. 2012. Effect of *Equisetum arvense* and *Stevia rebaudiana* extracts on growth and mycotoxin production by *Aspergillus flavus* and *Fusarium verticillioides* in maize seeds as affected by water activity. Int. J. Food Microbiol. 153: 21-27.

Gillott, C. 2018. Insect male mating systems, pp. 33-55. *In* S. R. Leather and J. Hardie [eds.]. Insect Reproduction. CRC Press Taylor and Francis, Boca Raton, FL.

Guedes, R. N. C., and G. C. Cutler. 2013. Insecticide-induced hormesis and arthropod pest management. Pest. Manag. Sci. 70: 690-697.

Guedes, R. N. C., L. C. Magalhães, and L. V. Cosme. 2009. Stimulatory sublethal response of a generalist predator to permethrin: hormesis, hormoligosis, or homeostatic regulation? J. Econ. Entomol. 102: 170-176.

Gurusubramanian, G., A. Rahman, M. Sarmah, S. Roy, and S. Bora. 2008. Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures. J. Environ. Biol. 29: 813-826.

Khaliq, A., M. H. Ahmad, R. Ullah, and M. Anas. 2015. Behavioral ecology of oviposition in insects – a dumpy review. SJAR 4: 1-7.

Kramer, W. L., and S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8: 1111-1117.

López-Bautista, E. 2014. Incidencia de daño y estrategias de control de *Tetranychus merganser* en el cultivo de papaya (*Carica papaya* L.). Ph.D. thesis, Colegio de Postgraduados Campus Montecillo, Texcoco, Estado de México, Mexico.

López-Bautista, E., M. T. Santillán-Galicia, J. Suárez-Espinosa, N. Cruz-Huerta, N. Bautista-Martínez, and J. A. Alcántara-Jiménez. 2016. Damage caused by mite *Tetranychus merganser* (Trombidiformes: Tetranychidae) on *Carica papaya* (Violales: Caricaceae) plants and effect of two species of predatory mite. Int. J. Acarol. 42: 303-309.

Luckey, T. D. 1968. Insecticide hormoligosis. J. Econ. Entomol. 61: 7-12.

Marazzi, C., and E. Städler. 2004. *Arabidopsis thaliana* leaf-surface extracts are detected by the cabbage root fly (*Delia radicum*) and stimulate oviposition. Physiol. Entomol. 29: 192-198.

Mir, S. A., A. K. Mishra, Z. A. Reshi, and M. P. Sharma. 2013. Preliminary phytochemical, screening of some pteridophytes from district Shopian (J & K). Int. J. Pharm. Pharm. Sci. 5: 632-637.

Montelongo-Ruiz, G., J. C. Chacón-Hernández, F. Reyes-Zepeda, P. Octavio-Aguilar, R. T. Q. Heinz-Castro, L. Juárez, and S. Ordaz-Silva. 2020. The stimulatory effect of *Chamaedorea radicalis* ethanolic extract on *Tetranychus merganser* Boudreaux (Acari: Tetranychidae). Int. J. Acarol. 46: 318-321.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reyes-Pérez, N., D. E. Platas-Rosado, J. A. Villanueva-Jiménez, M. Abato-Zárate, and M. Vargas-Mendoza. 2015. Rentabilidad de la reproducción de enemigos naturales de ácaros del papayo (*Carica papaya* L.). Agroproductividad 8: 44-51.

Riddick, E. W., Z. Wu, F. J. Eller, and M. A. Berhow. 2018. Do bioflavonoids in *Juniperus virginiana* heartwood stimulate oviposition in the ladybird *Coleomegilla maculata*? Int. J. Insect Sci. 10: 1-13.

Roy, S., G. Gurusubramanian, and S. K. Nachimuthu. 2011. Anti-mite activity of *Polygonum hydropiper* L. (Polygonaceae) extracts against tea red spider mite *Oligonychus coffeae* Nietner (Tetranychidae: Acarina). Int. J. Acarol. 37: 561-566.

Roy, S., G. Handique, N. Muraleedharan, K. Dashora, S. M. Roy, A. Mukhopadhyay, and A. Babu. 2016. Use of plant extracts for tea pest management in India. Appl. Microbiol. Biotechnol. 100: 4831-4844.

Stark, J. D., and J. E. Banks. 2003. Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48: 505-519.

Ullah, M. S., D. Moriya, M. H. Badii, G. Nachman, and T. Gotoh. 2011a. A comparative study of development and demographic parameters of *Tetranychus merganser* and *Tetranychus kanzawai* (Acari: Tetranychidae) at different temperatures. Exp. Appl. Acarol. 54: 1-19.

Ullah, M. S., D. Moriya, M. Kongchuensin, P. Konvipasruang, and T. Gotoh. 2011b. Comparative toxicity of acaricides to *Tetranychus merganser* Boudreaux and *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). Int. J. Acarol. 37: 535-543.

Chemical Composition of *Jatropha curcas*[1] Seed Extracts and its Bioactivity against *Copitarsia decolora*[2] under Laboratory and Greenhouse Conditions

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Abstract. The main objective of the study was to identify chemical compounds of extract of acetone of shell + almond (EACA) and nut of almond (EAA) of J*atrofa curcas* and evaluate bioactivity against *Copitarsia decolora* in laboratory and greenhouse conditions. In ingestion experiments, extracts at 250, 500, 1,000, 1,500, and 2,000 ppm were mixed into artificial diet. Checks were artificial diet mixed with a botanical insecticide Margosom® 0.3% EC (active ingredient [AI] 250 ppm, azadirachtin), and diet plus only water. EAA at 250 and 1,000 ppm inhibited larval weight and prolonged larval development of *C. decolora*. EAA at 500 ppm and EACA at 1,500 ppm caused most deformation in pupae and adults. As many as 50% of *C. decolora* larvae occurred with EAA at 500-. EAA was the most active extract in laboratory tests and later evaluated at 250, 500, and 1,000 ppm applied to cabbage plants infested with *C. decolora* larvae in a greenhouse. Two checks were cabbage (*Brassica oleracea* var. *capitata*) plants without extract (water only) and pyrethroid insecticide (Alfadex® AI 21.29% cypermethrin) at 500 ppm. Gas chromatography-mass spectrometry (GC-MS) analysis of the acetonic extract of *Jatropha curcas* seed identified oleic acid as the major compound. EAA at 1,000 ppm and Alfadex were most effective at protecting cabbage plants from attack by larval *C. decolora.*

Resumen. El objetivo principal de este estudio identificar los compuestos químicos del extracto de acetona de cáscara + almendra (EACA) y nuez de almendra (EAA) de *Jatrofa curcas* y evaluar su bioactividad contra *Copitarsia decolora*, en condiciones de laboratorio e invernadero. En las pruebas de ingestión, se mezclaron extractos a 250, 500, 1000, 1500, y 2000 ppm en una dieta artificial. Los controles consistieron en la dieta artificial mezclada con un insecticida botánico, Margosom® 0.3% EC (ingrediente activo [AI] 250 ppm, azadiractina) y agua más dieta solamente. EAA a 250 y 1000 ppm inhibió el peso larvario y prolongó el desarrollo larvario de *C.*

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decolora. EAA a 500 ppm y EACA a 1,500 ppm causaron el mayor porcentaje de deformaciones en pupas y adultos. Además, se produjo hasta un 50% de mortalidad de larvas de *C. decolora* con EAA a 500-. EAA fue el extracto más activo en pruebas de laboratorio y luego se evaluó a 250, 500, y 1,000 ppm cuando se aplicó a plantas de repollo infestadas con larvas de *C. decolora* en un invernadero. En este experimento se emplearon dos controles: plantas de repollo (*Brassica oleracea* var. *capitata*) sin extracto (solo agua) y un insecticida piretroide (Alfadex® AI 21,29% cipermetrina) a 500 ppm. El análisis de cromatografía de gases masas (CG-MS) del extracto acetónico de semilla de *Jatropha curcas* identificó al ácido oleico como el compuesto mayoritario. Estos resultados mostraron que EAA, a 1,000 ppm y Alfadex, fueron más efectivos para proteger las plantas de repollo del ataque de larvas de *C. decolora.*

Introduction

Crucifers are crops of great economic importance in México because they are a major food commodity exported to foreign countries such as the U.S., Canada, and Cuba (Suárez-Vargas et al. 2006, Moreno-Flores and Cerda-Lemus 2010). About 50,000 ha of vegetables in the Brassicacea family are planted in Mexico, of which 12.4% are cabbage (*Brassica oleracea* var. *capitata*) (Bujanos 2000). Cabbage-producing areas in México include the southern states of Chiapas, Oaxaca, Guerrero, Yucatán, and Tabasco. In the the center of the country the crop is produced in the states of Morelos, Puebla, Tlaxcala, and Veracruz. Puebla is the main producer of the vegetable in Mexico, with 2,704 ha cultivated representing 43% of the total area (Barrios-Díaz et al. 2004, SAGARPA 2011). However, the crop is susceptible to *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) that affects yield and quality of the commercial product, causing considerable economic loss to farmers (Bujanos 2000, Fernández and Vázquez 2003, Barrios-Díaz et al. 2004, Suárez-Vargas et al. 2006). To control the insect pest, synthetic insecticides are traditionally relied upon that often result in considerable environmental pollution in agricultural areas (Torres and Capote 2004). Insecticides negatively affect biodiversity, destroy natural ecosystems, poison wild species, and damage human health. Several insecticides currently used to control pests of cabbage lethally impact non-target species of vital importance in the ecosystem, such as pollinators, natural enemies, and soil-nutrient recyclers (Devine et al. 2008). It is necessary to use control alternatives less damaging to the environment and human health, such as botanicals that are biodegradable, specific, and economical.

Bello (2013) and Hernández-Miranda (2013) showed seeds of *Jatropha curcas* L. (Euphorbiaceae) could be used to control insect pests. Powdered seeds of the plant at 1, 2, 4, and 5% reduced larval weight, extended developmental times of larvae and pupae, deformed pupae and adults, and killed adults of *C. decolora*.

Powdered seeds of *J. curcas* have been reported to possess insecticidal properties against agave weevil, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), and fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) (Figueroa-Brito 2012). Extracts of *n*-hexane, ethyl acetate, and methanol from *J. curcas* had insecticidal effectiveness against the whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), mosquito, *Ochlerotatus triseriatus* Say (Diptera: Culicidae), corn earworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) (Georges et al. 2008). Phorbol esters of *J. curcas* seed oil also are active against *S. frugiperda* (Devappa et

al. 2012). The objective of the study was to evaluate the effect of acetonic extract of shell + almond nut or almond alone of *J. curcas* against larval *C. decolora* in laboratory and greenhouse studies.

Materials and Methods

Plants used in bioassays were collected in June 2014 at Chiapa de Corzo, Chiapas, México (16º42 "00"N, 93º11'00" W). Shell + almond nut (319.1 g) and almond nut (289.9 g) were dried and pulverized in an electric mill (SIEMENS) then sieved through a 1-mm mesh screen to obtain fine powder. The fine powder was macerated repeatedly for three replications at a photoperiod of 12:12 light:dark hours at room temperature (25 ± 3°C) for 72 hours. Each powder was dissolved separately in 500 ml of acetone (Mallinckrodt Baker® Griesheim, Germany). The solvent was separated in an R-3 BUCHI Rotavapor under reduced pressure (21 torr). Once extracts were concentrated, they were transferred to 250-ml Erlenmeyer flasks and placed in a LABHAU extractor-hood laminar flow chamber to remove solvent (Rao et al. 2012). Purity of the EACA and EAA extracts was 7.6 and 10.9%, respectively.

EACA and EAA extracts were analyzed using Agilent Technology (Santa Clara, CA) Gas Chromatography Model 7890B coupled to a mass selective detector model 5977. An HP-5MS capillary column 30 m long with 0.25-mm internal diameter and 0.25 µm particle size was used to separate extract. Two microliters of extract were dissolved with 1.5 ml of acetone. The injection volume was 2.5 µl, and the temperature at the injection port was 250°C in Splitless mode. The oven temperature initially was 60°C, with a first temperature ramp of 32°C minute^{-1} to 220°C for 5 minutes. This was followed by a second ramp of 4°C minute^{-1} to 240°C for 5 minutes that concluded with a third ramp of 5°C minute^{-1} to 260°C for 4 minutes. The post oven program was 280°C for 1 minute with a transfer-line temperature of 325°C. Spectra were collected at 70 eV ionization voltage and the mass range analyzed at 30-550 m/z with scan mode acquisition (2.8 scans/sec) in positive ionization. Compounds were identified by comparing their mass spectra with reference spectra of the NIST14 Library. In a second chemical analysis, compounds of acetonic extract of *J. curcas* seeds (i.e., EAC) were identified for chemical comparison to extracts EACA and EAA. The study was done the same way as the first chemical analysis.

Copitarsia decolora larvae were provided by the Entomology Laboratory of the Plant-Insect Interaction Department of the CeProBi-IPN in the Center for the Development of Biotic Products (San Isidro, Yautepec, Morelos, Mexico). Individuals were maintained under controlled conditions of 25 ± 3°C, 60 ± 5% relative humidity, and photoperiod of 12:12 light:dark hours. Sixty neonates were used for each treatment. Larvae were fed a general lepidopteran diet (500 g) proposed by Rojas et al. (1993) that contained 82.5 g corn flour (Maseca), 20.1 g wheat germ (Maxilu), 22 g beer yeast (Pronat Ultra), 1 g methylparaben, 0.80 g asorbic acid, 3 g ascorbic acid (Golden Bell Reagents), 3 g Ru Bi Otic (vitamins and antibiotic, Vitalab), 7.5 g agar-agar (Hycel, bacteriological grade), and 500 ml water.

EACA and EAA extracts of *J. curcas* were incorporated into artificial diet at 250, 500, 1,000, 1,500, and 2,000 ppm for use in laboratory ingestion bioassays. After preparation, the diet was allowed to cool to 45 ± 5°C for incorporation of extracts. A 1 cm^3 cube of diet was added to a 30-ml glass vial, and an artist's brush was used to put one *C. decolora* larva into each glass. Glasses with larvae and diet were put into a growth chamber with the conditions described. Two checks were one larva on diet only and another larva on diet with Margosom® 0.3% (Agri-Life, Andhra Pradesh,

India) EC botanical insecticide (active ingredient [AI] 250 ppm, azadirachtin/500 g diet) previously reported effective against *Plutella xylostella* L. (Lepidoptera: Plutillidae), another insect pest of cabbage (Singh et al. 2015).

A completely randomized experimental design was used where one larva comprised an experimental unit and was considered a replication. Response variables were weight and developmental time of larvae, pupae, deformation, and insects killed. Data were subjected to statistical analysis using SAS 9.0 software (SAS Institute, Cary, NC) that included one-way Analysis of Variance (ANOVA) and Tukey test. All reported values are mean ± standard error. Differences were considered significant at $P \leq 0.05$.

Cabbage seedlings were purchased at Los Reyes, Puebla, México and transplanted into plastic bags with soil for growth and development under controlled conditions for 60 days in a greenhouse. When plants were 20 cm tall, 12 plants were grouped for each treatment (total = 60 plants). EAA extracts were most active against larval *C. decolora* as identified from laboratory experiments. Aqueous solutions of 250, 500, and 1,000 ppm EAA were prepared for application. Two checks were pyrethroid insecticide Alfadex® (AI 21.29%, cypermethrin, Novastis, Guadalajara, Mexico) applied at 500 ppm in 250 ml water (this application rate of botanical insecticide was used to compare effect in relation to EAA that at 500 ppm was the most ingested treatment), and the other was only 250 ml water. A 500-ml airbrush (GONI® 315, Ferrecsa, Mexico) was used to spray extracts onto the plants allowed to dry for 2.5 hours. Afterward, each plant was infested with a single third-instar larva of *C. decolora* and maintained under controlled conditions of temperature and relative humidity (26 ± 3°C, 40 ± 5% relative) in a greenhouse of the University Autonomous of the State of Morelos (Chamilpa, Cuernavaca, Morelos, México) with a 3 x 5 x 3 m plastic cover. The percentage of cumulative damage during 19 days was recorded using a scale by Mihm (1984).

Results

Seventeen compounds were found in the acetonic extract of the shell+almond nut of *J. curcas* seeds (EACA), representing 99.6% of the extracted material (Table 1). Retention times ranged between 1,898 and 2,157 minutes. The compounds identified were fatty acids oleic (39.1%), linoleic (20.8%), vaccenic (19.4%), palmitic (10.7%), stearic (8.6%), and palmitoleic acid (1.0%). GC-MS analysis of the acetonic extract of almond nut alone (EAA) identified four compounds, representing 99.1% of the extracted material (Table 2) with retention time from 1,961 to 2,157 minutes. Compounds were the same fatty acids in EACA (except palmitoleic acid) and were oleic (50.3%), vaccenic (24.0%), linoleic (10.5%), palmitic (7.9%), and stearic acid (6.3%). Analysis of the acetonic extract shells (EAC) identified 16 compounds, representing 87.9% of extracted material with retention time range of 1,259 to 2,197 minutes. The compounds in the extract comprised the same fatty acids identified in EACA (except vaccenic acid) and were palmitic (9.1%), linoleic (5.1%), oleic (4.9%), palmitoleic (3.5%), and stearic acid (1.7%). Aldehyde 2-undecenal was also recovered from the extract and had a larger peak area (28.4%) than the fatty acids; this compound was not present in the other two extracts.

At 7 days and 500 ppm, EACA had the greatest phagostimulating effect on *C. decolora* larvae with a 157% significant increase in weight compared with larvae fed only check diet (water) (Table 2). The same effect occurred at 14, 21, and 28 days, where EACA at 250 ppm increased weight >185, 141, and 145%, respectively,

compared with water check. EACA at 1,000 ppm was the most active extract, with larval weight increasing by >200% by 21 days of exposure.

Table 1. Composition of Acetonic Extracts of *Jatropha curcas* Seeds

No.	Retention time (minutes)	Compound name	Area peak (%)*		
			EACA	EAA	EAC
1	1,259	2-decenal €-			9.3
2	1,312	2,4 decadienal (E,E)-			5.6
3	1,365	2-undecenal			28.4
4	1,479	Trans-2-undecenoic acid			3.7
5	1609	Tetradecanal			2.5
6	1,667	E-2-tetradecen-1-ol			1.9
7	1,696	2-pentadecanone			2.5
8	1,806	Cis-9-hexadecanal			1.7
9	1,860	δ-nonyl-δ-valeralactone			2.8
10	1,898	9-hexadecenoic acid (palmitoleic acid)	1.0		3.5
11	1,901	2-heptadecanone			2.6
12	19.61	Hexadecanoic acid (palmitic acid)	10.7	7.9	9.1
13	2,075	n-octadecanoic acid (stearic acid)	8.6	6.3	1.7
14	2,097	9-octadecenoic acid (Z)-(oleic acid)	39.1	50.3	4.9
15	21	Cis-13-octadecenoic (= vaccenic acid)	19.4	24.0	
16	2,157	Cis-9,cis-12-octadecadienoic acid (linoleic acid)	20.8	10.5	5.1
17	2,197	τ-Stearolactone			2.6
			99.6	99.1	88.6

EACA = acetonic acid of shell+almond; EAA = acetonic extract of almond; EAC = acetonic extract of shell+almond.
*Percentage of total area.

EAA at 250 and 1,000 ppm significantly inhibited weight by 53 and 44% at 21 days, to as much as 27 and 16% at 35 days, respectively, compared with the water check (Table 2). The extract was superior or similar in inhibiting larval weight as by the botanical insecticide. However, larvae fed diet with botanical insecticide weighed significantly less at Day 21, but there was no difference between weights at Day 35 compared with the water check. Larvae exposed to all EAA concentrations during their final developmental stage, at Day 35 managed to block any inhibitory effect on weight gain compared with the two checks.

Development of *C. decolora* larvae was prolonged 3 days when exposed to Margosom and 4 days by 1,000 ppm EAA extract compared with the water check (F = 14.95, p < 0.0001) (Fig. 1). Among surviving insects was a larger percentage of malformed pupae and adults (F = 53.87, p < 0.0001) when EACA, depending on concentration, produced as many as 30.7% deformed insects, respectively (Fig. 2). EAA at 500 and 1,500 ppm caused 60.0 and 34.8% deformed insects, respectively compared with water and botanical insecticide checks. At 63 days, EACA extract at 1,000 ppm produced 47% combined cumulative mortality (larval+pupae) (Fig. 3A). EAA at 500 and 2,000 ppm also caused 50 and 45% cumulative mortality, respectively (Fig. 3B). At 63 days, Margosom and check mortality averaged 18 and 20%, respectively (F = 14.95, p < 0.0001).

Table 2. Mean ± SD Weight of *Copitarsia decolora* Larvae Reared on Treated Diet Mixed with Varying Concentrations of Acetonic Extracts of *Jatropha curcas* Seeds

Extract (ppm)		Larval weight (mg ± SD)				
		Day 7	Day 14	Day 21	Day 28	Day 35
EACA	250	2.1 ± 0.2b	25.8 ± 2.0a	121.5 ± 11.6a	428.2 ± 50.8a	489.7 ± 60.0ab
	500	2.6 ± 0.0a	21.3 ± 9.9ab	55.0 ± 9.3cd	217.8 ± 30.4bc	524.9 ± 55.5a
	1000	1.8 ± 0.0bc	27.9 ± 8.6a	116.6 ± 13.9ab	395.9 ± 42.8a	520.3 ± 17.2a
	1500	1.8 ± 0.1bc	19.8 ± 2.2ab	98.6 ± 11.4ab	325.4 ± 14.4a	515.6 ± 20.4a
	2000	1.6 ± 0.1bc	14.6 ± 1.5b	70.6 ± 9.3c	260.2 ± 54.3b	513.3 ± 43.2a
EAA	250	1.6 ± 0.0c	16.1 ± 1.7b	47.7 ± 6.6d	170.5 ± 4.7c	497.7 ± 18.4a
	500	1.7 ± 0.2bc	20.6 ± 3.1ab	65.3 ± 11.5cd	227.2 ± 37.9bc	541.6 ± 43.2a
	1000	1.6 ± 0.1c	10.3 ± 0.9c	40.0 ± 3.8e	151.0 ± 9.4c	373.6 ± 29.6b
	1500	1.7 ± 0.1bc	16.0 ± 2.2b	62.7 ± 8.9cd	222.2 ± 32.7bc	428.8 ± 29.3b
	2000	1.7 ± 0.0c	19.0 ± 1.5ab	97.8 ± 7.3b	348.0 ± 42.7a	486.9 ± 47.3ab
Margosom®	25	2.0 ± 0.2bc	10.3 ± 0.8c	54.1 ± 4.7d	173.1 ± 20.5c	439.4 ± 64.0ab
Check	0	1.7 ± 0.2bc	13.9 ± 4.0bc	85.7 ± 20.0bc	294.2 ± 69.5b	513.0 ± 29.9a

Means followed by different letters in a column are significantly different (Tukey, $p < 0.05$). EACA = acetonic extract of shell + almond; EAA = acetonic extract of almond; Margosom® 0.3% EC (azadirachtin 25 ppm = 0.05-5.0/5.0g diet).

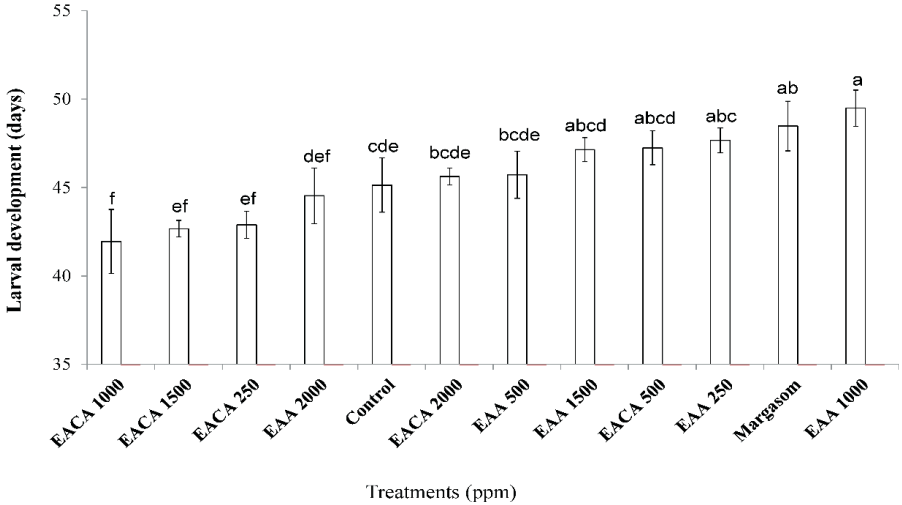

Fig. 1. Mean larval development (days) for *Copitarsia decolora* fed diet of added extracts of seeds of *Jatropha curcas*. EACA = acetonic extract of shell+almond; EAA = acetonic extract of almond, 250, 500, 1,000, 1,500, and 2,000 ppm concentration. Margasom = Margosom® 0.3% EC (botanical insecticide, azadirachtin 25 ppm = 0.05-5.0/5.0g diet). Means followed by the same letter are statistically the same ($F = 14.95$, $p < 0.0001$; Tukey test).

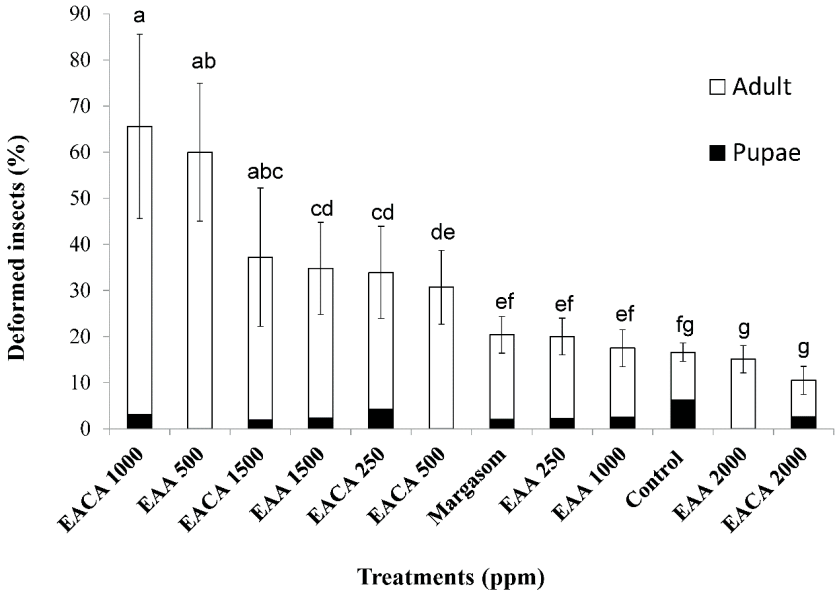

Fig. 2. Mean anatomical deformity (%) of *Copitarsia decolora* treated with acetonic extracts from *Jatropha curcas* seeds. EACA = acetonic extract of shell+almond; EAA = acetonic extract of almond, 250, 500, 1,000, 1,500, and 2,000 ppm concentration. Margosom® 0.3% EC (botanical insecticide, azadirachtin 25 ppm = 0.05-5.0/5.0g diet). Means with the same letter are statistically the same ($F = 53.87$, $p < 0.0001$; Tukey).

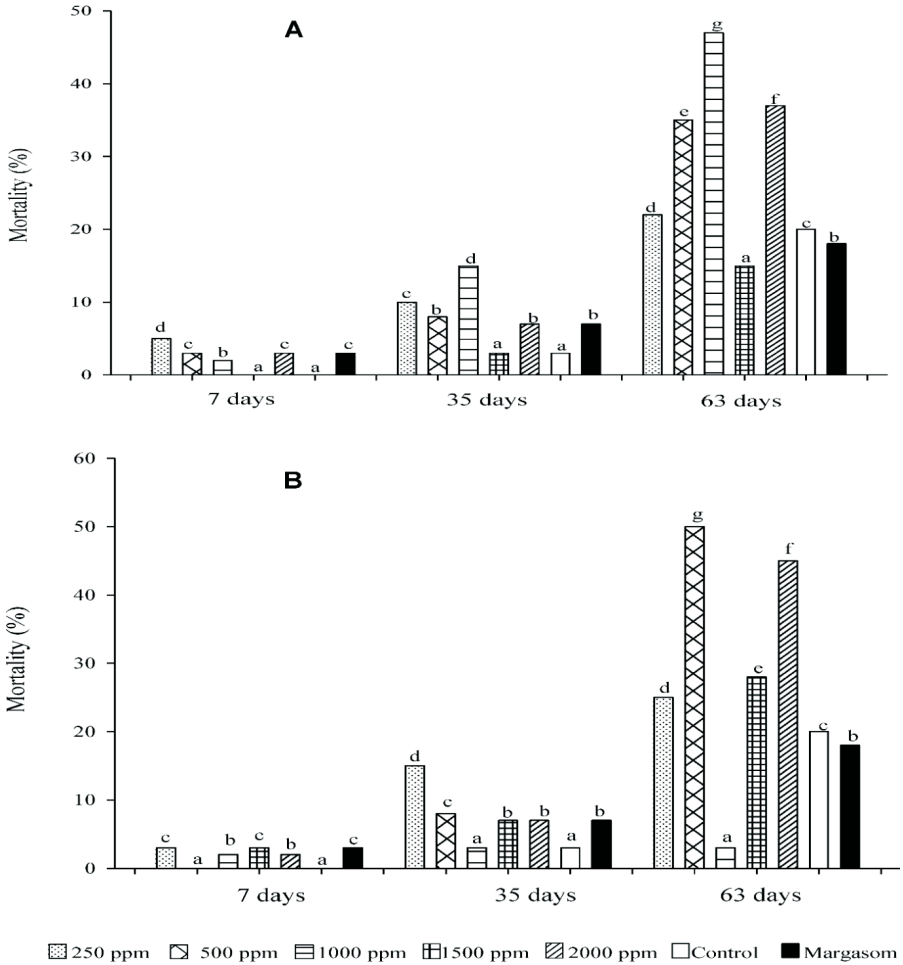

Fig. 3. Percentage mean mortality of *Copitarsia decolora* treated with extracts of *Jatropha curcas* seeds. A) acetonic extract of shell+almond (EACA) and B) acetonic extract of almond (EAA), 250, 500, 1,000, 1,500, and 2,000 ppm. Check and Margosom® 0.3% EC (botanical insecticide, azadirachtin 25 ppm = 0.05-5.0/5.0g diet). Means followed by the same letter are statistically the same (65 days: $F = 14.95$, $p < 0.0001$; Tukey).

On Day 2 of exposure, EAA at 1,000 ppm and Alfadex most protected cabbage plants from damage by *C. decolora* larvae ($F = 9.80$, $p < 0.0001$) (Table 3). At 250 ppm, larvae damaged 25% of plants and 36% damage of checks. On Days 4 and 6, cabbage plants treated with Alfadex were significantly less damaged (9.3%) compared with plants protected with 1,000 ppm EAA (18.6 and 22.1% for the same time periods, respectively ($F = 18.64$, $p < 0.0001$). At Day 8, insecticide treatment and 500 ppm EAA were most active in preventing damage by larvae ($F = 16.98$, $p < 0.0001$). From Day 10 through Day 18, Alfadex and 1,000 ppm EAA best controlled larvae as demonstrated by least percentage of damage ($F = 49.23$, $p < 0.0001$).

Table 3. Mean ± SD Percentage of Damage by *Copitarsia decolora* Larvae on Cabbage (*Brassica oleracea* var. *capitata*) Plants Treated with Acetonic Extract of Almond (EAA) from *Jatropha curcas*

Damage (%)	EAA treatment (ppm)				
Day	Check water	250	500	1,000	Alfadex® 500
2	25.6 ± 13.1ab	36.1 ± 11.1a	16.3 ± 18.7bc	8.16 ± 9.3c	9.3 ± 10.8c
4	33.8 ± 9.3ab	33.8 ± 9.3ab	21.1 ± 17.3bc	18.6 ± 15.0bc	9.3 ± 10.8c
6	44.3 ± 11.6a	44.3 ± 11.6a	26.8 ± 16.3b	22.16 ± 16.3bc	9.3 ± 10.8c
8	54.8 ± 13.9a	54.8 ± 13.9a	28.0 ± 15.7b	25.6 ± 22.2b	10.5 ± 12.1b
10	61.8 ± 11.1a	61.8 ± 11.1a	35.0 ± 23.5b	28.0 ± 23.8bc	10.5 ± 12.1c
12	65.3 ± 12.4a	65.3 ± 12.0a	40.8 ± 24.9b	28.0 ± 23.8bc	10.5 ± 12.1c
14	74.6 ± 9.1a	74.6 ± 9.1a	43.1 ± 25.6b	28.0 ± 23.8bc	10.5 ± 12.1c
16	92.0 ± 8.3a	92.0 ± 8.3a	43.1 ± 25.6b	28.0 ± 23.8bc	10.5 ± 12.1c
18	92.0 ± 8.3a	92.0 ± 8.3a	43.1 ± 25.6b	28.0 ± 23.8bc	10.5 ± 12.1c

Mean followed by the same letter in a column are not significantly different ($p < 0.05$, Tukey).

Discussion

EACA had a phagostimulating effect on *C. decolora* larvae. In contrast, EAA inhibited larval weight. This indicated the presence or absence of *J. curcas* shell determined its phagostimulatory or inhibitory effect. This might be caused by esterase proteins, lipase and curcin, that might cause phagostimulant effect in larvae (Staubmann et al. 1999). Ramos-Lopez et al. (2010, 2012) reported that fatty acids from *Ricinus communis* L. (another Euphorbiaceae) decreased larval weight of *S. frugiperda*. Therefore, it is necessary to evaluate oleic, linoleic, vaccenic, palmitic, stearic, and palmitoleic fatty acids isolated from EACA and EAA extracts to determine what compounds are responsible for inhibitory and phagostimulating effects on *C. decolora* larvae.

EAA also prolongs development of *C. decolora* larvae. This effect was reported previously by Bello (2013) with powder of the same shell + almond at 2% that prolonged *C. decolora* larval development 9 days. In our study, prolonged larval development was inversely related with inhibition of larval weight, with EAA (250 and 1,000 ppm) the extract that most inhibited larval weight and prolonged larval development. An insectistatic effect of the extract on *C. decolora* larvae is inferred. Bello (2013) found that 2% powder of shell+almond seed of Michoacán mixed into artificial diet of *C. decolora* extended larval development 5 days. EACA at 250 and 1,000 ppm prolonged *C. decolora* larval development 5 and 9 days, respectively.

Although EACA and EAA extracts did not adversely affect larvae and prepupa, they formed pupae with ruptured and damaged tissue with degradation of the cuticle. Deformed adults with folded, wrinkled, or reduced wings were produced by both extracts. Hernández-Miranda (2013) found that *C. decolora* larvae reared on diet containing 5% *J. curcas* shell+almond powder produced 4.3% deformed pupae and adults. Pupae from larvae reared on the same 2% powder extract had larval-stage heads and legs, as well as adults with deformed wings that either did not develop or fully extend (Bello 2013).

Our results showed that neither EACA nor EAA affected larvae of *C. decolora,* but both extracts killed >45% pupae. In previous studies, we evaluated shell+almond powder at two localities (Chiapa de Corzo, Chiapas, and Pueblillo, Michoacán). The

5% powder of shell + almond from Chiapa de Corzo killed 54% of larvae but 1% powder killed only 11% of larvae of the species (Hernández-Miranda 2013). Similarly, Bello (2013) reported that 4% shell+almond powder killed 62% of larvae, while 2% killed only 30%.

In greenhouse studies, 1,000 ppm of EAA extract protected 69% of cabbage plants from damage by *C. decolora* larvae for 18 days, whereas application of pyrethroid insecticide at 5.0 ppm protected 85.5%. Although the most bioactive extract, EAA at this concentration, did not protect plants from damage as Alfadex (applied at five times the maximum label rate) we concluded that *J. curcas* possesses compounds with insecticidal and insectistatic effect on the insect pest. More work is needed to determine the specific bioactive fatty acid(s) responsible for toxicity of EAA extract that might be isolated, thereby potentially increasing potency to control *C. decolora* larvae in the greenhouse and field.

Acknowledgment

This work was done during the sabbatical of Rodolfo Figueroa-Brito in collaboration with CEPROBI-IPN. It was financially supported by Project SIP 20170371 from IPN. We are grateful to the Gass Mass Laboratory, Benemerite University of Puebla, México, and to Edwin Javier Barrios-Gómez for CG-MS analysis and taxonomic identification, respectively. With this work, Axa Saron Tabárez-Parra, obtained his Bachelor's Degree in Environmental Science from the University of Morelos, and is part of his Master's thesis in Agroecological Management of Pests and Diseases of the Center for the Development of Biotic Products of the National Polytechnic Institute.

References Cited

Barrios-Díaz, B., R. Alatorre-Rosa, H.G. Calyecac-Cortero, and N. Bautista-Martínez. 2004. Identificación y fluctuación poblacional de plagas de col (*Brassica oleracea* var. capitata) y sus enemigos naturales en Acatzingo, Puebla. Agrociencia 38: 239-248.

Bello, V. S. 2013. Evaluación insecticida del polvo de *Jatropha curcas*, sobre el gusano del corazón de la col (*Copitarsia decolora*). Tesis de Licenciatura en Biotecnología Agrícola. Universidad Tecnológica De Izúcar de Matamoros. Izúcar de Matamoros, Puebla.

Bujanos, M. R. 2000. Manejo integrado de plagas en crucíferas, pp. 47-61. *In* M. N. Bautista, V. A. D. Suárez, y G. O. Morales [eds.], Temas Selectos en Fitosanidad de Hortalizas. Instituto de Fitosanidad, Colegio de Postgraduados, Texcoco, Estado de México.

Devappa, R. K., M. A. Angulo-Escalante, H. P. S. Makkar, and K. Becker. 2012. Potential of using phorbol esters as an insecticide against *Spodoptera frugiperda*. Ind. Crops Prod. 38: 50-53.

Devine, G. J., D. Eza, E. Ogusuku, and M. J. Furlong. 2008. Uso de insecticidas: contexto y consecuencias ecológicas. Rev. Peru Med. Exp. Salud Pública 25: 74-100.

Fernández, C. J. N., and O. J. Vázquez. 2003. *Copitarsia incommoda* Walker en cultivos de exportación para el Estado de Puebla, pp. 29-35. *In* M. N. Bautista y P. L. R. Flores [eds.], Memorias del Simposio Nacional sobre *Copitarsia incommoda* Walker. Colegio de Postgraduados, Montecillo, Estado de México.

Figueroa-Brito, R. 2012. Evaluación de extractos crudos del o los polvos activos de *Jatropha curcas* sobre insectos plagas (colectas del banco de SINAREFI, de Veracruz, Morelos y Puebla). Yautepec, Morelos.

Georges, K., B. Jayaprakasam, S. S. Dalavoy, and M. G. Nair. 2008. Pest-managing activities of plant extracts and anthraquinones from *Cassia nigricans* from Burkina Faso. Bioresour. Technol. 99: 2037-2045.

Hernández-Miranda, E. 2013. Actividad biológica de cinco especies vegetales sobre *Copitarsia decolora* (Lepidoptera: Noctuidae). Tesis de Maestría en Ciencias en Manejo Agroecológico del Plagas y Enfermedades, Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, Yautepec, Morelos.

Mihm, J. 1984. Técnicas eficientes para la crianza masiva e infestación de insectos, en la selección de las plantas hospederas para la resistencia al gusano cogollero (*Spodoptera frugiperda*). Centro Internacional para el Mejoramiento del Maíz y Trigo (CIMMYT), México. Folleto técnico.

Moreno-Flores, A. C. and M. E. Cerda-Lemus. 2010. La familia cruciferae en el Estado de Aguascalientes. Investigación y Ciencia 18: 12-19.

Ramos-López, M. A., G. S. Pérez, C. Rodríguez-Hernández, P. Guevara-Fefer, and M. A. Zavala-Sánchez. 2010. Activity of *Ricinus communis* (Euphorbiaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Afr. J. Biotechnol. 9: 1359-1365.

Ramos-López, M. A., M. M. González-Chávez, N. C. Cárdenas-Ortega, M. A. Zavala-Sánchez, and G. S. Pérez. 2012. Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. Afr. J. Biotechnol. 18: 4274-4278.

Rao, D. M., S. Anitha, A. Aranvida, B. Karunakar, and N. Devanna. 2012. Biopesticidal properties of seed, seed cake and oil of *Jatropha curcas* L. against the polyphagous lepidopteran pest *Helicoverpa armigera*, pp. 369-382. *In* T. Pullaiah, N. Carels, and M. Sujatha [eds.], Jatropha, Challenges for a New Energy Crop. Springer. New York.

Rojas, J. C. J., J. Cibrian-Tovar, J. Valdéz-Carrasco, and R. Nieto-Hernández. 1993. Análisis de la conducta de cortejo de *Copitarsia consueta* (Walker) y aislamiento de su feromona sexual. Agrociencia 4: 23-39.

SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación). 2011. Estudio de infraestructura logística hortofrutícula en el sur del país. http://www.sagarpa.gob.mx/agronegocios/Documents/ Estudios_ promercado/FDTAFT.pdf Accessed 26 July 2015.

Singh, K. I., P. S. Devi, M. K. Gupta, P. S. Devi, and G. Singh. 2015. Evaluation of plant extracts against insect pests of cabbage. Indian J. Entomol. 77: 109-114.

Staubmann, R., M. Schubert, A. Hiermann, and T. Kartnig. 1999. A complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2, 4-dione isolated from *Jatropha curcas*. Phytochemistry 50: 337-338.

Suárez-Vargas, A. D., N. Bautista-Martínez, J. Valdez-Carrasco, A. Angulo-Ormeño, R. Alatorre-Rosas, J. Vera-Graziano, A. Equihua-Martínez, and V. Manuel-Pinto. 2006. Fluctuación poblacional de *Copitarsia decolora* (Gueéne) y su asociación con crucíferas comerciales. Agrociencia 40: 501-509.

Torres, D., and T. Capote. 2004. Agroquímicos un problema ambiental global: uso del análisis químico como herramienta para el monitoreo ambiental. Ecosistemas 13: 2-6.

Diversity and Species Turnover of Insects in a *Jatropha curcas* Agroecosystem

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Abstract. *Jatropha curcas* L. hosts a wide variety of insects, but temporal variation in the composition of organisms (beta diversity) associated with crop farming has not been determined. Knowing the diversity and ecological role of its entomofauna are elements to develop management strategies for potential vulnerability of an agroecosystem. In this research, diversity, composition, and temporal turnover of insect species associated with cultivation of physic nuts were evaluated at Morelos, Mexico. During the second flowering period, 1,447 individuals in 13 orders, 106 families, 162 genera, and 190 species were collected; Hemiptera, Coleoptera, Diptera, and Hymenoptera comprised 89.9% of the individuals. Families Cicadellidae, Miridae, Chloropidae, Nitidulidae, and Chrysomelidae were most abundant. Insect alpha diversity, richness (0D), Shannon (1D), and Simpson (2D), did not show significant differences. Analysis of the composition of insect species using beta diversity (βSOR) showed a change of 0.37 ± 0.02 species. Piercing-sucking and chewing insects were the most diverse functional groups, of which at least 45 species are pests. The research should be complemented with more field observations in the *Jatropha curcas* crop. Results emphasized the need to consider different diversity aspects for appropriate crop management strategies.

Introduction

Jatropha curcas Linnaeus, 1753 (Euphorbiaceae) is an oilseed plant because of its ability to produce oil, a characteristic for which it is studied (López-Guillén et al. 2019, Méndez-López et al. 2020). The species known as physic nut or purge nut is distributed in tropical, subtropical, and semi-arid areas and gained economic value because of its oil quality that makes the plant an alternative for biodiesel (Heller 1996, Debnath and Bisen 2008, Sunil et al. 2013). Because of its potential, cultivation of *J. curcas* expanded rapidly in several countries including Brazil, China, India, and Mexico (Sorrell et al. 2010, Pecina-Quintero et al. 2014). The crop increase attracted a wide diversity of arthropods associated with the plant; some are beneficial pollinators or entomophagous insects, and some are phytophagous and harmful. A

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review by Alonso and Lezcano (2014) included 151 arthropod species associated with *J. curcas*, in 131 genera and 57 families. Diversity of chewing arthropods and piercing-sucking insects associated with the plant was reported by Lama et al. (2015), with 78 species in nine orders and 31 families. In México, 13 orders and 125 families were reported as associated with *Jatropha*; of those, 85% were Coleoptera, Diptera, Hemiptera, or Hymenoptera (Gómez-Ruíz et al. 2015). *J. curcas*, like any plant species, is associated with a wide diversity of organisms interacting with each other, but lacks studies evaluating the temporal variation of insect species composition (beta diversity) associated with the plant. It is important to evaluate beta diversity in a monocultural system with susceptibility to phytophagous attack, to overcome economic thresholds and defense barriers from plants, through expression of their secondary metabolites that generate unbalanced trophic relationships in a given space (Lama et al. 2015). Reports of economically important pest species are associated with *J. curcas* (Shanker and Dhyani 2006, Prabhakar et al. 2008, López-Guillén et al. 2019), in addition to sap-sucking insects with potential to transmit plant diseases (Grimm and Maes 1997, Méndez-López et al. 2018). Thus, identification of insect groups associated with the crop as well as their diversity and ecological role, constitute basic elements for implementation of management strategies to decrease agroecosystem vulnerability. The objective of the research was to evaluate diversity, composition, and temporary turnover of insect species associated with *J. curcas*.

Materials and Methods

The study occurred in the 4th year of an established *J. curcas* commercial plantation at latitude 18°43'46.8'' N and 99°22'29.99'' W and altitude 1,270 m above sea level, in Mazatepec County in the State of Morelos, Mexico. The climate is warm sub-humid with hot summers, mean annual temperature of 24ºC, and mean annual precipitation of 800 mm.

Sampling was once a month from September to November 2016, during the second flowering period of *J. curcas*. Arthropods were collected directly using a 59-cm-diameter entomological net to sweep plants. Ten double sweeps were made in each of 15 plants sampled during the study period, and the collected material was preserved in 70% alcohol, put into glass containers, and transferred to a laboratory at Universidad Autónoma Chapingo at Texcoco, Mexico, where the material was cleaned, separated, and identified. Identification to species was with the aid of taxonomic keys, and some genera were verified by site https://bugguide.net of the Department of Entomology, Iowa State University.

Insect diversity to species was obtained monthly. Abundance also was analyzed among the months sampled, and species diversity was analyzed with a non-parametric Kruskal-Wallis variance test. To determine if the number of sampled species was representative of the insect community at the study site, rarefaction curves were calculated based on sample size (Chao and Jost 2012), estimation of species proportion per sample, and comparisons among distinct populations in the community. To analyze alpha diversity during the 3-month sampling period, the effective number of species or Hill number, was calculated considering three orders of diversity (qD: 0, 1, and 2). Determined were 0D that indicated species richness, without abundance considerations; 1D (Shannon exponential) that considers the number of species equally common, and 2D (Simpson inverse) that considers the number of dominant species (Jost 2006). The three orders of diversity were compared among the months sampled, through rarefaction curves and extrapolation

(Chiu et al. 2014) with respective intervals of 95% confidence (100 bootstraps), calculated with the iNEXT package (Hsieh et al. 2016). For evaluation of changes in composition of insect species among the months sampled, a beta diversity partition method proposed by Baselga (2010) was used by calculating the Sorensen dissimilarity index (βsor) that explains the total variation of the sampling month including turnover and nesting; βsim indicated changes in species composition because of species turnover; βsne represented nesting (i.e., loss and/or gain in species), the result of difference between βsor and βsim (Baselga 2010). Metrics were calculated with the Betapart package (Baselga and Orme 2012), and R program version 3.4.2 (R Core Team 2017) was used for analysis.

Results

In total, 1,447 individuals of 190 species of arthropods were collected (Table 1), with the most abundant being *Draeculacephala* sp. (Ball, 1902) (Hemiptera: Cicadellidae), *Lygidea mendax* (Reuter, 1909) (Hemiptera: Miridae), and *Epichlorops* sp. (Becker, 1910) (Diptera: Chloropidae). Diversity was represented by 156 genera, with *Draeculacephala* (10.6%), *Lygidea* (7.1%), *Epichlorops* (6.7%), *Oncometopia* (Stål, 1869) (4.6%), and *Dikrella* (Oman, 1949) (4%) being most abundant (Fig. 1).

In total, 107 families were identified: Cicadellidae (Hemiptera) (25.7%), Miridae (Miridae) (8.7%), Chloropidae (Diptera) (6.8%), Nitidulidae (Coleoptera) (4.7%), and Chrysomelidae (Coleoptera) (3.1%) were most abundant (Fig. 2). A total of 13 orders was collected; of those, Hemiptera (48.5%), Coleoptera (17.8%), Diptera (14.9%), Hymenoptera (8.6%), Lepidoptera (4.8%), and Thysanoptera (2%) were most abundant (Fig. 3). However, there were no statistical differences among months sampled and population diversity of specimens identified (genera: H = 0.10, $P = 0.94$; families: H = 0.25, $P = 0.87$; orders: H = 0.60, $P = 0.73$, Kruskal-Wallis test).

Table 1. Diversity of Families, Species, and Individuals in Orders of Entomofauna Associated with Cultivated *Jatropha curcas* L.

	Families		Species		Individuals	
Order	Number	%	Number	%	Number	%
Coleoptera	18	16.822	42	22.222	252	17.415
Dermaptera	1	0.935	1	0.529	5	0.346
Diptera	20	18.692	25	13.228	219	15.135
Hemiptera	26	24.299	61	32.275	706	48.790
Hymenoptera	19	17.757	29	15.344	124	8.569
Lepidoptera	11	10.280	16	8.466	70	4.838
Mantodea	1	0.935	1	0.529	1	0.069
Neuroptera	3	2.804	3	1.587	12	0.829
Orthoptera	3	2.804	6	3.175	25	1.728
Psocoptera	1	0.935	1	0.529	2	0.138
Strepsiptera	1	0.935	1	0.529	1	0.069
Collembola	1	0.935	1	0.529	1	0.069
Thysanoptera	2	1.869	3	1.058	29	2.004
	107	100	190	100	1,447	100

% = percentage of families, species, and individuals of the collected aggregate.

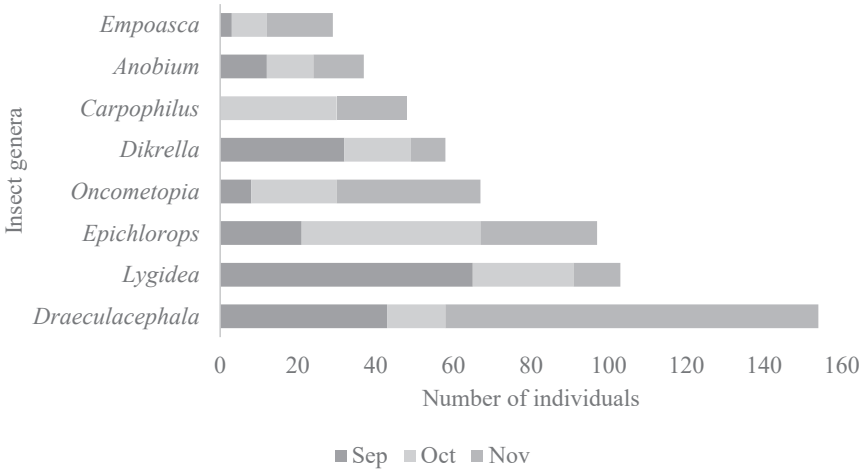

Fig. 1. Abundance of genera captured in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico. Only the most abundant genera are shown.

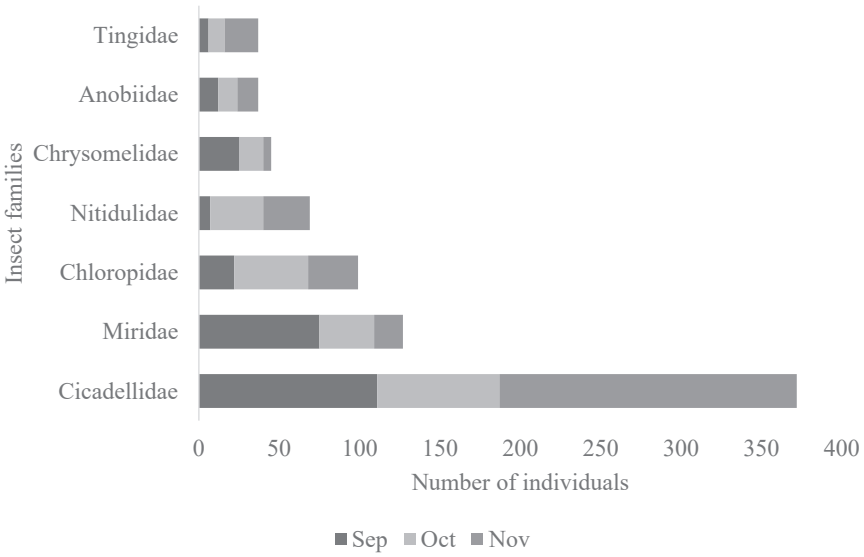

Fig. 2. Abundance of insect families captured in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico. The most abundant are shown.

Completeness was greater than 86% during the 3 months sampled. For insect alpha diversity, richness (0D), Shannon exponential (1D), and Simpson inverse (2D), there were no statistical differences among the months sampled (Fig. 4). However, composition of insect species analysis through diversity β_{sor}, showed species

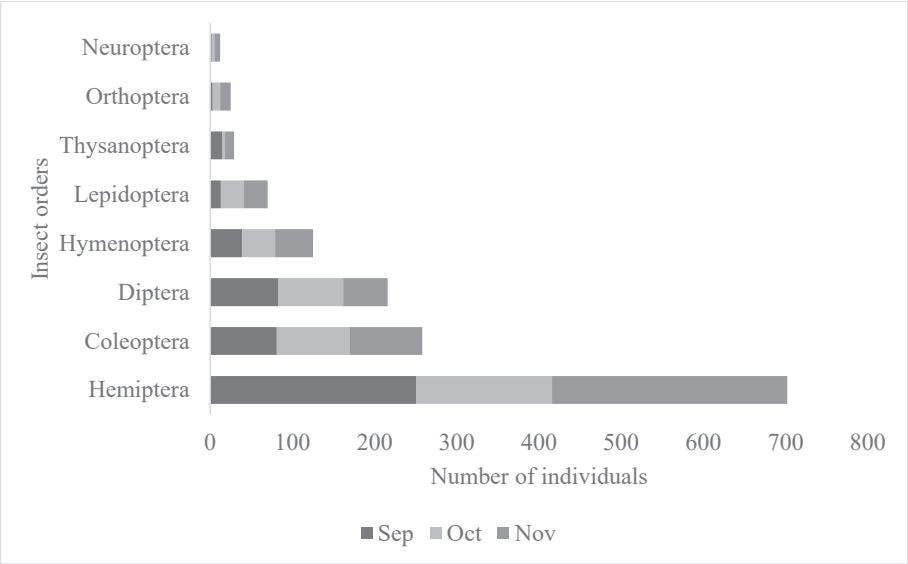

Fig. 3. Abundance of insect orders captured in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico.

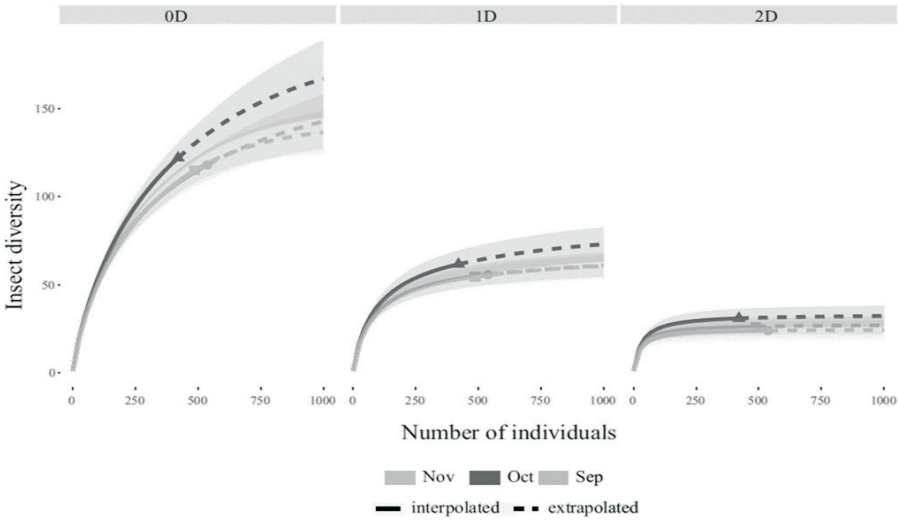

Fig. 4. Diversity of insect species captured in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico, during the months of September, October, and November sampled. Diversity estimated through Hill numbers (0D = Richness; 1D: exponent of Shannon diversity, 2D: inverse of Simpson's diversity index). The accumulation curves are interpolation (solid line) and extrapolation (dashed line) with 95% confidence intervals (shaded area) obtained with 100 randomizations.

turnover of 0.37 ± 0.02 among the months sampled (Fig. 5). The main factor was the spatial turnover of species (β_{sim}) among months (0.36 ± 0.01). In contrast, loss and/or gain of species (β_{sne}) contributed to a lesser extent to turnover (0.01 ± 0.005).

Five functional groups were identified in *J. curcas* monoculture, with importance as a guild in trophic relationships. The most abundant were piercing-sucking (39.4%), chewing (30.3%), predators (15.5%), parasitoids (14.4%), and least represented xylophages (0.3%) (Fig. 6, Table 2). Forty-five insect species are

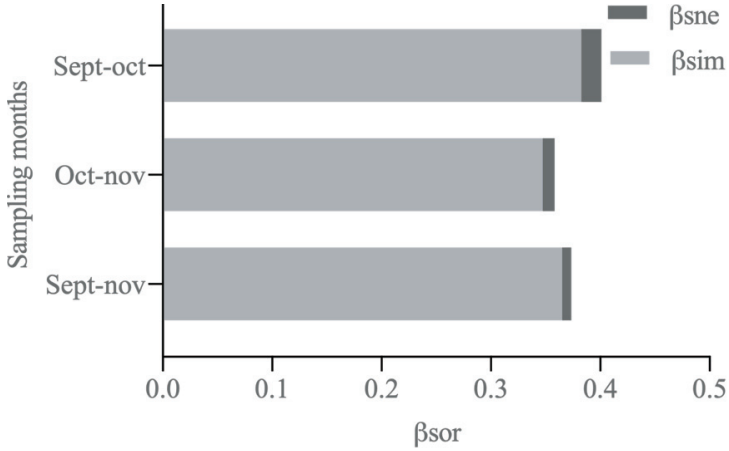

Fig. 5. Beta diversity of insects in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico. Turnover of species and interactions (β-diversity) among sampling months. A) Sorensen dissimilarity of species (βsor) and its components of turnover (βsim) and nestedness (βsne).

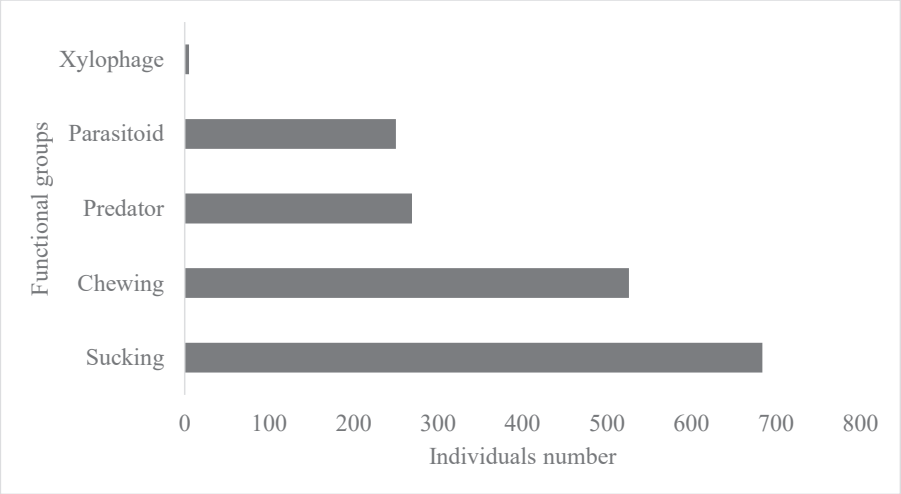

Fig. 6. Abundance of functional groups of insects captured in *Jatropha curcas* L. trees at Mazatepec, Morelos, Mexico.

Table 2. Abundance of Insect Species Captured in *Jatropha curcas* L. Trees at Mazatepec, Morelos, Mexico

Order	Family	Genus/Species	FG	sep	oct	nov	Tot
Coleoptera	Anobiidae	*Anobium* sp. (Fabricius, 1775)	Ch	12	12	13	37
	Attelabidae	*Rhynchites mexicanus* (Gyllenhal)*	Ch	10	4	12	26
	Bostrichidae	*Prostephanus truncatus* (Horn)	Ch, X	1	0	0	1
	Bruchidae	*Bruchus* sp. (Linnaeus)	Ch	4	3	5	12
	Cantharidae	*Chauliognathus* sp. (Hentz, 1830)	Ch	2	2	0	4
	Carabidae	*Lebia* sp. (Latreille 1802)*	P	2	1	1	4
	Cerambycidae	*Anoplodera canadensis* (Olivier)	Ch, X	0	0	1	1
		Desmocerus sp. (Audinet-Serville, 1835)	Ch, X	0	0	1	1
	Chrysomelidae	*Cryptocephalus* sp. (Geoffroy, 1762)	Ch	2	3	3	8
		Chalepus sp. (Thunberg, 1805)	Ch	1	0	0	1
		Disonychia sp. (Chevrolat, 1836)	Ch	7	1	0	8
		Diabrotica balteata (LeConte)*	Ch	4	1	0	5
		Megacerus sp. (Fåhraeus, 1839)	Ch	4	0	0	4
		Chalepus sp. (Thunberg, 1805)*	Ch, S	2	2	0	4
		Diachus sp. (LeConte, 1880)	Ch	0	3	1	4
		Colaspis sp. (Fabricius, 1798)*	Ch	1	1	1	3
		Diphaulaca aulica (Olivier, 1808)*	Ch	2	1	0	3
		Acalymma sp. (Barber, 1947)	Ch	0	1	0	1
		Anomoea sp. (Agassiz, 1846)	Ch	0	1	0	1
		Chaetocnema sp. (Stephens, 1831)	Ch	0	1	0	1
		Epitrix tuberis (Gentner)*	Ch	1	0	0	1
		Odontota sp. (Chevrolat, 1837)	Ch	1	0	0	1
	Cleridae	*Enoclerus* sp. (Gahan)	P	1	0	2	3
		Hippodamia convergens (Guérin, 1842)	P	1	1	1	3
	Coccinelidae	*Cycloneda sanguinea* (Linnaeus, 1763)	P	2	0	0	2
		Epilachna tredecimnotata (Latreille)	Ch	1	0	0	1
		Scymnus sp. (Kugelann, 1794)	P	0	1	0	1
		Apion godmani (Wagner, 1912)*	Ch	3	0	2	5
	Curculionidae	*Epicaerus* sp. (Schoenherr, 1834)*	Ch	0	1	0	1
		Nicentrites testaceipes (Champion, 1908)	Ch	0	0	1	1
	Elateridae	*Ctenicera* sp. (Latreille, 1829)	Ch	0	1	0	1
	Latridiidae	*Melanophthalma americana*(Mann., 1844)	Ch, X	1	0	0	1
	Melyridae	*Malachius aeneus* (Linnaeus, 1758)	Ch, P	2	3	4	9
	Mordellidae	*Mordella* sp. (Linnaeus, 1758)	Ch, P	1	0	1	2
		Carpophilus sp. (Stephens, 1830)	Ch	0	30	18	48
	Nitidulidae	*Lobiopa* sp. (Erichson, 1843)	Ch	7	2	8	17
		Conotelus mexicanus (Murray, 1864)	Ch, P	0	1	3	4
		Cotinis mutabilis(Gory and Perch., 1883)	Ch	2	2	6	10
	Scarabaeidae	*Euphoria* sp. (Burmeister, 1842)	Ch	0	5	3	8
		Cotinis nitida (Linnaeus, 1758)	Ch	2	2	1	5
		Cyclocephala sp. (Dejean, 1821)*	Ch	2	1	0	3
	Scolytidae	*Scolytus* sp. (Geoffroy, 1762)	Ch, X	0	1	0	1
	Staphylinidae	*Stenus* sp. (Latreille, 1797)	Ch, P	0	1	0	1
Dermaptera	Forficulidae	*Forficula aurucularia* (Linnaeus, 1758)*	Ch	1	3	1	5
Diptera	Agromyzidae	*Agromyza* sp. (Fallén, 1810)*	Ch	4	3	2	9
	Asilidae	*Efferia* sp. (Coquillet, 1893)*	P	2	3	1	6
	Bombyliidae	*Anthrax* sp. (Scopoli, 1763)	PA	0	2	0	2

	Cecidomyiidae	*Stenodiplosis sorghicola* (Coquillett, 1899)*	Ch	0	1	0	1
	Chamaemyiidae	*Chamaemyia* sp. (Meigen, 1803)	P	3	0	0	3
	Chironomidae	*Chironomus plumosus* (Linnaeus, 1758)	P	6	3	3	12
	Chloropidae	*Epichlorops* sp. (Becker)	Ch/X/P /PA	21	46	30	97
		Thaumatomyia sp. (Zenker, 1833)	Ch, P	1	0	1	2
	Culicidae	*Anopheles* sp. (Meigen, 1818)*	S	7	0	1	8
	Curtonotidae	*Curtonotum* sp. (Macquart, 1843)	PA	5	0	0	5
	Dolichopodidae	*Dolichopus* sp. (Latreille, 1796)	P	7	3	3	13
	Heleomycidae	*Amoebaleria* sp. (Garrett, 1921)	Ch	0	1	1	2
	Muscidae	*Musca domestica* (Linnaeus, 1758)	Ch	0	0	2	2
	Mycetophilidae	Not identified	Ch, P	0	1	0	1
	Phoridae	*Metopina subarcuata* (Borgmeier, 1963)	P, Ch, PA	2	4	6	12
	Stratiomyidae	*Stratiomys laticeps* (Loew, 1866)	Ch	0	1	0	1
	Syrphidae	*Eristalis* sp. (Latreille, 1804)	Ch	2	0	0	2
	Tabanidae	*Tabanus atratus* (Fabricius, 1775)	S	0	2	2	4
	Tachinidae	*Aplomyiopsis* sp. (Villeneuve, 1933)	PA, P	15	0	0	15
		Archytas sp. (Jaennicke, 1867)	PA	1	1	1	3
		Winthemia sp. (Robineau, 1830)	PA	0	3	0	3
		Euphorocera sp. (Townsend, 1892)	Ch, PA	2	0	0	2
	Tephritidae	Not identified	Ch	3	1	1	5
		Rhagoletis sp. (Loew, 1862)*	Ch	2	0	0	2
	Ulidiidae	*Euxesta* sp. (Loew, 1867)*	Ch	0	4	0	4
Hemiptera	Achilidae	*Catonia* sp. (Uhler, 1895)*	S	1	0	0	1
	Anthocoridae	*Orius tristicolor* (White, 1879)	P	17	7	5	29
	Aphididae	*Sitobion avenae* (Fabricius, 1775)*	S	0	6	4	10
	Cercopidae	*Aeneolamia albofasciata* (Lallemand, 1939)*	S	1	1	1	3
		Clastoptera sp. (Germar, 1839)*	S	2	0	1	3
	Cicadellidae	*Aeneolamia* sp. (Fennah, 1949)*	S	1	1	0	2
		Draeculacephala sp. (Ball, 1902)	S	37	15	86	138
		Oncometopia sp. (Stål, 1869)*	S	8	22	37	67
		Dikrella sp. (Oman, 1949)*	S	32	17	9	58
		Empoasca fabae (Harris, 1841)*	S	3	9	13	25
		Draeculacephala minerva (Pelota, 1927)*	S	6	0	10	16
		Tylozygus sp. (Fieber, 1865)	S	9	3	2	14
		Hecalus sp. (Stål, 1864)	S	3	1	7	11
		Homalodisca vitripennis (Germar, 1821)*	S	2	2	7	11
		Circulifer tenellus (Baker, 1896)*	S	2	1	3	6
		Tylozygus fasciatus (Walker, 1851)	S	5	1	0	6
		Empoasca spp. (Walsh, 1862)*	S	0	0	4	4
		Erythroneura spp. (Fitch , 1851)	S	0	0	4	4
		Homalodisca sp. (Stål, 1869)*	S	0	2	2	4
		Apogonalia sp. (Evans, 1947)*	S	2	1	0	3
		Circulifer sp. (Zachvatkin, 1935)*	S	0	2	1	3
		Apogonalia stali (Signoret, 1855)*	S	2	0	0	2
	Cixiidae	*Oecleus* sp. (Stål, 1862)*	S	0	3	0	3
		Cixius angustatus (Caldwell, 1938)*	S	0	2	0	2
	Coccidae	Not identified	S	0	0	1	1
	Coreidae	*Anasa* sp. (Amyot and Serville, 1843)	S	5	0	3	8
		Leptoglossus zonatus (Dallas, 1852)	S	0	0	7	7
	Delphacidae	*Stenocranus* sp. (Fieber, 1866)	S	0	1	2	3

	Dictyopharidae	*Scolops* sp. (Schaum, 1850)	S	2	2	1	5
	Flatidae	Not identified	S	0	0	2	2
	Fulgoridae	*Cyrpoptus* sp. (Stål, 1862)	S	1	0	0	1
	Geocoridae	*Geocoris* sp. (Fallén, 1814)	P	1	0	0	1
	Membracidae	*Aconophora projecta* (Funkhouser, 1846)	S	1	0	4	5
		Entylia concisa (Walker, 1851)	S	0	3	0	3
	Miridae	*Archasia* sp. (Stål, 1867)	S	1	0	0	1
		Lygidea mendax (Reuter, 1909)	S	65	26	12	103
		Lygus lineolaris (Palisot de Beauvois, 1818)*	S	9	4	4	17
		Adelphocoris sp. (Reuter, 1896) *	S	1	4	1	6
		Halticus sp. (Hahn, 1832)	S	0	0	1	1
	Nabidae	*Nabis americoferus* (Carayon, 1961)	P	1	1	0	2
	Ortheziidae	*Orthezia* sp. (Morrison 1952)	S	0	0	2	2
	Pachygronthidae	*Phlegyas abbreviatus* (Uhler, 1876)	S	2	0	0	2
	Pentatomidae	*Thyanta* sp. (Stål, 1860)	S	4	1	3	8
		Nezara viridula (Linnaeus, 1758)*	S	1	0	3	4
		Murgantia histrionica (Hahn, 1834)	S	0	1	2	3
		Not identified	S	1	0	2	3
		Podisus maculiventris (Say, 1832)	P	2	0	0	2
	Pseudococcidae	Not identified	S	0	2	0	2
	Reduviidae	*Sinea diadema* (Fabricius, 1776)	P	4	3	6	13
		Barce uhleri (Banks, 1909)	P	0	1	0	1
	Rhopalidae	*Arhyssus* sp. (Stål, 1870)	S	1	1	4	6
	Rhyparochromidae	*Eremocoris* sp. (Fieber, 1860)	S	1	0	1	2
	Scutelleridae	*Myodocha* sp. (Latreille, 1807)	S	1	1	0	2
		Pachycoris sp. (Jean-Baptiste, 1843)	S	1	7	5	13
	Tingidae	*Acalypta* sp. (Westwood, 1840)	S	1	5	13	19
		Corythucha sp. (Stål, 1873)*	S	3	5	4	12
		Acalypta lillianis (Torre-Bueno, 1916)*	S	0	0	3	3
		Not identified	S	2	0	0	2
		Atheas sp. (Champion, 1898)	S	0	0	1	1
	Triozidae	*Trioza anceps* (Tuthill, 1944)*	S	7	1	3	11
Hymenoptera	Ampulicidae	*Ampulex* sp. (Jurine, 1807)	PA	3	2	0	5
	Anthophoridae	*Anthophora* sp. (Latreille, 1803)	Ch, S	0	2	1	3
		Melipona sp. (Illiger, 1806)	S	0	0	5	5
	Apidae	*Apis mellifera* (Linnaeus, 1758)	S	0	0	1	1
		Melecta sp. (Latreille, 1802)	S	0	1	0	1
	Bethylidae	Not identified	PA	1	1	2	4
	Braconidae	*Phanomeris phyllotomae* (Muesebeck 1932)	P, PA	4	1	3	8
		Apanteles diatraeae (Muesebeck, 1921)	PA	2	1	3	6
		Phanomeris sp. (Förster, 1862)	P, PA	1	1	2	4
		Apanteles sp. (Förster, 1862)	PA	1	0	0	1
		Chelonus texanus (Cresson, 1872)	P, PA	1	0	0	1
	Chalcididae	*Chalcis* sp. (Fabricii 1787)	PA	2	2	0	4
	Colletidae	*Hylaeus* sp. (Fabricius, 1793)	PA	2	0	0	2
	Diaprididae	Not identified	PA	2	0	1	3
	Encyrtidae	Not identified	PA	1	0	8	9
	Eulophidae	Not identified	PA	2	3	3	8
		Solenopsis sp. (Westwood, 1840)	Ch	1	1	0	2
	Formicidae	Not identified	Ch, P	0	1	0	1
		Lasius alienus (Foerster, 1850)	P	1	0	0	1

	Halictidae	*Agapostemon* sp. (Guérin, 1844)	PA	1	1	1	3
		Sphecodes sp. (Latreille, 1804)	PA	0	1	2	3
	Ichneumonidae	*Phobocampe* sp. (Förster, 1869)	PA	0	0	1	1
	Megachilidae	*Coelioxys* sp. (Latreille, 1809)	PA	0	0	1	1
		Megachile sp. (Latreille, 1802)	PA	0	1	0	1
	Melittidae	Not identified	Ch, S	0	3	0	3
	Mymaridae	Not identified	PA	2	2	2	6
	Pteromalidae	Not identified	PA	5	4	5	14
	Scelionidae	Not identified	PA	1	0	3	4
	Torymidae	*Torymus* sp. (Dalman, 1820)	PA	6	12	2	20
Lepidoptera	Arctiidae	Not identified	Ch	0	3	2	5
	Crambidae	*Crambus* sp. (Fabricius, 1798)	Ch	0	3	0	3
	Gelechiidae	*Pectinophora gossypiella* (Saunders, 1844)*	Ch	3	1	4	8
		Keiferia lycopersicella (Walsingham, 1897)	Ch	0	2	3	5
		Phthorimaea operculella (Zeller, 1873)	Ch	2	1	1	4
		Not identified	Ch	5	4	7	16
	Geometridae	*Nepytia canosaria* (Walker, 1862)	Ch	0	5	3	8
		Perthophlebia sp. (Capps, 1943)*	Ch	0	0	6	6
	Hesperiidae	*Thorybes pylades* (Scudder, 1870)	Ch	0	2	0	2
		Ancyloxypha sp. (Felder, 1863)*	Ch	0	0	1	1
	Lycaenidae	*Thecla* sp. (Fabricius, 1807)	Ch	0	1	0	1
	Lymantriidae	Not identified	Ch	0	0	1	1
	Noctuidae	*Spodoptera* sp. (Guenée, 1852)	Ch	1	1	1	3
	Pyralidae	*Pyralis* sp. (Linnaeus, 1758)*	Ch	1	3	0	4
	Sesiidae	*Synanthedon* sp. (Hübner, 1819)	Ch	1	0	0	1
	Sphingidae	Not identified	PA	0	2	0	2
Mantodea	Mantidae	*Stagmomantis* sp. (Saussure, 1869)	P	0	0	1	1
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens, 1836)	P	0	3	5	8
	Hemerobiidae	*Hemerobius* sp. (Linnaeus, 1758)	P	0	0	1	1
	Mantispidae	*Mantispa cincticornis* (Banks, 1911)	P	2	0	1	3
Orthoptera	Acrididae	*Sphenarium purpureaceae* (Charpentier, 1845)	Ch	1	2	3	6
	Gryllidae	*Oecanthus* sp. (Serville, 1831)	P Ch	0	2	1	3
		Scudderia sp. (Stål, 1873)	Ch	0	3	5	8
	Tettigoniidae	*Conocephalus* sp. (Zenker, 1833)	Ch	2	0	2	4
		Scudderia furcata (Brunner von Wattenwyl, 1878)	Ch	0	1	2	3
		Microcentrum rhombifolium (Saussure, 1859)	Ch	0	1	0	1
Psocoptera		Not identified	Ch	0	1	1	2
Strepsiptera	Halictophagidae	*Halictophagus serretus* (Bohart, 1943)	PA	0	0	1	1
Symphypleona	Bourletiellidae	*Bourlettiella* sp. (Banks, 1899)	Ch, S	0	1	0	1
Thysanoptera	Phlaeothripidae	*Gynaikothrips ficorum* (Marshall, 1908)	Ch	15	1	10	26
	Thripidae	*Thrips tabaci* (Lindeman, 1889)	Ch	0	2	0	2
		Caliothrips sp. (Daniel, 1904)	Ch	0	0	1	1

Species of insects considered pests (*). FG: functional group, P: predator, PA: parasitoid, Ch: chewing (masticadores), S: piercing-sucking (chupadores), and X: xylophage.

harmful to crops in the area, with the most abundant being *Oncometopia* sp., *Dikrella* sp. (Hemiptera: Cicadellidae), *Rhynchites mexicanus* (Coleoptera: Attelabidae), and *Empoasca fabae* (Harris, 1841) (Hemiptera: Cicadellidae).

Discussion

Analysis of diversity of arthropods associated with *J. curcas* showed the monthly turnover of a notorious insect species, as well as association of diverse functional groups that could be beneficial or harmful to the production system. *J. curcas* recently was established as an agricultural system (Saturnino et al. 2005, Achten et al. 2010, Li et al. 2010). Although resistant to disease and insect pests, the plant is not exempt from attack by biotic agents in the agroecosystem (Saturnino et al. 2005, Anitha and Varaprasad 2012, Terren et al. 2012, Lama et al. 2015, López-Guillén et al. 2019).

All parts of the plant are vulnerable to a wide variety of arthropods, mostly piercing-sucking insects that infest flowers, fruits, seeds, foliage, and to a lesser extent roots, stems, and branches (Zamarripa and Solís 2013). Organisms in *J. curcas* include defoliators, visitors, predators, and pollinators (Regupathy and Ayyasamy 2011, García-Alonso et al. 2018). Lama et al. (2015) reported that 78 insects in nine orders and 31 families feed on the plant, and identified 34 phytophagous species in Central and South America, 30 species in Asia, and 21 species in Africa, whereas, in Australia, 60 species in 21 families were reported (Shanker and Dhyani 2006). In Nigeria, 16 genera of Acrididae and Pyrgomorphidae (Orthoptera) feed on leaves and seedlings, and Cetoniidae (Coleoptera), Pentatomidae (Hemiptera), Coreidae (Hemiptera), and Scutelleridae (Hemiptera) feed on flowers and fruits (Abdoul et al. 2014).

Ecological production systems of *J. curcas* show evidence of a biodiverse and slightly disturbed entomofauna (Cañarte et al. 2017). In the research, a large diversity of arthropods was represented by 1,447 individuals, distributed in 10 orders, 177 genera, and 190 species. However, no significant differences were found in insect alpha diversity, probably because of monoculture that provides the same amounts of resources to associated species during the months sampled. This coincides with studies on *Eucalyptus*, where richness of other taxonomical groups (i.e., vertebrates, invertebrates, and plants) is maintained (Barlow et al. 2007), possibly because monocultures homogenize species diversity. In the research, however, evidence showed remarkable temporal turnover (beta diversity), in which composition of insect species changed on a monthly basis. Harrison and Berenbaum (2013) in a study on monocultures for biodiesel found similar turnover of moth species (almost 30%). This was in contrast to the importance in natural sites where species turnover was greater compared to those managed by humans (Suguituru et al. 2011).

Plant feeders were the most diverse functional group, which agrees with results of other studies (Lama et al. 2015, Cañarte et al. 2017). However, the group could have negative implications, with production of *J. curcas* being susceptible to pest attack (Saturnino et al. 2005, Anitha and Varaprasad 2012, Lama et al. 2015), which is the main reason for abandonment by farmers in southern Mexico (Soto et al. 2018). In the functional groups, 45 species are pests affecting several agricultural crops, and some such as *Oncometopia* sp., *Dikrella* sp., and *Empoasca fabae* specifically affect *J. curcas*. Ranga-Rao et al. (2010) reported about 40 species of insect pests specifically damage *J. curcas.* Nielsen (2010) mentioned that the main

species considered pests varied according to geographical area; therefore, the number of pests seems to be much larger.

In the *Jatropha* agroecosystem, chewing and piercing-sucking insects were the most diverse functional groups, representing a phytosanitary risk to crop production; beta diversity or temporary species turnover presented dynamism, that is, entomofauna that change monthly, increasing risk. Findings from this research emphasize the importance of considering different aspects of diversity to obtain more appropriate conclusions about management of monocultures and ecological interactions in agroecosystems, especially those important for sustainable energy. Results were important in *J. curcas* crop management and biological conservation of entomofauna. It is the first report evaluating diversity of arthropods with emphasis on temporal turnover of species (beta diversity) in *J. curcas* monoculture.

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References Cited

Abdoul, H. Z., A. Toudou, E. Haubruge, G. Mergeai, and F. J. Verheggen. 2014. Insects associated with *Jatropha curcas* Linn. (Euphorbiaceae) in West Niger. J. Insect Sci. 14: 255.

Achten, W. M. J., W. H. Maes, R. Aerts, L. Verchot, A. Trabucco, E. Mathijs, ..., and B. Muys. 2010. Jatropha: from global hype to local opportunity. J. Arid Env. 74: 164-165.

Alonso, O., and J. C. Lezcano. 2014. Arthropods associated to *Jatropha curcas* Linnaeus. Functions and strategy for their management. Pastos y Forrajes 37: 3-16.

Anitha, K., and K. S. Varaprasad. 2012. Jatropha pests and diseases: an overview. *In* N. Carels, M. Sujatha, and B. Bahadur [eds.], Jatropha, Challenges for a New Energy Crop. Springer, New York.

Barlow, J., T. A. Gardner, I. S. Araujo, T. C. Ávila-Pires, A. B. Bonaldo, J. E. Cost, and M. S. Hoogmoed. 2007. Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. Proc. Nat. Acad. Sci. 104: 18555-18560.

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography 19: 134-143.

Baselga, A., and C. D. L. Orme. 2012. Betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution 3: 808-812.

Cañarte Bermúdez, E., O. Valarezo Cely, and J. B. Navarrete Cedeño. 2017. Study of the fauna of arthropods associated with physic nut (*Jatropha curcas* L.) In Manabi, Ecuador. Revista Científica Ecuatoriana 4: 1-9.

Chao, A., and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology 93: 2533-2547.

Chiu, C. H., L. Jost, and A. Chao. 2014. Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers. Ecological Monographs 84: 21-44.

Debnath, M., and P. S. Bisen. 2008. *Jatropha curcas* L., a multipurpose stress resistant plant with a potential for ethnomedicine and renewable energy. Current Pharmaceutical Biotechnology 9: 288-306.

García-Alonso, F., E. García-Pérez, A. Pérez-Vázquez, O. Ruiz-Rosado, and R. Martínez-Martínez. 2018. Insects associated with *Jatropha curcas* L., in the central region of the state of Veracruz, Mexico. Agroproductividad 11: 9-14.

Gómez-Ruíz, J., G. López-Guillen, E. Herrera-Parra, M. Hernández-Arenas, M. Martínez-Bolaños, and J. F. Barrera. 2015. Plagas y enfermedades del piñón (*Jatropha curcas* L.) en el trópico mexicano [Pests and diseases of the pinion (*Jatropha curcas* L.) in the Mexican tropics]. Ecosur-Inifap, Folleto Técnico 14. Primera edición. Tapachula, Chiapas, México.

Grimm, P. C., and J. M. Maes. 1997. Insects associated with physic nut (*Jatropha curcas* L.) (Euphorbiaceae) in the Pacific region of Nicaragua. Revista Nicaragüense de Entomología 39: 13-26.

Harrison, T., and M. R. Berenbaum. 2013. Moth diversity in three biofuel crops and native prairie in Illinois. Insect Sci. 20: 407-419.

Heller, J. 1996. Physic nut: *Jatropha curcas* L., pp. 24-29. *In* IPGRI IPK Italy Germany© International Plant Genetic Resources Institute.

Hsieh, T., K. Ma, and A. Chao. 2016. iNEXT online: interpolation and extrapolation (Version 1.0) [Software]. http://chao.stat.nthu.edu.tw/blog/software-downlod

Jost, L. 2006. Entropy and diversity. Oiko 113: 363-375.

Lama, A. D., T, Vuorisalo, and P. Niemel. 2015. Global patterns of arthropod herbivory on an invasive plant, the physic nut (*Jatropha curcas* L.). J. Appl. Entomol. 139: 1-10.

Li, Z., L. Lin, X. Zhao, M. Sagisaka, and R. Shibazaki. 2010. System approach for evaluating the potential yield and plantation of *Jatropha curcas* L. on a global scale. Env. Sci. Techn. 44: 2204-2209.

López-Guillén, G., J. L. Solís Bonilla, B. B. Martínez Valencia, E. Herrera Parra, and C. A. Zamarripa. 2019. Agronomy of *Jatropha curcas* in Mexico. *In* S. Mulpuri, N. Carels, and B. Bahadur [eds.] Jatropha, Challenges for a New Energy Crop. Springer, Singapore.

Méndez-López, A., L. Córdova-Téllez, M. Sánchez-Vega, J. C. Salazar-Torres, and O. García-Martínez. 2018. Diversity and abundance of leafhoppers in *Jatropha curcas* L., in Mazatepec, Morelos, Mexico. Southwest. Entomol. 43: 733-741.

Méndez-López, A., L. Córdoba-Téllez, and M. Sánchez-Vega. 2020. Accelerated aging affects the physiological and biochemical quality of the seed of *Jatropha curcas*. Tropical Subtropical Agroecosystems 23.

Nielsen, F. 2010. Pest and diseases, pp. 23-27. *In* The *Jatropha* Handbook: from Cultivation to Applications. FACT-Foundation, Eindhoven, Netherlands.

Pecina-Quintero, V., J. L. Anaya-López, A. Zamarripa-Colmenero, C. A. Núñez-Colín, N. Montes-García, J. L. Solís-Bonilla, and M. F. Jiménez-Becerri. 2014. Genetic structure of *Jatropha curcas* L. in Mexico and probable centre of origin. Biomass and Bioenergy 60: 147-155.

Prabhakar, M., Y. G. Prasad, G. R. Rao, and D. Ramakrishna. 2008. Pests of economic importance on *Jatropha curcas* L., a biodiesel plant in Andhra Pradesh, India. Entomon 33: 83-86

R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranga-Rao, G. V., S. Marimuthu, S. P. Wani, and V. P. Rao. 2010. Insect pests of *Jatropha curcas* L. and their management. Information Bulletin.

Regupathy, A., and R. Ayyasamy. 2011. Ants in biofuel, *Jatropha* ecosytem: pollination and phoresy. Hexapoda 18: 168-175.

Saturnino, H. M., D. D. Pacheco, J. Kakida, T. Nagashi, and N. P. Gonçalves. 2005. Cultura do pinhão-manso (*Jatropha curcas* L.). Informe Agropecuário, Belo Horizonte 26: 44-78.

Shanker, C., and S. K. Dhyani. 2006. Insect pests of *Jatropha curcas* L. and the potential for their management. Current Sci. 91: 162-163.

Sorrell, S., R. Miller, R. Bentley, and J. Speirs. 2010. Oil futures: a comparison of global supply forecasts. Energy Policy 38: 4990-5003.

Soto, I., C. Ellison, M. Kenis, B. Diaz, B. Muys, and E. Mathijs. 2018. Why do farmers abandon *Jatropha* cultivation? The case of Chiapas, Mexico. Energy for Sustainable Development 42: 77-86.

Suguituru, S. S., R. R. Silva, D. R. D. Souza, C. D. B. Munha, and M. S. D. C. Morini. 2011. Ant community richness and composition across a gradient from *Eucalyptus* plantations to secondary Atlantic forest. Biota Neotropical 11: 369-376.

Sunil, N., V. Kumar, and K. S. Varaprasad. 2013. Origin, domestication, distribution and diversity of *Jatropha curcas* L., pp. 137-151. *In* Bahadur et al. [eds.], *Jatropha,* Challenges for a New Energy Crop. Vol. 2. Springer Science.

Terren, M., J. Mignon, C. De Clerck, H. Jijakli, S. Savery, P. Jacquet de Haveskercke, ..., and G. Mergeai. 2012. Principal disease and insect pests of *Jatropha curcas* L. in the lower valley of the Senegal River. Tropicultura 30: 222-229.

Zamarripa, C. A., and B. J. Solís. 2013. *Jatropha curcas* Alternativa Bioenegética en México [*Jatropha curcas* Bioenegetic Alternative in Mexico]. INIFAP, Izapa, México.

Population Dynamics of the Predator Mite *Bdella longistriata*[1] Collected by Malaise Trap at Tula, Tamaulipas, Mexico

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Abstract. *Bdella longistriata* Atyeo is a mite with restricted distribution that has been registered in the United States of America (Texas) and Mexico (Colima, State of Mexico, Guerrero, Jalisco, Morelos, San Luis Potosí, and Tamaulipas). Were colected 130 mites in different stages of development, except larvae and eggs. Population structure collected by the Malaise trap were 85% females, 9% males, and 6% immature stages. The mites were compared with those collected in a Berlese funnel from a tropical deciduous forest in the State of Jalisco.

Introduction

Bdellid mites tolerate cold weather, heat, and low humidity. They are found on the surface of organic soil or in more specialized habitats such as mosses, lichens, and tree bark, and have been found even under mosses and rocks near waterfalls, in pine cones, in arid soil, on grasses in swamps, and on agricultural products (Atyeo 1960, Fisher et al. 2011, Hernandes et al. 2016). The genus *Bdella* is known worldwide; some members of the genus are biological control agents for mites, springtails, and insect eggs (Gerson et al. 2003).

Seven species of the genus *Bdella* occur in Mexico: *Bdella cardinalis* Banks, *Bdella distincta* Baker & Balock, *Bdella longicornis* Linnaeus, *Bdella longistriata* Atyeo, *Bdella muscorum* Ewing, *Bdella tlascalana* Vitzthum, and *Bdella tropica* Atyeo (Baker and Balock 1944; Atyeo 1960; Mejía-Recamier 1986, 1997; Mejía-Recamier and Cutz 2007; Hernandes 2013; Hernandes et al. 2016). *B. longistriata* is an endemic species distributed only in Mexico and the United States on the American Continent (Hoffmann and López-Campos 2000, Hernandes et al. 2016). In Mexico, it was recorded on leaf litter, moss, epiphytic moss, corticicoles mosses, epiphytes, and under logs in the states of Colima (Municipality Manzanillo), Guerrero (Taxco), Jalisco (La Huerta), State of Mexico, Morelos (Tepoztlán), San Luis Potosí (Ciudad de Maíz), and Tamaulipas (Antiguo Morelos, El Mante, and Llera de Canales) (Atyeo 1960; Mejía-Recamier 1986, 1997; Mejía-Recamier and Cutz 2007). Considering this, more data are needed on the distribution, biology, and ecology of predator bdellid

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mites. Their importance in the dynamics and structure of communities needs to be evaluated. The objective of the study was to record population dynamics of *B. longistriata* collected in a Malaise trap for 1 year in a tropical deciduous forest at Tula, Tamaulipas, Mexico.

Materials and Methods

From 30 January 2016 until 3 June 2017, a Malaise trap was at Ejido Gallitos, municipality of Tula, in the State of Tamaulipas (22.887973 N, 99.551983 W, in a low deciduous tropical forest 979 m above sea level). The Malaise trap was Standard (BT1004) 1.1 m tall x 1.1 m wide, black with a white top (Fig. 1). The trap trademark was MegaView Science Co.®, with a collection bottle with 96% ethyl alcohol on top. The bottle was changed approximately every 15 days to preserve specimens. The total number of samples was 23.

Fig. 1. Malaise traps Standard model (BT1004). A) Collecting and B) Malaise traps in a low deciduous tropical forest.

Mites from samples preserved in refrigerated plastic bottles were separated with the aid of a Leica MZ12 stereomicroscope. Mites were separated from samples in 70% ethyl alcohol in Eppendorf tubes, quantified, and recorded in an Excel database. Mites were put into lactic acid for 7 days to make them transparent, rinsed with distilled water, and mounted permanently in polyvinyl alcohol (Walter and Krantz 2009). To identify species, the original description by Atyeo (1960) was used and compared with keys by Hernandes et al. (2016). Permanent preparations were deposited in the Acarological Collection of the Insect Museum of the Facultad de Ingeniería y Ciencias (MIFA) of the Universidad Autonoma de Tamaulipas (UAT), at Ciudad Victoria, Tamaulipas, Mexico.

Results and Discussion

Trapped during sampling for more than 1 year were 130 mites in different stages of development, except larvae and eggs: 112 ♀, 11 ♂, four tritonymphs (Tn), two deutonymphs (Dn), and one protonymph (Pn) (Table 1). This was the first record

in Mexico of *B. longistriata* with population structure differentiated and collected for 1 year in Malaise traps.

Table 1. Number of Mites and Collembola Collected by Malaise Traps at Tula, Tamaulipas

Collection date (2016-2017)	*Bdella longistriata* ♀	♂	Tn	Dn	Pn	Collembola	Other mites
30 January-14 February	3					51	9
15 February-27 February						191	23
29 February-14 March						193	37
15 March-31 March						173	93
1 April-14 April	2					361	156
15 April-28 April						918	66
29 April-14 May						4,407	111
15 May-31 May	3			1		6,365	226
1 June-15 June						4,310	314
16 June-30 June	29		1			3,320	121
1 July-15 July	2		2			280	21
16 July-31 July	1					655	132
1 August-14 August	10		1			455	76
15 August-25 August	6					399	34
26 August-15 September	21	5				194	8
16 September-30 September	3	2		1	1	210	36
1 October-15 October						172	37
16 October-31 October						127	23
1 November-15 November	20	2				165	39
16 November-30 November	7	2				270	15
1 December-15 December	2					156	42
16 December-31 December	3					51	69
1 January-21 January						67	37
23 samples	112	11	4	2	1	23,667	1,725

In Mexico, the mite species was in different types of climates and vegetation. Collecting in different states and localities with different types of vegetation could help expand the distribution record. Bdellid mites were collected more frequently at the beginning of development of vegetation when organic matter was scarce (Wallwork et al. 1985). They were collected in leaf litter and epiphytic environments, so abundance increased as the dry season ended. Most specimens were collected at the end of spring (June and July) and in August and September during summer (Table 1) when the rainy season began. Snetsinger (1956) said the mites usually hibernated in spring and were scarce, which agreed with results of the current study (Fig. 2). Alberti (1973) and Mejía-Recamier (1997) said the species was an important regulator of abundance of other species with which it shares a niche, causing them to coexist in equilibrium. In addition to Bdellidae, organisms such as Collembola (Entomobrydae and Symphypleona), Anystidae mites (adults and nymphs), larvae of Erythraeidae, Eupodidae (adults and nymphs), Trombididae (nymphs), and some Phytoseiidae (adults) were found throughout the year. Abundance of organisms and the predator increased at the same time. Mejía-Recamier (1997) documented the

species as a predatory mite that fed on Eupodidae mites (Eupodoidea), which were abundant in samples in the trap in the study, and also springtails (Collembola). Predators increased in abundance when numbers of prey, usually springtails, and soft-bodied mites were increasing (Kethley 1990; Wallace and Mahon 1971, 1972, 1976). Bdellidae mites have been used for efficient biological control in some crops (Wallace and Walters 1974).

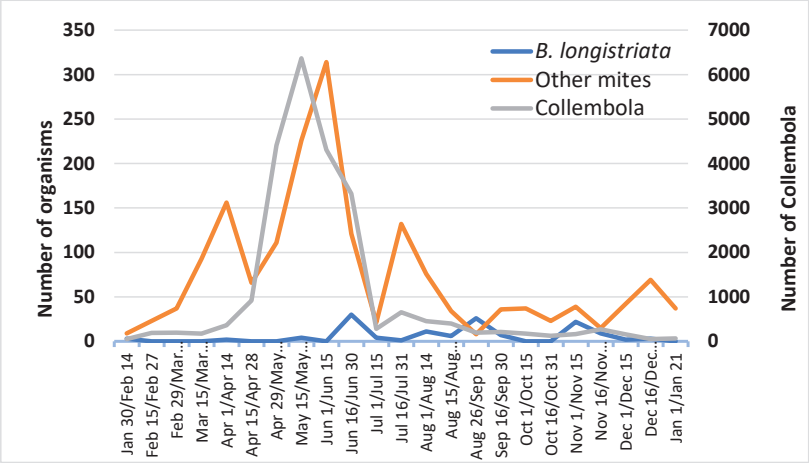

Fig. 2. Number of mites and Collembola collected in one Malaise trap at Tula, Tamaulipas, Mexico.

The most specimens were collected (130) in 14 of 23 samples from the low deciduous forest (Tula) (Fig. 3A), which might have been caused by a greater amount of organic matter. Biotic and abiotic factors influenced the abundance of predators. Alberti (1979) mentioned the genital papillae of bdellid mites were used for uptake of water, which allows them to inhabit places with low humidity, although they were more abundant when the environment was humid (Mejía-Recamier 1997), which denotes the influence of climatic factors on abundance.

Badejo (1990) and Chen et al. (2011) found seasonal differences in abundance of soil arthropods. They reported that abundance of microarthropods greatly fluctuated, being susceptible to small changes in microenvironment, and that water was the primary factor influencing abundance.

Population structure collected by the Malaise trap differed throughout the year: females were most abundant, 85%, while males constituted only 9%, and immature stages (tritonymphs, deutonymphs, and protonymphs) were 6%. No larvae or eggs were found. Results probably reflected that immature stages hibernated in places such as fissures, holes, cracks, bark of the trees, and rocks (Atyeo 1960, Soliman and Mohamed 1972, Alberti 1973), while adults were active. Adults were more abundant in June, September, and November, unlike what Mejía Recamier (1997) found that abundance at Jalisco was homogeneous throughout the year, with most in November and March.

That more organisms were in the Berlese funnel probably was because all developmental stages were in soil from which samples were collected, and when a sample was put into a funnel, the fauna in the sample continued to move. Berlese funnels are used to extract arthropods from soil and leaf litter. They work on the principle that insects and other arthropods that normally live in soil and litter respond negatively to light that forces arthropods to move downward where they fall through a funnel and into a container with ethanol (Edgar 1992, Chaires et al. 2013).

Unlike a Berlese funnel, a Malaise trap commonly is used for sampling low-flying insects (Sheikh et al. 2016). The presence of mites in traps is not accidental, because predatory organisms have rapid movement and sometimes move great distances to find prey (mites, collembola, and such microorganisms as nematodes).

Several factors seemed to result in more females than males in samples. Some species are parthenogenic (Soliman and Mohamed 1972). Another factor is reproduction whereby males deposit spermatophores sought by females (Alberti 1974, Alberti and Storch 1976). In the samples, 70% of females were ovigerous. They might have been in Malaise traps because they were looking for food.

In Mexico, only two studies used a full year of sampling with a Berlese funnel (Mejía-Recamier 1986, 1997), including collection of adults without differentiating males from females. In this study, males were collected only in September and October, tritonymphs between June and August, deutonymphs in May and September, protonymphs in September, and females for 7 months (Table 2, Fig. 3B). More specimens were obtained with a Berlese funnel (Fig. 3C,D) at Jalisco (Chamela), unlike in this study in a tropical deciduous forest. The mites normally were associated with Bromeliaceae and Orchidaceae plants or in leaf litter or moss at ground level 20 to 3,900 m above sea level (Mejía-Recamier 1997). This is the first time *Bdella* mites were in collecting bottles in Malaise traps 1.1 m from the ground.

Table 2. Number of *B. longistriata* Collected by Two Sampling Methods

	Malaise trap (this study)	Berlese funnel (Mejía-Recamier 1997)
Adult	112♀/11♂	37
Tritonymph	4	160
Deutonymph	2	92
Protonymph	1	69
Larva	0	37
Total	130	395

Mites were collected in natural areas or with little disturbance or anthropogenic activity, a factor considered relevant to locating them, which is why they can be considered a bioindicator and efficient population regulator and might be a promising biological control agent (Wallace 1974, Mejía-Recamier 1997). Ejido Gallitos where mites were collected in a Malaise trap has only 144 inhabitants and although it is at 960 m above sea level, access to the collection site was difficult.

In conclusion, *B. longistriata* is a species with restricted distribution that has been registered in the United States of America and seven states of Mexico. The mites are a new record at the Municipality of Tula in the State of Tamaulipas. A Berlese funnel usually is used to extract soil mites, but this is the first time systematic collection with a Malaise trap has been used to collect mites and other arthropods in a detailed study.

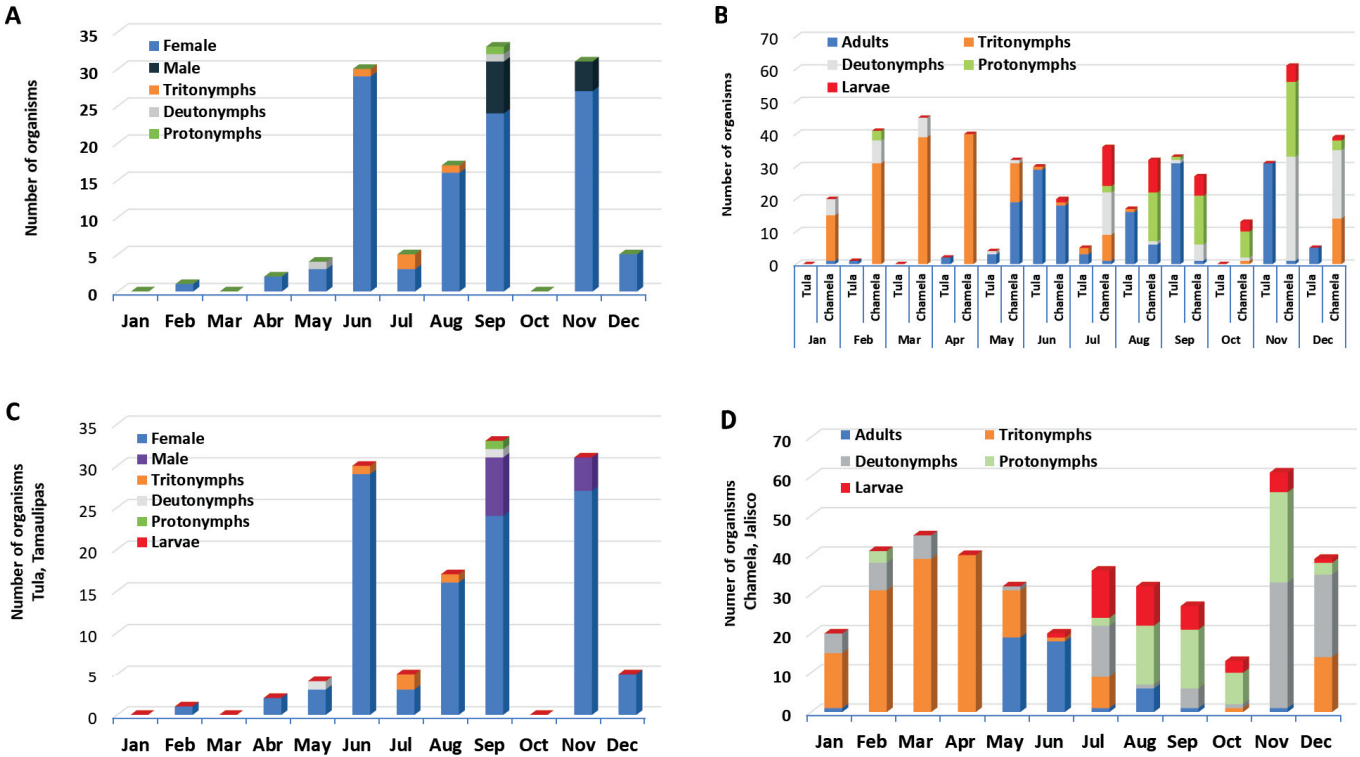

Fig. 3. A) Number of *Bdella longistriata* specimens captured by sampling date at Tula, Tamaulipas. B) Number of mites captured per month at Tula, Tamaulipas (current study), and Chamela, Jalisco by Mejía-Recamier (1997). C and D) Immatures and adults captured by Malaise trap (Tamaulipas) and Berlese funnel per month (Jalisco: Mejía-Recamier 1997).

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References Cited

Alberti, G. 1973. Ernährungsbiologie und Spinnvermögen der Schabelmilben (Bdellidae, Trombidiformes). Z. Morph. Tiere. 76: 285-338.

Alberti, G. 1974. Fortpflanzungsverhalten und fortpflanzungsorgane der schnabelmilben (Acarina: Bdellidae, Trombidiformes). Z. Morph. Tiere. 78: 111-157.

Alberti, G. 1979. Fine structure and probable function of genital papillae and Claparède organs of Actinotrichida, pp. 501-507. *In* J. Rodriguez [ed.], Recent Advances in Acarology, Vol. II. Academic Press, New York.

Alberti, G., and V. Storch. 1976. Spermiocytogenese, spermien und spermatophore von schnabelmilben (Bdellidae, Acari). Acta Zool. 57: 177-188.

Atyeo, W. T. 1960. A revision of the family Bdellidae in North and Central America (Acarina: Prostigmata). Univ. Kans. Sci. Bull. 40: 345-499.

Badejo, M. 1990. Seasonal abundance of soil mites (Acarina) in two contrasting environments. Biotropica 22: 382-390.

Baker, E. W., and J. W. Balock. 1944. Mites of the family Bdellidae. Proc. Entomol. Soc. Wash. 46: 176-184.

Chaires-Grijalva, M. P., E. G. Estrada-Venegas, J. A. Acuña-Soto, y A. Equihua-Martínez. 2013. Comparación de tres tipos de muestreo para ácaros mesostigmados en suelo agrícola, pp. 98-109. *In* E. Estrada-Venegas, M. P. Chaires-Grijalva, J. A. Acuña-Soto, y A. Equihua-Martínez [eds.], Fauna de Suelo II. Micro, Meso y Macrofauna, Sociedad Mexicana de Entomología. México.

Chen, X., Y.-X. Zhang, Y.-P. Zhang, J. Ji, and J.-Z. Lin. 2011. Influence of temperature on development of *Bdella tropica* Atyeo (Acari: Bdellidae) with *Tetranychus urticae* Koch (Acari: Tetranychidae) as its prey. Int. J. Acarology. 37(Sup.1): 34-39.

Edgar, A. L. 1992. A quantitative study of litter and soil invertebrates utilizing the Berlese funnel, pp. 73-89. *In* C. A. Goldman, S. E. Andrews, P. L. Hauta, and R. Ketchum [eds.], Tested Studies for Laboratory Teaching, Vol. 6. Proceedings of the 6th Workshop/Conference of the Association for Biology Laboratory Education (ABLE).

Fisher, J. R., M. J. Skvarla, G. R. Bauchan, R. Ochoa, and A. P. Dowling. 2011. *Trachymolgus purpureus* sp. n., an armored snout mite (Acari, Bdellidae) from the Ozark highlands: morphology, development, and key to *Trachymolgus* Berlese. ZooKeys 125: 1-34.

Gerson, U., R. L. Smiley, and R. A. Ochoa. 2003. Mites (Acari) for pest control. Blackwell, Oxford.

Hernandes, F. A. 2013. Revision of Nathan Banks' type specimens of Bdellidae Dugès (Acari: Trombidiformes) of the Museum of Comparative Zoology, Cambridge. Int. J. Acarology 39: 1-9.

Hernandes, F. A., M. J. Skvarla, J. R. Fisher, A. P. G. Dowling, R. Ochoa, E. A. Ueckermann, and G. R. Bauchan. 2016. Catalogue of snout mites (Acariformes: Bdellidae) of the world. Zootaxa 4152: 1-83.

Hoffmann, A., y M. G. López-Campos. 2000. Biodiversidad de los ácaros de México. Primera ed. CONABIO, México.

Kethley, J. 1990. Acarina: Prostigmata (Actinedida), pp. 667-756. *In* D. L. Dindal [ed.], Soil Biology Guide. John Wiley and Sons, New York.

Mejía-Recamier, B. E. 1986. Sistemática, Distribución Geográfica y Aspectos Ecológicos de los Bdellidae (Acari: Prostigmata) Mexicanos. Tesis de Licenciatura en Biología, Facultad de Ciencias, Universidad Nacional Autónoma de México.

Mejía-Recamier, B. E. 1997. Aspectos Ecológicos de los Ácaros de la Familia Bdellidae de Chamela, Jalisco. Tesis de Maestría, Facultad de Ciencias, Universidad Nacional Autónoma México, D.F., México.

Mejía-Recamier, B. E., and L. Q. Cutz-Pool. 2007. Diversidad altitudinal de Bdellodea (Prostigmata) en musgos cortícícolas en el Estado de México. Entomología Mexicana 6: 54-59.

Sheikh, A. H, M. Thomas, R. Bhandari, and H. Meshram. 2016. Malaise trap and insect sampling: Mini review. Bio Bulletin 2: 35-40.

Snetsinger, R. 1956. Biology of *Bdella depressa,* a predaceous mite. J. Econ. Entomol. 49: 745-746.

Soliman, Z. R., and M. I Mohamed. 1972. Biological studies on the soil-inhabiting bdellid mite*, Spinibdella bifurcata* (Acarina: Bdellidae) in U.A.R. Z. Angew. Entomol. 70: 15-23.

Wallace, M. M. H. 1974. An attempt to extend the biological control of *Sminthurus viridis* (Collembola) to new areas in Australia by introducing a predatory mite, *Neomolgus capillatus* (Bdellidae). Aust. J. Zool. 22: 519-529.

Wallace, M. M. H., and J. A. Mahon. 1971. The ecology of *Sminthurus viridis* (Collembola) III. The influence of climate and land use on its distribution and that of an important predator, *Bdellodea lapidaria* (Acari: Bdellidae). Aust. J. Zool. 19: 177-188.

Wallace, M. M. H., and J. A. Mahon. 1972. The taxonomy and biology of Australian Bdellidae (Acari). 1. Subfamilies Bdellinae, Spinibdellinae and Cytinae. Acarologia 14: 544-580.

Wallace, M. M. H., and J. A. Mahon. 1976. The taxonomy and biology of Australian Bdellidae (Acari). 2. Subfamily Odontoscirinae. Acarologia 18: 65-123.

Wallace, M. M. H., and M. C. Walters. 1974. The introduction of *Bdellodes lapidaria* (Acari: Bdellidae) from Australia into South Africa for the biological control of *Sminthurus viridis* (Collembola). Aust. J. Zool. 22: 505-517.

Wallwork, J. A., B. W. Kawil, and W. G. Whitford. 1985. Distribution and diversity patterns of soil mites and another microarthropods in a Chihuahua desert sites. J. Arid Environ. 9: 215-231.

Walter, D. E., and G. W. Krantz. 2009. Collecting, rearing and preparation specimens, pp. 83-96. *In* G. W. Krantz and D. E. Walter [eds.], A Manual of Acarology. Texas Tech University Press, Lubbock, TX.

Tomato Russet Mite, *Aculops lycopersici* (Massee)[1], Abundance and Effect on Tomato Yield

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Abstract. *Aculops lycopersici* (Massee) causes plant desiccation and severe damage to tomato (*Lycopersicon esculentum* Mill) fruit, resulting in serious economic loss. The objective of the study was to determine the incidence of damage to fruit by *A. lycopersici*. Three varieties of tomato (SUN 7705, CID, and V305 F1) were used. Variety V305 F_1 had the greatest index of damaged fruit (1.6852a), lowest index of useful fruit, (2.1878a) and least average weight per fruit (302.97a) of the three varieties. CID was least affected. The middle stratum had the greatest index of damaged fruit (2.3070a), lowest index of useful fruit, (1.5660b), and least average weight per fruit (254.89b) of the three strata. The lower stratum was least affected.

Resumen. *Aculops lycopersici* (Massee) provoca la desecación de las plantas y daños graves a los frutos del tomate (*Lycopersicon esculentum* Mill), lo que genera graves pérdidas económicas. El objetivo de este estudio fue determinar la incidencia de daño a frutos por *A. lycopersici*. Se utilizaron tres variedades de tomates (SUN 7705, CID, y V305 F1). De las tres variedades, la variedad V305 F1 presentó el mayor índice de frutos dañados (1.6852a), el menor índice de frutos útiles (2.1878a), y el menor peso promedio por fruto (302.97a). La variedad CID fue la menos afectada. De los tres estratos, el estrato medio presentó el mayor índice de frutos dañados (2.3070a), el menor índice de frutos útiles (1.5660b), y el menor peso promedio por fruto (254.89b). El estrato inferior fue el menos afectado.

Introduction

Among many pests affecting tomato (*Lycopersicon esculentum* Mill), the tomato russet mite, *Aculops lycopersici* (Massee), is one of the most damaging. The pest was first detected in Australia (Masse 1937) and has since dispersed worldwide (Jepson et al. 1975, Bastos 1981, Perring and Farrar 1986). Tomato is its main host (Jeppson et al. 1975), but it also affects other Solanaceae plants such as potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.), tobacco (*Nicotiana tabacum* L.), chili (*Capsicum* sp.), petunia (*Petunia* sp.), and nightshade (*Solanum nigrum* L.). It also affects species of Convolvulaceae such as bindweed (*Convolvulus arvensis* L.) and morning glory (*Ipomoea purpurea* L. Roth) (Bastos 1981, Gómez and Rivera 1987). *A. lycopersici* has extreme polyphagia, proliferates optimally in a

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large number of varieties of the genus *Lycopersicum*, and is cosmopolitan in distribution (López-Bautista et al. 2016).

The life cycle of *A. lycopersici* has four development stages and lasts an average of 7 days (Navarro et al. 2011, Schuster 2013). Adults are fusiform and robust, whitish to yellow-orange. Their main identifying characteristic is a tubercle of the seta of the dorsal shield directed posteriorly and divergent. The front lobe of the prodorsal shield has a lobulated edge; feather-claws on the legs have four rays (Jeppson et al. 1975, Keifer et al. 1982). Female adults live approximately 22 days and male adults live 16 days (Mau and Lee 1994). Females lay approximately 52 eggs (Haque and Kawai 2003), and Fernández (2011) reported the species does not diapause.

Optimal developmental conditions for the species were 27ºC and 30% relative humidity (Saini and Alvarado 2001, Haque and Kawai 2003). Under these conditions, a generation is completed in 6 to 7 days. They are most abundant in spring and fall (Saini and Alvarado 2001, Navarro et al. 2011).

A. lycopersici feeds on epidermal cells by using three types of stylets (15 µm long) that circle the mouth: chelicerae, labrum, and infra-capitulum. Chelicerae deposit saliva on epidermal cells, while the labrum and infra-capitulum form a feeding preorally-directed channel to suck cell contents (Nuzzaci and Alberti 1996). Feeding destroys epidermal cells, induces formation of insensitive tissues in damaged areas, and reduces photosynthesis and respiration (Royalty and Perring 1988). *A. lycopersici* establishes on the underside of leaves on the lower third of a plant (Fernandez 2011, Navarro et al. 2011). As abundance increases, it moves upward, and symptoms normally appear in the basal part of the plant and quickly propagate upward. The tomato russet mite is a major pest severely damaging all stages of tomato (Perring 1996) and causing serious economic loss (Royalty and Perring 1987, Duso et al. 2010). Typical symptoms of tomato russet mites are silver-colored rings on the lower surface of leaves that turn yellow to tannish-brown with a silvery appearance on the underside when the leaves dry and then fall off (Jeppson et al. 1975, Doreste 1984). Stems become bronze or cinnamon colored; the lower part loses its trichomes, cracking longitudinally, and eventually dies. Skin of green and ripe fruit becomes rough and brown. Fruit can become deformed and lose weight (Saini and Alvarado 2001, Kawai and Haque 2004, Navarro et al. 2011), and also is sunburned after leaves fall off (Jeppson et al. 1975, Doreste 1984).

A. lycopersici is microscopic in size (0.1 to 0.2 mm) and usually is not noticed until symptoms appear about 10 days after infestation, when abundant and difficult to control (Oliveira et al. 1982, Saini and Alvarado 2001, Navarro et al. 2011). Using tomato varieties with resistance to the pest is important for integrated management to reduce the need for insecticide. The aim of the study was to determine resistance of three varieties of tomato (SUN 7705, CID, and V305 F1) against *A. lycopersici.*

Materials and Methods

The study was done in a greenhouse at Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico (19º 27´ N; 98º 53´ W). Tomato varieties V305 F_1, CID, and SUN 7705 were selected for the study because they are the most commonly grown commercial varieties in the region. Seeds of all varieties were treated with *Bacillus subtilis* strain QST713 (Ehrenberg) Cohn 1872 and planted in plastic trays. Four weeks later, seedlings were transplanted into red volcanic

(tezontle) substrate in polyethylene bags. Water and nutritive solution (Steiner) were provided (8 liters per hour) through an automated drip irrigation system.

The experimental unit was 10 tomato plants selected randomly from each variety. Three strata were used, with different plant height from base to apex: lower (0-13.77 inches), middle (13.78-27.55 inches), and upper (27.55-41.33 inches) with three replications of each variety, for 30 plants per variety and a total of 90 plants. Tomato plants were infested with mites 60 days after transplanting by putting an infested 5-cm stem into each plant stratum. The experimental design was randomized blocks.

Results of the experiment were obtained 60 days after infestation. Different racemes of tomato fruit on each plant stratum were evaluated. The measurement of the stratum changed because the plant grew from infestation to evaluation: lower (0-23.62 inches), middle (23.63-47.24 inches), and upper (23.64-70.86 inches). Each fruit of each raceme was divided into categories of: 1) damaged or not marketable when more than 20% of fruit was damaged and 2) acceptable for market when less than 20% of the fruit surface was damaged. The amount of damage per fruit was determined visually. All fruit in the "acceptable for market" category was weighed. An index of damaged fruit per raceme was calculated by dividing the total number of fruit by the number of damaged fruit (more than 20% damaged surface). The index of useful fruit was the total number of fruit divided by the number of acceptable fruit per raceme. Infestation density was determined by counting all live *A. lycopersici* on a square centimeter per damaged fruit, observed with a 10X magnifying glass.

Analysis of variance with sub-sampling was done for each variable by using a general linear model and Tukey test ($\alpha = 0.05$) in SAS v. 9.4. Correlation analysis also was done.

Results

Results of analysis of variance indicated damage to fruit in at least one stratum was significantly different ($F_{2,4} = 32.91$; $P = 0.003$). A Tukey test indicated the middle stratum had significantly more damaged fruit, followed by the upper stratum, and then the lower stratum (Table 1).

Fruit of variety V305 F_1 was most damaged ($F_{2,4} = 6.20$; $P = 0.0595$; $\alpha = 0.1$) (Fig. 1). Variety CID had the fewest damaged fruit and was more resistant to damage by *A. lycopersici*. Fruits of varieties CID and SUN 7705 from the three strata were damaged, but damage was most evident on V305 F_1. Correlation analysis indicated fruit that was more damaged ($\rho = 0.793$) was more infested by tomato russet mites.

At least one strata resulted in a greater useful fruit index ($F_{2,4} = 32.92$; $P = 0.0033$). The Tukey test indicated the lower stratum had the most useful fruit, followed by the middle and upper strata (Table 1). The number of *A. lycopersici* increased on the middle and upper parts where damage was significantly more ($P > F$, 0.0033). V305 F_1 had the lowest index of useful fruit ($P > F$, 0.0595) and CID had the greatest proportion in the lower and upper strata (Fig. 2). The middle stratum of variety CID and upper stratum of V305 F_1 had similar useful fruit indices. Correlation analysis ($\alpha = 0.1$) indicated the larger the number of useful fruit, the less the index of damage ($\rho = -1.000$).

Results of analysis of variance indicated the middle stratum had the least proportion of useful fruit per raceme ($F_{2,4} = 21.23$; $P = 0.0074$), followed by the upper and lower strata. V305 F_1 had the least number of useful fruit per raceme ($F_{2,4} = 7.98$; $P = 0.0401$) (Fig. 3). CID was the variety that yielded most, and had the greatest

proportion of useful fruit per raceme. The middle stratum of the CID and upper stratum of Sun 7705 were not significantly different in terms of useful fruit per raceme because of vegetative development of the varieties. Correlation analysis (α = 0.1) indicated that the more useful fruit per raceme, the lower the index of damaged fruit (ρ = -0.929). Likewise, the greater the number of useful fruit per raceme, the greater the index of useful fruit (ρ = 0.928).

The middle stratum had the least average weight per fruit ($F_{2,4}$ = 11.64; P = 0.0215), followed by the upper and lower strata (Fig. 4). Comparing varieties at α =

Table 1. Results of Damaged Fruit indices of Tomato by Varieties (SUN 7705, V305 F_1) and Strata (lower, middle, upper) using the Test Comparing Means of Tukey (α = 0.05)

Variety and stratum	IDF	IUF	NUF	Weight	UWR	Density
SUN 7705	1.1711a	2.7020 a	12.910ab	373.83a	1530.9ab	205.19b
V305 F1	1.6852a	2.1878a	10.887b	302.97a	1074.8b	406.89a
CID	0.7927a	3.0803a	17.644a	460.31a	2739.3a	90.48b
Lower stratum	0.2549b	3.6181a	19.193a	502.40a	2626.4a	105.86b
Middle stratum	2.3070a	1.5660b	7.918b	254.89b	1198.5b	350.15a
Upper stratum	1.2549b	2.7860a	14.330a	379.82ab	1520.0ab	246.54ab

IDF: index of damaged fruit, IUF: index of useful fruit, NUF: average number of useful fruit, UWR: useful weight per raceme.

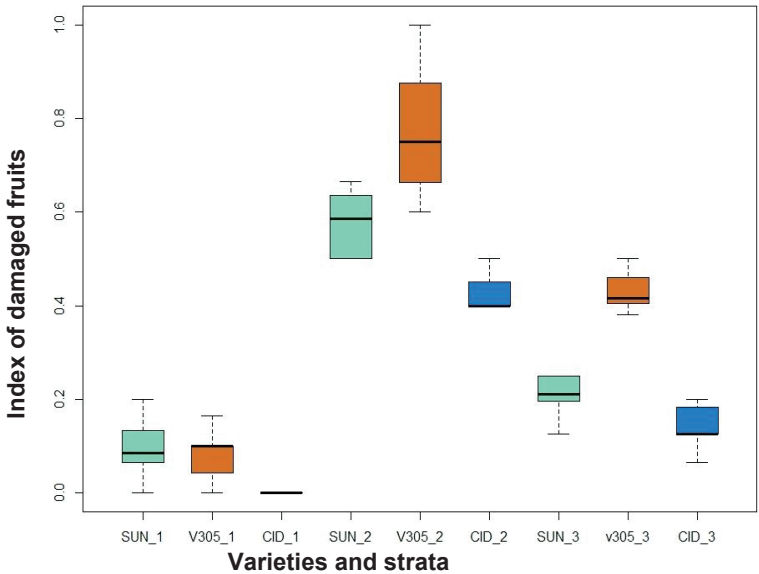

Fig. 1. Distribution of damaged fruit indices of tomato varieties SUN 7705 (box green), V305 F_1 (box orange) and CID (box blue) in three strata: 1 (lower), 2 (middle), and 3 (upper).

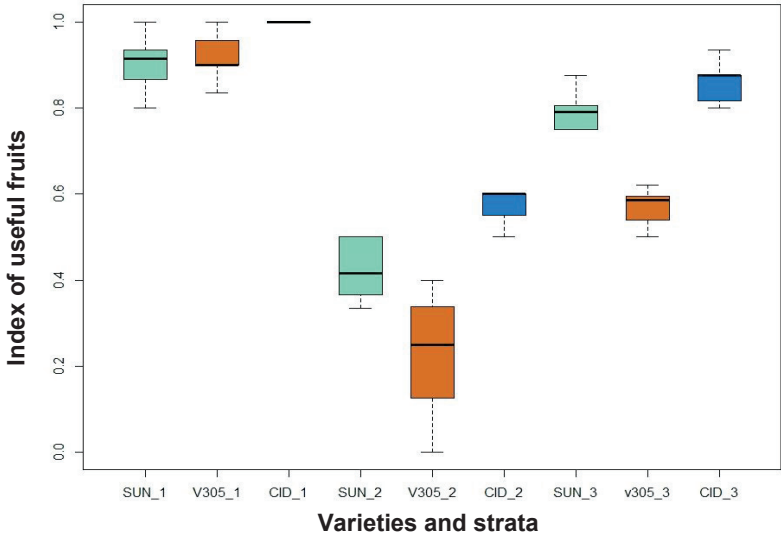

Fig. 2. Distribution of useful fruit indices of tomato varieties SUN 7705 (box green), V305 F_1 (box orange), and CID (box blue) in the three strata: 1 (lower), 2 (middle), and 3 (upper).

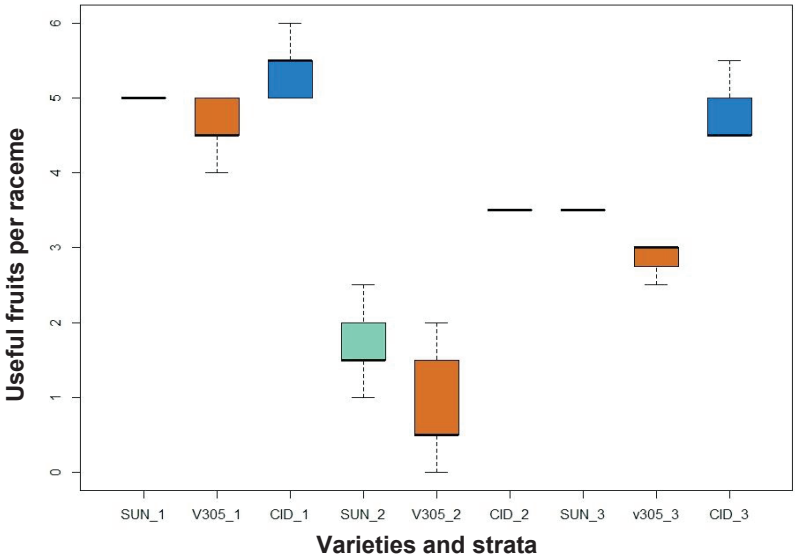

Fig. 3. Distribution of indices of useful fruit per raceme of tomato varieties SUN 7705 (box green), V305 F1 (box orange), and CID (box blue) in three strata: 1 (lower), 2 (middle), and 3 (upper).

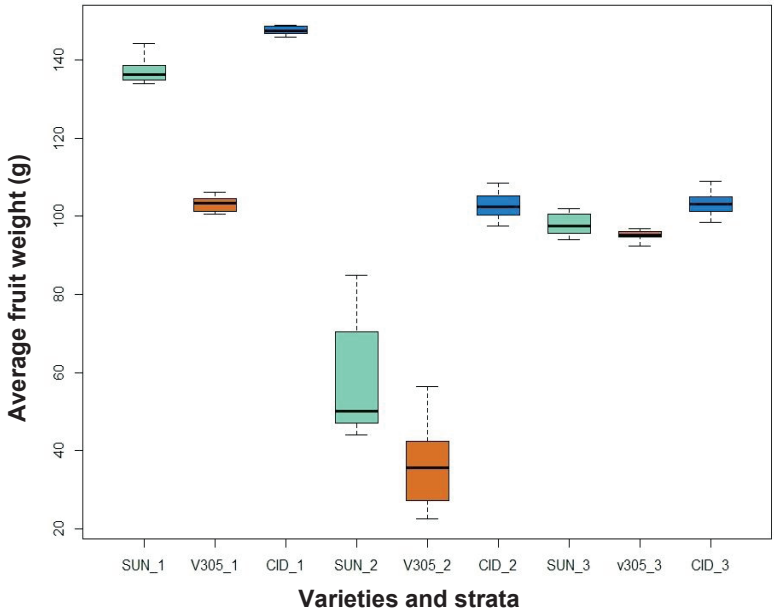

Fig. 4. Distribution of average fruit weight of tomato varieties SUN 7705 (box green), V305 F_1 (box orange), and CID (box blue) in three strata: 1 (lower), 2 (middle), and 3 (upper).

0.1, V305 F_1 had least fruit weight ($F_{2,4}$ = 4.72; P = 0.0886). CID had the greatest fruit weight and also had similar results in the middle and upper strata. A Tukey test indicated V305 F_1 was the variety with least average weight of fruit in the three strata. Correlation analysis showed that greater average fruit weight resulted in a lower index of damaged fruit (ρ = -0.846), and greater indices of useful fruit (ρ = 0.844) and useful fruit per raceme (ρ = 0.902).

Only useful fruit per raceme was considered in average raceme weight. Analysis of variance indicated the middle stratum had the least average weight per raceme ($F_{2,4}$ = 9.32; P = 0.0312), followed by upper and lower strata (Fig. 5). V305 F_1 had the least average fruit weight ($F_{2,4}$ = 12.29; P = 0.0196) of the three varieties. A Tukey test indicated the variety with the greatest fruit weight per raceme in the three strata was CID (Table 1). The lower stratum of the three tomato varieties had greater useful weight per raceme; *A. lycopersici* did not damage this plant stratum.

Correlation analysis at α = 0.1 determined the greater the average weight of useful fruit per raceme, the less the damage index (ρ = -0.753). The greater the average weight of useful fruit per raceme, the greater the useful fruit index (ρ = 0.750), number of useful fruit per raceme (ρ = 0.861), and average weight per fruit (ρ = 0.894). Analysis of variance (α = 0.05) of abundance of *A. lycopersici* in fruit indicated statistically significant differences ($F_{2,4}$ = 11.79; P = 0.020) among the three strata of tomato varieties. Results showed significantly more *A. lycopersici* in the middle stratum, followed by the upper stratum, while mites were least abundant in the lower stratum (Fig. 6). Tomato russet mites were significantly most abundant on V305 F_1

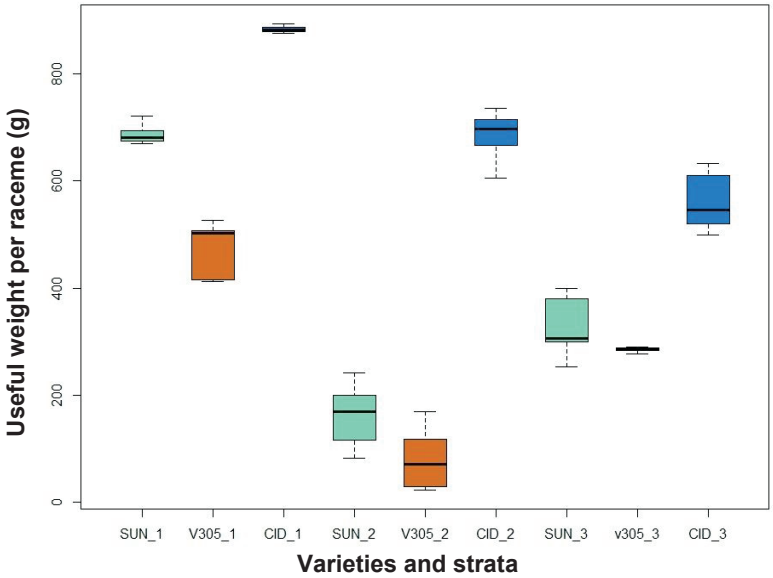

Fig. 5. Distribution of useful weight per raceme of tomato varieties SUN 7705 (box green), V305 F_1 (box orange), and CID (box blue) in three strata: 1 (lower), 2 (middle), and 3 (upper).

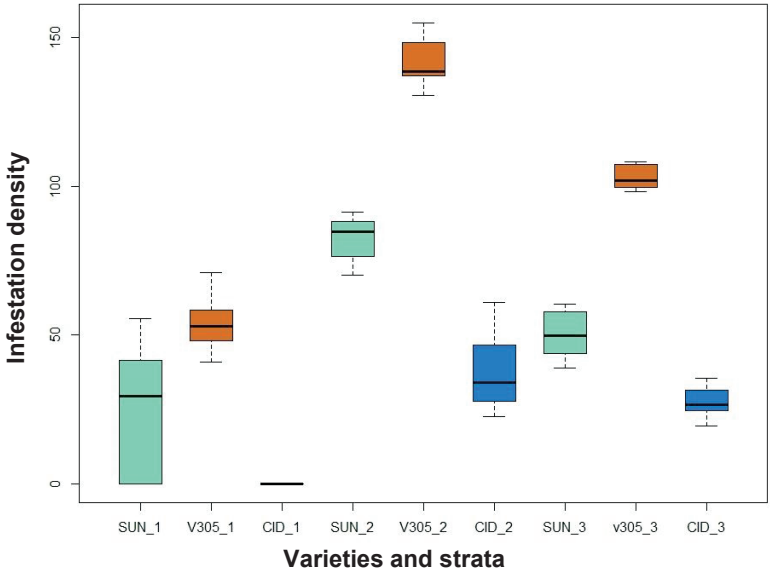

Fig. 6. Distribution of abundance of infestation by *Aculops lycopersici* in tomato fruit of varieties SUN 7705 (box green), V305 F_1 (box orange), and CID (box blue) in three strata: 1 (lower), 2 (middle), and 3 (upper).

($F_{2,4}$ = 20.12; P = 0.008; α = 0.1) and V305 F_1 in the three strata (lower, middle, and upper). A Tukey test indicated CID followed by SUN 7705 had fewest tomato russet mites in the three strata (Table 1).

Correlation analysis showed that the greater the abundance of *A. lycopersici*, the greater the index of damage (ρ = 0.793). More individual tomato russet mites, resulted in smaller numbers of useful fruit (ρ = -0.791), useful fruit per raceme (ρ = -0.802), average fruit weight (ρ = -0.769), and average raceme weight (ρ = -0.820).

Discussion

Tomato russet mites were most abundant on V305 F_1 that had a greater index of damaged fruit, lower index of useful fruit, and least average weight per fruit and per raceme. V305 F_1 was more susceptible to damage by tomato russet mite than was CID or SUN 7705. Earliness of V305 F_1 made it more susceptible because the mites had more time to feed and cause damage. According to Fernández (2011), *A. lycopersici* does not diapause phase and is present year around. For this reason, V305 F_1 is not recommended for production of tomatoes in a greenhouse, especially with history of mites. Under greenhouse conditions and in terms of commercialization, CID had the highest index of useful fruit and average fruit weight, low abundance of *A. lycopersici*, and better crop yield. *A. lycopersici* impacted yield of the varieties, and there was large correlation between mites and variables in the study. Lack of statistically significant differences in abundance of *A. lycopersici* in the three strata of the varieties was caused by mite saturation of the space where the data were collected. The short life cycle of *A. lycopersici* (7 days) was associated with high fecundity (51.7 eggs per female), which meant abundance increased exponentially in a few days (Haque and Kawai 2003, Navarro et al. 2011, Schuster 2013).

Results concurred with those of Oliveira et al. (1982), Saini and Alvarado (2001), and Navarro et al. (2011) in that symptoms by tomato russet mite were evident, in this case, damaged fruit, when mites were abundant. Damage in three strata of tomato plants agreed with Perring (1996) in that tomato russet mites damaged both old (lower) or young (upper) strata at any developmental stage of the crop. The fewer tomato russet mites on the tomato crop, the greater the number of fruit that can be marketed, as found by Oliveira et al. (1982), Saini and Alvarado (2001), and Navarro et al. (2011). The largest proportion of damaged fruit in the middle stratum and greatest useful fruit index in the lower stratum concurred with results of Doreste (1984), Saini and Alvarado (2001), Fernández (2011), and Navarro et al. (2011) because *A. lycopersici* abundance and damage moves upward, so mites in the lower part of the tomato plant were not abundant enough to damage fruit, while the number of *A. lycopersici* increased in the middle and upper parts where damage was significantly greater. Low weight of the tomatoes was caused by damage by *A. lycopersici*, concurring with Saini and Alvarado (2001), Kawai and Haque (2004), and Navarro et al. (2011). According to Royalty and Perring (1988) and Nuzzaci and Alberti (1996), low fruit weight was caused by tomato russet mite feeding: they feed on epidermal cells of the plant, reducing photosynthesis and respiration, which in turn decreases weight. The low average weight of fruit from the middle and upper strata means economic losses for a grower because it lowers crop production (Berlinger et al. 1982, Royalty and Perring 1987).

Our objective in determining the incidence of damage by *A. lycopersici* and how it affects yields of three tomato varieties was achieved. Abundant *A. lycopersici*

caused visible damage on tomato fruit and reduced fruit weight, mainly in the middle stratum of the plant. Of the three varieties (CID, SUN 7705, and V305 F_1), V305 F_1 was most infested by *A. lycopersici*, followed by SUN 7705, and finally CID that was most resistant to damage by mites. V305 F_1 was most susceptible, having the greatest index of damaged fruit and greatest abundance of *A. lycopersici* as well as the lowest index of useful fruit and least average fruit weight. Variety CID is recommended for commercial tomatoes in a greenhouse when problems with *A. lycopersici* are recurrent, because in the study it had least incidence of damage, greatest index of useful fruit, more average fruit weight, and least mites. The variety yielded 45% more than did SUN 7705 and 61% more than V305 F_1. Of the three strata of the three varieties, the lower strata had least damage and infested fruit, as well as the greatest index of useful fruit and average useful fruit weight. This was because the plant continued to develop, and when old leaves were removed, *A. lycopersici* tended to move up to the next stratum. Less abundance of tomato russet mites in that stratum resulted in considerably less damage, while damage was greater in the middle and upper strata.

Acknowledgment

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References Cited

Bastos, M. J. A. 1981. Principais pragas das culturas seus controles. Nobel, Sao Paulo.

Berlinger, M. J., R. Dahan, and S. Cohen. 1982. Greenhouse tomato pests and their control in Israel. Proc. Working Group Integrated Control in Glasshouses. Darmstadt 7-11.

Doreste, S. E. 1984. Acarología. Instituto Interamericano de Cooperación para la Agricultura. Serie investigación y desarrollo, No. 11. San José, Costa Rica.

Fernández, B. J. 2011. *Aculops lycopersici*. Ficha Técnica de Sanidad Vegetal. Núm. 060. Gobierno de Extremadura. Consejería de Agricultura, Desarrollo rural, medio ambiente y energía.

Gómez, A. A., y H. Rivera. 1987. Descripción de malezas en plantaciones de café. Centro Nacional de Investigaciones del Café: CERNICAFE. Carvajal, Chinchiná, Colombia.

Haque, M. M., and A. Kawai. 2003. Effect of temperature on development and reproduction of the tomato russet mite, *Aculops lycopersici* Massee (Acari: Eriophydae). Entomology and Zoology 38: 97-101.

Jeppson, L. R., H. B. Keifer, and E. W. Baker. 1975. Mites injurious to economic plants. University of California Press.

Kawai, A., and M. M. Haque. 2004. Population growth of tomato russet mite, *Aculops lycopersici* (Acari: Eriophyidae) and its injury effect on the growth of tomato plants. Journal of Acarology Society Japón 11: 1-10.

Keifer, B. H., E. W. Baker, T. Kono, M. Delflnado, and W. E. Styer. 1982. An illustrated guide to plant abnormalities caused by eriophyid mites in North America.

López-Bautista, E., M. T. Santillán-Galicia, J. Suárez-Espinosa, N. Cruz-Huerta, N. Bautista-Martínez, and J. A. Alcántara-Jiménez. 2016. Damage caused by mite *Tetranychus merganser* (Trombidiformes: Tetranychidae) on *Carica papaya* (Violales: Caricaceae) plants and effect of two species of predatory mite. International Journal of Acarology 42: 303-309.

Massee, A. M. 1937. An eriophyid mite injurious to tomato. Bull. Entomol. Res. 28: 403.

Mau, R. F. L., and S. G. Lee. 1994. *Aculops lycopersici* (Massee). Crop Knowledge Master. http://www.extento.hawaii.edu/kbase/crop.

Navarro Cuesta, V., L. A. Lidia, y F. F. Milagros. 2011. Colección de fichas de plagas en cultivos hortícolas protegidos. Córdoba. Consejería de Agricultura y Pesca, Instituto de Investigación y Formación Agraria y Pesquera 1-36.

Nuzzaci, G., and G. Alberti. 1996. Internal anatomy and physiology, pp. 101-150. *In* E. E. Lindquist, M. W. Sabelis, and J. Bruin [eds.], Eriophyoid Mites - Their Biology, Natural Enemies and Control. Elsevier Science Publ., Amsterdam.

Oliveira, C. A. L., D. Eschtapati, D. Velho, y O. I. Sponchiado. 1982. Daños cuantitativos causados pelo microácaro A*culops lycopersici (*Massaee 1937) en cultura de tomatero. Ecossistema 7: 14-18.

Perring, T. M. 1996. Vegetables, pp. 593-610. *In* E. E. Lindquist, M. W. Sabelis, and J. Bruin [eds.], Eriophyoid Mites Their Biology, Natural Enemies and Control. World Crop Pest 6.

Perring, T. M., and C. A. Farrar. 1986. Historical perspective and current world status of the Tomato Russet Mite (Acari: Eriophyidae). Entomol. Soc. Am. 63: 19.

Royalty, R. N., and T. M. Perring. 1987. Comparative toxicity of acaricides to *Aculops lycopersici* and *Homeopronematus anconal* (Acari: Eriophyidae. Tydeidae). J. Econ. Entomol. 80: 348-351.

Royalty, R. N., and T. M. Perring. 1988. Morphological analysis of damage to tomato leaflet by tomato russet mite (Acari: Eriophyidae). J. Econ. Entomol. 81: 816-820.

Saini, E., y L. Alvarado. 2001. Insectos y Ácaros perjudiciales al cultivo de tomate y sus enemigos naturales. INTA y SAGPyA. Buenos Aires, República Argentina.

Schuster, D. 2013. Tomato russet mite: *Aculops lycopersici*. UF/IFAS GCREC-Balm, University of Florida, Wimauma, FL.

Cicadomorpha[1] in a Vineyard at Parras, Coahuila, Mexico, and Vectors of Diseases

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Víctor Enrique Robles-Hernández[2], and Sergio R. Sánchez-Peña[2]

Abstract. Information is provided on Auchenorryncha entomofauna from a grape (*Vitis vinifera* L.) vineyard at the Municipality of Parras, Coahuila. Two families, five subfamilies, 14 genera, and 17 species were collected, and are the first data provided for this region. No species tested positive for *Xylella fastidiosa* (Wells et al., 1987) (Xanthomonadales: Xanthomonadaceae) bacteria. However, *Balclutha mexicana* (Blocker, 1967) (Hemiptera: Cicadellidae) was positive for *Candidatus* Phytoplasma asteri (Lee et al. 2004) (Acholeplasmatales: Acholeplasmataceae) and *Candidatus* Phytoplasma trifolii (Hiruki y Wang 2004) (Acholeplasmatales: Acholeplasmataceae), and this is the first report of grapevine for Mexico.

Introduction

In the State of Coahuila, Mexico, grapes (*Vitis vinifera* L.) have been cultivated for many years, mainly in the southwestern municipalities that constitute the Comarca Lagunera (Torreón, San Pedro, Matamoros, and Francisco I. Madero), central (Cuatrociénegas), southeastern (Arteaga and Saltillo), and southern (General Cepeda and Parras). Currently 594 ha are planted with grapevines in the State, from which 468 are in the valley of Municipality of Parras (SIAP 2016), where grapes have been cultivated since 1598, and wines produced have recognized prestige for their quality (Páez 2009).

Pierce's disease is in vineyards in Europe, America, and Australia, generating significant economic losses. It is caused by *X. fastidiosa* (Wells et al., 1987) bacteriatransmitted by Cicadellidae (Díaz Ortíz 2003, Aguilar et al. 2005, EPPO 2009) Cercopidae and Membracidae (Hopkins 1989, Lópes et al. 1996, Almeida and Purcell 2003, Redak et al. 2004). The microorganism is in the xylem, affecting physiology of the grapevines, and can kill large percentages of plants. *X. fastidiosa* is in commercial vineyards at Parras, Coahuila, damaging plants, and consequently production of grapes.

In the world, 7.1 million hectares of grapevines are cultivated, of which 909 thousand are in the Americas (OIV 2016). During 2016, the vineyards of Mexico occupied 31 thousand hectares that generated 351.3 thousand tons of grapes valued at 5,704 million pesos (SIACON-SIAP 2016). The crop, has great economic importance because of great and diversified consumption. Grapes are attacked by approximately 89 arthropod species (González et al. 2014) and also microorganisms

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including *X. fastidiosa* (Hopkins and Purcell 2002). The bacterium attacks more than 30 families of mono- and dicotyledonous plants, which include 153 species of host plants and is transmitted by vector insects from the families Cicadellidae, Cercopidae, Aphophoridae, Cicadidae, and Membracidae.

In the Cicadellinae subfamily are 39 species and 19 genera that vector *X. fastidiosa* (EFSA 2015) on the American continent. In the family Ahophoridae, *Philaenus spumarius* is an efficient vector distributed in America and Europe, and potential vector, *Neophilaenus campestris* is distributed only in Europe. Also, reported as possible vectors are *Cicada orni* (Hemiptera: Cicadidae), *Cercopis sanguinolenta* (Hemiptera: Cercopidae), and *Euscelis lineolatus* (Hemiptera: Cicadellidae) (Cornara et al. 2017).

In Membracidae, only *Cyphonia clavigera* is an efficient vector and *Entylia carinata* as a potential vector in Argentina. Therefore, the objectives of the study were to determine which species of the insect families mentioned are in a commercial vineyard at the municipality and to know which are potential vectors of disease.

Materials and Methods

The study was done from March to December 2017, in a vineyard at San Lorenzo, Coahuila. An entomological net with a 30-cm diameter was used randomly every 15 days to make 500 sweeps to collect adults of Cicadellidae, Cercopidae, and Membracidae from foliage of grapevines and undergrowth that grew on the banks and between rows of the crop. Five blankets each 2.5 x 2.5 m (6.25 m^2) were placed randomly on the ground below the drip-line of grapevines in different places in the vineyard. A pyrethroid insecticide (knock-down) was applied with a hand pump to foliage of the grapevines. Thirty minutes after application, a brush and forceps were used to collect insects that had fallen on the blanket. Insects caught with the entomological net and on the blankets at each sampling were put into a 1,000-cc glass bottle containing 75% ethyl alcohol diluted with distilled water and labelled with the date, name of the collector, and collection location. The bottles with samples were transported the same day to the Departmento de Parasitología Agrícola at the Universidad Autónoma Agraria Antonio Narro, Campus Saltillo, where they were kept at room temperature to later separate the adults by family of interest to the study. Adults of Cicadellidae and Cercopidae were counted, recorded, and kept in 75% ethyl alcohol diluted with distilled water in bottles labelled with field data. No adult Membracidae was collected. All adults of leafhoppers and spittlebugs were taken to the Laboratorio de Entomologia y Acarologia del Centro Nacional de Referencia Fitosanitaria (CNRF) at 37.5 km of the Federal Highway Mexico-Pachuca, Tecámac, Estado de Mexico, where they were morphologically and molecularly identified to genus and species. Primers RST31 (5'-GCGTTAAT TTTCGAAGTGATTCGATTGC-3') and RST33 (5'-CACCATTCGTATCC CGGTG-3') (Minesavage et al. 1994) were used.

Results and Discussion

Eighty adults of Cicadellidae and two of Cercopidae were collected in the subfamilies, genera, and species of Cicadellidae: *Acinopterus* sp. (Van Duzee, 1892) (Deltocephalinae), *Aceratagallia* sp. (Kirkaldy, 1907) (Agalliinae), *Balclutha mexicana* (Blocker, 1967) (Deltocephalinae), *Bolotheta* sp. (Kramer, 1963) (Deltocephalinae), *Cuerna* sp. (Melichar, 1925) (Cicadellinae), *Draeculacephala minerva* (Ball, 1927)

(Cicadellinae), *Empoasca* sp. (Walsh, 1862) (Typhlocybinae), *Exitianus* sp. (Ball, 1929) (Deltocephalinae), *Exitianus escavatus* (DeLong & Hershberger, 1947) (Deltocephalinae), *Exitianus picatus* (Gibson, 1919) (Deltocephalinae), *Graminella nigrifrons* (Forbes, 1885) (Deltocephalinae), *Graminella sonora* (Ball, 1900), (Deltocephalinae), *Neokolla hieroglyphica* (Say, 1830) (Cicadellinae), *Scaphytopius* sp. Ball, 1931 (Deltocephalinae), *Texananus* sp. Ball, 1918 (Deltocephalinae), and *Xyphon nudum* (Nottinham, 1932) (Cicadellinae). From Cercopidae, *Prosapia ignífera* Hamilton, 1977 (Tomaspidinae) was collected. In total, 14 genera, five subfamilies, and 17 species were identified.

Although Cicadellidae was not abundant in the vineyard, diversity included groups of economic importance. The genus *Cuerna* included *C. occidentalis, C. yuccae*, and *C. costalis* species that vector Pierce's disease in vineyards in California and Georgia. Unfortunately, in this work, the species of *Cuerna* obtained could not be identified. In the genus *Xyphon*, the species *X. triguttatum, X. fulgidum*, and *X. flaviceps* transmit the bacterium to grapevines and vegetables, and *Draeculacephala minerva* is recognized as a vector of Pierce's disease (Daane et al. 2011). *Graminella nigrofrons* transmits phytoplasmas (Beanland et al. 2006, Arocha et al. 2011) 16SrI-W *Candidatus* Phytoplasma asteri (Lee et al. 2004) and 16rVII-A *Candidatus* Phytoplasma fraxini (Griffiths et al. 1999) to *Prunus armeniaca* L., *Prunus persica* L., *Prunus domestica* L., and *Pyrus communis* L. The fruit trees coexist with grapevines in the Valle de Parras. This implied an endless number of interactions of different microorganisms with grapevine, and as Arocha et al. (2011) commented, they generate a complex ecological panorama. No adult of *Homalodisca vitripennis* was obtained (Germar 1821). *Carneocephala fulgida* Nottinham 1932 and *Graphocephala atropunctata* Signoret 1854 are cicadellids that have been reported as vectors of *X. fastidiosa*, without considering adjacent vegetation (walnut trees, mesquites, undergrowth) or surrounding areas, such as a stream near the vineyard where abundant vegetation grows that could shelter cicadellids. Only two individuals from Cercopidae were obtained, indicating almost zero presence of the family in the vineyard. The genera *Acinopterus, Aceratagalia, Bolotheta, Cuerna, Scaphytopius,* and *Texananus* and the species *Balclutha mexicana, Draeculacephala minerva, Exitanus escavatus, Exitanus picatus,* and *Graminella nigrifrons* are new records, as is *Xyphon nudum* (Cicadellidae), for the State of Coahuila, on grapevine.

Results from amplification and sequencing of DNA indicated no species of Cicadellidae nor Cercopidae were positive for *X. fastidiosa* (primer RST31 / RST33). Although it was not the objective of the study, it is important to note that two of 16 adults of *Balclutha mexicana* were positive for *Candidatus Phytoplasma trifolii* (Hiruki y Wang, 2004) (primer R16F2n / R16R2), and two of 16 adults of the same species also were positive for *Candidatus* Phytoplasma asteri (primer fUS / rU3) (Table 1). *Candidatus* Phytoplasma trifolii was reported in cultivars of chili (*Capsicum annuum* L.) in the State of Zacatecas (Mauricio et al. 2015). This is the first report of the phytoplasma on grapevine for Mexico.

The fact that only four of 16 specimens of *B. mexicana* were positive for phytoplasmas indicated that not all individuals in the population are vectors, which could also occur with vectors of *X. fastidiosa*. This possibly depend on whether they feed or not on diseased plants, which might explain why the specimens of *Draeculacephala minerva* reported as a vector were not positive for *X. fastidiosa*. It is possible that Cicadellidae vectors of *X. fastidiosa* are also acting on other hosts, and from there they arrive at the vineyard. Considering that *B. mexicana* was positive for two phytoplasmas indicated that not only *X. fastidiosa* was in the vineyard, a fact

that must affect the symptomatology and health of the grapevines. It is unknown if the organisms interact with each other, and in any case, how and to what extent.

Table 1. Results of the PCR Test in Insects for the Detection of *Xylella fastidiosa*, *Ca.* Phytoplasma trifolii, and *Ca.* Phytoplasma asteris

Species	PCR results		
Cicadellidae/ Cercopidae	*X. fastidiosa*	*Ca.* Phytoplasma trifolii	*Ca.* Phytoplasma asteris
Aceratagallia sp	Negative		
Acinopterus sp	Negative		
Balcluttha mexicana	Negative	Positive	Positive
Balcluttha sp	Negative		
Bolotheta sp.	Negative		
Cuerna sp	Negative		
Empoasca sp.	Negative		
Exitianus escavatus	Negative		
Exitianus picatus	Negative		
Exitianus sp	Negative		
Graminella nigrifons	Negative		
Graminella sonora	Negative		
Neokolla hieroglyphica	Negative		
Scaphytopius sp	Negative		
Texananus sp	Negative		
Xyphon nudum	Negative		
Prosapia ignifera	Negative		

In conclusion, it is possible that other species of cicadellids and spittlebugs in the vineyard have not been obtained, so this is a first reference in this regard. It is useful and operative to sample areas surrounding the vineyard and consider collecting in the afternoon and night to detect possible movement into the crop, which would support monitoring the entomofauna of these suckers to locate their ethology, ecology, and role, for which it also is necessary to identify to species all individuals collected. Detection of two phytoplasmas in the crop indicate other problems that lead to consider comprehensive approaches in the study, monitoring, and management of the complex.

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References Cited

Aguilar, E., W. Villalobos, L. Moreira, C. M. Rodríguez, E. W. Kitajima, and C. Rivera. 2005. First report of *Xylella fastidiosa* infecting citrus in Costa Rica. Plant Disease 89: 687-687.

Almeida, R. P., and A. H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 96: 264-271.

Beanland, L., R. Noble, and T. K. Wolf. 2006. Spatial and temporal distribution of North American grapevine yellows disease and potential vectors of the causal phytoplasmas in Virginia. Environ. Entomol. 35: 332-344.

Cornara, D., M. Saponari, A. R. Zeilinger, A. de Stradis, D. Boscia, G. Loconsole, ... and F. Porcelli. 2017. Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J. Pest Sci. 90: 521-530.

Daane, K. M., C. M. Wistrom, E. B. Shapland, and M. S. Sisterson. 2011. Seasonal abundance of *Draculacephala minerva* and other *Xilella fastidiosa* vectors in California almond orchards and vineyards. J. Econ. Entomol. 104: 367-374.

Díaz Ortíz, B. E. 2003. Vectores de la enfermedad de Pierce, pp. 1-37. Seminario Internacional de Vitivinicultura, Memorias del Seminario.

EFSA. 2015. Scientific opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 13: 1-262.

EPPO/CABI. 2009. Data Sheets on Quarantine Pests: *Xylella fastidiosa*. http://www.eppo.org/QUARANTINE/bacteria/Xylella_fastidiosa/XYLEFA_ds.pdf Consultado 26 April 2019.

González, G. E., B. M. Borja, M. L. Reyes, M. A. Galind, V. R. Velásquez, L. R. Sánchez, y R. F. Tafoya. 2014. Principales insectos plaga de los viñedos en el mundo, México y Aguascalientes. INIFAP, Folleto Técnico 55.

Hopkins, D. L. 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27: 271-290.

Hopkins, D. L., y A. H. Purcel. 2002. *Xylella fastidiosa* cause of Pierce´s disease of grapevine and other emergent diseases. Plant Dis. 86: 1056-1066.

Lópes, J. R. S., M. J. G. Beretta, R. Harakava, R. P. P. Almeida, R. Krugner, y A. Garcia Júnior. 1996. Confirmação da transmissão por cigarrinhas do agente causal da clorose variegada dos citros, *Xylella fastidiosa*. Fitopatología Brasileira 21(Supl.): 343.

Mauricio, C. J. A., M. S. Salas, V. R. Velázquez, G. S. Ambri, y T. L. R. Raveles. 2015. ´*Candidatus* Phytoplasma trifolii´ (16SrV1) en chile mirasol (*Capsicum annum*) cultivado en Zacatecas, México. Rev. Fitotec. Mex. 38(4).

OIV (Organización Internacional del Vino y la Viña). 2016. Aspectos de la Coyuntura Mundial. OIV. Paris, France. 3-5. http://www.oiv.int/public/medias/4588/oiv-noteconjmars2016-es.pdf

Páez, S. A. C. 2009. El Valle de Parras y el desastre de la filoxera, 1870-1910. Génesis y consolidación de una empresa vitivinícola en tiempos de crisis. Mundo Agrario 9: 1-20.

Redak, R. A., A. H. Purcell, J. R. Lopes, M. J. Blua, R. F. Mizell Iii, and P. C. Andersen. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annu. Rev. Entomol. 49: 243-270.

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2016. http://www.siap.sagarpa.gob.mx/ Accessed 9 October 2018.

Landscape Structure and its Effects on Crapemyrtle Bark Scale, *Acanthococcus lagerstreoemiae*[1], and its Natural Enemies

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Abstract. Knowledge of factors influencing abundances and distributions of species on host plants could facilitate effective, sustainable management of the non-native crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae). We characterized habitat variables associated with crapemyrtles, *Lagerstroemia* spp. (Myrtlaes: Lythraceae), widely planted in Texas landscapes receiving minimal cultural inputs such as insecticide, irrigation, fertilization, or pruning. Arthropod species on individual trees and landscape cover in a 32.4-m-radius area around each of 100 *Lagerstroemia* spp. (40 of 'Natchez' hybrid cultivar and 60 of various other cultivars) were surveyed in Brazos County and Tarrant County during six consecutive seasons starting in spring 2018 and ending in summer 2019. Data were analyzed using principal component analysis to create a reduced set of independent variables. Principal component analysis consistently generated three principal components (PC1, PC2, and PC3) that cumulatively described at least 90% of the variance among the landscape variables. Factor weightings were similar between the two locations, with areas covered by turf and hardscape explaining the greatest amount of habitat variation in PC1 with the two factors negatively related to each other. PC2 was composed of area covered by *Lagerstroemia* spp., and PC3 the area covered by other tree species, and both factors had positive weighting. A similar pattern was detected for cultivar type ('Natchez' versus non- 'Natchez') except area covered by *Lagerstroemia* spp. and the area covered by other tree species best represented by PC2. Factor weights associated with seasonal principal component analysis were similar to results from analyses by location. The seasonal-specific factor weightings remained constant, suggesting a lack of seasonal effect on habitat variables included in the study. The principal components were included in a least-squares stepwise multiple progression procedure to test for the influence of landscape composition on the scale and select associated arthropod populations. Although $p < 0.05$ for some variables, no models had adjusted $R^2 > 0.10$. In the minimal input landscapes, trees, turf, and hardscape were the dominant habitat variables. Landscape variables were not a strong predictor of *A. lagerstroemiae* populations or natural enemies, thus suggesting manipulation of the parameters would have minimal effects on abundance and distribution of the species.

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Introduction

Crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is a non-native pest from Asia that was first discovered in Texas, at Richardson in 2004 (Wang et al. 2016). The scale has now spread to 14 southern and southwestern U.S. states where it affects value and health of crapemyrtles, specifically *Lagerstroemia indica* (L.) (Myrtales: Lythraceae), *Lagerstroemia fauriei* (Koehne), and hybrid *Lagerstroemia indica x fauriei* (EDDMaps 2020). The ornamental trees are ubiquitous features of the southern U.S. landscape because of aesthetic appeal and relative lack of diseases and other pests (Pooler 2007, Chappell et al. 2012). Dozens of cultivars encompass a wide range of flower colors and ornate bark types. Infestation by the scale causes branch dieback and heavy coverage by black sooty mold that grows on honeydew excreted by *A. lagerstreomiae* (Jiang and Xu 1998, Luo et al. 2000, Ma 2011, Chappell et al. 2012).

Intensive surveys of *A. lagerstroemiae* infesting *Lagerstroemia* spp. in Texas documented a large number of scale insect predators and competitors, as well as a number of non-*A. lagerstroemiae* parasitoids. Hence, there is keen interest in biological control exerted by indigenous and other exotic natural enemies. Among the different biological control agents, insects such as coccinellids and chrysopids frequently eat scale insects. Their augmentation might be worth investigating to control *A. lagerstroemiae* (Pappas et al. 2011, Wang 2016). Classical biological control efforts might focus on *A. lagerstroemiae* parasitoids such as those in China (Wang 2016). Conservation biological control seeks to maximize the effect of natural enemies by manipulating their environment (Barbosa 1998). Known *A. lagerstroemiae* predators in the United States are *Hyperaspis bigeminata* (Randall), *Chilocorus cacti* (L.), *Chilocorus stigma (Say)*, and introduced *Harmonia axyridis* (Pallas) (Wang 2016). All four coccinellids also are found in Texas. Known predators of *A. lagerstroemiae* are in the southern U.S. landscape, but what influences their presence on *Lagerstroemia* spp. has not been examined

Lagerstroemia spp. are in diverse urban, suburban, and rural habitats. Occurrence of *Lagerstroemia* spp. results from direct planting of nursery-grown stock or growth from winged seeds adapted for aeolian dispersal. The seeds can be blown considerable distances from the parent tree (Graham and Graham 2014), and growth from seed might best explain the occurrence of *Lagerstroemia* spp.

Research indicates insects with sucking mouthparts and limited mobility such as scales increase along an urbanization gradient (Raupp et al. 2012). As habitat fragmentation, human disturbance, and habitat modification increase, sucking insects are more likely than chewing insects such as lepidopteran larvae to outbreak in urban areas (Martinson and Raupp 2013). Research found that increased diversity of the urban landscape might reduce scales on trees, possibly because of other landscape characteristics such as edge effects, patch sizes, and habitat fragmentation (Bāders er al. 2018).

Because the pest was only recently discovered in the U.S., little is known about faunistic and habitat factors influencing its abundance and distribution. The objective of this research was to determine factors influencing *A. lagerstroemiae* and its natural enemies at two Texas counties 190 km apart: Brazos (30°39'36.00" N -96°17'60.00" W) and Tarrant (32°46'12.00" N -97°17'24.00" W). Information about the surrounding landscape and arthropod fauna on *Lagerstroemia* spp. was collected and analyzed using principal component analysis followed by stepwise multiple regression to identify landscape factors that influence *A. lagerstroemiae* and its natural enemies.

Materials and Methods

Sampling was in two Texas counties, Brazos and Tarrant. The counties are more than 190 km apart and in different plant hardiness zones defined by average annual extreme minimum temperature. Brazos is in Zone 8b with -9.4 to -6.7°C and Tarrant is in 8a with -12.2 to -9.4°C (USDA 2012). Tarrant County has been infested longer than Brazos County, because it is closer to the initial introduction of *A. lagerstroemiae* in adjacent Dallas County in 2004. Because quantification of geographical, cultivar, and habitat structure on arthropod abundance and composition within *Lagerstroemia* spp. was the focus of the study, sampling protocol was based on statistical evaluation of the appropriate number of trees to sample per county and foliage samples to collect per tree. Considerable variance was observed between populations on branches on the same tree and between different trees. To obtain reliable data on *A. lagerstroemiae* and its natural enemies, preliminary data were collected on mean/variance relationships and scale populations. Ten 30-cm branches each were taken from 40 trees. With 40 trees, the change in standard error was less than 0.005 for each additional tree in the sample. Results were similar when standard error was taken between branches on the same tree. To avoid excessive damage that would affect health of trees and skew data, only 10 branches were collected per tree.

Arthropods were sampled and habitat assessed in spring 2018 (8 May-9 June), summer 2018 (9 August-9 September), fall 2018 (10 November-11 December), winter 2019 (9 February-12 March), spring 2019 (8 May-9 June), and summer 2019 (9 August-9 September). Multiple seasons were sampled to determine if seasonality affected landscape factors influencing the scale and natural enemy populations. Six seasons were sampled to try to detect any difference between successive years. Trees were sampled from a variety of landscapes, including roadsides, parks, schools, government buildings, hotels, apartment complexes, and medical facilities. On sample dates, foliage was sampled from 40 trees of the Natchez cultivar and 60 trees from a mixture of non-Natchez cultivars. By comparing a known cultivar with a mixture of unknown cultivars, it was hoped any differences in infestation by *A. lagerstroemiae* between *Lagerstroemia* spp. cultivars would be detected. The crowns of sample trees were ideally separated from the crowns of neighboring *Lagerstroemia* spp. However, if the number of *Lagerstroemia* spp. in contact with one another was three trees or less, they were sampled as one tree. Ten 30-cm branch tips as sub-samples were collected from each tree by using a telescoping long-handled 2.7-m pruner. An assistant caught the branches in Sure Fresh® rectangular deep storage containers, 10 cm x 35 cm (Greenbrier International, Chesapeake, VA). Samples were labelled by tree, each tree was given a unique number, and GPS data were collected using a cellphone application (GPS Status & Toolbox Pro 9.0.183 2019). GPS coordinates were recorded to use the same trees every season.

Non-migratory dispersal distances for natural enemies were obtained from literature review. The review focused on 24-hour dispersal distances that reflected dispersal between short landscape distances and excluded seasonal migration and long-distance dispersal. Twenty-four-hour dispersal distances were examined for arthropod morphotypes *Hippodamia convergens* Guérin-Méneville, *Orius* spp. Wolff, Araneae, *Scymnus* spp. Kugelann, *Aphis gossypii Glover*, *Myzus persicae* (Sulzer), *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus fallacis* Garman, *Neoseilus californicus* (Chant), *Kampimodromus aberrans* (Oudemans), and *Euseius*

finlandicus Oudemans (Heinz 1998, Prasifka et al. 1999, Jung and Croft 2001). The average dispersal distance of the 10 species was 11.057 m with a standard error of 4.363. The maximum distance for dispersal in applicable field studies with applicable insect species was 32.4 m. *Orius* spp. dispersed farthest in 24 hours. Because natural enemies such as *Orius* spp. were of special interest in the study, landscape composition within a 32.4-m radius of each tree was measured on each arthropod sampling date.

Landscape cover measurements were estimated using GPS imagery checked by ground observations every season. If more than one plant type covered a space, the cover was subdivided equally among the overlapping plant types. For example, the area of tree cover over turf grass was divided equally between the categories of tree cover and turf grass.

In the laboratory, plastic containers containing branch samples were kept at 6°C in a cold room for at least 24 hours to slow arthropods and for preservation during sample processing. After 24 hours, the containers were removed, shaken vigorously by hand for 10 seconds, and opened. Branches were examined carefully and transferred from the capture container to a clean container of the same kind, and back again. The branches were shaken individually into the containers during transfer. The purpose was to dislodge any arthropods clinging to *Lagerstroemia* spp. branches while also preventing as few as possible from escaping. The displaced arthropods were aspirated into 12-dram vials. The vials were filled with 70% ethyl alcohol to preserve the specimens. With the aid of 10-63× magnification of a stereo-microscope (Olympus SZ60, Tokyo, Japan), the organisms captured in alcohol were counted and identified to the lowest taxon possible. Adult scales and nymphs attached firmly to the branches also were counted with the aid of microscopes in the laboratory. At Tarrant County where microscopes were not available, illuminated 30× magnifying glasses were used (Ylanfer 30x Handheld Lighted Magnifier, Extra Large Double Glass Lens Magnifying Glass with 12 LED Lights, Shenzhen, China). Eggs hidden beneath the waxy covering of atrophied adult females were not counted. The branches were put into sealed, white paper bags to allow parasitoid emergence. After 3 months, the bags were opened and the contents shaken into a Petri dish containing 70% ethanol to kill living arthropods and aid in examination of detritus from the bottom of the bag. Using a stereo-microscope with 10-63× magnification, parasitoids were counted and identified to the lowest taxonomic level possible before put into labelled vials with 70% ethanol for preservation. Dr. Jim Woolley at Texas A&M University provided taxonomic assistance, and voucher specimens were put into the TAMU insect collection.

Factors influencing abundance and distribution of select insect taxa were determined using principal component analysis and stepwise multiple regression. This proven method has been applied in entomology, horticulture, and ecology (Iezzoni and Pritts 1991, Brazzle et al. 1997, Janžekovič and Novak 2012). Principal component analysis creates a reduced number of orthogonal variables or factors, described by factor-loading values, by mathematically maximizing spatial separation of the original variables (Brazzle et al. 1997). Using this method creates a reduced set of variables more conducive to interpretation. Brazzle and colleagues (1997) used the approach in their study of *Bemisia argentifolli* Bellows & Perring (Hemiptera: Aleyrodidae) infesting cotton (*Gossypium hirsutum* L.) in the Imperial Valley of California in 1993 and 1994. Values from the statistically independent variables were used in a least-squares multiple regression procedure to test the influence of agronomic factors on abundance of immature *B. argentifolii.*

When analyzing *Lagerstroemia* spp. landscape, variables used in principal component analysis represented greater than 1% of landscape area sampled. Area of turf grass, *Lagerstroemia* spp. cover, other tree cover, and hardscape were included in the analysis. Flowerbeds containing annuals, small streams, and native brush were rarely found at sampling locations and were not included. Of 200 trees, only two were near a body of water and only four trees coexisted with flowering annuals. Analysis was limited to three principal components derived from landscape measurements. The principal components were saved and used as independent variables in a series of stepwise multiple regression analyses. The target pest, *A. lagerstromiae*, and separately, its natural enemies were used as response variables for stepwise multiple regression. Natural enemies were from the ecosystem survey. The category included the family Anthocorridae (Hemiptera), *Axion plagiatum* (Olivier) (Coleoptera: Coccinellidae), *Chilocorus* spp. (Coleoptera: Coccinellidae), *Exochomus* spp. (Redtenbacher) (Coleoptera: Coccinellidae), lacewings in the families Chrysopidae and Hemerobiidae (Neuroptera), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Hyperaspis* spp. (Coleoptera: Coccinellidae), and *Scymnus* spp. (Coleoptera: Coccinellidae). JMP® 14.3 software was used for statistical analysis (SAS Institute Inc., Cary, NC).

Results

Three principal components cumulatively described at least 90% of variance among four variables. Composition of the new independent variables generated by the analysis was affected by geographic location and cultivar (Tables 1-3). Results were discussed by location (Brazos versus Tarrant County), cultivar (Natchez versus non-Natchez), and season (collections in spring, summer, and fall 2018 and winter, spring, and summer 2019) to determine which class variables contributed most to variation in principal components and subsequent regression analyses. Stepwise multiple regression models did not produce high adjusted R^2 values when abundance of *A. lagerstroemiae* or natural enemies of *A. lagerstroemiae* was the response variable.

Table 1. Principal Component Analysis of Landscape Values Surrounding Sampled Lagerstroemia spp. in Brazos County and Tarrant County, Texas, 2018-2019. Numbers represent factor loadings of the principal components. Numbers in the final column represent the percentage of variance explained by the principal component. Factor loadings >0.5 are highlighted.

Brazos County	*Lagerstroemia* spp. cover	Other tree cover	Turf	Hardscape	Percentage explained
PC1	-0.26344	**0.71304**	**0.92118**	**-0.96324**	58.856
PC2	**0.95879**	0.18353	-0.00018	-0.12654	24.224
PC3	-0.10556	**0.67578**	-0.36421	0.18080	15.829
Total					98.909
Tarrant County	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	0.25610	0.48459	**0.94407**	**-0.95373**	52.532
PC2	**0.84592**	**-0.58043**	0.03878	-0.02938	26.437
PC3	0.46778	**0.65436**	-0.25122	0.20941	18.806
Total					97.740

Table 2. Principal Component Analysis of Landscape Values Surrounding Sampled 'Natchez' and Non-'Natchez' Cultivar *Lagerstroemia* spp. in Texas in 2018-2019. Numbers represent factor loadings of the principal components. Numbers in the final column represent the percentage of variance explained by the principal component. Factor loadings >0.5 are highlighted.

'Natchez' cultivar	*Lagerstroemia* spp. cover	Other tree cover	Turf	Hardscape	Percentage explained
PC1	-0.04940	**0.58140**	**0.91276**	**-0.95022**	51.913
PC2	**0.89546**	**0.56729**	-0.23355	-0.07620	29.601
PC3	-0.44207	**0.58191**	-0.22987	0.15822	15.298
Total					96.811
Non-'Natchez' cultivar	*Lagerstroemia* spp. cover	Other tree cover	Turf	Hardscape	Percentage explained
PC1	-0.30436	0.36805	**0.94459**	**-0.97091**	51.575
PC2	**0.74772**	**0.72619**	-0.12976	-0.08536	27.764
PC3	**0.59006**	**-0.57953**	0.25798	-0.15367	19.355
Total					98.694

Location. Principal Component 1 represents negative correlation between turf and hardscape in the landscape area. Principal Component 2 represents the amount of area occupied by other *Lagerstroemia* spp. trees in the landscape. In Principal Component 3, the prominent factor was non-*Lagerstroemia* spp. tree cover.

In stepwise multiple regression analyses, the adjusted $R^2 < 0.01$ for both Brazos County and Tarrant County (Table 4). In Brazos County the area of *Lagerstroemia* spp. measured in the landscape was positively correlated with *A. lagerstroemiae* collected with more than 95% confidence. In Tarrant County the area of *Lagerstroemia* spp. was negatively correlated with *A. lagerstroemiae* with more than 85% confidence. Natural enemy analysis using stepwise multiple regression was inconsistent between the two counties. In Brazos County, PC3 was the most significant with $p = 0.19855$ but adjusted $R^2 = 0.0011$. In Tarrant County PC2 was most significant, with $p = 0.02073$ and $R^2 = 0.0073$.

Cultivar. As in the county comparison, Principal Component 1 represented negative correlation between turf and hardscape in the landscape area measured. Principal Component 2 represented a positive correlation between area occupied by other *Lagerstroemia* spp. trees and other trees in the landscape. Principal Component 3 was composed of varying interactions depending on the cultivar, but in both cases explained less than 20% of the data.

Multiple regression analysis produced $R^2 < 0.05$ for both cultivars. Considering *p*-values, PC2 was significant with $p < 0.05$ for both cultivars (Table 5). It was positively correlated with the number of *A. lagerstroemiae*. In cultivar analysis of natural enemies of *A. lagerstroemiae*, results were relatively consistent between cultivars. The *p* values of PC2 were <0.05 and <0.09 for PC3. Natural enemies were negatively correlated with PC2 and PC3 on the 'Natchez' cultivar. However, on the non-'Natchez' cultivar, they were negatively correlated with PC2 but positively correlated with PC3. Adjusted R^2 values for 'Natchez' and non-'Natchez' were 0.0150 and 0.0080, respectively.

Season. In the seasonal principal component analysis, principal components remained relatively steady across seasons because landscape area values in the study did not change unless altered by human modification (Table 6). Principal

Table 3. Principal Component Analysis of Landscape Values Surrounding Sampled *Lagerstroemia* spp. in Texas, spring 2018. Numbers represent factor loadings of the principal components. Numbers in the final column represent the percentage of variance explained by the principal component. Factor loadings >0.5 are highlighted.

Spring 2018	*Lagerstroemia* spp. cover	Other tree cover	Turf	Hardscape	Percentage explained
PC1	-0.17939	0.46314	**0.935293**	**-0.96953**	51.543
PC2	**0.83808**	**0.62705**	-0.16460	-0.01507	28.072
PC3	**-0.51508**	**0.62599**	-0.25165	0.15077	18.581
Total					98.195
Summer 2018	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	-0.17939	0.46314	**0.935293**	**-0.96953**	51.543
PC2	**0.83808**	**0.62705**	-0.16460	-0.01507	28.072
PC3	**-0.51508**	**0.62599**	-0.25165	0.15077	18.581
Total					98.195
Fall 2018	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	-0.18156	0.46352	**0.93610**	**-0.97015**	51.632
PC2	**0.83810**	**0.62546**	-0.25398	-0.15055	28.014
PC3	**-0.51508**	**0.62599**	-0.25165	0.15077	18.607
Total					98.252
Winter 2019	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	-0.18156	0.46352	**0.93610**	**-0.97015**	51.632
PC2	**0.83810**	**0.62546**	-0.16336	-0.01564	28.014
PC3	**-0.51430**	**0.62657**	-0.25398	0.15055	18.607
Total					98.252
Spring 2019	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	-0.16060	0.46476	**0.93454**	**-0.97050**	51.426
PC2	**0.84223**	**0.61861**	-0.17298	-0.00970	28.051
PC3	**-0.51450**	**0.63236**	-0.25183	0.14547	18.729
Total					98.206
Summer 2019	***Lagerstroemia* spp. cover**	**Other tree cover**	**Turf**	**Hardscape**	**Percentage explained**
PC1	-0.20102	0.48179	**0.93278**	**-0.97008**	52.092
PC2	**0.83972**	**0.62361**	-0.16126	-0.01935	28.010
PC3	**0.50436**	**-0.61437**	0.26861	-0.15135	18.172
Total					98.274

Table 4. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled *Lagerstroemia* spp. Using Numbers of *A. lagerstroemiae* from Sampled Trees as a Dependent Variable, 2018-2019, Brazos County and Tarrant County

Brazos County	Partial correlation coefficient	*F* (df = 598)	*P*	R^2 adjusted
PC2	3.2110687	4.068	0.04415	0.0051
Tarrant County	**Partial correlation coefficient**	***F* (df = 598)**	***P***	**R^2 adjusted**
PC2	-1.2481216	2.255	0.13371	0.0021

Table 5. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled 'Natchez' and Non-'Natchez' Cultivar *Lagerstroemia* spp. Using Number of *A. lagerstroemiae* from Sampled Trees as a Dependent Variable, 2018-2019, Texas

'Natchez' cultivar	Partial correlation coefficient	*F* (df = 476)	*P*	R^2 adjusted
PC1	2.1375641	3.578	0.0592	
PC2	6.1537886	16.908	<0.001	
PC3	3.9444391	3.590	0.0587	
				0.0481
Non-'Natchez' cultivar	Partial correlation coefficient	*F* (df = 717)	*P*	R^2 adjusted
PC2	1.9172471	4.077	0.04385	
PC3	-1.3945836	1.504	0.22052	
				0.0050

Table 6. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled *Lagerstroemia* spp. Using Numbers of *A. lagerstroemiae* from Sampled Trees as a Dependent Variable in Spring 2018, Texas

Spring 2018	Partial correlation coefficient	*F* (df = 196)	*P*	R^2 adjusted
PC1	1.3855466	1.519	0.21929	
PC2	-3.4949266	5.263	0.02284	
PC3	-2.8769606	2.360	0.12606	
				0.0299
Summer 2018	Partial correlation coefficient	*F* (df = 197)	*P*	R^2 adjusted
PC2	16.8319101	21.501	<0.000001	
PC3	7.807940824	2.518	0.11419	
				0.0996
Fall 2018	Partial correlation coefficient	*F* (df = 196)	*P*	R^2 adjusted
N/A	N/A	N/A	N/A	N/A
Winter 2019	Partial correlation coefficient	*F* (df = 198)	*P*	R^2 adjusted
PC1	-1.1613293	7.893	0.00546	0.0335
Spring 2019	Partial correlation coefficient	*F* (df = 198)	*P*	R^2 adjusted
PC2	-0.55382264	3.433	0.06538	0.0121
Summer 2019	Partial correlation coefficient	*F* (df = 198)	*P*	R^2 adjusted
PC2	5.15414386	5.748	0.01743	0.0233

Component 1 represents negative correlation between turf and hardscape in the landscape area measured. Principal Component 2 represents positive correlation between area occupied by other *Lagerstroemia* spp. trees and other trees in the landscape, but with somewhat more emphasis on *Lagerstroemia* spp. Principal Component 3 is composed of negative interactions between the area composed of *Lagerstroemia* spp., and other tree species. However, Principal Component 3 consistently described less than 20% of the data.

In spring and summer, when *A. lagerstroemiae* was most abundant, stepwise multiple regression results were similar to results for location and cultivar analyses; Principal Component 2 was the only component with $p < 0.1$. In fall and winter, the

regression analysis differed. In fall 2018, analysis found no components with $p < 0.25$. In winter 2019, Principal Component 1 was the most significant predictor of numbers of *A. lagerstroemiae*. As in the analyses for county and cultivar, R^2 values were low; none exceeded 0.0996. Regression analyses for natural enemies of *A. lagerstroemiae* across seasons were more inconsistent. However, PC1 was more consistently significant in the stepwise multiple regression than in any other regression analyses in the study. R^2 did not exceed 0.0832 in any season.

Discussion

The multivariate analysis methods could not adequately explain why *A. lagerstroemiae* was more common on some trees than others. Principal component analysis paired with stepwise multiple regression produced models with low adjusted R^2. Although $p < 0.05$ for some variables, no models had adjusted $R^2 \geq 0.10$. This was true for analyses by season, cultivar, and location.

All *Lagerstroemia* spp. trees sampled were in non-residential urban and suburban environments. In North America, *Lagerstroemia* spp. are non-native ornamentals that most often occur in these environments. Past research indicated that scale insects of other species might increase along an urbanization gradient (Raupp et al. 2012). However, not all *Lagerstroemia* spp. trees in the study were colonized by large numbers of *A. lagerstroemiae* regardless of surroundings. It was common to find heavily colonized trees only a few meters from trees with only a few individuals. Even within trees, some branches were covered in scales, and other branches ignored.

Measurements of the landscape were taken within a 32.4-m circle surrounding the tree. Measurements of landscape variables within the area could vary considerably, but the landscape typically consisted of concrete, turf grass, *Lagerstroemia* spp. trees, other trees, and buildings. Other variables were rare and not analyzed. Notably, two sample trees were near a wide creek, and water composed a significant portion of the landscape area. Flowering annuals were planted in a 32.4-m radius of four trees. Considering limited occurrence of these factors, it was not possible to judge how water features and flowering annuals would have affected the analysis in the study if they had been common enough to analyze. A manipulative experiment of the factors was not done because of landscape and landscape infrastructure modifications a manipulative experiment would entail, e.g., irrigation systems for flowering annuals and large water features. Properties were not managed by the researchers but entities such as state and city governments, or private businesses.

Multivariate analysis of natural enemies of *A. lagerstroemiae* did not produce adequate or consistent results (Tables 7-9). Although $p < 0.05$ for some variables, no

Table 7. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled *Lagerstroemia* spp. Using Numbers of *A. lagerstroemiae* Natural Enemies from Sampled Trees as a Dependent Variable, 2018-2019, Brazos County and Tarrant County

Brazos County	Partial correlation coefficient	*F* (df = 598)	*P*	R^2 adjusted
PC3	-0.0619465	1.657	0.19855	0.0011
Tarrant County	Partial correlation coefficient	*F* (df = 598)	*P*	R^2 adjusted
PC2	0.15670772	5.378	0.02073	0.0073

model had adjusted $R^2 \geq 0.10$. This might be a result of sporadic numbers of *A. lagerstroemiae*. Prey populations influence predator populations, and *A. lagerstroemiae* was the most common scale insect and most common herbivore in samples (Lotka 1925). Many *A. lagerstroemiae* predators were in the environment. However, no parasitoids of *A. lagerstroemiae* were collected despite efforts to do so that resulted in collection of parasitic Hymenoptera from 10 families.

A biological control strategy for augmenting abundance of natural enemies is planting flowering plants near plants of interest (Buchanan et al. 2018). The flowering plants attract and support natural enemy species such as coccinellids, chrysopids,

Table 8. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled 'Natchez' and Non-'Natchez' Cultivar *Lagerstroemia* spp. Using Numbers of *A. lagerstroemiae* Natural Enemies from Sampled Trees as a Dependent Variable, 2018-2019, Texas

'Natchez' cultivar	Partial correlation coefficient	*F* (df = 477)	*P*	R^2 adjusted
PC2	-0.177487	6.029	0.01443	
PC3	-0.1821285	3.281	0.07072	
				0.0150
Non-'Natchez' cultivar	Partial correlation coefficient	*F* (df = 717)	*P*	R^2 adjusted
PC2	-0.0841436	4.764	0.02938	
PC3	0.08090885	3.071	0.08014	
				0.0080

Table 9. Stepwise Multiple Regression Analysis of Principal Components Derived from Landscape Measurements Surrounding Sampled *Lagerstroemia* spp. Using Numbers of *A. lagerstroemiae* Natural Enemies from Sampled Trees as a Dependent Variable across Seasons in Texas

Spring 2018	Partial correlation coefficient	*F* (df = 198)	*P*	R^2 adjusted
PC2	-0.405411	7.024	0.00869	0.0294
Summer 2018	Partial correlation coefficient	*F* (df = 196)	*P*	R^2 adjusted
PC1	0.10058732	3.107	0.07950	
PC2	-0.1561718	4.080	0.04476	
PC3	-0.2781173	8.564	0.00383	
				0.0602
Fall 2018	Partial correlation coefficient	*F* (df = 197)	*P*	R^2 adjusted
PC1	0.03899781	2.651	0.10510	
PC2	0.13564622	17.400	<0.000001	
				0.0832
Winter 2019	Partial correlation coefficient	*F* (df = 196)	*P*	R^2 adjusted
N/A	N/A	N/A	N/A	N/A
Spring 2019	Partial correlation coefficient	*F* (df = 198)	*P*	R^2 adjusted
PC1	-0.2365337	10.108	0.00171	0.0438
Summer 2019	Partial correlation coefficient	*F* (df = 197)	*P*	R^2 adjusted
PC1	0.06426231	2.712	0.10116	
PC2	-0.1713291	10.367	0.00150	
				0.0527

and parasitic Hymenoptera by providing resources such as pollen and nectar (Patt 1997, Johanowicz and Mitchell 2000). In the study, flowering annuals were within a 32.4-m radius of only four trees. However, if planting annuals is widely used, many sites in the study, and by extension elsewhere in Texas, would need more management as well as installment of irrigation systems. Furthermore, while there already are many natural enemies in the environment, they are not adequately controlling *A. lagerstroemiae*.

The study highlights the sporadic and clumped colonization habits of *A. lagerstroemiae*. Infestations and outbreaks of the pest are difficult to predict. Initial colonization of new trees by *A. lagerstroemiae* is not well understood: adult males can fly, but females cannot. Females of some Coccoidea species disperse by phoresy as they cling to other animals, while in others dispersal is aeolian as they are blown by wind (Hill 1980, Mow et al. 1982, Barras et al. 1994, Magsig-Castillo et al. 2010). Understanding the way females disperse might provide insight into distribution of *A. lagerstroemiae*.

The study analyzed landscape factors influencing *A. lagerstroemiae* and its natural enemies on *Lagerstroemia* spp. in non-residential urban and suburban environments in Texas, and also characterized the environments. *Lagerstroemia* spp. trees are typically near other trees of the same species, at locations dominated by turf grass and concrete, often with other tree genera nearby. Plant species diversity and water sources are lacking. This is a harsh artificial habitat where management consists of mowing the grass and little else. Despite this, the *Lagerstroemia* spp. survey revealed a diverse and complex arthropod community.

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References Cited

Bāders, E., Ā. Jansons, R. Matisons, D. Elferts, and I. Desaine. 2018. Landscape diversity for reduced risk of insect damage: a case study of sprucebud scale in Latvia. Forests 9: 545.

Barbosa, P. A. [ed.]. 1998. Conservation Biological Control. Elsevier.

Barrass, I. C., P. Jerie, and S. A. Ward. 1994. Aerial dispersal of first- and second-instar longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) (Pseudococcidae: Hemiptera). Aust. J. Exp. Agr. 34: 1205-1208.

Brazzle, J. R., K. M. Heinz, and M. P. Parrella. 1997. Multivariate approach to identifying patterns of *Bemisia argentifolii* (Homoptera: Aleyrodidae) infesting cotton. Environ. Entomol. 26: 995-1003.

Buchanan, A., M. Grieshop, and Z Szendrei. 2018. Assessing annual and perennial flowering plants for biological control in asparagus. Biol. Control 127: 1-8.

Chappell, M. R., S. K. Braman, J. Williams-Woodward, and G. Knox. 2012. Optimizing plant health and pest management of *Lagerstroemia* spp. in commercial production and landscape situations in the southeastern United States: a review. J. Environ. Hortic. 30: 161-172.

EDDMapS. 2020. Early Detection & Distribution Mapping System. The University of Georgia - Center for Invasive Species and Ecosystem Health. http://www.eddmaps.org/ Accessed 18 September 2020.

GPS Status & Toolbox Pro 9.0.183. 2019. MobiWIA Ltd. https://play.google.com/store/apps/details?id=com.eclipsim.gpsstatus2&hl=en_US

Graham, S. A., and A. Graham. 2014. Ovary, fruit, and seed morphology of the Lythraceae. Int. J. of Plant Sci. 175: 202-240.

Heinz, K. M. 1998. Dispersal and dispersion of aphids (Homoptera: Aphididae) and selected natural enemies in spatially subdivided greenhouse environments. Environm. Entomol. 27: 1029-1038.

Hill, M. G. 1980. Wind dispersal of the coccid *Icerya seychellarum* (Margarodidae: Homoptera) on Aldabra Atoll. J. Anim. Ecol. 939-957.

Iezzoni, A. F., and M. P. Pritts. 1991. Applications of principal component analysis to horticultural research. HortScience 26: 334-338.

Janžekovič, F., and T. Novak. 2012. PCA–a powerful method for analyze ecological niches. Principal component analysis-multidisciplinary applications: 127-142. Books on Demand.

Jiang, N., and H. Xu. 1998. Observation on *Eriococcus lagerstroemiae* Kuwana. J. Anhui Agri. U. 25: 142-144.

Johanowicz, D. L., and E. R. Mitchell. 2000. Effects of sweet alyssum flowers on the longevity of the parasitoid wasps *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae). Fla. Entomol. 41-47.

Jung, C., and B. A. Croft. 2001. Aerial dispersal of phytoseiid mites (Acari: Phytoseiidae): estimating falling speed and dispersal distance of adult females. Oikos 94: 182-190.

Lotka, A. J. 1925. Elements of Physical Biology. Williams and Wilkins.

Luo, Q., X. Xie, L. Zhou, S. Wang, and X. Zongyi. 2000. A study on the dynamics andbiological characteristics of *Eriococcus lagerstroemiae* Kuwana population in Guiyang. Acta Entomol. Sin. 43: 35-41.

Ma, J. 2011. Occurrence and biological characteristics of *Eriococcus lagerostroemiae* Kuwana in Panxi district. S. China Fruits 5: 3.

Magsig-Castillo, J., J. Morse, G. Walker, J. Bi, P. Rugman-Jones, and R. Stouthamer. 2010. Phoretic dispersal of armored scale crawlers (Hemiptera: Diaspididae). J. Econ. Entomol. 103: 1172-1179.

Martinson, H. M., and M. J. Raupp. 2013. A meta-analysis of the effects of urbanization on ground beetle communities. Ecosphere 4: 1-24.

Mow, V. C., B. H. Gunn, and G. H. Walter. 1982. Wind dispersal and settling of first-instar crawlers of the cochineal insect *Dactylopius austrinus* (Homoptera: Coccoidea: Dactylopiidae). Ecol. Entomol. 7: 409-419.

Pappas, M. L., G. D. Broufas, and D. S. Koveos. 2011. Chrysopid predators and their role in biological control. J. Entomol. 8: 301-326.

Patt, J. M., G. C. Hamilton, and J. H. Lashomb. 1997. Impact of strip-insectary intercropping with flowers on conservation biological control of the Colorado potato beetle. Adv. Hortic. Sci.: 175-181.

Pooler, M. 2007. Crapemyrtle, pp. 439-457. *In* Flower Breeding and Genetics. Springer, Dordrecht.

Prasifka, J. R., P. C. Krauter, K. M. Heinz, C. G. Sansone, and R. R. Minzenmayer. 1999. Predator conservation in cotton: using grain sorghum as a source for insect predators. Biol. Control 16: 223-229.

Raupp, M. J., P. M. Shrewsbury, and D. A. Herms. 2012. Disasters by design: outbreaks along urban gradients. Insect Outbreaks Revisited: 311-340.

USDA. 2012. USDA Plant Hardiness Zone Map. https://planthardiness.ars.usda.gov/PHZMWeb/Default.aspx 18 September 2020

Wang, Z., Y. Chen, M. Gu, E. Vafaie, M. Merchant, and R. Diaz. 2016. Crapemyrtle bark scale: a new threat for crapemyrtles, a popular landscape plant in the US. Insects 7: 78.

Rickettsioses and other Tick-borne Diseases across the Border: Regional Focus in Chihuahua

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Abstract. Rocky Mountain spotted fever is of public importance from clinical and veterinary perspectives. During the last decade, cases have increased throughout Mexico, along with other tick-borne diseases. The number of cases reported has increased in northern states of the country that border the United States of America. Studies to understand the current state of affairs with the tick vector and identification of etiological agents also have increased. From the six border states, two in the northwest and central region are of special interest because of the close proximity of the populations from both regions -- Baja California (Tijuana-San Diego and Mexicali-Calexico) and Chihuahua (Ciudad Juárez-El Paso). However, not much information is available on the current situation of Rocky Mountain spotted fever and other rickettsiosis in the State of Chihuahua. Because of increased clinical cases reported, where Chihuahua was the State with the second most number of cases nationwide in 2020, the purpose of this study was to identify methods of detection and vector-collection strategies in the region.

Introduction

Tick-borne diseases are of clinical and veterinary importance in Mexico, especially Rocky Mountain spotted fever caused by *Rickettsia rickettsii*, a neglected disease important to public health. Despite Rocky Mountain spotted fever being a disease requiring immediate follow-up by the National Directorate of Epidemiology, with a total of 449 cases reported nationwide in 2019, the State of Chihuahua reported most cases, with 98 confirmed, of which 39 were caused by Rocky Mountain spotted fever and 59 by other rickettsiosis (Direccion General de Epidemiología 2019). In 2018, Chihuahua was second in the number of cases of rickettsiosis nationwide, behind the western neighboring State of Sonora with 77 cases, according to the state laboratory of Public Health. Despite multi-institutional efforts to prevent tick-borne diseases, emergence of Rocky Mountain spotted fever and other tick-borne diseases in different municipalities across the State has been a challenge since the first two

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confirmed cases were reported in 2013. Little is known of the current prevalence of ticks or identification of other pathogens related to vector-borne diseases such as *Anaplasma phagocytophilum* (Foggie), other *Rickettsia* spp., and *Borrelia burgdorferi* (Johnson). Literature on diagnostic tests approved by the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) for tick-borne pathogens worldwide will not be reviewed here, but is available from Luce-Fedrow et al. (2015), Portillo et al. (2017), and Yazid et al. (2018).

The geographical location of the State that borders New Mexico and Texas in the US, and is the second largest binational metropolitan area at the US-Mexico border called el "Paso del Norte", emphasizes the importance of research on ticks and identification of etiological agents of clinical and veterinary importance in the region. Proper detection is highlighted by the overwhelming amount of work at the centralized National Institute of Epidemiological Reference (INDRE, in Spanish) and state-run laboratories. According to the National Center for Disease Prevention and Control (CENAPRECE, in Spanish) through the Program of Prevention and Control of Rickettsiosis, by November 2019 only 16% of probable cases of rickettsiosis were confirmed by a laboratory, of a total of 1,804 or 64% of 2,786 probable cases. Chihuahua was the State with most deaths, a total of 21. It is important to consider no official acknowledgment or updated knowledge of prevalence of other diseases similar to rickettsiosis with relevance in public health, such as Lyme disease, ehrlichiosis, and anaplasmosis. We reviewed current information on tick-borne disease in the State of Chihuahua in northern Mexico, specifically geographical distribution, and reported infections by ticks and diagnostic methods used.

Materials and Methods

For current information on Rocky Mountain spotted fever and other tick-borne diseases, we referred to PubMed and SciELO (for articles in Spanish) and searched "Chihuahua Rocky Mountain spotted fever", "Chihuahua ticks", "Chihuahua rickettsiosis", "Chihuahua tick-borne diseases", "Rocky mountain spotted fever in Chihuahua", "tick-borne disease(s) Mexico", "ticks in Chihuahua", and "rickettsiosis Mexico". The search of literature focused on tick-borne diseases in Mexico (Fig. 1), with a total of 373 articles from PubMed. We excluded 353 publications not related to the topic, leaving 20 full-text publications. Another publication from SciELO, two publications from bibliographies, three review papers from PubMed, one website, and seven historical papers not in PubMed also were included.

Results

Taxonomic Identification. Since the early 20th Century, researchers like Hoffman (1925), Bustamante and Varela (1943), and Tovar (1944, 1945) began to report tick-borne diseases in Mexico where all effort was focused on rickettsiosis. One of most significant works for identification of ticks from North America was by Bishopp and Trembley (1945). Ticks identified in northern Mexico were *Amblyomma americanum* (Linnaeus), *A. cajennense* (Fabricius), *A. dissimile* (Koch), and *A. maculatum* (Koch), especially abundant in states bordering the Gulf of Mexico. *Dermacentor albipictus* (Packard) was found in northern Mexico and *D. andersoni* (Stiles) was identified in Arizona and New Mexico. *D. nitens* was abundant on the east coast of Mexico.

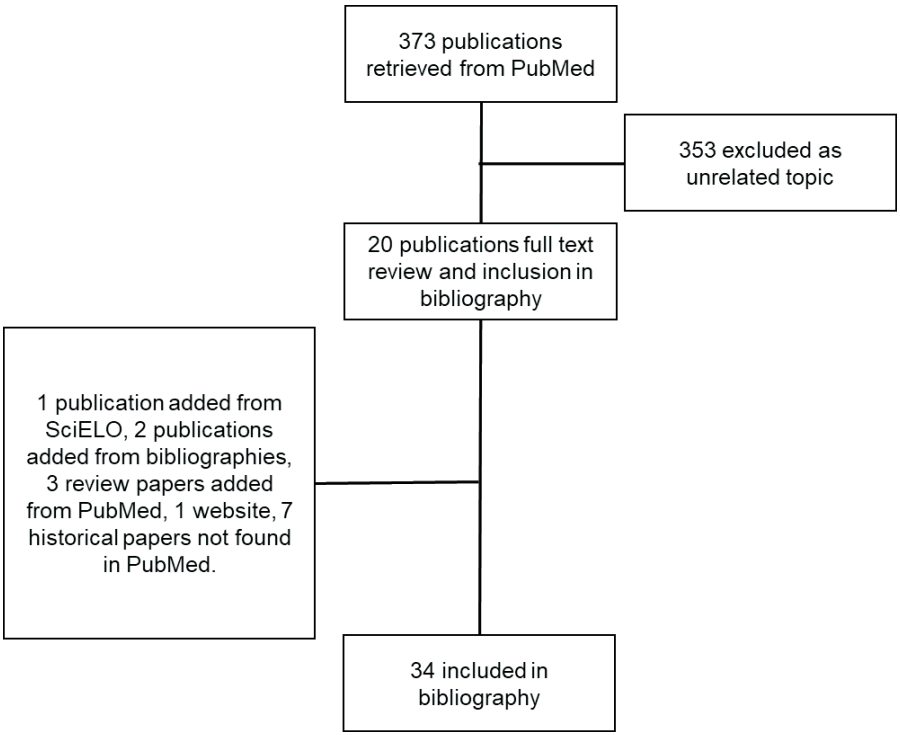

Fig. 1. Selection process for literature on "tick-borne disease(s) Mexico, Rocky Mountain spotted fever, rickettsiosis Mexico, Chihuahua Rocky Mountain spotted fever, Chihuahua ticks, Chihuahua rickettsiosis, Chihuahua tick-borne diseases" focused on Rocky Mountain spotted fever and other rickettsiosis in the State of Chihuahua, North Mexico.

The presence of *Rhipicephalus sanguineus* (Latreille) in South Texas also was highlighted. In the State of Chihuahua, reports by Chavarria (1941) identified *D. andersoni*, but the information still is not certain. Entomological surveys to collect ticks and report prevalence in Chihuahua are becoming more important. Recently, *D. parumapertus* (Neumann), *Ixodes hearlei* (Gregson), *I. kingi* (Bishopp), *Ornithodoros* sp., and *R. sanguineus* s.l. were reported (López-Pérez et al. 2019).

Rocky Mountain Spotted Fever in North Mexico. Refer to Table 1 and Figs. 2-3 for a summary. Rocky Mountain spotted fever is a potentially fatal disease caused by *Rickettsia rickettsii* (Brumpt), an intracellular obligate bacterium transmitted to humans by ticks (Harrell 1949). In the past decade, studies on the prevalence of *R. rickettsii* in dogs, *Canis lupus familiaris* (Linnaeus), and ticks were reported for Baja California (Tinoco-Gracia et al. 2009). In 2009, there was an outbreak of Rocky Mountain spotted fever among residents who reported *R. sanguineus* and tick bites at a community in Mexicali, Baja California, Mexico.

Table 1. Rocky Mountain Spotted Fever and Other Rickettsiosis Analysis by Sample and Diagnostic Test. Colors and numbers indicate location in Fig. 2.

Sample	Geographic area	Diagnostic test	(Author), color/ number, Fig. 2
Tick	Janos Biosphere, Chihuahua, Mexico	Samples analyzed using PCR amplification gltA gene for *R. rickettsii* and flaB for *Borrelia* spp.	(López-Pérez et al. 2019) (Green, 1)
Dog	Cochise, Santa Cruz, Yuma, Arizona, USA	Serum samples tested by IFA.	(Yaglom et al. 2018) (Red, 1)
Dog	Imperial County, California, USA	Blood samples tested using IFA to detect *R. rickettsii*, *E. canis*, and *A. phagocytophilum*.	(Estrada et al. 2019) (Red, 2)
Dog and tick	Baja California, Coahuila, Sonora, Mexico	IFA in serum samples from dogs and RT-PCR targeting ompA in ticks.	(Pieracci et al. 2019) (Red, 3)
Human adult	Ensenada, Baja California, Mexico	Blood samples to detect antibodies against *R. rickettsii* measured with *R. rickettsii* ELISA in dogs, adapted to humans with anti-human IgG conjugate.	(Field-Cortazares et al. 2015) (Blue, 1)
Human adult (veterinary worker)	Ciudad Juarez, Chihuahua, Mexico	Blood samples evaluated using IFA and PCR targeting 16S rRNA to detect *R. rickettsii, Ehrlichia* spp., and *A. phagocytophilum*.	(Escárcega-Ávila et al. 2019) (Blue, 2)
Tick	South of Coahuila, Mexico	PCR using 23S-5S rRNA intergenic spacer and ompA *R. rickettsii.*	(Ortega-Morales et al. 2019) (Green, 2)
Pregnant woman	Hermosillo, Sonora	Confirmatory diagnoses made by PCR targeting gltA for *R. rickettsii* or serological reactivity by IFA.	(Licona-Enriquez et al. 2017) (Blue, 4)
Human adult	Hermosillo, Sonora	Blood samples analyzed by PCR targeting gltA gene or IFA to detect *R. rickettsia*	(Delgado-De la Mora et al. 2018) (Blue, 5)
Child	Hermosillo, Sonora, Mexico	Diagnosis of RMSF by IFA.	(Alvarez-Hernandez et al. 2015) (Blue, 3)
Tick	Calexico and El Centro, California, USA	RT-PCR targeting rOmpA gene from *R. rickettsii*.	(Fritz et al. 2012) (Red, 2)
Tick	Mexicali, Mexico	Sequencing 12S mitochondrial rRNA on DNA from ticks. *R. rickettsii* genetic typing of intergenic fragments RR0155-rpmB, cspA- ksgA, and RR1240-tlc5.	(Eremeeva et al. 2011) (Green, 3)
Tick and dog	Ciudad Juarez, Chihuahua, Mexico	Blood samples tested by PCR aimed at 16S rRNA gene for *Ehrlichia* spp. and *A. phagocytophilum*.	(Escárcega Ávila et al. 2018) (Red, 4)

Tick	Chihuahua City, Mexico	Samples tested using PCR.	(Prado et al. 2018) (Green, 6)
Horse and tick	Ciudad Juarez, Chihuahua, Mexico	Blood samples from horses and soft ticks analyzed by endpoint or nested PCR.	(Medrano-Bugarini et al. 2019) (Black, 1)
Human adult	Coahuila, Nuevo Leon, Tamaulipas, Mexico	Serum samples analyzed by ELISA to detect antibodies against *B. burgdorferi* and Western Blot to confirm.	(Gordillo-Pérez et al. 2003) (Blue, 6)
Tick	Texas, USA; Tamaulipas, Nuevo Leon, and Coahuila, Mexico	Samples tested using PCR amplification of 16SrRNA-23SrRNA to detect *B. burgdorferi.*	(Feria-Arroyo et al. 2014) (Green, 7)
White-tailed deer and coyote	South Texas	TickPath Layerplex qPCR targeting 23S/5S rRNA gene for *Borrelia*; 16SrRNA for *Rickettsia* and *Ehrlichia*; rrs gene for *Anaplasma*, 18SrRNA for *Babesia*, and 18SrRNA for *Theileria*.	(Yu et al. 2020) (Black, 2)
Human adult and tick	Allende and Linares, Nuevo Leon, and Mexico	Serum samples from humans tested using IFA for IgG antibodies against *Rickettsia prowazekii*, *R. typhi*, and *R. parkeri*. Ticks analyzed by PCR amplification of 17-kDa gene.	(Medina-Sanchez et al. 2005) (Blue, 7)

Fig. 2. Geographic representation of study areas with tick-borne pathogens at the Mexico-US border between 2003 and 2019. Legend: Color code by type of study. Green triangles and asterisk (*) indicate studies of ticks. Blue triangles, studies with blood human samples. Red triangles indicate studies with dog blood samples. Black triangles indicate studies of wild animals and horses. Maps were from Google Maps.

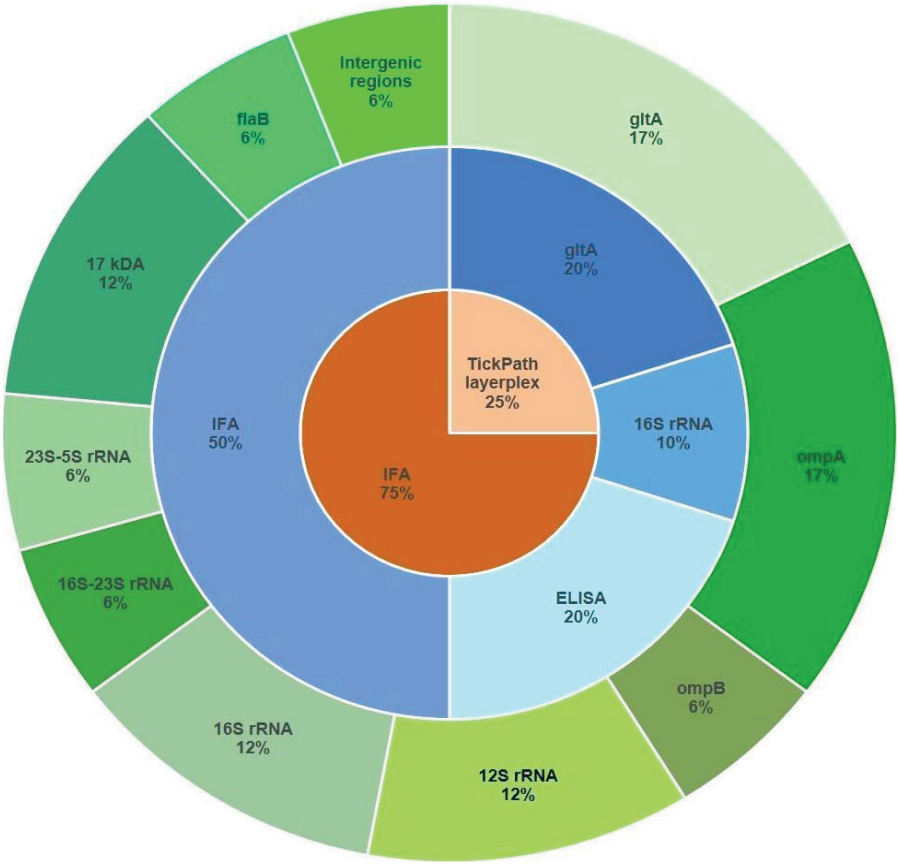

Fig. 3. Most common diagnostic tests used on blood samples from dogs/wild animals (orange circle), humans (blue circle), and ticks (green circle). All genes are used to detect *R. rickettsia* except flab and 16SrRNA-23SrRNA used for *Borrelia burgdorferi.*

A later study determined the prevalence of vector ticks as a possible cause of outbreak on 45 dogs at the Calexico shelter and 71 dogs at El Centro, CA. In total, 200 ticks were collected among the dogs. All the ticks were morphologically identified as *R. sanguineus*. All were tested by real-time PCR targeting the 154-bp fragment of the ompA gene from *R. rickettsii*, but none was detected (Fritz et al. 2012).

In contrast, a previous study at Mexicali collected 96 adult ticks on 11 stray dogs in a neighborhood where one patient was diagnosed with Rocky Mountain spotted fever. The 12S mitochondrial rRNA was sequenced from DNA from ticks, and *R. rickettsii* was detected by genetic typing of intergenic fragments RR0155-rpmB, cspA-ksgA, and RR1240-tlc5. Thirty of 96 ticks (31%) were positive for *R. rickettsii* (Eremeeva et al. 2011). This was one of the first studies to show *R. rickettsii* in ticks where Rocky Mountain spotted fever had been reported. Between 2016 and 2017, seroprevalence was surveyed in 752 dogs from Imperial County, a US county

that borders Mexicali, Mexico. Blood samples were collected and tested using immunofluorescence antibody assay (IFA) to detect *R. rickettsii*, *Ehrlichia canis* (Moshkovski), and *A. phagocytophilum*. Total prevalence was 12% for *R. rickettsia* in dogs (Estrada et al. 2019). To the west during the same period, animals in shelters and rabies clinics in three southern Arizona counties (Cochise, Santa Cruz, and Yuma) were studied near the Mexican border with the State of Sonora. In total, serum from 217 owned and stray dogs was tested by IFA and showed total prevalence of 5% for spotted fever group rickettsia (SFGR) IgG antibodies, with seropositivity ranging from 3 to 12% across the counties (Yaglom et al. 2018). Later studies found similar prevalence among dogs from Arizona and Baja California. In Mexico, one study of ticks from five municipalities in the State of Coahuila confirmed *R. rickettsii* in engorged female ticks from 253 free-roaming dogs. In total, 1,238 ticks were morphologically identified as *R. sanguineus*, of which 86% were engorged females and 14% engorged nymphs. The ticks were put into 30 groups, their DNA was purified, and conventional PCR assays were done using 23S-5S rRNA intergenic spacer and the outer membrane protein (ompA) gene of *R. rickettsii*. The six groups (each with six females) were positive for *R. rickettsii* DNA, with minimum infection of 3% (Ortega-Morales et al. 2019). In another study at Baja California, Coahuila, and Sonora, seroprevalence of *R. rickettsii* was evaluated in 1,136 serum samples from dogs and 942 ticks (413 adult females, 313 adult males, 215 nymphs, and one larva morphologically identified as *R. sanguineus* s.l.*)* using IFA antibody assays in dogs and real-time PCR assays targeting the first ompA gene in ticks. In total, 6% (69 dogs) had antibodies to *R. rickettsii*, with most in Baja California (12%), followed by Coahuila (4%), and then by Sonora (4%). Positive samples on ticks were sequenced using assays for genus-specific *Rickettsia*, showing evidence of *R. massiliae* (Beati and Raoult), *R. parkeri* (Lackman), and *R. rickettsii* by ompA PCR with further sequencing (Pieracci et al. 2019).

Most studies (three of six) of rickettsiosis in humans were from the State of Sonora (Table 1). Analysis of 104 children diagnosed with Rocky Mountain spotted fever by IFA at a hospital at Sonora, indicated 20% died (Alvarez-Hernandez et al. 2015). During 2015 and 2016, four pregnant women were infected by Rocky Mountain spotted fever. Diagnoses were confirmed by PCR targeting a conserved fragment of 805 bp of the citrate synthase (gltA) gene for *R. rickettsii* or by serological reactivity to *R. rickettsii* antigens by IFA (Licona-Enriquez et al. 2017). Blood samples analyzed by PCR targeting the gltA gene or IFA in 47 human deaths from 2013 to 2016 showed all were by *R. rickettsii* (Delgado-De la Mora et al. 2018). At Ensenada, Baja California, a cross-sectional study between 2009 and 2011 collected 384 human blood samples from patients older than 1 year. Antibodies against *R. rickettsii* were measured in samples using *R. rickettsia* ELISA in dogs but adapted to humans with an anti-human IgG conjugate. Sensitivity and specificity were determined using IFA in 32 samples. Results showed adjusted seroprevalence of 4% of rickettsiosis for *R. rickettsii* not associated with the sex, age, or occupation of humans or mobility of dogs between the exterior and interior of houses according to seropositivity (Field-Cortazares et al. 2015). Four years later, two more studies were published: one at Ciudad Juarez, Chihuahua, that evaluated blood samples from 106 veterinarians, 36 veterinary assistants, 19 pet groomers, and six veterinary administrative workers by IFA and PCR targeting 16S rRNA to detect *R. rickettsii, Ehrlichia* spp., and *A. phagocytophilum*. Results showed 54% of the participants were seropositive for at least one of the pathogens. The percentage of coinfection with two or three pathogens was remarkable. In total, 3% had co-exposure to *R. rickettsii* and *A.*

phagocytophilum, 9% to *Ehrlichia* spp. and *A. phagocytophilum*, 2% to *Ehrlichia* spp. and *R. rickettsii*, and 2% were positive for all three pathogens. Veterinarians and pet groomers were at great risk of being infected with *R. rickettsii* (Escárcega-Ávila et al. 2019).

Other Tick-borne Diseases. Other tick-borne diseases were reported recently in the region. Etiological agents were reported for Lyme disease, anaplasmosis, ehrlichiosis, and other rickettsiosis detected in humans, ticks, dogs, or wild animals. Most findings were reported from northeastern states, in contrast to the few publications from the northwestern region.

Researchers of a cross-sectional descriptive study at Mexico City, Coahuila, Nuevo León, and Tamaulipas between 1987 and 1988 used ELISA to test 2,346 human serum samples to detect antibodies against *B. burgdorferi*, and Western Blot to confirm positive samples. Twelve percent of the samples were positive by ELISA, and 122 of the samples were confirmed by Western Blot. Seroprevalence by region was 3% for Mexico City and 6% for the northeastern area of the country (Gordillo-Pérez et al. 2003). A decade later, analysis focused on Texas and partly the states of Tamaulipas, Nuevo León, and Coahuila where 1,235 samples of ticks were collected from vertebrate hosts. In total, 109 (9%) of ticks were identified as *Ixodes scapularis* (Say). *B. burgdorferi* was detected by PCR targeting 16SrRNA-23SrRNA and was in 45% of the ticks (Feria-Arroyo et al. 2014). In counties from South Texas near the Mexican border where the purpose was to know molecular prevalence of tick-borne pathogens, 245 white-tailed deer, *Odocoileus virginianus* (Zimmermann), and 122 coyotes, *Canis latrans* (Say), were analyzed. Whole-blood samples were evaluated by TickPath Layerplex qPCR test, targeting multiple genes for *Borrelia*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Babesia*, and *Theileria* genera. Coyotes had total prevalence of 9% for *Babesia vogeli* (Reichenow) and 0.8% for *Borrelia turicatae* (Brumpt), while white-tailed deer had total prevalence of 0.4% for *Anaplasma platys* (Dumler), 1.6% for *Ehrlichia chaffeensis* (Anderson), and 7% for *Theileria cervi* (Denier). The study indicated wild animals could be sentinels for a number of zoonotic tick-borne pathogens (Yu et al. 2020).

The approach has been used in other regions of Mexico, in the Yucatan Peninsula where overall prevalence of tick-borne rickettsial agents was 20% in white-tailed deer and 50% in *Mazama* sp. (Ojeda-Chi et al. 2019). Some tick-borne diseases can be confused with each other. In the State of Nuevo Leon in 2001, the Public Health Department collected sera from 345 human febrile patients at Allende and Linares that were suspected to have dengue fever. IFAs were used for IgG antibodies against *Rickettsia prowazekii* (da Rocha-Lima), *R. typhi* (Wolbach and Todd), and *R. parkeri* (Lackman). In total, 25% had antibodies reactive with typhus group rickettsiae and 16% against *R. parkeri*. Also, 190 ticks (*A. cajennense* and *A. imitator* (Kohls)) from livestock and tested in groups showed 50% were positive for *R. prowazekii* (Medina-Sanchez et al. 2005). One of the most ambitious analyses of tick-borne pathogens in Mexico to date was of samples collected between 1997 and 2013. About 1,107 ticks were collected from a range of mammalian hosts in forests and ecotourism parks in 22 of 32 states of the Republic of Mexico. *R. sanguineus* was collected most frequently (43%). PCR was used on the 16S rRNA gene for *A. phagocytophilum*, *E. chaffeensis*, and *E. canis*; gltA and 17 kDa protein-encoding genes for *Rickettsia* spp. were used to assay 477 groups of ticks. *A. phagocytophilum* was the most common pathogen followed by *E. canis* in 45 and 42 groups evaluated. *R. rickettsii*, *E. chaffeensis*, and *Ca. R. amblyommii* also were found in 4.2% of the total evaluated (Sosa-Gutierrez et al. 2016).

Tick-borne Diseases and Ticks at Chihuahua. At Chihuahua, in the "Paso del Norte" region, *Ehrlichia* spp. and *A. phagocytophilum* were reported in 30 dogs from Ciudad Juarez, Chihuahua infested by at least five female engorged *R. sanguineus*. In the study, blood samples from dogs and ticks were tested using PCR by amplification of the 16S rRNA gene for *Ehrlichia* spp. and *A. phagocytophilum*. *Ehrlichia* spp. was found in 40% of dogs and 66% of ticks. *Anaplasma* spp. was detected in 27% of dogs and 66% of ticks (Escárcega Ávila et al. 2018). Results might suggest more prevalence of bacteria in the ticks collected. However, follow-up studies need to clarify this contrast with reports from other regions.

At the city of Chihuahua (capital of the State) between August and November 2015, 664 ticks were extracted from 99 dogs in neighborhoods with reported clinical cases of Rocky Mountain spotted fever. Results showed 99.5% of the ticks were *R. sanguineus*. Ticks were grouped and evaluated by PCR. All were negative for *R. rickettsii* ompA, but two were positive for *A. phagocytophilum*, for total prevalence of 7% of the grouped samples tested (Prado et al. 2018). To date, this has been the only report of another tick-borne disease bacteria related to Rocky Mountain spotted fever from endemic neighborhoods in the State of Chihuahua. Recently, a study at Janos Biosphere Reserve in northwest Chihuahua collected 45 hard and soft ticks from wild animals. All were tested using PCR amplification of the gltA gene for *R. rickettsii* and a fragment of 470 bp of the flagellin (flaB) gene for *Borrelia* spp. Results showed *B. burgdorferi s.s*. in an *I. kingi* collected from a kit fox, *Vulpes macrotis* (Merriam), and *R. massiliae* was in 6.5% of 31 *R. sanguineus* from free-roaming dogs (López-Pérez et al. 2019). At Ciudad Juarez, a study with 144 blood samples from horses and soft ticks on them were analyzed by endpoint or nested PCR. Total prevalence of *Babesia caballi* was 2.8% in horse blood and 6% in soft ticks (Nuttall and Strickland). One sample tested positive for *A. phagocytophilum*, indicating prevalence of 0.8% in horse blood (Medrano-Bugarini et al. 2019).

In conclusion, despite efforts focused on molecular diagnosis of tick-borne disease in the State of Chihuahua, it is fundamental to develop strategies according to tools available by human and financial resources that could impact the prevalence and mortality of the exposed population in endemic counties. For example, in the neighboring State of Sonora, it was possible to decrease the prevalence of tick-infested dogs from 32.5 to 8.8% by using long-acting acaricidal collars, applying acaricides, and educating people about Rocky Mountain spotted fever (Alvarez-Hernandez et al. 2019). It is important to maintain ongoing collection and taxonomic identification of ticks throughout the State, which will aid in understanding current cases of Rocky Mountain spotted fever. To date (47th week of epidemiological surveillance in 2020), 34 cases of FMMR and other rickettsiosis have been reported, and Chihuahua is the second state with the most cases nationwide. Intersectoral participation of Public Health Departments at national and state levels and participation of the two public Autonomous Universities in the State are encouraged to address the diseases.

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References Cited

Alvarez-Hernandez, G., C. Murillo-Benitez, M. del Carmen Candia-Plata, and M. Moro. 2015. Clinical profile and predictors of fatal Rocky Mountain spotted fever in children from Sonora, Mexico. J. Pediatr. Infect. Dis. 34: 125-130.

Alvarez-Hernandez, G., N. Drexler, C. D. Paddock, J. D. Licona-Enriquez, J. D. la Mora, A. Straily, M. del Carmen Candia-Plata, D. I. Cruz-Loustaunau, and V. A. Arteaga-Cardenas. 2019. Community-based prevention of epidemic Rocky Mountain spotted fever among minority populations in Sonora, Mexico, using a One Health approach. Trans. R. Soc. Trop. Med. Hyg. 00: 1-8.

Bishopp, F. C., and H. L. Trembley. 1945. Distribution and hosts of certain North American ticks. J. Parasitol. 31: 1.

Bustamante, M., y G. Varela. 1943. Una nueva rickettsiosis en México. Existencia de la fiebre manchada americana en los Estados de Sinaloa y Sonora. Rev. del Inst. de Salubridad y Enf. Tropicales. 4: 189-210.

Chavarría, M. 1941. Garrapatas determinadas en México. Caracteres genéricos de las más comunes. Revista del Instituto Pecuario 1: 18-24.

Delgado-De la Mora, J., J. D. Licona-Enríquez, M. Leyva-Gastélum, D. Delgado-De la Mora, A. Rascón-Alcantar, y G. Álvarez-Hernández. 2018. Una serie de casos fatales de fiebre manchada de las Montañas Rocosas en Sonora, México. Biomedica 38: 69-76.

Direccion General de Epidemiología. 2019. Boletin Epidemiológico: Semana 52. 36: 28-32. https://www.gob.mx/cms/uploads/attachment/file/522437/ BSEMANAL _52.pdf.

Eremeeva, M. E., M. L. Zambrano, L. Anaya, L. Beati, S. E. Karpathy, M. M. Santos-Silva, B. Salceda, D. Macbeth, H. Olguin, G. A. Dasch, et al. 2011. *Rickettsia rickettsii* in *Rhipicephalus* ticks, Mexicali, Mexico. J. Med. Entomol. 48: 418-421.

Escárcega Ávila, A. M., B. S. Luna Flores, A. De la Mora Covarrubias, y F. Jiménez. 2018. Análisis exploratorio de enfermedades Rickettsiales transmitidas por garrapatas en perros de Ciudad Juárez, Chihuahua, México. Acta Univ. 28: 72-78.

Escárcega-Ávila, A. M., A. de la Mora-Covarrubia, A. Quezada-Casasola, and F. Jiménez-Vega. 2019. Occupational risk for personnel working in veterinary clinics through exposure to vectors of rickettsial pathogens. Ticks Tick Borne

Estrada, I., C. Balagot, M. Fierro, P. Kriner, E. Iniguez-Stevens, A. Kjemtrup, and J. Foley. 2019. Spotted fever group rickettsiae canine serosurveillance near the US-Mexico border in California. Zoonoses Public Health 00: 1-8.

Feria-Arroyo, T. P., I. Castro-Arellano, G. Gordillo-Perez, A. L. Cavazos, M. Vargas-Sandoval, A. Grover, J. Torres, R. F. Medina, A. A. Pérez de León, and M. D. Esteve-Gassent. 2014. Implications of climate change on the distribution of the tick vector *Ixodes scapularis* and risk for Lyme disease in the Texas-Mexico transboundary region. http://www.parasitesandvectors.com/content/7/ 1/199.

Field-Cortazares, J., A. Escárcega-Avila, G. López-Valenci, A. Barreras-Serrano, and L. Tinoco-Gracia. 2015. Seroprevalence and risk factors associated with rickettsiosis (*Rickettsia rickettsii*) in humans from Ensenada, Baja California, Mexico. Gac. Med. Mex. 151: 38-42.

Fritz, C. L., P. Kriner, D. Garcia, K. A. Padgett, A. Espinosa, R. Chase, R. Hu, and S. L. Messenger. 2012. Tick infestation and spotted-fever group rickettsia in shelter dogs, california, 2009. Zoonoses Public Health 59: 4-7.

Gordillo-Pérez, G., J. Torres, F. Solórzano-Santos, V. Garduño-Bautista, R. Tapia-Conyer, e O. Muñoz. 2003. Estudio seroepidemiológico de borreliosis de Lyme en la Ciudad de México y el noreste de la República Mexicana. Salud Publica Mex. 45: 351-355.

Harrell, G. 1949. Rocky Mountain spotted fever. Medicine (Baltimore) 28: 333-368.

Hoffman, C. 1925. La fiebre manchada de Choix. Boletín del Departamento de Salubridad Pública de México 1925 1: 35-37.

Licona-Enriquez, J. D., J. Delgado-De La Mora, C. D. Paddock, C. A. Ramirez-Rodriguez, M. Del Carmen Candia-Plata, and G. Á. Hernández. 2017. Case report: Rocky Mountain spotted fever and pregnancy: four cases from Sonora, Mexico. Am. J. Trop. Med. Hyg. 97: 795-798.

López-Pérez, A. M., S. Sánchez-Montes, J. Foley, C. Guzmán-Cornejo, P. Colunga-Salas, E. Pascoe, I. Becker, J. Delgado-de la Mora, J. D. Licona-Enriquez, and G. Suzan. 2019. Molecular evidence of *Borrelia burgdorferi* sensu stricto and *Rickettsia massiliae* in ticks collected from a domestic-wild carnivore interface in Chihuahua, Mexico. Ticks Tick Borne Dis. 10: 1118-1123. https://doi.org/10.1016/j.ttbdis.2019.05.018.

Luce-Fedrow, A., K. Mullins, A. P. Kostik, H. K. St. John, J. Jiang, and A. L. Richards. 2015. Strategies for detecting rickettsiae and diagnosing rickettsial diseases. Future Microbiol. 10: 537-564.

Medina-Sanchez, A., D. H. Bouyer, V. Alcantara-Rodriguez, C. Mafra, J. Zavala-Castro, T. Whitworth, V. L. Popov, I. Fernandez-Salas, and D. H. Walker. 2005. Detection of a typhus group *Rickettsia* in *Amblyomma* ticks in the State of Nuevo Leon, Mexico. Ann. N Y Acad. Sci. 1063: 327-332.

Medrano-Bugarini, R. A., J. V. Figueroa-Millán, B. E. Rivera-Chavira, J. J. Lira-Amaya, C. A. Rodríguez-Alarcón, D. M. Beristain-Ruiz, and J. R. Adame-Gallegos. 2019. Detection of *Theileria equi*, *Babesia caballi*, and *Anaplasma phagocytophilum* DNA in soft ticks and horses at Ciudad Juarez, Mexico. Southwest. Entomol. 44: 647-657.

Ojeda-Chi, M. M., R. I. Rodriguez-Vivas, M. D. Esteve-Gasent, A. Pérez de León, J. J. Modarelli, and S. Villegas-Perez. 2019. Molecular detection of rickettsial tick-borne agents in white-tailed deer (*Odocoileus virginianus yucatanensis*), mazama deer (*Mazama temama*), and the ticks they host in Yucatan, Mexico. Ticks Tick Borne Dis. 10: 365-370.

Ortega-Morales, A. I., E. Nava-Reyna, V. Ávila-Rodríguez, V. H. González-Álvarez, A. Castillo-Martínez, Q. K. Siller-Rodríguez, A. Cabezas-Cruz, F. Dantas-Torres, and C. Almazán. 2019. Detection of *Rickettsia* spp. in *Rhipicephalus sanguineus* (sensu lato) collected from free-roaming dogs in Coahuila state, northern Mexico. Parasites and Vectors 12: 1-7.

Pieracci, E. G., J. D. P. De La Rosa, D. L. Rubio, M. E. S. Perales, M. V. Contreras, N. A. Drexler, W. L. Nicholson, J. J. P. De La Rosa, I. H. Chung, C. Kato, et al. 2019. Seroprevalence of spotted fever group rickettsiae in canines along the United States-Mexico border. Zoonoses Public Health 66: 918-926. https://onlinelibrary.wiley.com/doi/abs/10.1111/zph.12642

Portillo, A., R. De Sousa, S. Santibáñez, A. Duarte, S. Edouard, I. P. Fonseca, C. Marques, M. Novakova, A. M. Palomar, M. Santos, et al. 2017. Guidelines for the detection of *Rickettsia* spp. Vector-Borne Zoonotic Dis. 17: 23-32.

Prado, R., Q. Rascon, D. Beristain, y J. Adame. 2018. Agente etiológico de la anaplasmosis granulocítica humana en la garrapata café de perro en Chihuahua, México. Salud Publica Mex. 60: 377-378.

Sosa-Gutierrez, C. G., M. Vargas-Sandoval, J. Torres, and G. Gordillo-Pérez. 2016. Tick-borne rickettsial pathogens in questing ticks, removed from humans and animals in Mexico. J. Vet. Sci. 17: 353-360.

Tinoco-Gracia, L., H. Quiroz-Romero, M. T. Quintero-Martínez, T. B. Rentería-Evangelista, Y. González-Medina, A. Barreras-Serrano, S. Hori-Oshima, M. H. Moro, and J. Vinasco. 2009. Prevalence of *Rhipicephalus sanguineus* ticks on dogs in a region on the Mexico-USA border. Vet. Rec. 164: 59-61.

Tovar, R. 1944. Clasificación de ectoparásitos encontrados en liebres en el Estado de Nuevo León y Tamaulipas. An. del Inst. de Inv. Científicas.

Tovar, R. 1945. Rickettsiosis exantemáticas transmitidas por garrapata en América. Revista Medicina México 3: 65-92.

Yaglom, H. D., W. L. Nicholson, M. Casal, N. C. Nieto, R. Z. Branch, C. Epidemiology, and F. Officer. 2018. Serologic assessment for exposure to spotted fever group rickettsiae in dogs in the Arizona-Sonora border region. Zoonoses Public Health 65: 984-992.

Yazid, M., R. Abou, P. Fournier, J. Stenos, and S. Vasoo. 2018. A concise review of the epidemiology and diagnostics of rickettsioses: *Rickettsia* and *Orientia* spp. J. Clin. Microbiol. 56: 1-10.

Yu, S., J. Modarelli, J. M. Tomeček, J. T. French, C. Hilton, and M. D. Esteve-Gasent. 2020. Prevalence of common tick-borne pathogens in white-tailed deer and coyotes in South Texas. Int. J. Parasitol. Parasites Wildl. 11: 129-135.

Characterization of Sites with Mosquito[1] Larvae in the Metropolitan Region of Puebla, Mexico

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Abstract. Immature stages of mosquitoes (Diptera: Culicidae) were studied with the objective to characterize diversity and sites at the metropolitan region of Puebla, Mexico. Three weather periods (dry-cold, dry-hot, and wet) and two sampling methods (ovitraps and direct collection at larval habitats) were used. Seven species of mosquitoes were identified. Greatest richness and abundance were during the wet season with both sampling methods, but few mosquitoes were in ovitraps. *Aedes epactius* Dyar & Knab was dominant in the ovitraps. In larval habitats, dominant species were *Ae. epactius* and *Culex quinquefasciatus* Say. Sixty-one sites with developing mosquitoes were found and classified according to origin, location, and type. Characteristics such as insolation, organic matter, vegetation, and associated fauna of the sites were considered. Correspondence analysis determined preference patterns between species and sites. *Ae. epactius*, *Culex restuans* Theobald, and *Cx. quinquefasciatus* were opportunistic species, because they were found in a variety of larval habitats, whereas *Culiseta particeps* (Adams) and *Culex stigmatosoma* Dyar were generalist species. *Cs. particeps* was related to sites with accompanying fauna, and most individuals were collected in tires; *Cx. stigmatosoma* was associated with sites with vegetation in temporary puddles. Results can be important for improvement of entomological control and surveillance programs in the metropolitan region of Puebla.

Resumen. Se realizó un estudio sobre los estados inmaduros de mosquitos (Diptera: Culicidae) con la finalidad de caracterizar la diversidad de mosquitos y sus criaderos dentro de la zona metropolitana de Puebla. El trabajo considero tres temporadas climáticas (seca-fría, seca-cálida, y lluvias) empleando dos métodos de colecta, colocación de ovitrampas y colecta directa en sitios de crianza. En total se identificaron siete especies de mosquitos. Durante la temporada de lluvias se obtuvó la mayor riqueza y abundancia con ambos métodos de muestreo; sin embargo, la proporción de ovitrampas positivas en general fue baja. *Aedes epactius* Dyar & Knab fue la especie dominante encontrada en ovitrampas. En los sitios de crianza las especies dominantes fueron *Ae. epactius* y *Culex quinquefasciatus* Say. En total se encontraron 61 criaderos, clasificados de acuerdo con el origen, ubicación, y tipo;

[1]Diptera: Culicidae

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además, se tomaron en cuenta características propias de los criaderos como la insolación, materia orgánica, vegetación, y fauna asociada. Mediante un análisis de correspondencia, se encontraron patrones de preferencia entre las especies y los sitios de crianza. *Ae. epactius*, *Cx. quinquefasciatus*, y *Culex restuans* Theobald se consideraron especies oportunistas, ya que se encuentran en gran variedad de criaderos, mientras que *Culiseta particeps* (Adams) y *Culex stigmatosoma* Dyar se consideraron especialistas. *Cs. particeps* se relacionó con criaderos con fauna acompañante y la mayoría se colectaron en llantas; *Cx. stigmatosoma* se asoció a criaderos con vegetación en charcos temporales. Estos resultados pueden ser importantes para mejorar los programas de control y vigilancia entomológica en la zona metropolitana de Puebla.

Introduction

Study of mosquitoes (Diptera: Culicidae) is focused mainly on medical and sanitary importance because species vector pathogenic agents transmitted by female mosquitoes (Clements 2012) to complete the gonotrophic cycle and produce viable eggs (Foster and Walker 2002). Females choose a body of water for oviposition, a choice not necessarily random, because a suitable choice determines the abundance, developmental success, and distribution of adults that do not generally disperse far from places where larval and pupal stages developed (Ibáñez-Bernal and Martínez-Campos 1994). The places are called rearing sites, which are mainly freshwater and low-mobility habitats, but also can be bodies of saltwater (Rejmánková et al. 2013). The most common sites are swamps, lakes, dams, marshes, rice fields, puddles, tree-holes, plant axils, snail shells, fallen leaves, small artificial containers such as buckets, tires, domestic utensils, and almost any container where water accumulates (Silver 2007).

Juvenile stages of mosquitoes are studied mostly for control and surveillance of vector species (Service 1993), because it is easier to access rearing sites and a large number of species regardless of the habits of the adult stage of each species (Zapata-Peniche et al. 2007, Mendoza et al. 2008). Ovitraps also can be placed, but are used mostly for entomological surveillance and control programs that focus on dengue and other viruses associated with *Aedes aegypti* (Linnaeus 1762). Nevertheless, species of the genera *Anopheles*, *Culex,* and *Haemagogus* use ovitraps as rearing sites (Chadee and Ritchie 2010, De la Cruz-Francisco et al. 2012, Wu et al. 2013).

Mosquito rearing sites are diverse. Therefore, their classification requires study and understanding their role in mosquito populations. Each classification depends on the author and objectives of the study. Verna (2015) classified rearing sites as natural and artificial. Rejmánková et al. (2013) classified them as natural or artificial, temporary or permanent, large or small, and fresh or saltwater. Zapata-Peniche et al. (2007) classified sites according to the place they were located (intradomiciliary or peridomiciliary), their size (large, medium, small), and origin (natural or artificial), and if it was a disposable or useful container. The SSA (2014) classified mosquito-rearing sites as controllable (those where physical action can be taken to stop development of larvae), controlled (those where larvae do not develop as a result of larval control), disposable (objects that accumulate water but can be disposed of), seasonal (those that contain water only during a period of time when larvae develop), structural (parts of construction), and permanent (those that contain water throughout the year and allow development of larvae). Apart from the type of

rearing site, biotic (predation, competition) and abiotic (temperature, light, pH, salinity, quantity of nutrients, etc.) factors also influence development, abundance, and species richness (Mendoza-Palmero 2007). For example, Verna (2015) reported 44 species at natural rearing sites and 19 species in artificial ones, of which some species had restricted habits, whereas others were opportunistic. Zapata-Peniche et al. (2007) reported 12 species restricted to only one urban area at Merida, Yucatan, Mexico, thus, most of the rearing sites were artificial. At urban areas with human settlements, finding species of medical relevance is more likely. Humans are at greater risk of acquiring vector-borne disease because of rearing sites inside or near homes.

Most studies focused on the wet season because of rainfall during that time of year. However, there are few studies on immature stages of mosquitoes during different weather periods. The study aimed to characterize diversity of larvae and rearing sites on a spatio-temporal scale in the metropolitan region of Puebla, Mexico and intended to provide information about the relationship between larvae and rearing sites to contribute to improvement of disease prevention strategies and vector surveillance and control programs, mainly in the State of Puebla.

Materials and Methods

The study area was at the metropolitan region of the State of Puebla that comprises the municipalities of Puebla (19°02´ N; 98°11´ W; 2,147 m above sea level) with a population of 1,692,181; San Pedro Cholula (19°01´ N; 98°19´ W; 214 m above sea level) with a population of 138,433; San Andrés Cholula (18°59´ N; 98°15´ W; 2140 m above sea level) with a population of 154,448; Cuautlancingo (19°05´ N; 98°14´ W; 2,159 m above sea level) with a population of 137,435; and Amozoc (19° 59´ N; 97° 58´ W; 2320 m above sea level) with a population of 125,876 (INEGI 2021) (Fig. 1). The area has temperate subhumid climate with rainfall in the summer and two seasons: the dry season from November through April and wet season from May through October, with most rainfall during June. In the dry season, two sub-seasons are a cold season, with January being the coldest month, and a hot season, with April being hottest. Sampling in 2017 was during three weather periods: January was dry-cold, April was dry-hot, and August was wet.

Ovitraps were used during the three seasons. The location of each ovitrap was determined with the help of volunteers through the Facebook social network. We obtained help from 70 volunteers, and 150 ovitraps were homogeneously distributed at the north (50), center (50), and south (50) of the study area (Fig. 1). Ovitraps were black plastic 1-liter containers with a piece of 10 x 20 cm pellon (non-woven uniform formation and rigid polyester fiber) inside, and two holes at the middle of the container to drain rainwater. The ovitraps were filled with tap water and put in domiciliary and peridomiciliary areas, gardens, planters, or open shade sites that attract females. Each ovitrap was used for 10 days. Afterward, ovitraps were transported to a laboratory and extensively searched for eggs and first-stage larvae. All biological material was put into individual vials for subsequent rearing.

Mosquitoes were collected directly at rearing sites only during the wet season. Nine sites were selected from directed selection of collection sites: three each at the north, center, and south of the metropolitan region (Fig. 1). A 1-ha (10,000 m^2) quadrant was established at each site where potential rearing sites in domiciliary and peridomiciliary areas were searched. Immature mosquitoes were collected with a 350-ml mosquito-dipper; sample collection was standardized using a method by

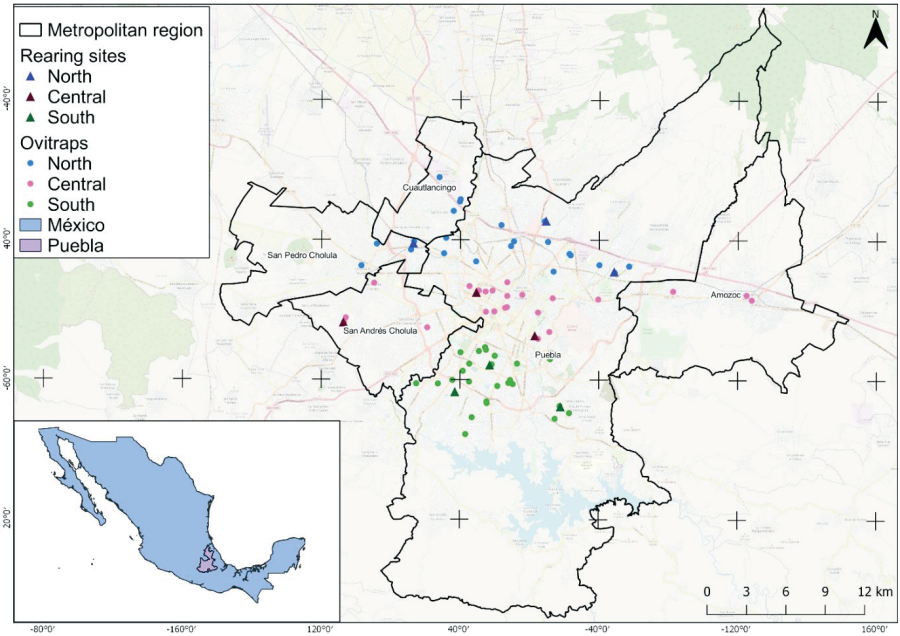

Fig. 1. Map of area where mosquitoes were studied at metropolitan region of Puebla, Mexico.

Mendoza et al. (2008), in which the number of dips depended on the surface (m^2) of the body of water. For small containers, a large pipette was used to fill the dipper.

Location of larval habitats, organic matter, vegetation, insulation (complete, partial, or shade), and associated fauna were used to characterize rearing sites. Rearing sites were classified as artificial or natural, which were divided into domiciliary or peridomiciliary, disposable, or controllable, and permanent or seasonal. Classification was based on studies by Gómez-Dantés and Méndez-Galván in Fernández and Flores (1995), Zapata-Peniche et al. (2007), and Verna (2015).

All material collected was transported live to a laboratory. Half the material was used for taxonomic identification of fourth-stage larvae killed by thermal shock and preserved in 70% alcohol. The remainder of the larvae were individually reared until adults emerged and killed with chloroform fumes. Rearing and mounting larvae and adults used methods by Belkin et al. (1967). Identification was with the aid of a Leica S4E stereomicroscope using dichotomous keys by Carpenter and LaCasse (1955), Bram (1967), Ibáñez-Bernal and Martínez-Campos (1994), and Darsie and Ward (2005). The material was deposited in the Arthropods of Medical and Veterinary Importance collection at the Laboratory of Arthropodology and Health at the Facultad de Ciencias Biológicas of the Benemérita Universidad Autónoma de Puebla (BUAP). Hill numbers for the effective number of species in a sample (alpha diversity) were calculated only for rearing sites because insufficient data were obtained from ovitraps. Two correspondence analyses were done to establish relationship between larval sites and mosquito species and determine association of species with different characteristics of rearing sites.

Results

In total, 2,013 individuals were collected, 512 in ovitraps and 1,501 by direct collection at larval rearing sites and were grouped into seven species: *Aedes epactius* Dyar & Knab, *Culex nigripalpus* Theobald, *Culex quinquefasciatus* Say, *Culex restuans* Theobald, *Culex salinarius* Coquillett, *Culex stigmatosoma* Dyar, and *Culiseta particeps* (Adams). *Ae. epactius*, *Cx. quinquefasciatus,* and *Cx. stigmatosoma* were recorded during the dry-cold period. *Ae. epactius*, *Cx. quinquefasciatus,* and *Cx. restuans* were recorded in the dry-hot period. During the wet period were recorded *Ae. epactius*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. salinarius*, *Cx. nigripalpus,* and *Cs. particeps*.

Hill numbers for the metropolitan region were N0 = 6, N1 = 3.7, and N2 = 3.1. This indicated the effective species number was three: *Ae. epactius*, *Cx. quinquefasciatus*, and *Cx. restuans*, indicating dominant species and that species were not distributed evenly. An index for the three regions (north, center, and south) was recorded; the largest number was the center (N0 = 6) followed by the south (N0 = 5) and the north (N0 = 4). *Ae. epactius*, *Cx. quinquefasciatus*, *Cx. restuans*, and *Cx. stigmatosoma* were recorded in the three regions. *Cx. salinarius* was only in the center and *Cs. particeps* was in the center and south. When calculated N2 was calculated, we obtained decrease in effective species N2 = 4 for the center, N2 = 3 for the south, and N2 = 1 for the north, with *Ae. epactius*, *Cx. quinquefasciatus*, and *Cx. restuans* most abundant in the three regions.

Few mosquitoes were in ovitraps. No mosquitoes were in ovitraps during the dry-cold season, 2% of only *Ae. epactius* were during the dry-hot season, and most were during the wet season with 18% and six species (*Ae. epactius*, *Cx. nigripalpus*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. salinarius,* and *Cx. stigmatosoma*).

Larvae were collected directly only during the wet season in which there were 61 larval habitats with *Ae. epactius*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. salinarius*, *Cx. stigmatosoma,* and *Cs. particeps. Ae. epactius* was most abundant with 701 individuals, followed by *Cx. quinquefasciatus* and *Cx. restuans* with 291 and 231 individuals, respectively.

Rearing sites with most mosquitoes were tires, 200-liter drums, and buckets (Fig. 2). Other larval habitats had frequencies of one or two, and were grouped in categories of Other (containers such as kitchen utensils, trays, bins, toilet tanks, basins, or water tanks) and Garbage (plastic cup and beer bottle not disposed of and remaining to accumulate water in a public road). Most larval habitats were artificial, and only one natural one (puddle) was recorded during the wet season (Table 1).

Correspondence analysis for association of mosquito species with larval habitats explained 72.9% of the association ($p < 0.05$) (Fig. 3). *Ae. epactius* and *Cx. quinquefasciatus* had generalist habits, as observed in the graph where nodes of the species were clustered at the center and surrounded by nodes of rearing sites where they were found (buckets, tires, drums, water tanks, among others). *Ae. epactius* was not associated with a specific larval habitat but is well adapted to artificial rearing sites. *Cx. quinquefasciatus* showed a tendency for buckets. Although the *Cs. particeps* node was at the center of the graph, it should not be interpreted as a generalist species, because it shows preference for tires but shares sites with *Cx. quinquefasciatus* and *Ae. epactius*. *Cx. stigmatosoma* and *Cx. restuans* were at the edges of Fig. 3 which indicated they were not generalists, since they were at specific rearing sites (puddle, pan, and bin). *Cx. salinarius* was close to nodes of generalist species because they were the only individuals collected in a construction block.

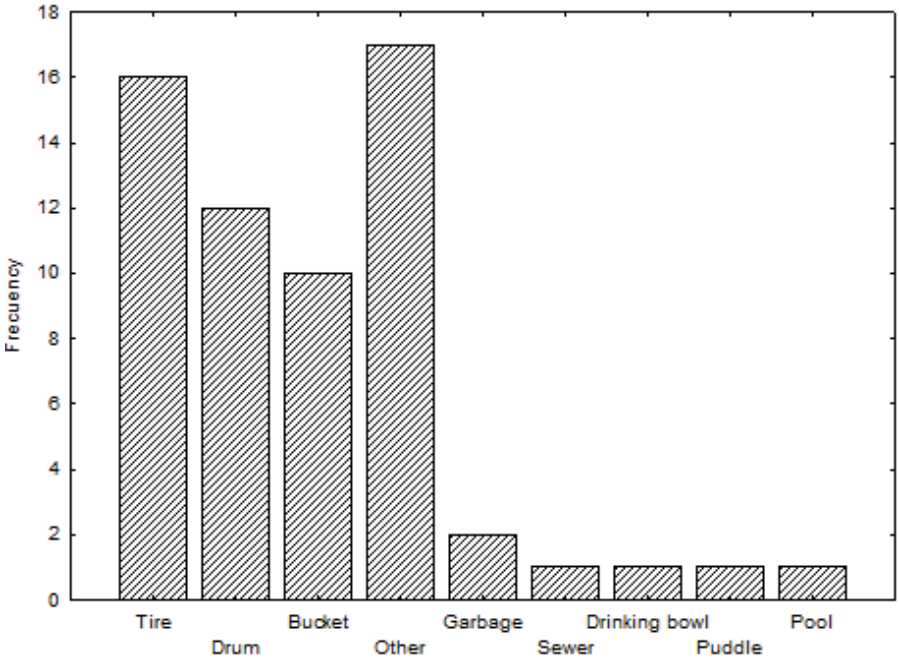

Fig. 2. Frequency of mosquitoes at rearing sites sampled during the wet season at metropolitan region of Puebla, Mexico.

Table 1. Classification and Number of Rearing Sites where Mosquitoes Were Sampled at Metropolitan Region of Puebla, Mexico

Origin	Location	Type	Number
Artificial	Domiciliary	Disposable	11
		Controllable	38
	Peridomiciliary	Disposable	2
		Controllable	7
		Structural	1
Natural	Domiciliary	Permanent	0
		Seasonal	0
	Peridomiciliary	Permanent	0
		Seasonal	1

Correspondence analysis for association between characteristics of rearing sites and species showed 89.2% of inertia ($p < 0.05$) (Fig. 4). Analysis showed that although *Ae. epactius* was a species with generalist habits, it preferred sites with turbid water in the shade and without fauna, vegetation, or organic matter. However, this was the only species that used rearing sites with clean water, such as that in water tanks, pools, and drums. *Cu. quinquefasciatus* and *Cx. restuans* are related species and prefer sites with organic matter and partial insulation. *Cu. Particeps* shared the same preferences but also preferred sites with fauna and colored water.

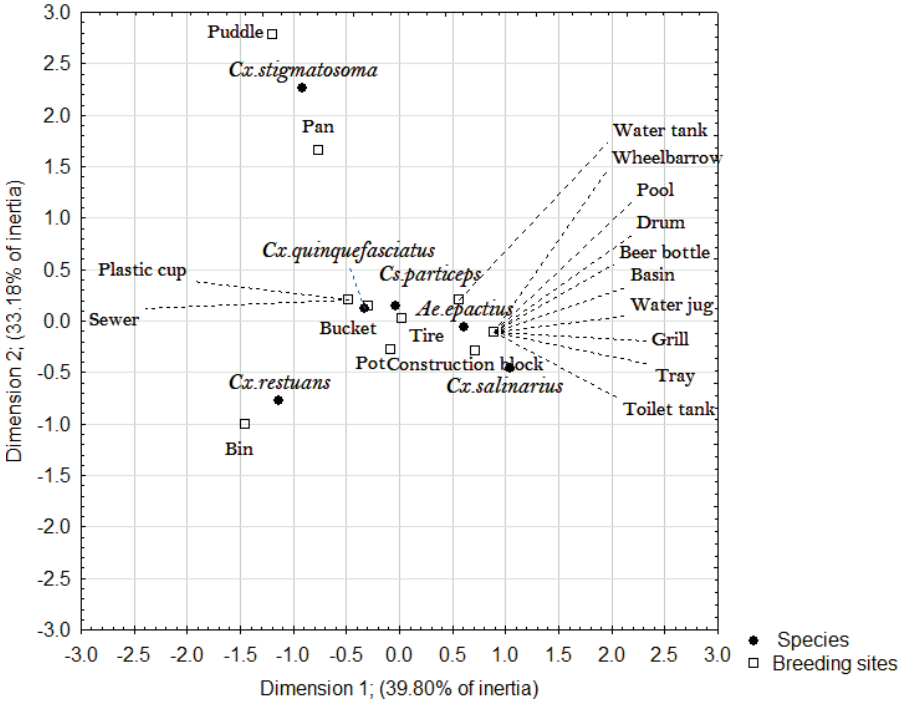

Fig. 3. Correspondence analysis of species-rearing sites at metropolitan region of Puebla, Mexico. Opportunistic species and their rearing sites are at the center, while species with more specific habits are at the periphery.

Cx. stigmatosoma was the only species at a site with vegetation. *Cx. salinarius* was not associated with any variable, and no preferences were observed because few individuals were collected.

Discussion

The only mosquito studies for the metropolitan region of Puebla were by Mendez-Andrade (2016) and Viveros-Santos and Sandoval-Ruiz (2018) who reported *Ae. epactius*, *Aedes trivittatus* (Coquillett), *Culex coronator* Dyar & Knab, *Cx. salinarius*, *Cx. stigmatosoma,* and *Cs. particeps*. We did not find *Cx. coronator* or *Ae. trivittatus*. However, there was an increase in species at the capital of the State of Puebla with records of *Cx. nigripalpus*, *Cx. quinquefasciatus,* and *Cx. restuans*. Both studies mentioned adult mosquitoes, and results for *Ae. epactius*, *Cx. quinquefasciatus,* and *Cx. salinarius* differed from ours because *Cx. salinarius* was considerably more abundant (>500) (Mendez-Andrade 2016) compared to the 13 specimens in our study. We found *Ae. epactius* the dominant species, which contrasts with results by Viveros-Santos and Sandoval-Ruiz (2018) who did not report it as dominant although it occurred during three weather periods. *Cx. quinquefasciatus* was reported as a cosmopolitan species at all states of Mexico (Ibáñez-Bernal and Martínez-Campos 1994); however, Mendez-Andrade (2016) and

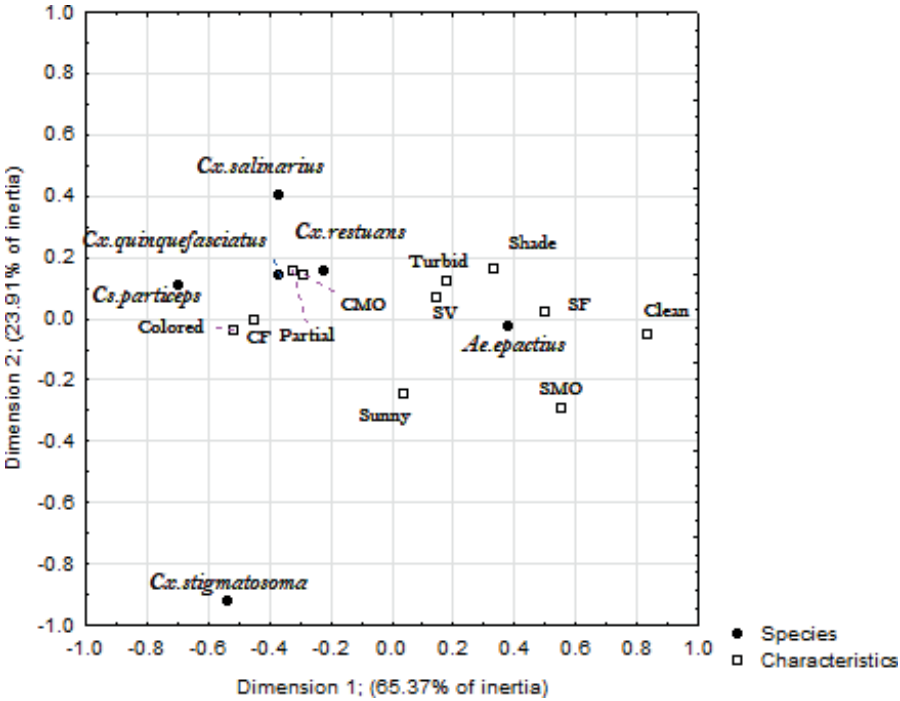

Fig. 4. Correspondence analysis of characteristics of mosquito species-rearing sites at metropolitan region of Puebla, Mexico. SMO (without organic matter), CMO (with organic matter), SF (without fauna), CF (with fauna), SV (without vegetation), and CV (with vegetation).

Viveros-Santos and Sandoval-Ruiz (2018) did not report the species. This indicated the importance of considering immature and adult stages of species, because abundance of adults can be calculated from abundance and survival of immature stages (Silver 2007). Future studies could explain what is occurring with the species at the study site.

We obtained few mosquitoes from ovitraps. This was not unexpected since Wu et al. (2013) and Kuri-Morales et al. (2017) used 10,380 and 2,253 ovitraps, respectively, and reported mosquitoes in fewer than 1% of ovitraps, even for *Ae. aegypti,* and even less for other culicid species. Consequently, we concluded that ovitraps were not efficient for diversity studies, because they are of little use because of preference of each species for particular sites. Ovitraps also competed with a great variety of potential rearing sites usually found in domiciliary and peridomiciliary areas, and females were not likely to choose ovitraps over usual rearing sites. In vector surveillance and control programs, ovitraps are useful only for dengue disease and not entirely efficient.

Independent of the sampling method, mosquitoes were most diverse during the wet season. Although *Ae. epactius* and *Cx. quinquefasciatus* were the only species during the three weather periods, we asserted that all species found in the

wet season persisted throughout time in some form, probably because of resistance of eggs to desiccation (Farnesi et al. 2015).

In urban areas, it is more common to find artificial rearing sites, with buckets and tires being most common (Zapata-Peniche et al. 2007, Verna 2015). This is supported by our results and explained by urbanization generating change in mosquito dynamics because of modification of the environment, mostly changes in temperature, precipitation, and humidity, which impacted the biology of vectors by accelerating development time and increasing survival, thus causing emergence of infectious diseases (Li et al. 2014, Marquetti-Fernández et al. 2014). Species also have adapted to urban environments by using new artificial rearing sites instead of natural ones. Of most concern are "disposable" sites such as pots, pans, bins, and water jugs that would not generate new rearing sites if they were properly disposed of instead of being accumulated. "Controllable" rearing sites (buckets, drums, basins, pools, and tires) are used occasionally or permanently to store water where mosquito proliferation can be prevented if they were covered, upside down, or indoors to avoid water accumulation. For better vector prevention and control, the "social" factor (Argüello 2014) and an "eco-bio-social" approach should be considered. This means that, apart from biology of culicids especially those of medical importance, different variables such as health services, sanitation, water supplies, demography, and knowledge and attitudes shaped by different factors such as poverty, social inequality, and community dynamics should be included (Quintero et al. 2014).

Ae. epactius was dominant and had opportunistic habits, because it was in natural and artificial rearing sites. Lozano-Fuentes et al. (2012) found *Ae. epactius* in a large number of artificial sites at Puebla City, mainly in tires (232) and buckets (123), because they are the most abundant rearing sites at the study area. The authors also reported co-occurrence of the species with *Ae. aegypti* and proposed additional studies to exempt *Ae. epactius* as a transmitter of dengue virus, which should be considered because it is the most important and only species in the study area capable of developing in containers with clean water used daily by humans.

Cx. quinquefasciatus and *Cx. restuans* were the next most abundant species. Verna (2015) reported the species as the only ones at natural and artificial rearing sites. This could be because the two species have similar preferences as shown by correspondence analysis. It is common to find them at the same site with similar abundance (Stough and Wallace 2016, Harbison et al. 2017). The preferred rearing sites of the species are tires, buckets, drinking bowls, and small plastic containers, according to this study and studies by Muñoz-Cabrera et al. (2006) and Zapata-Peniche et al. (2007).

Cx. stigmatosoma does not have generalist habits like the other species. According to correspondence analysis, the species prefers sites with colored water and vegetation, such as temporary puddles, which agrees with Muñoz-Cabrera et al. (2006) and Ortega-Morales et al. (2011) who reported the species frequently was in temporary puddles or flooded fields and less frequently at artificial rearing sites, with a preference for sites with polysaprobic and contaminated water.

Cs. particeps develops in marshes, stream margins, and puddles (Schreieber et al. 1989), as well as in artificial containers such as cemetery flower pots or tanks, in sites with wastewater or clean water with or without vegetation, and in the shade or complete insulation (Muñoz-Cabrera et al. 2006). In the study area, according to correspondence analysis, the species was found mostly in tires, preferring sites with colored water, organic matter, partial insulation, and fauna. Associated fauna we found were Odonata larvae and larvae such as Chironomidae and Psychodidae.

Only eight specimens of *Cx. salinarius* were collected in a construction block, and it was not possible to obtain definite results about its preference for rearing sites. Rochlin et al. (2008) reported larvae of the species in marshes, wetlands, coastal reservoirs, and artificial containers, but the latter were not specified. Zapata-Peniche et al. (2007) found it in buckets, drinking bowls, and small garbage objects, whereas Ortega-Morales et al. (2011) reported it in tires. Because these results do not agree with ours, it is not possible to determine with certainty at what sites the species develops at Puebla City. However, because we did not record the species at artificial rearing sites, and according to Rochlin et al. (2008), it is possible it is found in temporary puddles, pond-like bodies of water, or sewers.

We recommend surveying *Ae. epactius* and *Cx. quinquefasciatus* because they are dominant species in the study area and are of medical importance. Studies suggest the species can transmit dengue or zika viruses, respectively (Lozano-Fuentes et al. 2012, Franca et al. 2016, Kenney et al. 2017). It is important to create a well-established disease surveillance and control program for species of the genus *Culex*.

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References Cited

Argüello, S. 2014. Ecosalud y la participación social en el campo de las enfermedades transmitidas por vectores ETVs. Espacio Abierto 23: 339-348.

Belkin, J. M., C. L. Hogue, P. Galindo, T. H. Aitken, R. X. Schick, and W. A. Powder. 1967. Estudios sobre mosquitos (Diptera, Culicidae) IIa. Métodos para coleccionar, criar y preservar mosquitos. Contrib Am Entomol. 2: 20-89.

Carpenter, S. J., and W. J. LaCasse. 1955. Mosquitoes of North America, North of Mexico. University of California Press, Los Angeles.

Chadee, D. D., and S. A. Ritchie. 2010. Efficacy of sticky and standard ovitraps for *Aedes aegypti* in Trinidad, West Indies. J. Vector Ecol. 35: 395-400.

Clements, A. N. 2012. The Biology of Mosquitoes Transmission of Viruses and Interactions with Bacteria. Vol. 3. CABI publishing, New York.

Darsie, R. F., and R. A. Ward. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida.

De la Cruz-Francisco, V., D. I. Veda-Moreno, and A. Valdés-Murillo. 2012. Aspectos ecológicos de la incidencia larval de mosquitos (Diptera: Culicidae) en Tuxpan, Veracruz, México. Rev. Colomb. Entomol. 38: 128-133.

Farnesi, L. C., R. F. Menna, A. J. Martins, D. Valle, and G. L. Rezende. 2015. Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: connection with distinct levels of resistance to desiccation. J. Insect Physiol. 83: 43-52.

Foster, W. A., and E. D. Walker. 2002. Mosquitoes (Culicidae), pp. 204-256. *In* L. Durden and G. Mullen [eds.], Medical and Veterinary Entomology. Academic Press, United States.

Franca, R. F. O., M. H. Neves, C. F. Ayres, O. P. Melo-Neto. and S. P. Filho. 2016. First International Workshop on Zika Virus Held by Oswaldo Cruz Foundation FIOCRUZ in Northeast Brazil March 2016 – A Meeting Report. PLoS Negl. Trop. Dis. 10: e0004760

Harbison, J. E., B. Hulselbosch, J. Buczek, A. Runde, and R. Nasci. 2017. Reduced productivity of *Culex pipiens* and *Cx. restuans* (Diptera: Culicidae) mosquitoes in parking area catch basins in the northeast Chicago metropolitan area. J. Vector Ecol. 42: 148-154.

Ibáñez-Bernal, S., and C. Martínez-Campos. 1994. Clave para la identificación de larvas de mosquitos comunes en las áreas urbanas y suburbanas de la República Mexicana (Diptera: Culicidae). Folia Entomol Mex. 92: 42-73.

INEGI, 2021. Censo de Población y Vivienda 2020, Principales resultados por localidad (ITER). Puebla. Instituto Nacional de Estadística y Geografía. https://www.inegi.org.mx/programas/ccpv/2020/default.html#Datos_abiertos

Kenney, J. L., H. Romo, N. Duggal, W. P. Tzeng, K. L. Burkhalter, A. C. Brault, and H. M. Savage. 2017. Transmission incompetence of *Culex quinquefasciatus* and *Culex pipiens pipiens* from North America for zika virus. J. Trop. Med. Hyg. 95: 1235-1240.

Kuri-Morales, P., F. Correa-Morales, C. González-Acosta, G. Sánchez-Tejeda, E. Dávalos-Becerril, M. F. Juárez-Franco, A. Díaz-Quiñonez, A. Huerta-Jimenez, M. D. Mejía-Guevara, M. Moreno-García, and J. F. González-Roldán. 2017. First record of *Stegomyia aegypti* (= *Aedes aegypti*) in Mexico City, Mexico. Med. Vet. Entomol. 31: 240-242.

Li, Y., F. Kamara, G. Zhou, S. Puthiyakunnon, C. Li, Y. Liu, Y. Zhou, L. Yao, G. Yan, and X. G. Chen. 2014. Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. PLoS Negl. Trop. Dis. 8: e331.

Lozano-Fuentes, C., S. Hayden, M. H. Welsh-Rodríguez, C. Ochoa-Martínez, B. Tapia-Santos, K. C. Kobylinski, C. K. Uejio, E. Zielinski-Gutierrez, L. Delle-Monache, A. J. Monaghan, D. F. Steinhoff, and L. Eisen. 2012. The dengue virus mosquito vector *Aedes aegypti* at high elevation in México. Am. J. Trop. Med. Hyg. 87: 902-909.

Marquetti-Fernández, M. C., C. Fuster-Callaba, and I. Martín-Díaz. 2014. Distribución espacial y temporal de los sitios de cría de *Aedes albopictus* y *Aedes aegypti* (Diptera: Culicidae) en Pinar del Río, Cuba. Rev Biomed. 25: 54-67.

Mendez-Andrade, A. 2016. Relación Microclimática del Ritmo de Picadura de Mosquitos (Diptera: Culicidae) Silvestres y Urbanos. Tesis de Licenciatur Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, Puebla, México.

Mendoza, F. S., S. Ibáñez-Bernal, and F. Cabrero-Sañudo. 2008. A standardized sampling method to estimate mosquito richness and abundance for research and public health surveillance programmes. Bull. Entomol. Res. 98: 323-332.

Mendoza-Palmero, F. S. 2007. Diversidad y Hábitats Acuáticos de Mosquitos (Diptera: Culicidae) en la Región Central de Veracruz, México. Tesis de Doctorado, Instituto de Ecología, A.C. Veracruz, México.

Muñoz-Cabrera, L. O., S. Ibáñez-Bernal, and M. C. Corona-Vargas. 2006. Los mosquitos (Diptera: Culicidae) de Tlaxcala, México. I: Lista comentada de especies. Folia Entomol. Mex. 45: 223-271.

Ortega-Morales, A. I., H. Huerta, D. Strickman, F. J. Sánchez-Ramos, J. Landeros-Flores, and E. Cerna-Chávez. 2011. Registro de mosquitos en México: *Culex stigmatosoma* Dyar y *Culex thriambus* Dyar (Diptera: Culicidae) con notas taxonómicas para ambas especies. Southwest. Entomol. 36: 177-196.

Quintero, J., H. Brochero, P. Manrique-Saide, M. Barrera-Pérez, C. Basso, S. Romero, A. Caprara, J. C. De Lima-Cunha, E. Beltrán-Ayala, K. Mitchell-Foster, A. Kroeger, J. Sommerfeld, and M. Petzold. 2014. Ecological, biological and social dimensions of dengue vector breeding in five urban settings of Latin America: a multicountry-study. BMC Infect. Dis. 14: 38.

Rejmánková, E., J. Grieco, N. Achee, and D. R. Roberts. 2013. Ecology of larval habitats, pp. 397-446. *In* S. Manguin [ed.] *Anopheles* Mosquitoes New Insights into Malaria Vectors. Intech, Rijeka.

Rochlin, I., M. E. Dempsey, S. R. Campbell, and D. V. Ninivaggi. 2008. Salt marsh as *Culex salinarius* larval habitat in coastal New York. J. Am. Mosq. Control Assoc. 24: 359-367

Schreieber, E. T., J. D. Chaney, M. S. Mulla, and W. E. Walton. 1989. Bionomics of *Culiseta particeps* in southern California. J. Am. Mosq. Control Assoc. 5: 434-435.

Service, M. W. 1993. Mosquito Ecology, Field Sampling Methods. Springer Science+Business Media B. V, Liverpool, UK.

Silver, J. B. 2007. Mosquito Ecology, Field Sampling Methods. Springer Science+Business Media B.V., New York.

SSA (Secretaría de Salud). 2014. Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE). Guía metodológica para las acciones de control larvario.

Stough, J. E., and J. R. Wallace. 2016. Oviposition activity patterns of *Culex pipiens* and *Culex restuans* in Pennsylvania. J. Am. Mosq. Control Assoc. 32: 156-159.

Verna, T. N. 2015. Species composition and seasonal distribution of mosquito larvae (Diptera: Culicidae) in southern New Jersey, Burlington County. J. Med. Entomol. 52: 1165-1169.

Viveros-Santos, V., and C. A. Sandoval-Ruiz. 2018. Spatio-temporal diversity of mosquitoes from the central area of Puebla state, México. Southwest. Entomol. 43: 357-367.

Wu, H. H., C. Y. Wang, H. J. Teng, C. Lin, L. C. Lu, S. W. Jian, N. T. Chang, T. H. Wen, J. W. Wu, D. P. Liu, L. J. Lin, D. E. Norris, and H. S. Wu. 2013. A dengue vector surveillance by human population-stratified ovitrap survey for *Aedes* (Diptera: Culicidae) adult egg collections in high dengue-risk areas of Taiwan. J. Med. Entomol. 50: 261-269.

Zapata-Peniche, A., P. Manrique-Saide, E. A. Rebollar-Téllez, A. Che-Mendoza, and F. Dzul-Manzanilla. 2007. Identificación de larvas de mosquito (Diptera: Culicidae) de Mérida Yucatán, México y sus principales criaderos. Rev. Biomed. 18: 3-17. Mendoza, F. S., S. Ibáñez-Bernal, and F. Cabrero-Sañudo. 2008. A standardized sampling method to estimate mosquito richness and abundance for research and public health surveillance programmes. Bull. Entomol. Res. 98: 323-332.

New Host Record and Distribution of *Dicarnosis ripariensis* Kerrich[1] in Mexico

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Abstract. From collections in central and northern Mexico, *Dicarnosis ripariensis* (Hymenoptera: Encyrtidae) was registered for the first time as a parasitoid of two species of mealybugs of economic importance, *Phenacoccus madeirensis* and *P. solenopsis* (Hemiptera: Pseudococcidae).

Resumen. De colectas realizadas en el centro y norte de México, se registra por primera vez a *Dicarnosis ripariensis* (Hymenoptera: Encyrtidae) como parasitoide de dos especies de piojos harinosos de importancia económica, *Phenacoccus madeirensis*, y *P. solenopsis* (Hemiptera: Pseudococcidae).

The genus *Dicarnosis* (Hymenoptera: Encyrtidae) was established by Mercet (1921) when describing *D. superbus* Mercet from the Canary Islands, Spain. To date, nine species of the genus have been described (Noyes 2020), the last of which was *D. lisenchiae* Fusu from Cyprus (Fusu 2012). Most species have been identified and reported from Europe and to a lesser extent from Asia. Only two species are registered in America (Table 1). Although the species parasitize mealybugs (Hoffer and Trjapitzin 1977), only *D. ripariensis* Kerrich was recorded on *Phenacoccus gossypii* Townsend and Cockerell (Hemiptera: Pseudococcidae) (Kerrich 1978).

Parasitoids that emerged from mealybugs were collected on 24 October 2019 by Héctor González Hernández at a commercial cotton (*Gossypium hirsutum* L.) farm at Ejido Reforma, Mexicali, Baja California, México (32.616 N; 114.823 O; 22 m above sea level) and on 10 January 2020 by Isela Hernández on bell peppers (*Capsicum annuum* L.) in a greenhouse at Montecillo, Texcoco, State of Mexico (19.455 N; 98.907 W; 2,220 m above sea level). Keys by Noyes et al. (1997) were used to determine the Encyrtidae parasitoids belonged to the *Dicarnosis* genus.

For preservation and identification, adult parasitoids were dehydrated gradually (80, 90, and 95% ethanol) at 30-minute intervals in each solution. After the last alcohol solution, the specimens were put into amyl acetate for 24 hours. Subsequently, the specimens were dried at room temperature for 24 hours, then put on 200 g Bristol cards (1 X 0.5 cm) to preserve diagnostic structures for easier recognition. Keys and descriptions by Kerrich (1978) were used to identify the parasitoid species.

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Table 1. Distribution of Species of *Dicarnosis* Registered in Noyes Database (2020)
Cuadro 1. Distribución de las Especies de *Dicarnosis* Reportadas en la Base de Datos de Noyes (2020)

Species	Distribution
Dicarnosis erythrocephala Kerrich, 1978	United States of America
Dicarnosis helena Hoffer, 1952	Austria, Czech Republic; France, Hungary, Italy, Romania, Serbia, Slovakia, Spain, Sweden, Yugoslavia
Dicarnosis hofferi Trjapitzin, 1965	Czech Republic, South Korea, Mongolia, Russia
Dicarnosis lisenchiae Fusu, 2012	Cyprus
Dicarnosis ripariensis Kerrich, 1978	United States of America
Dicarnosis sinuates Xu, 2000	Peoples' Republic of China
Dicarnosis sugonjaevi Myartseva, 1977	Azakhstan, Mongolia, Tadzhikistan, Tselinograd Obl., Turkey, Turkmenistan
Dicarnosis superba Mercet, 1921	Algeria, Azerbaijan, Bulgaria, Canary Islands, Croatia, Cyprus, France, Greece, Italy, Kuwait, Spain, Turkey, Yugoslavia
Dicarnosis vicina Trjapitzin and Hoffer, 1977	Afghanistan, Armenia, Azerbaijan, Turkmenistan

Four adult female mealybugs collected on bell pepper plants were processed by macerating in a 1.5-ml Eppendorf tube with 10% KOH and heating to 80°C for 10 to 15 minutes in a thermoblock (AccuBlock, Labnet, Edison, NJ). The Kosztarab technique (1963) was used for processing including clarification, staining, dehydration, and slide-mounting adult females.

Mealybugs collected on bell peppers at Texcoco, State of Mexico, were identified as *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), and 20 specimens of parasitoids (10 females and 10 males) were obtained from the mealybugs. The species of mealybug collected in cotton at Mexicali, Baja California previously was identified as *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) Tinsley (González-Hernández 2019); 15 adult parasitoid specimens emerged from the mealybug (10 females and five males). The mealybug specimens were identified using the method of Williams and Granara de Willink (1992). Material from processed and preserved parasitoids was kept in the Insect Collection of the Posgrado en Fitosanidad at Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de Mexico (CEAM-CP).

All parasitoid specimens were *Dicarnosis ripariensis* (Fig. 1). The species was described by Kerrich (1978) based on specimens deposited in the Entomology Research Museum at the University of California, Riverside, that were collected in Arkansas and California, in 1960 and 1964, respectively. Trjapitzini and Ruiz-Cancino (2003) also reported *D. ripariensis* from material deposited at the University of California, Riverside, and at the Insect Collection of the Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México. J. Pinto, E. Fischer, and J. LaSalle collected the material 2 km northeast of Uruapan, Michoacán in 1980. A. González H. also collected the material at Fortín de las Flores, Veracruz, México in 1997. No host was recorded in either collection. This is the first record of *D. riparienis* as a parasitoid of *P. madeirensis* and *P. solenopsis*. Its distribution is expanding in Mexico, to Baja California and State de Mexico.

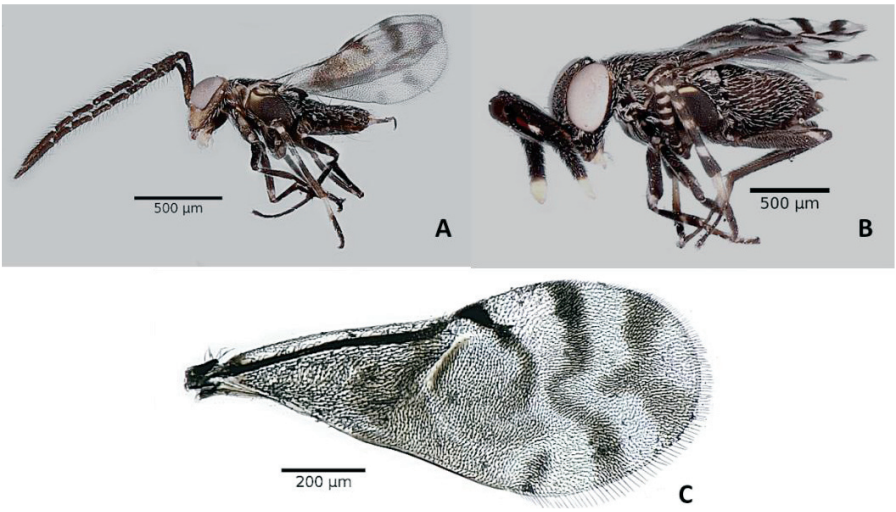

Fig. 1. *Dicarnosis ripariensis* Kerrich, 1978. A) male, B) female, C) female frontal wing (Photographs by Jorge M. Valdez Carrasco, Colegio de Postgraduados).
Fig. 1. *Dicarnosis ripariensis* Kerrich, 1978. A) macho, B) hembra, C) ala anterior de la hembra (Fotografías tomadas por Jorge M. Valdez Carrasco, Colegio de Postgraduados).

Both mealybug species are pests of great economic importance and of American origin (Ferris 1953, McKenzie 1967, Williams and Granara de Willink 1992). *P. solenopsis* is distributed worldwide and causes severe damage to agricultural, ornamental, and greenhouse crops (Arif et al. 2009, Abbas et al. 2010, García-Morales 2016, Spodek et al. 2018). In Texas, it was found on cotton and 29 other hosts (Fuchs et al. 1991), while at Mexicali, Baja California, the northern part of México, it recently was one of the main pests of cotton (González-Hernández et al. 2019). *Phenacoccus madeirensis* is more common in Central and South America, but of worldwide distribution, attacking cassava (*Manihot esculenta* Crantz) and potato (*Solanum tuberosum* L.) (Williams and Granara de Willink 1992), and is a common pest in peppers grown in greenhouses in Mexico (J. Lima-Espindola personal communication).

Although, some commercially available natural enemies such as *Leptomastix algirica* Trjapitzin (Hymenoptera: Encyrtidae) are available for *P. solenopsis* (van Lenteren 2012), the parasitoid is not present in Mexico and cannot be imported. Moreover, parasitoids to control *P. solenopsis* are not yet commercially available in the northern part of México. However, the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is commercially available for both pests, and is effective in controlling some species of mealybugs such as pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae), in several crops and wild plant species (Kairo et al. 2000, Santiago-Islas et al. 2008). Unfortunately, it is very expensive, and its effectiveness depends on abundance of the prey, making it an unfeasible alternative in management of mealybug pests.

Dicarnosis ripariensis is a natural enemy of at least two species of mealybugs that attack cotton in the field or vegetables in greenhouses. Because of its natural discovery in the different environments, from the dry warmth of Mexicali to sub-humid temperate climate at Texcoco, it seems reasonable to assume it is environmentally adaptable. Studies are needed to determine its potential as a biological control agent against *P. madeirensis* and *P. solenopsis*.

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References Cited

Abbas, G., M. J. Arif, M. Ashfaq, M. Aslam, and S. Saeed. 2010. Host plants, distribution and overwintering of cotton mealybug (*Phenacoccus solenopsis*; Hemiptera: Pseudococcidae). Int. J. Agric. Biol. 12: 421-425.

Arif, M. I., M. Rafiq, and A. Ghaffar. 2009. Host plant of cotton mealybug (*Phenacoccus solenopsis*): a new menace to cotton agroecosystem of Punjab. Int. J. Agric. Biol. 11: 163-167.

Ferris, G. F. 1953. Atlas of the Scale Insects of North America. VI. The Pseudococcidae. Stanford University Press, Stanford, CA.

Fuchs, T. W., J. W. Stewart, R. Minzenmayer, and M. Rose. 1991. First record of *Phenacoccus solenopsis* Tinsley in cultivated cotton in the United States. Southwest. Entomol. 16: 215-221.

Fusu, L. 2012. A new species of *Dicarnosis* Mercet (Hymenoptera, Encyrtidae) from the Island of Cyprus: the missing link in the evolution of the group. Deut. Entomol. Z. 58: 243-246.

García-Morales, M., B. D. Denno, D. R. Miller, G. L. Miller, Y. Ben-Dov, and N. B. Hardy. 2016. ScaleNet: A literature-based model of scale insect biology and systematics. Database. doi: 10.1093/database/bav118. http://scalenet.info.

González-Hernández, H., J. R. Lomeli-Flores, J. Suárez-Espinosa, R. T Mora-Armanta, H. C. Arredondo-Bernal, C. A. Catzim, H. M. Santoyo-Cuevas, J. García-Feria, y R. Gómez-Mercado. 2019. Parasitismo en el piojo harinoso del algodón (Pseudococcidae) en el Valle de Mexicali, pp. 128-132. *In* Memorias XLII Congreso Nacional de Control Biológico, Sociedad Mexicana de Control Biológico, Boca del Río, Veracruz.

Hoffer, A., and V. A. Trjapitzin. 1977. Survey of Palearctic species of *Dicarnosis* Mercet, 1921 (Hymenoptera: Chalcidoidea Encyrtidae) with the description of a new species. Studia Ent. Forest 2: 247-257.

Kairo, M. T. K., G. V. Pollard, D. D. Peterking, and V. F. Lopez. 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) in the Caribbean. Integr. Pest Manag. Rev. 5: 241-254.

Kerrich, G. J. 1978. A revision of the Dinocarsiine Encyrtidae with a study of the genus *Pelmatencyrtus* De Santis (Hymenoptera: Chalcidoidea). Zool. J. Linn. Soc. 62: 117-118.

Kosztarab, M. 1963. The armored scale insect of Ohio (Homoptera: Coccoidea: Diaspididae). Bull. Ohio Biol. Surv. 2: 1-120.

McKenzie, H. L. 1967. Mealybugs of California. With Taxonomy, Biology, and Control of North *American* Species (Homoptera: Coccoidea: Pseudococcidae). University of California Press, California.

Mercet, R. G. 1921. Fauna Iberica. Himenópteros Fam. Encírtidos. Museo Nacional de Ciencas Naturales, Madrid, España.

Noyes, J. S. 2020. Universal Chalcidoidea Database. The Natural History Museum, London. http://www.nhm.ac.uk/research–curation/projects/chalcidoids/.

Noyes, J. S., J. B. Woolley, and G. Zolnerowich. 1997. Family Encyrtidae. Chapter 8, pp. 170-320. *In* G. A. P. Gibson, J. T. Huber, and J. B. Woolley [eds.], Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). National Research Council Canada, NRC Research Press, Ottawa.

Santiago-Islas, T., A. Zamora-Cruz, E. A. Fuentes-Temblador, L. Valencia-Luna, y H. Arredondo-Bernal. 2008. Cochinilla rosada del hibiscus, *Maconellicoccus hirsutus* (Hempitera: Pseudococcidae). Cap. 15, pp. 177-190. *En* H. C. Arrendondo-Bernal y L. A. Rodriguez del Bosque [eds.], Casos de Control Biológico en México. Mundi Prensa, México, DF México.

Spodek, M., Y. Ben-Dov, L. Mondaca, A. Protasov, E. Erel, and Z Mendel. 2018. The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in Israel: pest status, host plants and natural enemies. Phytoparastitica https://doi.org/ 10.1007/s12600-018-0642-1.

Trjapitzini, V. A., y E. Ruiz Cancino. 2003. *Dicarnosis ripariensis* Kerrich (Hymenoptera: Encyrtidae) en los estados de Michoacán y Veracruz, México. BioTam, Nueva Serie 14: 69-71.

Van Lenteren, J. C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57: 1-20.

Williams, D. J., and M. C. Granara de Willink. 1992. Mealybugs of Central and South America. C. A. B. International Institute of Entomology, London.

New Species of *Vaejovis* C.L. Koch, 1836 (Scorpiones: Vaejovidae) with Comments on Lateral Aculear Serrations

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Abstract. We describe a new species of *Vaejovis* C.L. Koch from a forest of pine (*Pinus* Linnaeus) and oak (*Quercus* Linnaeus) in the State of Guerrero in Mexico. In addition, the lateral aculear serration (LAS) was examined during the life of several specimens from the species, showing gradual deterioration, ending with complete loss of the structure before the adult stage. Phylogenetic and taxonomic implications of the new findings were discussed, both for the family and for the genus.

Resumen. Nosotros describimos una nueva especie de *Vaejovis* de un bosque de pino y encino del estado de Guerrero en México. Adicionalmente se examinaron las Serraciones Laterales del Aculeo (LAS, por sus siglas en inglés) durante la historia de vida de varios especímenes de esta especie, mostrando la gradual desaparición, terminando en la ausencia completa de estas estructuras en los estadios adultos. Las implicaciones filogenéticas y taxonómicas de este nuevo descubrimiento son discutidas, tanto para la familia como del género.

Introduction

Scorpions, with 2,400 species described worldwide do not match the diversity in other orders of Arachnida, such as spiders, Opiliones, or Acari (Santibáñez-López et al. 2016). But, what they lack in diversity, they compensate with other interesting features such as a high rate of endemism (Contreras-Félix and Francke 2019).

In North America, the most diverse family is Vaejovidae with 221 species, and *Vaejovis* C. L. Koch, 1836 is the most diverse genus in the family (Santibáñez-López et al. 2016). In the family, life history or morphological changes that occur during successive molts have been studied for several species (Smith 1966; Matthiesen 1970; Vachon et al. 1970; Francke 1976, 1981, 1984; Francke and Jones 1982; Lourenço 1979; Armas 1982; Sissom and Francke 1983; Francke and Sissom 1984). Most studies included only species in the subfamily Syntropinae, which left the other two subfamilies in Vaejovidae lacking information. Thus, it is imperative to fill gaps in information to better understand the family Vaejovidae.

The lateral aculear serration (LAS) was first described by Fet et al. (2006) as a “minute serrated row located on each side of the base of the scorpion’s aculeus”. The authors indicated the character had been overlooked in all previous systematic studies of the family Vaejovidae and claimed that 11 of 68 species examined had the

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character. However, all specimens examined were adults, hence no variation was given or discussed regarding other life stages of the species.

We described a new species from the Mexican state of Guerrero. In addition, development of the LAS throughout the life history of the species was studied with the aid of scanning electronic microscopy. Implications derived from variation in the structure for systematics of the family and genus are discussed relative to the importance of LAS as a taxonomic character.

Materials and Methods

Nomenclature and measurements used were those by Stahnke (1970), except for trichobothrial terminology after Vachon (1974); metasomal and pedipalpal carination after González-Sántillan and Prendini (2013); number of metasomal setae, modified from Santibáñez-López and Sissom (2010); telotarsal spination and number were those of Contreras-Félix et al. (2015); terminology for the lateral eyes from Loria and Prendini (2014); LAS terminology follows Fet et al. (2006); hemispermatophores were dissected following the method of Vachon (1952), and cleared using the technique of Alvarez-Padilla and Hormiga (2008). Higher-level taxonomy of scorpions follows Sharma et al. (2015) and Prendini and Wheeler (2005). Photographs of each segment were made using ultraviolet-light following recommendations of Prendini (2003) and Volschenk (2005). Electronic scanning photographs were made using Hitachi SU1510SEM (LANABIO) following methodology by Acosta et al. (2007). Photographs were edited with Adobe Photoshop CS6 software. The map was generated with ESRI ArcGIS online suite. Measurements were made with an ocular micrometer calibrated at 10X and given in millimeters. The specimens were deposited in Colección Nacional de Arácnidos, Instituto de Biología UNAM (CNAN), and Arachnid collection in the American Museum of Natural History (AMNH).

Results

Systematics.
Family Vaejovidae.
Genus *Vaejovis* C. L. Koch, 1836.
Vaejovis mendozai n.sp. (Figs. 1-5).

Holotype. MEXICO: Guerrero, Municipio: Ixcateopan de Cuauhtemoc, 4 km south from Ixcateopan de Cuauhtemoc (18.53347ºN, 99.73361ºW, 2308 m above sea level). 25-VI-2013. Cols: C. Santibáñez, J. Mendoza, and G. Contreras. 1M# (CNANT-01211). ***Paratypes.*** Same data as the holotype, 8 F# (CNANT-01212), 2 F# (AMNH).

Other Material. MEXICO: Guerrero, Municipio: Ixcateopan de Cuauhtemoc, 4 km south from Ixcateopan de Cuauhtemoc (18.53347ºN, 99.73361ºW, 2308 m above sea level). 25-VI-2013. Cols: J. Mendoza, R. Monjaraz, D. Ortíz, and G. Contreras. 1M# 6F#. MEXICO: Guerrero, Municipio: Ixcateopan de Cuauhtemoc, 2 km south from Ixcateopan de Cuauhtemoc (18.50384ºN, 99.7776ºW, 1930 m above sea level). 21-IV-2012. Cols: J. Mendoza, R. Monjaraz, D. Ortíz, and G. Contreras. 1F#. MEXICO: Guerrero, Municipio: Ixcateopan de Cuauhtemoc, 5 km northeast from Ixcateopan de Cuauhtemoc (18.53016ºN, 99.74167ºW, 2309 m above sea level). 23-X-2010. Cols: J. Cruz, D. Barrales, A. Valdez, and G. Contreras. 1F#.

Etymology. The specific epithet is a patronym honoring Jorge Mendoza, for his contribution to taxonomy and phylogeny of theraphosid spiders and for collecting efforts with this species in particular.

Diagnosis. *Vaejovis mendozai* n. sp. belongs to the "mexicanus" group of *Vaejovis* because of several characters: six rows of denticles on fixed finger of chela; trichobothrias *ib* and *it* on base of fixed finger of chela and hemispermatophore without mating plug. This medium-sized scorpion is 33.5 to 37.7 mm long (Table 2). Carapace on males is shorter than metasomal segment V (C L/MSV L: 0.9), but on females tends to be slightly longer than segment V (C L/MSV L [1.08 +/-0.4]). Sternite V on males with a notable triangular white patch on posterior third. Sternite VII brown to dark brown, with a faint white patch medially on the posterior. Vesicle slender, about twice as long as wide (L/W 2.23 [2.24+-0.07), and thin (W/D 1.5 [1.39+-0.6]); with a white vesicular patch dorsally on posterior third. Dentate margin of pedipalp chela fingers slightly scalloped and fixed finger with a feeble proximal notch. Lamella simple, without distal crest and with a weak median lobe.

Vaejovis mendozai n. sp. is similar to *Vaejovis pusillus* Pocock, 1898, but easily separated by the characters: *V. mendozai* n. sp. is at least two times larger than *V. pusillus*; a distinct white patch is on the dorsal face of the vesicle of *V. mendozai* n. sp., but small in *V. pusillus*; two telotarsal distal spinules on *V. pusillus*, and four in *V. mendozai* n. sp.; and large difference in the number of pectinal teeth in *V. pussilus* (11-12 on males, 12-12 on females) compared with *V. mendozai* n. sp. (18-19 males, 16-18 on females*). V. mendozai* n. sp. also is morphologically similar to *Vaejovis smithi* Pocock, 1902, but can be separated by the characters: white patch on sternite VII present in *V. mendozai* n.sp. and absent in *V. smithi*; absence of coloration on the dorsal intercarinal spaces in *V. smithi* and present in *V. mendozai* n.sp.; greater number of teeth on *V. smithi* (22 on males, 20 on females) compared with *V. mendozai* n. sp. (18-19 males, 16-18 on females). *V. mendozai* n. sp. is morphologically similar to *Vaejovis mexicanus* C.L Koch, 1836, but differs in the characters: vesicle is less than twice longer than wide on *V. mexicanus* (V L/W 1.6), whereas on *V. mendozai* n. sp. it is more than twice longer than wide (V L/W 2.23); fewer pectinal teeth on *V. mexicanus* (14-15 on males, 16 17 on females) than on *V. mendozai* n. sp. (18-19 males, 16-18 on females); dentate margins of pedipalp chela fingers on males of *V. mexicanus* are straight, but scalloped on *V. mendozai* n. sp.

Description of Holotype Male (Fig. 1a,b). ***Prosoma.*** Chelicerae: Pale brown color with a reticulated, dark brown pattern. Serrula present and well-developed; dorsal margin of movable finger with two small denticles, flanked by two bigger teeth; ventral edge of movable finger smooth; fixed finger with only one denticle. Carapace (Fig. 2a): pale brown with underlying fusco-piceous pattern; anterior margin concave; anteromedian longitudinal sulcus present; surface granular, except posterior lateral zones smooth. Ocular tubercle, superciliary carinae lower than the median ocelli; lateral ocelli type 3B (Loria and Prendini 2014).

Mesosoma. Tergites pale brown with underlying fusco-piceous pattern. Tergites I-II with granulations located laterally and on posterior margin, the rest smooth. Tergites III-VI shagreened anteriorly, becoming granular on posterior half. Tergite VII sparsely granular, with median and lateral carinae strong, not reaching posterior margin. Sternites III-VI smooth; pale brown with diffuse fusco-piceous pattern, usually only around the setae; Sternite V with a conspicuous triangular white patch on posterior third. Sternite VII brown to dark brown, with a faint white patch medially on posterior third; median region smooth, becoming shagreened to sparsely

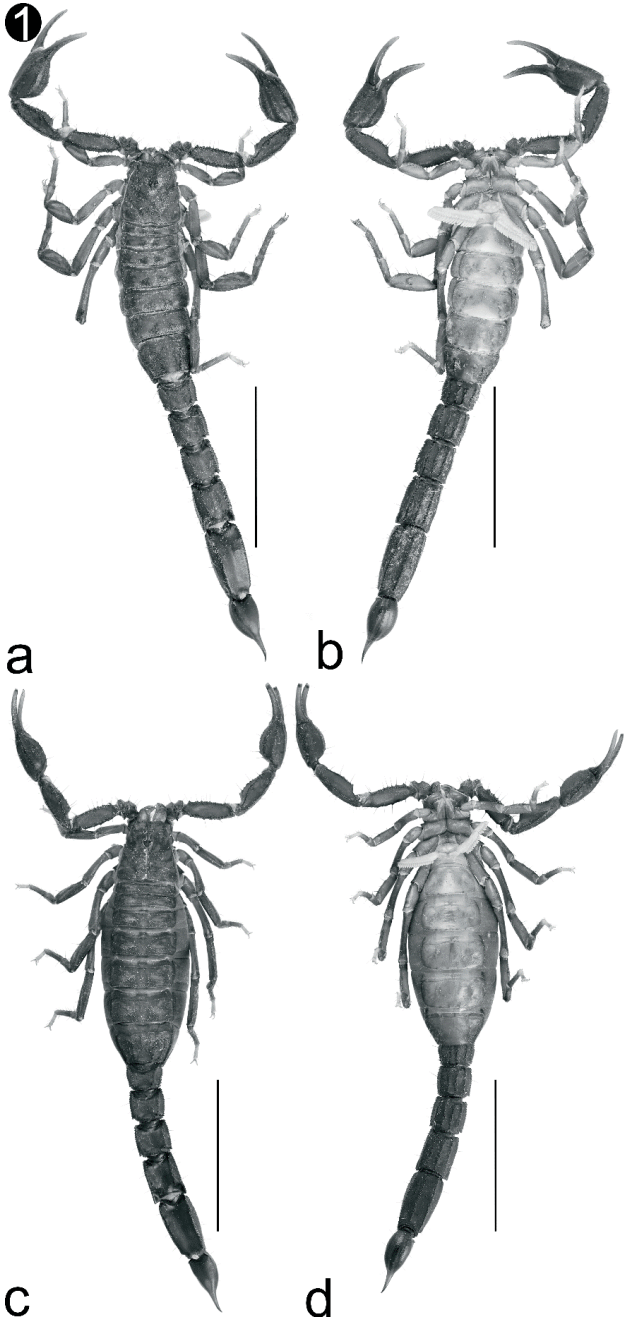

Fig. 1. a. Habitus of *Vaejovis mendozai* n. sp. dorsal view; b. Habitus of *Vaejovis mendozai* n. sp. ventral view.

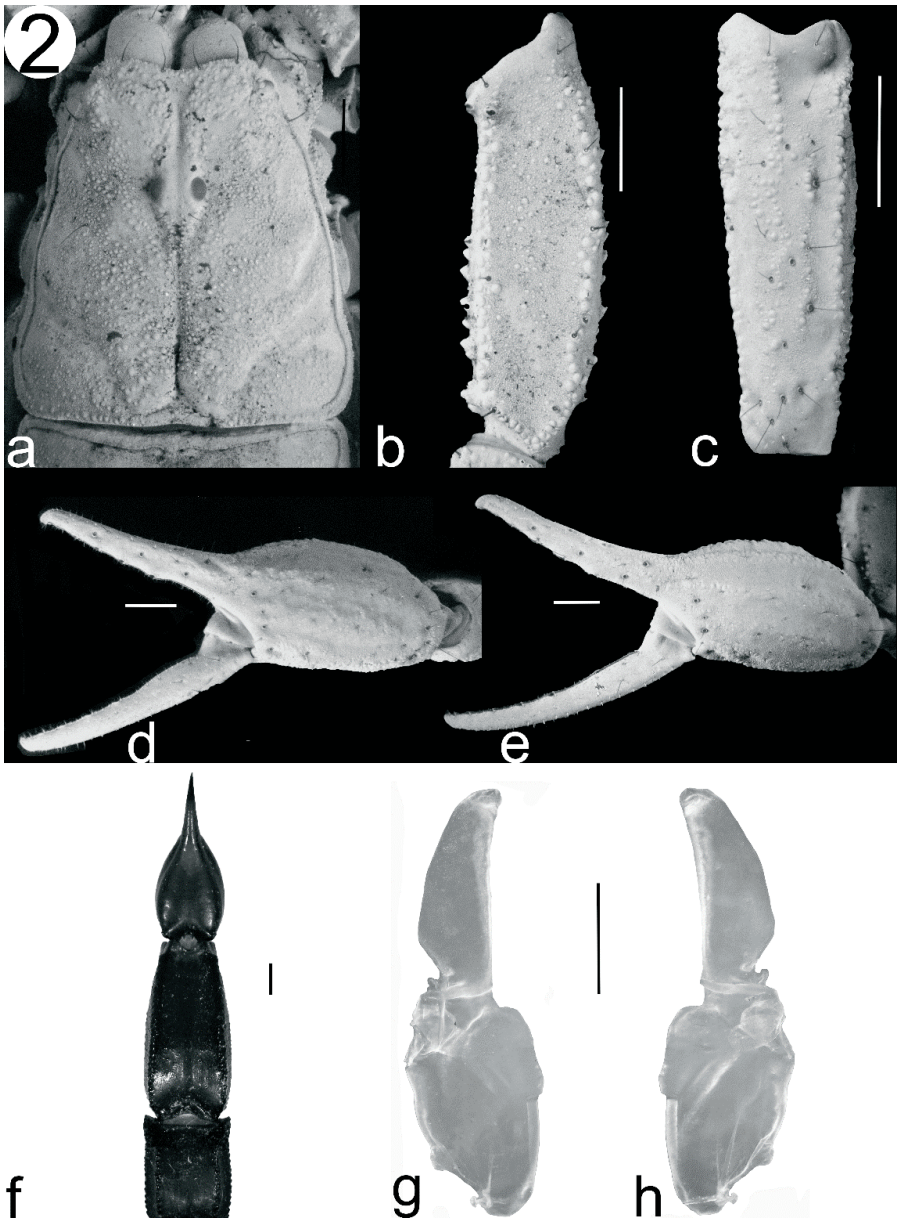

Fig. 2. a. Carapace of *Vaejovis mendozai* n. sp. dorsal view; b. Femur of *V. mendozai* n. sp. dorsal view; c. Patella of *V. mendozai* n. sp. retrolateral view; d. Chela of male *V. mendozai* n. sp. retrolateral view; f. Detail on vesicular gland of *V. mendozai* n. sp. dorsal view; g and h. Detail on hemispermatophore of *V. mendozai* n. sp. ental and mesal view.

granular laterally; median carinae absent; lateral carinae well-developed; with 10 setae. Pectinal teeth: 18-19.

Metasoma. Segments I-IV dorsally pale brown, with diffuse fusco-piceous color; ventrally dark brown with a predominant black color; segment V dorsally brown and ventrally predominantly black. Dorsal lateral carinae on I-IV, strong, composed by a single line of granules, with a prominent pointed granule distally; lateral median carinae strong, composed by a single row of granules, on I-III with a prominent pointed granule distally; lateral inframedian carinae on I strong, complete; on II and III, vestigial, present only by some granules distally; on segment IV absent; ventral lateral carinae on I-IV strong, composed on a single row of granules; ventral submedian carinae on I-IV, strong and composed by a single row of granules. Intercarinal surfaces on segments I-IV smooth. Dorsal lateral carinae on V vestigial, composed by several rows of small granules; lateral median carinae vestigial, composed of several rows of minute granules and only on anterior two-thirds; ventral lateral carinae strong, composed by a single row of granules; ventral median carina strong, composed by a single row of granules and not reaching posterior margin. Setae count on I-IV: DL: 1/1/1/2; LM: 1/2/2/2; LI: 2/0/0/0; VL: 2:3/3/3; VS: 2/3/3/3. Segment V: DL: 4; LM: 2; VL: 4, and VM: 4.

Telson (Fig. 2f). Vesicle slender, about twice as long as wide (L/W 2.23), and thin (W/D 1.5). Color reddish brown with a fusco-piceous pattern; dorsally with a vesicular gland patch on posterior third; ventrally with two submedian stripes without black. Dorsal surfaces smooth, and ventral surface sparsely granular. LAS absent.

Pedipalps. Femur pale brown, with diffuse infuscations throughout; patella and chela reddish brown color with underlying fusco-piceous color. Orthobothriotaxia type “C”. Femur (Fig. 2b): More than three times longer than wide (L/W: 3.5), as wide as deep (W/D: 1). Dorsal retrolateral carina strong, composed by single row of granules; dorsal prolateral carina strong, with a single row of granules; prolateral ventrosubmedian and prolateral ventral carinae vestigial, only present by some scattered big granules; ventral prolateral carina strong, comprised by a line of aggregated granules; ventral median and ventral retrosubmedian carinae strong, comprised of aggregated granules; retrolateral ventral carina weak, with small granules and almost smooth; ventral retrolateral carina weak, smooth; retrolateral dorsosubmedian carina weak, comprised by a row of scattered granules. Intercarinal spaces smooth to shagreened, dorsally may have some scattered granules. Patella (Fig. 2c): dorsal retrolateral carina strong, composed of several rows of aggregated granules; dorsal prolateral carina strong, comprised of a single row of granules; prolateral subdorsal carina absent; prolateral median carina strong, composed of a row of scattered granules; ventral prolateral carina strong, composed of a single row of granules; ventral median carina vestigial, composed of a single row of small granules, almost smooth; ventral retrolateral carina strong, composed of several rows of aggregated granules; retrolateral median carina strong, complete, composed of several rows of aggregated granules; retrolateral dorsosubmedian carina weak, composed of few scattered granules. Intercarinal spaces shagreened. Chela (Fig. 2d): dorsal retrolateral carina weak, almost smooth, slightly elevated and with some scattered small granules; retrosubmedian accessory carina vestigial, only present as elevation of cuticle; dorsal median carina weak, slightly elevated and with some scattered small granules; dorsal prosubmedian and dorsal prolateral carinae strong, composed of several rows of granules; prolateral dorsal absent; prolateral median and prolateral ventrosubmedian carinae smooth, only present as an elevation of the cuticle and with some scattered big granules; ventral prolateral and prolateral ventral

carinae vestigial, smooth and slightly elevated; ventral median and ventral prosubmedian vestigial, only present as a small row of small granules; ventral retrosubmedian and ventral retrolateral carinae, weak, comprised of several rows of granules; retrolateral subventral and accessory carinae weak, smooth, only present as a small elevation of the cuticle; retrolateral ventral and dorsal carinae absent; retrolateral median carina smooth, present as slight elevation of the cuticle. Pedipalp chela movable fingers have dentate margins with slight lobe basally, fixed finger with a feeble proximal notch.

Legs. Base color pale yellow, with large portions with dark brown infuscation. Telotarsi I-IV with single row of spinules ventrally, with four distal spinules on almost every leg (Table 2). Telotarsal setae on prolateral and retrolateral aspects as follows: (pi/ri) 1/2:2/2:2/2:X/X, 1/2:2/2:X/X:2/2.

Hemispermatophore (Fig. 2fg). Lamelliform (TL: 3.5; LL: 1.7; W: 0.6 mm). Lamella very wide medially and narrowing near the hooks; two hooks rounded. Median lobe weak and translucent.

Variation. Full measurements and intraspecific variation see Tables 1 and 2; variation in the life cycle on the LAS of the species is shown on Figs. 3 and 4.

Table 1. Measurements (millimeters) of *Vaejovis mendozai* n. sp.

V. mendozai n. sp	Holotype ♂	Paratype ♀	Paratype ♀	Paratype ♀	Paratype ♀
Total L	34	36.2	37.7	43	33.5
Carapace L/W	4.5/2.4	4.8/2.7	4.8/2.7	5.6/3.1	4.4/2.4
Mesosoma L	9.5	12.5	13.1	14.7	11.5
SM I L/WD	2/2.6/2.1	2/2.9/2.3	2/3/2.4	2.4/3.4/2.7	1.08/2.8/2.2
SM II length	2.4	2.2	2.4	2.7	2.1
SM III length	2.7	2.4	2.6	3	2.4
SM IV length	3.2	3	3.2	3.6	2.8
SM V L/W/D	5/2.5/2	4.7/2.8/2.3	5/2.9/2.2	5.5/3.3/2.6	4.1/2.5/2
Metasoma L	15.3	14.3	15.2	17.2	13.2
Vesicle L/W/D	4.7/2.1/1.4	4.6/2/1.5	4.6/2.2/1.5	5.5/2.4/1.8	4.4/1.9/1.3
Femur L/W/D	4.3/1.2/1.2	4.3/1.4/0.9	4.5/1.4/0.9	5.1/1.5/1	4.2/1.3/0.8
Patella L/W/D	4.5/1.5/0.8	4.7/1.7/1.3	4.9/1.6/1.3	5.4/1.8/1.5	4.4/1.5/1.2
Chela length	4.1/2.1/2.3	4.1/2/2	4.3/2.1/2.2	4.8/2.4/2.6	4/1.9/2.1
Movable finger L	4.4	4.4	4.5	5.5	4.1
Fixed finger L	3.4	3.5	3.5	4.6	3.4
Pectinal teeth	18-19	16-17	17-17	16-17	18-17

Table 2. Variation in the setation in *V. mendozai* n. sp.

Metasomal setation				
Holotype ♂	Paratype♀	Paratype ♀	Paratype ♀	Paratype ♀
1/1/1/2/4	1/1/1/2/4	1/1/1/2/5/	1/1/1/2/4	1/2/2/2/5
1/2/2/2/2	1/2-3/2/2/2	1/2/2/2/3	1/1/2/2/2	1/2/2/2/2-3
2/0/0/0	1/0/0/0	2-1/0/0/0	2/0/0/0	2/0/0/0
2/3/3/3/4	2-3/3/2-3/3/4	2/3/2-3/3/5	3/3/3/3/4	2/3/3/3/4
2/3/3/3/4	3/3/3/3/3	3/3/3/4/4	3/3/3/3/4	3/3/3/3/3
Distal terminal leg spinules				
2-4-4-X	2-4-4-X	4-4-4-4	5-4-4-4	4-4-4-2
4-4-4-4	2-4-4-4	4-4-4-5	4-4-4-4	2-4-4-4
Ventral prolateral and retrolateral setation				
1/1:2/2:2/2:X/X	1/1:2/2:2/2:X/X	1/1:2/2:2/1:2/2	1/1:2/2:2/2:2/2	1/1:2/2:2/2:2/2
1/2:2/2:X/X:2/2	1/1:2/2:2/2:2/2	1/1:2/1:2/2:2/2	1/1:2/1:2/2:2/2	2/1:2/2:2/2:2/2

Figs. 3ab represents the second molt in the life of *V. mendozai* n. sp. showing how the LAS are fully developed with seven noticeable denticles; Figs. c-d represent the third instar, starting to show very reduced LAS, shown only by three denticles; in Figs. e-f, representing the fourth molt, the LAS already are absent; on Figs. g-h, representing the fifth molt, the LAS still are absent; on Figs. i-j, representing the sixth molt, LAS are absent.

Fig. 4 is another specimen of *V. mendozai* n. sp. and shows the disappearance of the LAS as follows: second molt was ruined and the telson could not be found; Figs. a-b shows the third molt in the life of the species and the LAS is present as six reduced denticles; Figs. c-d represents the fourth instar, shows the LAS with four very reduced denticles; in Figs. e-f, representing the fifth molt, the LAS are already absent; in Figs. g-h, representing the sixth molt, the LAS are absent.

Distribution. The species is known only from four nearby locations in the southern part of the Sierra Madre del Sur in the State of Guerrero (Fig. 5).

Natural History. The species inhabits mixed pine (*Pinus* Linnaeus), and oak (*Quercus* Linnaeus*)* forest. Under laboratory conditions, the species can survive longer periods of drought than other closely related species (i.e., *V. mexicanus* and *V. smithi*).

Life History. Parturition takes place in August and a litter can consist of as many as 54 newborns.

Discussion

It is well known that different types of organisms have a change in body structure, either by addition (legs on centipedes and millipedes), regression or

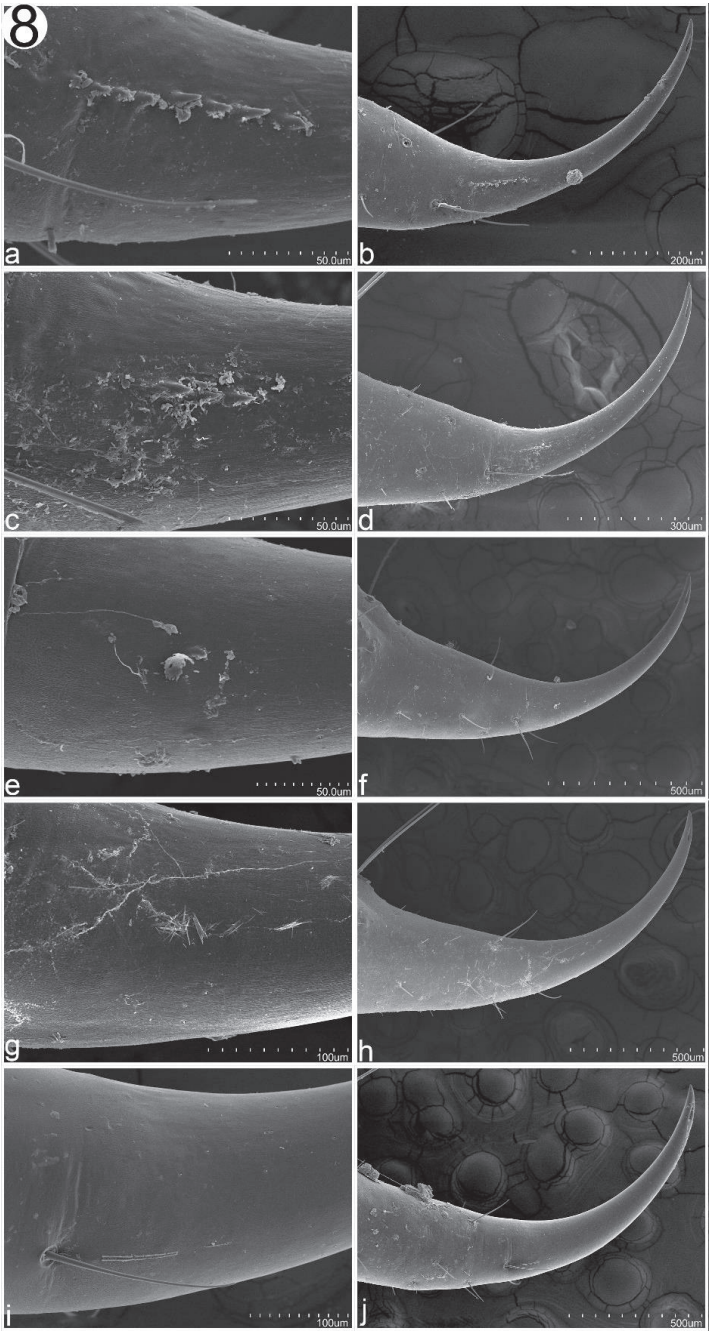

Fig. 3. *Vaejovis mendozai* n. sp. lateral aculear serration detail on the second molt (a-b), third molt (c-d), fourth molt (e-f), fifth molt (g-h), and sixth molt (i-j).

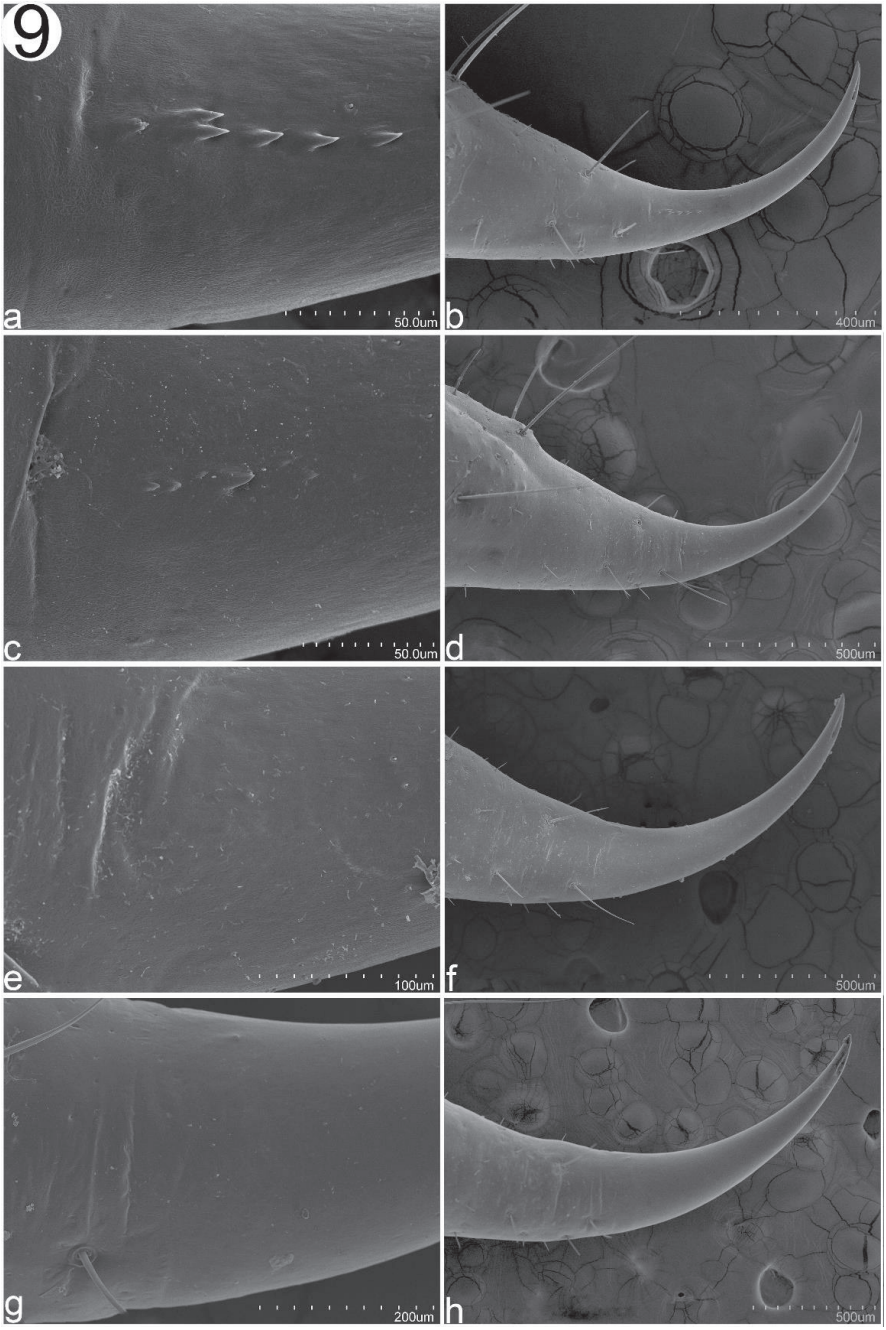

Fig. 4. Lateral aculear serration on another specimen of *Vaejovis mendozai* n. sp. detail on the third (a-b), fourth (c-d), fifth (e-f), and sixth molts (g-h).

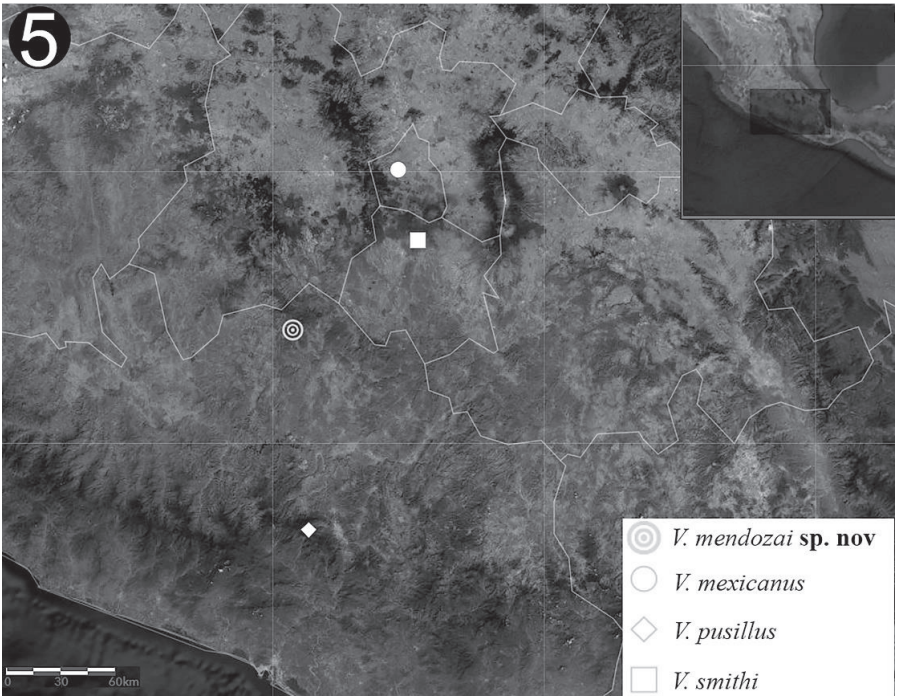

Fig. 5. Map of *Vaejovis mendozai* n. sp. and geographically close species.

atrophy (atrophy of mouthparts of Ephemeroptera) of different body structures during its life cycle (Andersson 1976, 1984; Kluge 2004; Voigtländer 2006) and most develop all sexual characters by the time they are adults. Little is known of species that lose characters that are not sexually related, and less is known of the reduction of the body while advancing its life cycle, in a process named anti-grow by regression of specific structures accompanying a molt (Kluge 2004, Minelli and Fusco 2013).

Previous work on the life cycle of some species of vaejovids made the statement that vaejovids need six to eight molts to reach maturity (Francke 1976, Sissom and Francke 1983, Francke and Sissom 1984), but never before a change in a character other than allopatric growth has been mentioned. We present evidence of the development of the LAS until the sixth molt in a new species of *Vaejovis*; a character described by Fet et al. (2006), but we discuss this character at length considering this new insight.

Absence in adulthood in vaejovids is not a new feature; hints on change of the character have been made before when it was said, "we encountered the LAS structure in all species, except *V. mexicanus,* which was represented by adults only" (Fet et al. 2006). This short mention provides a step for change of the LAS, but is difficult to prove without evidence we presented in this work.

Additionally, LAS are vulnerable to damage which might explain absence or presence (Fet et al. 2006), but we decided against it, because it is clearly seen on the images 3c and 4c; weakness of the structure is gradual. Because we kept the

specimens in captivity during the life cycle, damage is assumed to be much less than in natural conditions, and we correlate continuous weakness of LAS to growth and not with damage during life.

Phylogenetic implications refer to the structure, such as random non-random apparition of LAS; the synapomorphic state of LAS on the Vejovids tree of life, absence of the structure in the genus *Uroctonites* which the authors consider as a basal sister group of vaejovids and this made the LAS synapomorphic (Fet et al. 2006). Considering this in light that the phylogeny on which the authors take these assumptions has been criticized (Prendini and Wheeler 2005) and recently presented big changes (González-Santillán and Prendini 2013), we rather wait until a better resolved phylogeny to do such statements about the LAS. Nevertheless, some species aggregations can be made on the presence or absence on the adulthood of the LAS; as in the mexicanus group (no mating plug group), where *V. mendozai* n. sp. belongs and those species does not present LAS; meanwhile on the so called vorhiesi group (sometimes within the mexicanus group) this are present and on the nitidulus/nigrescens group they are present. Sure, it will be interesting mark the outcome of this character in a phylogeny and maybe codify this character as dependent, where the absence comes after the presence.

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References Cited

Acosta, L. E., A. Pérez-González, and A. L. Tourinho. 2007. Methods for taxonomic study, pp. 494-510. *In* R. Pinto-da-Rocha, G. Machado, and G. Giribet [eds.], The Biology of Opiliones. Harvard University Press, Cambridge, MA.

Álvarez-Padilla, F., and G. Hormiga. 2008. A protocol for digesting internal soft tissues and mounting spiders for scanning electron microscopy. J. Arachnology 35: 538-542.

Andersson, G. 1976. Post-embrionic development of *Lithobius forficatus* (L.), (Chilopoda: Lithobiidae). Entomologica Scandinavica 7: 161-168.

Andersson, G. 1984. Post-embrionic development of *Lamyctes fulvicornis* Meinert (Chilopoda: Henicopidae). Entomologica Scandinavica 15: 9-14.

Contreras-Félix, G. A., and O. F. Francke. 2019. Taxonomic revision of the "*mexicanus*" group of the genus *Vaejovis* C.L. Koch, 1836 (Scorpiones: Vaejovidae). Zootaxa 4596: 1-100.

Contreras-Félix, G. A., O. F. Francke, and R. Bryson Jr. 2015. A new species of the "mexicanus" group of the genus Vaejovis C. L. Koch, 1836 from the Mexican state of Aguascalientes (Scorpiones: Vaejovidae). Zootaxa 3936: 131-140.

De Armas, L. F. 1982. Desarrollo de *Didymocentrus trinitarius* (Franganillo) (Scorpiones, Diplocentridar). Miscel. Zool. (Cuba) 16: 3-4.
Fet, V., M. E. Soleglad, and M. S. Brewer. 2006. Laterobasal aculear serrations (LAS) in scorpion Family Vaejovidae (Scorpiones: Chactoidea). Euscorpius 45: 1-19.
Francke, O. F. 1976. Observations on the life history of *Uroctonus mordax* Thorell (Scorpiones, Vaejovidae). Bull. British Arachnological Soc. 3: 256-260.
Francke, O. F. 1979. Observations on the reproductive biology and life history of *Megacormus gertschi* Diaz (Scorpiones, Chactidae, Megacorminae). J. Arachnology 7: 223-230.
Francke, O. F. 1981. Birth behavior and life history of *Diplocentrus spitzeri* Stahnke (Scorpiones, Diplocentridae). Southwest. Naturalist 25: 517-523.
Francke, O. F. 1984. The life history of *Diplocentrus bigbendensis* Stahnke (Scorpiones, Diplicentridae). Southwest. Naturalist 29: 387-393.
Francke, O. F., and S. K. Jones. 1982. The life history of *Centruroides gracilis* (Scorpiones, Buthidae). J. Arachnology 10: 223-239.
Francke, O. F., and W. D. Sissom. 1984. Comparative review of the methods used to determine the number of molts to maturity in scorpions (Arachnida), with analysis of the post-birth development of *Vaejovis coahuilae* Williams (Vaejovidae). J. Arachnology 12: 1-20.
González-Santillán, E., and L. Prendini. 2013. Phylogeny of the North American vaejovid scorpion subfamily Syntropinae Kraepelin, 1905, based on morphology, mitochondrial and nuclear DNA. Cladistics 2015: 341-405.
Kluge, N. J. 2004. Larval/pupal leg transformation and a new diagnosis for the taxon Metabola Burmeister, 1832 = Oligoneoptera Martynov, 1923. Russian Entomological Journal 13: 189-229.
Loria, S. F., and L. Prendini. 2014. Homology of the lateral eyes of Scorpions: a six-ocellus model. Plosone 9: 1-30.
Lourenço, W. R. 1979. Le scorpion Buthidae: *Tytius mattogrossensis* Borelli, 1901 (Morphologie, ecologie, biologie et development postembryonnaire). Bulletin of the museum nationale of historie Naturalle of Paris, 4e serie 1: 95-117.
Matthiesen, F. A. 1970. Le développement post-embryonnaire du scorpion Buthidae: *Tityus bahiensis* (Perty, 1834). Bulletin of the Museum Nationale of Historie Naturalle of Paris serie 2 41: 1367-1370.
Minelli, A., and G. Fusco. 2013. Arthropod post-embryonic development, pp. 91-122. *In* A. Minelli, G. Boxshall, and G. Fusco [eds.], Arthropod Biology and Evolution Molecules, Development, Morphology. Springer, Dordrecht London.
Prendini, L. 2003. Revision of the genus Lisposoma Lawrence, 1928 (Scorpiones: Bothriuridae). Insect Systematics and Evolution 34: 241-264.
Prendini, L., and W. Wheeler. 2005. Scorpions higher phylogeny and classification, taxonomic anarchy, and standards for peer review in online publishing. Cladistics 21: 446-494.
Santibáñez-López, C. E., and W. D. Sissom. 2010. A new species of the *Vaejovis eusthenura* group in Oaxaca, Mexico (Scorpiones: Vaejovidae). Zootaxa 2493: 49-58.
Santibáñez-López, C. E., O. F. Francke, C. Ureta, and L. D. Possani. 2016. Scorpions from Mexico: from species diversity to venom complexity. Toxins 8: 1-18.
Sharma, P. P., R. Fernandez, L. A. Esposito, E. González-Santillán, and L. Monod. 2015. Phylogenomic resolution of scorpions revels multilevel discordance with

morphological phylogenetic signal. Proc. Royal Society B: Biological Sciences 282: 2-10.
Sissom, W. D., and O. F. Francke. 1983. Post-birth development of *Vaejovis bilineatus* Pocock (Scorpiones, Vaejovidae). J. Arachnology 11: 69-75.
Smith, G. T. 1966. Observations on the life history of the scorpion *Urodacus abruptus* Pocock (Scorpionidae), and an analysis of its home sites. Australian Journal Zoology 14: 383-398.
Stahnke, H. L. 1970. Scorpion nomenclature and mensuration. Entomological News 81: 297-316.
Vachon, M. 1952. Étude sur les Scorpions. Institut Pasteur d'Algérie, Alger, pp. 482.
Vachon, M. 1974. Étude des caracteres utilisés pour classer les familes et les genres de Scorpions (Arachnides) 1. La trichobothriotaxie en Arachnologie, Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. Bulletin du Muséum National d'Histoire Naturelle, Series 3, 140 (Zool. 104), mai-juin 1973, 857-958. [Paris, Cover date 1973, published 31 January 1974: See footnote on p. 958].
Vachon, M., R. Roy, and M. Condamin. 1970. Le development post-embryonnaire du scorpion *Pandinus gambiensis* Pocock. Bulletin de l'Institut Français d'Afrique Noire, série A 32: 412-432.
Voigtländer, K. 2006. The life cycle of *Lithobius mutabilis* L. Koch, 1862 (Myripoda: Chilopoda). Bonner Zoologische Beiträge 55: 9-25.
Volschenk, E. S. 2005. A new technique for examining surface morphosculpture of scorpions. J. Arachnology 33: 820-825.

Resistencia a *Anthonomus eugenii*[1] en Poblaciones Criollas y Silvestres de Chile de México

Resistance of Mexican Landrace and Wild Pepper Populations to *Anthonomus eugenii*[1]

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Resumen. Se estudió bajo condiciones controladas el número de insectos muertos, número de marcas por alimentación, y el número de adultos emergidos de *Anthonomus eugenii* en trece poblaciones de chile (*Capsicum annuum*) silvestres, diez criollas y dos cultivares comerciales colectadas en México, así como la relación de estos parámetros de resistencia con el largo, ancho y grosor de la pared de los frutos. Las poblaciones silvestres y criollas tuvieron significativamente un mayor número de insectos muertos, un menor número de marcas por alimentación, y un menor número de insectos emergidos comparado con los cultivares comerciales, indicando que estas poblaciones poseen genes de resistencia a este insecto. Diez poblaciones de chile silvestre y 3 criollas mostraron una resistencia alta y dos poblaciones silvestres y una criolla tuvieron una resistencia intermedia porque tuvieron significativamente un mayor número de insectos muertos, un menor número de marcas por alimentación, y un menor número de insectos emergidos de *A. eugenii,* en comparación con el resto de las poblaciones y los cultivares comerciales que fueron altamente susceptibles porque fueron severamente afectados por *A. eugenii*. Estos resultados indican que estas poblaciones tienen mecanismos de resistencia tipo antibiosis a *A. eugenii*. Estas poblaciones de chiles con diferentes niveles de resistencia pueden utilizarse para producir cultivares de chile resistentes a *A. eugenii.*

Abstract. The number of dead insects, feeding punctures, and emerged insects of *Anthonomus eugenii* in 13 wild populations, 10 landraces, and two commercial cultivars of pepper (*Capsicum annuum*) collected in Mexico, were studied in controlled conditions, as was the relationship of these resistance parameters with morphological traits of fruits and length, width, and thickness of the wall. Wild and landrace populations had significantly greater number of dead insects, fewer feeding punctures, and fewer emerged insects compared to the commercial cultivars,

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indicating that these populations possess genes for resistance against the insect. Ten populations of wild pepper and three landraces showed high resistance and two wild populations and one landrace had intermediate resistance because they had significantly greater number of dead insects, fewer feeding punctures, and fewer emerged insects of *A. eugenii*, compared to the other populations and commercial cultivars that were very susceptible because they were severely affected by the insect. The results indicated the populations have mechanisms of antibiosis resistance to *A. eugenii*. These populations of pepper with different levels of resistance could be used to produce pepper cultivars resistant to *A. eugenii*.

Introducción

El picudo del chile, *Anthonomus eugenii* Cano, originario de México (Cano 1894), es una plaga clave en la producción de chiles en Estados Unidos, México, y Centro América (Rodríguez-Leyva et al. 2012). *Anthonomus eugenii* es polífago y holometábolo con tres instares larvales, los cuales se alimentan y desarrollan completamente en el interior de botones floreales y frutos de todas las especies de chile cultivadas, causando su prematura absición (Patrock y Schuster 1992). Los adultos se alimentan de hojas, botones florales, flores, y frutos (Rodríguez-Leyva et al. 2007).

El control de *A. eugenii* se ha basado en estrategias: culturales y el uso continuo de asperjaciones de insecticidas sintéticos, las cuales han demostrado ser parcialmente efectivas (Avendaño-Meza et al. 2016). Una alternativa de manejo sustentable es el uso de cultivares resistentes (Smith 2005).

Los parientes silvestres y domesticados de plantas han sido utilizadas exitosamente en los programas de mejoramiento genético de plantas cultivadas con la finalidad de mejorar sus rasgos agronómicos deseables y su resistencia a plagas (Blum 1988, Allard 1999). Se ha descrito una amplia variación genética en los centros de origen de los diferentes cultivos (Harlan 1971, Vavilov 1992). México es considerado el centro de origen del chile domesticado *C. annuum*, el cual es la especie de chile más cultivada, y poseedora de la mayor variación genética a nivel mundial (Pickersgill 1997, Kraft et al. 2014). Se ha reportado una amplia variación genética de chile en diferentes regiones de México (Hernández-Verdugo et al. 2001, Pacheco-Olvera et al. 2012, Retes-Manjarrez et al. 2016, 2017, 2018). Los parientes silvestres y domesticados de los cultivos de chile han demostrado ser importantes fuentes de resistencia para insectos clave en la producción de esta hortaliza como *Frankliniella occidentalis* (Fery y Schalk 1991), *Bemisia tabaci* (Ballina-Gómez et al. 2013, Hernández-Alvarado et al. 2019), y *Liriomyza trifolii* (Abdala-Roberts et al. 2015).

Pocos intentos se han realizado con la finalidad de detectar fuentes de resistencia a *A. eugenii* (Seal y Bondary 1999, Berny-Mier y Teran et al. 2013, Seal y Martin 2016, Wu et al. 2019). Sin embargo, a pesar de estos intentos, los programas de mejoramiento genético para la resistencia a *A. eugenii* carecen de fuentes de resistencia, y se han concentrado en evaluar la preferencia (antixenosis) del insecto en líneas de mejoramiento y cultivares comerciales. A la fecha, no hay información sobre estudios de resistencia tipo antibiosis a *A. eugenii*. Basados en estos estudios y en la biología del insecto, el uso de parámetros como el número de marcas por alimentación, insectos muertos y emergidos de los frutos podrían ser importantes variables para determinar la resistencia genética de las poblaciones de chile. Adicionalmente, las investigaciones donde se ha analizado la resistencia tipo

antixenosis han mostrado que el daño de los insectos está altamente correlacionado con los caracteres morfológicos de los frutos. Por lo tanto, importantes rasgos físicos de los frutos como longitud, ancho, y grosor de la pared son importantes parámetros a considerar para la caracterización de la resistencia. Con base en lo anterior, los objetivos de este estudio fueron identificar fuentes de resistencia tipo antibiosis a *A. eugenii* que puedan utilizarse para complementarse para el desarrollo de cultivares resistentes a este insecto, y analizar la interacción de los parámetros de resistencia con los caracteres morfológicos de los frutos como longitud, anchura y grosor de la pared.

Materiales y Métodos

Material Vegetal. Se recolectaron frutos de 10 plantas en 23 poblaciones silvestres y criollas de *Capsicum* spp., durante la primavera-verano en Guerrero, Chiapas, Veracruz, Tabasco, Campeche, Yucatán, y Quintana Roo, México (Cuadro 1). Las especies de *Capsicum* fueron identificadas utilizando los descriptores morfológicos del Instituto Internacional de Recursos Fitogenéticos (IPGRI 1995). Los cultivares comerciales de *C. annuum* 'Fascinato' (Syngenta) y 'Maccabi' (Hazera) fueron usados como controles susceptibles.

Colonia de Insectos. Se recolectaron 2,000 frutos del suelo de campos comerciales de chile jalapeño con síntomas de haber sido infestados por *A. eugenii* (Avendaño-Meza et al. 2016). Los frutos fueron confinados en contenedores transparentes de plástico (500 ml) y mantenidos en una cámara de crecimiento a 28 ± 2°C, 70% ± 5% humedad relativa, y 12 h luz y 12 h obscuridad. La identificación del insecto fue corroborada con base en las características morfológicas de las claves taxonómicas de Soto-Hernández et al. (2013).

Ensayos de Resistencia. Se hicieron dos experimentos de resistencia a *A. eugenii* en 2018 y 2019. Se utilizó el método de no libre elección del insecto con los frutos de las 23 poblaciones silvestres y criollas bajo condiciones de invernadero. Las semillas fueron germinadas en charolas de poliestireno de 200 cavidades (Frigocel, ICAPSA, México) con sustrato de turba (peat moss) y vermiculita a una proporción 5:1. Las charolas ya sembradas se colocaron en una cámara de germinación a 32 ± 2ºC. Las charolas se pasaron a un invernadero de producción de plantas y se transplantaron las plantas de chile directamente en el suelo de otro invernadero a los 40 días después de la siembra en surcos de 25 m de largo y 1.50 m entre surcos, con una separación entre plantas de 0.33 m. Previo a la infestación de los frutos, se tomaron tres muestras al azar de 50 adultos de *A. eugenii* de la colonia de insectos y se encontró un promedio 70.9% de hembras y 29.1% de machos. Con base en estos resultados, los frutos se infestaron con cinco adultos de *A. eugenii* sexualmente maduros (4-7 días post emergidos) en contenedores de plástico de 118 ml con dos frutos inmaduros de 8-14 mm de largo de chile. Se utilizó un diseño completamente al azar con 25 tratamientos (23 poblaciones + 2 cultivares de chile), donde cada tratamiento fue una población silvestre-criolla o un cultivar comercial de chile. Cada tratamiento tuvo ocho plantas y cada planta fue considerada como una repetición. Se infestaron dos frutos por planta/repetición en contenedores de plástico. Los frutos en los contenedores fueron expuestos al insecto por siete días. Ambos ensayos fueron desarrollados en el invernadero a 22-32°C.

Para analizar la resistencia de los frutos a *A. eugenii*, el número de insectos muertos fue registrado diariamente por siete días, el número de marcas por

alimentación e insectos emergidos fueron registrados a los 7 y 30 días después de la infestación. Adicionalmente, se midió la longitud y el ancho (cm) de todos los frutos antes de iniciar los ensayos, así como el grosor de la pared de frutos (cm) 30 días después de la infestación con un vernier digital para analizar la interacción entre todos estos parámetros.

Análisis Estadístico. Los datos obtenidos de ambos ensayos fueron sometidos a un análisis de varianza no paramétrico con la prueba de Kruskal-Wallis y la prueba de medianas de Mood para determinar diferencia estadística entre poblaciones ($P \leq 0.05$). Se utilizó la prueba de correlaciones de Spearman para determinar la interacción entre parámetros de resistencia y caracteres morfológicos de los frutos. Todos los análisis se realizaron en el programa estadístico Minitab 17.

Resultados y Discusión

La identificación y desarrollo de variedades de chile resistentes a *A. eugenii* es crucial para el control efectivo y sustentable de este importante insecto plaga de Estados Unidos, México, y Centro América. El primer paso para el desarrollo de cultivares resistentes es el escrutinio de recursos genéticos silvestres y/o domesticados para posteriormente ser utilizados en los programas de mejoramiento (Pickersgill 1997).

Los datos obtenidos en ambos ensayos de resistencia a *A. eugenii* no fueron significativamente diferentes, por lo tanto fueron combinados considerados como uno sólo. En ambos experimentos, los tres grupos con diferente grado de domesticación no mostraron diferencia significativa en el número de insectos muertos (NIM) en los primeros 2 días después de la infestación (ddi). Sin embargo, a los 3, 4, 5, 6, y 7 ddi se observó diferencias significativas entre grupos. El grupo silvestre mostró el mayor NIM seguido por el grupo criollo el cual tuvo significativamente un mayor NIM que el grupo comercial ($P \leq 0.001$) (Fig. 1).

Adicionalmente, el grupo silvestre tuvo el menor número de marcas por alimentación (NMA) seguido por el grupo criollo, el cual a su vez tuvo significativamente un menor NMA que el grupo comercial 7 días ddi ($P \leq 0.001$) (Fig. 2A). Respecto al número de insectos emergidos (NIE), el grupo silvestre tuvo el menor NEI seguido por el grupo criollo, el cual a su vez tuvo significativamente un menor NIE que el grupo comercial 30 ddi ($P \leq 0.001$) (Fig. 2B).

Estos resultados indican, que las poblaciones de chile silvestres tuvieron un mayor nivel de resistencia a *A. eugenii* seguidas por las criollas en comparación con los cultivares comerciales. Estos resultados coinciden con los reportados por Freitas et al. (2002), Tripathi y Varma (2002), y Sanchez-Peña et al. (2006), quienes indican que las especies cultivadas de plantas son más susceptibles a plagas en comparación con las poblaciones silvestres y criollas. Dado que estas poblaciones mexicanas de chile han cohabitado con *A. eugenii,* es probable que hayan estado expuestas a una presión de selección por parte del insecto. Esto podría explicar parcialmente los diferentes niveles de resistencia exhibidos por las poblaciones contra este insecto. cEstas poblaciones de chile son fuentes prometedoras de resistencia y podrían ser incorporadas en futuros programas de mejoramiento orientados en el desarrollo de cultivares comerciales resistentes a *A. eugenii.* Estudios previos, han mostrado que las poblaciones silvestres y criollas de *Capsicum* son fuentes de resistencia a diferentes fitopatógenos como el Virus huasteco vena amarilla del chile (Retes-Manjarrez et al. 2016, 2017, 2018), nematodos (Carrillo-Fasio et al. 2020), e insectos como *Frankliniella occidentalis* (Fery y Schalk 1991),

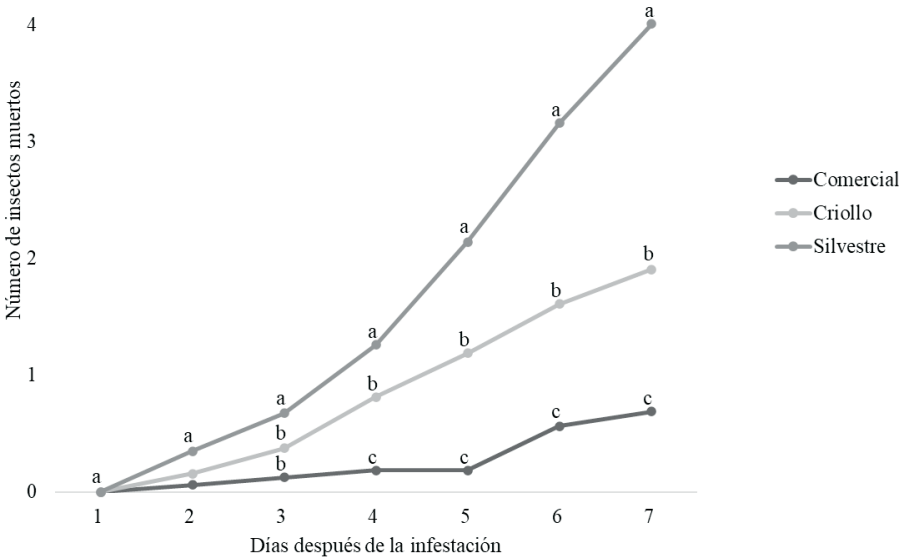

Fig. 1. Medias diarias del número de insectos muertos agrupados por grado de domesticación. Medias con diferente literal indican diferencia significativa de acuerdo con la prueba de Mood ($P \leq 0.05$).
Fig. 1. Daily means of number of dead insects grouped per domestication degree. Means with different letters indicate significant differences with the Mood test ($P \leq 0.05$).

Bemisia tabaci (Ballina-Gómez et al. 2013, Hernández-Alvarado et al. 2019), *Liriomyza trifolii* (Abdala-Roberts et al. 2015), y *Anthonomus eugenii* (Seal y Bondary 1999, Berny-Mier y Teran et al. 2013, Seal y Martin 2016, Wu et al. 2019).

En ambos experimentos, diez poblaciones de chile silvestre y tres criollas mostraron una resistencia alta y dos poblaciones silvestres y una criolla tuvieron una resistencia intermedia debido a que mostraron significativamente un mayor número de insectos muertos, un menor número de marcas por alimentación, y un menor número de insectos emergidos de *A. eugenii,* en comparación con el resto de las poblaciones y los cultivares comerciales que fueron altamente susceptibles porque fueron severamente afectados por *A. eugenii* 30 ddi ($P \leq 0.001$) (Cuadro 1). Estos resultados indican que el insecto no pudo completar su ciclo biológico en los frutos de estas poblaciones, sugiriendo la presencia de mecanismos de resistencia tipo antibiosis en estas plantas. Los diferentes niveles de resistencia a *A. eugenii* encontrados en estas poblaciones concuerdan con Bailey et al. (1967), Stephens y Lee (1961), y Douglas (1966), quienes encontraron diferentes niveles de resistencia tipo antibiosis al picudo del algodón *A. grandis* en diferentes poblaciones de algodón. Aunque, este es el primer reporte de fuentes de resistencia tipo antibiosis en poblaciones de chile silvestres y criollas de México, es necesario realizar estudios posteriores en condiciones de campo abierto bajo presiones naturales del insecto para analizar el nivel de resistencia de estos chiles en condiciones naturales. Estudios de caracterización y heredabilidad de los mecanismos de resistencia también serán necesarios.

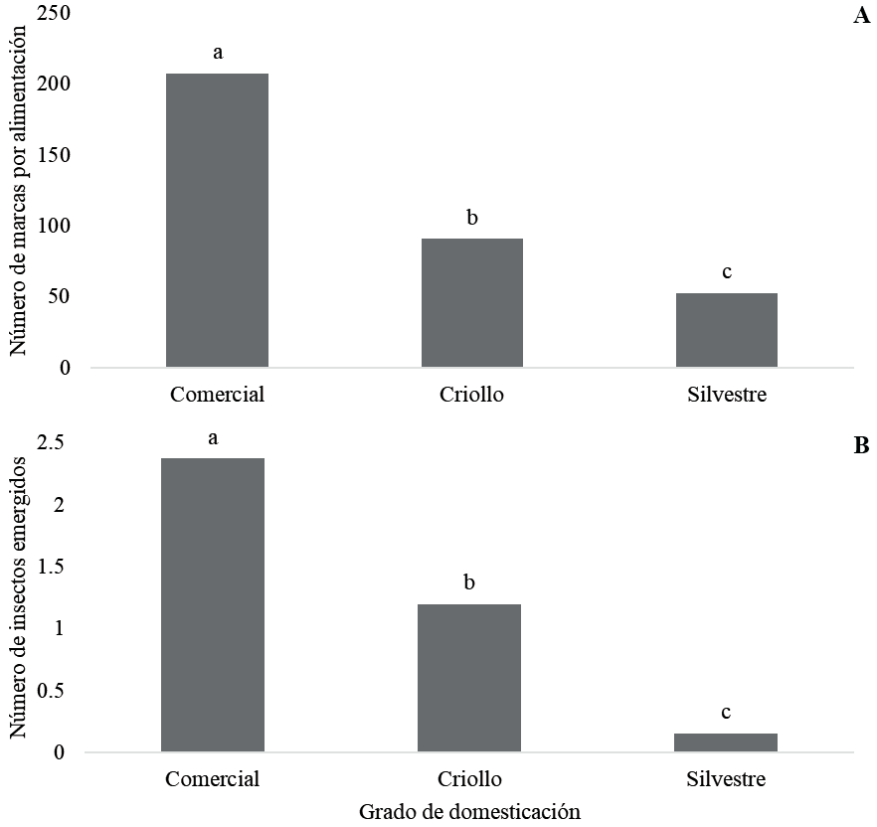

Fig. 2. Valor promedio del número de marcas por alimentación (A) e insectos emergidos (B) agrupados por grado de domesticación 7 y 30 días después de la infestación, respectivamente. Medias con diferente literal indican diferencia significativa de acuerdo con la prueba de Mood ($P \leq 0.05$).
Fig. 2. Average value of number of feeding punctures (A) and emerged insects (B) grouped per domestication degree 7 and 30 days after infestation, respectively. Means with different letters indicate significant differences with the Mood test ($P \leq 0.05$).

En ambos experimentos, diez poblaciones de chile silvestre, y tres criollas mostraron una resistencia alta y dos poblaciones silvestres y una criolla tuvieron una resistencia intermedia debido a que mostraron significativamente un mayor número de insectos muertos, un menor número de marcas por alimentación, y un menor número de insectos emergidos de *A. eugenii,* en comparación con el resto de las poblaciones y los cultivares comerciales que fueron altamente susceptibles porque fueron severamente afectados por *A. eugenii* 30 ddi ($P \leq 0.001$) (Cuadro 1). Estos resultados indican que el insecto no pudo completar su ciclo biológico en los frutos de estas poblaciones, sugiriendo la presencia de mecanismos de resistencia tipo antibiosis en estas plantas. Los diferentes niveles de resistencia a *A. eugenii*

Cuadro 1. Resultados de dos Ensayos de Resistencia a *Anthonomus eugenii* en 13 Poblaciones Silvestres, 10 Domesticadas de Chile Colectadas en México, más dos Cultivares Comerciales

Table 1. Results of Two Resistance Assays to *Anthonomus eugenii* in 13 Wild, 10 Landrace Pepper Populations Collected from Mexico, and Two Commercial Cultivars

O	P	E	T	G	NIM	NMA	NIE
Guerrero	UTC15	*C. annuum*	Chiltepin	Silvestre	98 a	33.9 h	0.0 a
Tabasco	UTC06	*C. annuum*	Chiltepin	Silvestre	92 ab	40.0 h	0.0 a
Chiapas	UTC04	*C. annuum*	Chiltepin	Silvestre	90 ab	60.0 gh	0.0 a
Chiapas	UTC23	*C. pubescens*	Manzano	Criollo	90 ab	63.3 gh	0.0 a
Yucatán	UTC12	*C. chinense*	Habanero	Criollo	88 ab	49.5 h	0.0 a
Chiapas	UTC16	*C. annuum*	Chiltepin	Silvestre	88 ab	50.0 h	0.0 a
Yucatán	UTC03	*C. annuum*	Chiltepin	Silvestre	86 ab	47.1 h	0.0 a
Quintana Roo	UTC22	*C. annuum*	Chiltepin	Silvestre	86 ab	51.8 h	0.0 a
Campeche	UTC01	*C. annuum*	Chiltepin	Silvestre	86 ab	39.6 h	0.0 a
Tabasco	UTC17	*C. annuum*	Chiltepin	Silvestre	86 ab	45.9 h	0.0 a
Campeche	UTC08	*C. annuum*	Pico Paloma	Criollo	86 ab	65.6 gh	0.0 a
Oaxaca	UTC02	*C. annuum*	Chiltepin	Silvestre	78 ab	61.4 gh	0.0 a
Yucatán	UTC21	*C. annuum*	Chiltepin	Silvestre	78 ab	46.1 h	0.0 a
Chiapas	UTC05	*C. annuum*	Chiltepin	Silvestre	72 bc	74.4 ef	0.0 a
Oaxaca	UTC14	*C. annuum*	Chiltepin	Silvestre	70 bc	73.1 ef	0.0 a
Tabasco	UTC13	*C. chinense*	Habanero	Criollo	69 bc	80.6 ef	0.0 a
Oaxaca	UTC09	*C. annuum*	Jalapeño	Criollo	40 de	82.3 de	2.0 bc
Guerrero	UTC18	*C. annuum*	Serrano	Criollo	40 de	84.6 de	2.0 bc
Quintana Roo	UTC10	*C. chinense*	Habanero	Silvestre	38 de	84.3 de	2.0 bc
Yucatán	UTC07	*C. annuum*	Pico Paloma	Criollo	38 de	88.4 de	2.0 bc
Veracruz	UTC20	*C. pubescens*	Manzano	Criollo	26 e	109.0 bc	2.0 bc
Veracruz	UTC19	*C. annuum*	Serrano	Criollo	20 e	91.1 cd	2.0 bc
Veracruz	UTC11	*C. frutescens*	Tabasco	Criollo	18 e	100.0 bc	2.0 bc
Not apply	UTC24	*C. annuum*	Bell	Comercial	12 e	197.4 a	2.5 c
Not apply	UTC25	*C. annuum*	Lamuyo	Comercial	10 e	217.6 a	2.3 c

Origen (O), población (P), especie (E), tipología (T), genealogía (G), número de insectos muertos (NIM), número de marcas por alimentación (NMA), y número de insectos emergidos (NIE). Medias con diferente literal en columnas indican diferencia estadística significativa de acuerdo con la prueba de Mood (P = 0.05).

encontrados en estas poblaciones concuerdan con Bailey et al. (1967), Stephens y Lee (1961), y Douglas (1966), quienes encontraron diferentes niveles de resistencia tipo antibiosis al picudo del algodón *A. grandis* en diferentes poblaciones de algodón. Aunque, este es el primer reporte de fuentes de resistencia tipo antibiosis en poblaciones de chile silvestres y criollas de México, es necesario realizar estudios posteriores en condiciones de campo abierto bajo presiones naturales del insecto para analizar el nivel de resistencia de estos chiles en condiciones naturales. Estudios de caracterización y heredabilidad de los mecanismos de resistencia también serán necesarios.

El NIM se correlacionó negativamente con el NMA, NIE, longitud, anchura, y grosor de la pared de frutos, el NMA se correlacionó positivamente con el NIE, anchura y grosor de la pared de frutos, y el NIE se correlacionó positivamente con la anchura y grosor de la pared de frutos ($P \leq 0.05$) (Cuadro 2). Estas correlaciones indican que las poblaciones con mayor mortalidad tuvieron significativamente un

Cuadro 2. Correlaciones entre Parámetros de Resistencia y Caracteres Morfológicos de los Frutos
Table 2. Correlations between Resistance Parameters and Morphological Traits of the Fruits

	NIM	NMA	NIE	LF	AF
NMA	-0.560 0.001				
NIE	-0.401 0.001	0.499 0.001			
LF	-0.167 0.018	0.118 0.096	0.053 0.458		
AF	-0.299 0.001	0.175 0.013	0.310 0.001	0.437 0.001	
GPF	-0.452 0.001	0.402 0.001	0.420 0.001	0.500 0.001	0.763 0.001

Número de insectos muertos (NIM), número de marcas por alimentación (NMA), número de insectos emergidos (NIE), longitud de frutos (LF), anchura de frutos (AF), y grosor de la pared de frutos (GPF). Interacciones realizadas con la prueba de correlaciones de Spearman ($P \leq 0.05$).

menor daño por alimentación y emergencia de adultos de *A. eugenii*, corroborando que existen mecanismos de resistencia en estas poblaciones de chile silvestre y criollas. Este resultado concuerda con Painter (1951) y Smith (2005), quienes mencionan que las plantas con mecanismos tipo antibiosis afectan negativamente la biología y el ciclo de vida de los insectos, ayudando al manejo de insectos agrícolas al reducir sus poblaciones. El número de marcas por alimentación fue positivamente correlacionado con el número de insectos emergidos, indicando que los frutos más dañados carecen de mecanismos de resistencia y son hospederos más adecuados para que el insecto complete su ciclo biológico. Este resultado concuerda con Seal y Bondari (1999), Berny-Mier y Teran et al. (2013), Seal y Martin (2016), y Wu et al. (2019), quienes determinaron que las poblaciones carentes de mecanismos de resistencia fueron más dañadas por *A. eugenii*. Adicionalmente, se encontró que el número de insectos emergidos fue positivamente correlacionado con los caracteres morfológicos anchura y grosor de la pared de frutos, indicando que *A. eugenii* completa su ciclo biológico en frutos con mayor biomasa.

El significativo mayor número de insectos vivos, daño por alimentación, e insectos adultos emergidos en los frutos de los controles susceptibles en ambos ensayos de resistencia indica que la metodología utilizada fue adecuada para discriminar entre poblaciones resistentes y susceptibles. Este resultado concuerda con Porter et al. (2007), quienes encontraron que *A. eugenii* puede completar perfectamente su ciclo de vida en el interior de micro jaulas con frutos de chiles tipo jalapeño de 10-30 mm.

La amplia variación genética mostrada por las poblaciones silvestres y criollas de México utilizadas en este estudio permitieron detectar fuentes potenciales de resistencia con diferentes niveles a *A. eugenii,* indicando que esta variación debe ser

estudiada y preservada. Las poblaciones resistentes detectadas en esta investigación son fuentes potenciales de resistencia que podrían ser utilizadas en los programas de mejoramiento genético para el desarrollo de cultivares de chile resistentes a *A. eugenii.*

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Referencias Citadas

Abdala-Roberts, L., J. C. Berny-Mier y Terán, X. Moreira, A. Durán-Yáñez, and F. Tut-Pech. 2015. Effects of pepper (*Capsicum chinense*) genotypic diversity on insect herbivores. Agr. Forest Entomol. 17: 433-438.

Allard, R. W. 1999. Principles of Plant Breeding, 2nd ed. John Wiley & Sons, New York.

Avendaño-Meza, F., J. L. Corrales-Madrid, S. Parra-Terraza, R. Medina-López, S. S. Gaspar-Aguilar, y F. D. Avendaño-Jatomea. 2016. Líneas base de susceptibilidad a tres insecticidas en poblaciones de picudos del chile *Anthonomus eugenii* Cano, 1894 (Coleopera: Curculionidae) del estado de Sinaloa. Entomología Mexicana 3: 775-780.

Ballina-Gómez, H., E. Ruiz-Sánchez, W. Chan-Cupul, L. Latournerie-Moreno, L. Hernández-Alvarado, I. Islas-Flores, and J. J. Zúñiga-Aguilar. 2013. Response of *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) biotype B to genotypes of pepper *Capsicum annuum* (Solanales: Solanaceae). Neotrop. Entomol. 42: 205-210.

Bailey, J. C., F. G. Maxwell, and J. N. Jenkins. 1967. Mortality of boll weevils in squares of genotypically different lines of cotton. J. Econ. Entomol. 60: 1279-1280.

Berny-Mier y Teran, J. C., L. Abdala-Roberts, A. Durán-Yáñez, and F. Tut-Pech. 2013. Variation in insect pest and virus resistance habanero peppers (*Capsicum chinense* Jacq.) in Yucatán, México. Agrociencia 47: 471-482.

Blum, A. 1988. Plant Breeding for Stress Environments. CRC Press, Florida.

Cano, A. D. 1894. El barrenillo. La Naturaleza 2: 377-379.

Carrillo-Fasio, J. A., J. A. Martínez-Gallardo, F. Ayala-Tafoya, C. A. López-Orona, R. Allende-Molar, and J. E. Retes-Manjarrez. 2020. Screening for resistance to *Meloidogyne enterolobii* in *Capsicum annuum* landraces from Mexico. Plant Dis. 104: 817-822.

Douglas, A. G. 1966. Selection in cotton for antibiosis to the boll weevil, *Anthonomus grandis*. J. Econ. Entomol. 59: 32-34.

Fery, R. L., and J. M. Schalk. 1991. Resistance in pepper (*Capsicum annuum* L.) to western flower thrips [*Frankliniella occidentalis* (Pergande)]. HortScience 26: 1073-1074.

Harlan, J. R. 1971. Agricultural origins: centers and noncenter. Science 174: 468-474.

Hernández-Alvarado, L. A., E. Ruíz-Sánchez, L. Latournerie-Moreno, R. Garruña-Hernández, D. González-Mendoza, and W. Chan-Cupul. 2019. Resistance

of *Capsicum annuum* genotypes to *Bemisia tabaci* and influence of plant leaf traits. Rev. Fitotec. Mexi. 42: 251-257.

Hernández-Verdugo, S., R. Luna-Reyes, and K. Oyama. 2001. Genetic structure and differentiation of wild and domesticated populations of *Capsicum annuum* (Solanaceae) from Mexico. Plant Syst. Evol. 266: 129-142.

International Plant Genetic Resources Institute (IPGRI). 1995. Descriptors for Capsicum (*Capsicum* spp.). https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Descriptors_for_capsicum__*Capsicum*_spp.__345.pdf. Accessed April 2018.

Kraft, K. H., C. H. Brown, G. P. Nabhanc, E. Luedelingd, R. J. J. Luna, G. C. d'Eeckenbruggef, R. J. Hijmansg, and P. Geptsa. 2014. Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. Proc. Nat. Acad. Sci. 111: 6165-6170.

Pacheco-Olvera, A., S. Hernández-Verdugo, V. Rocha-Ramírez, A. González-Rodríguez, and K. Oyama. 2012. Genetic diversity and structure of pepper (*Capsicum annuum* L.) from northwestern Mexico analyzed by microsatellite markers. Crop Sci. 52: 231-241.

Painter, R. H. 1951. Insect Resistance in Crop Plants. Macmillan Company, New York.

Patrock, R. J., and D. J. Schuster. 1992. Feeding, oviposition and development of the pepper weevil, (*Anthonomus eugenii* Cano), on selected species of Solanaceae. Trop. Pest. Manage. 38: 65-69.

Pickersgill, B. 1997. Genetic resources and breeding of *Capsicum* spp. Euphytica 96: 129-133.

Porter, P., B. E. Lewis, R. Scanlon, and L. Murrays. 2007. Pepper weevil infestation of cv. early jalapeno peppers of different size classes. Southwest. Entomol. 32: 1-6.

Retes-Manjarrez, J. E., S. Hernández-Verdugo, A. Evrard, and J. A. Garzón-Tiznado. 2017. Heritability of the resistance to pepper huasteco yellow vein virus in wild genotypes of *Capsicum annuum*. Euphytica 213: 275.

Retes-Manjarrez, J. E., S. Hernández-Verdugo, B. Pariaud, L. A. Hernández-Espinal, S. Parra-Terraza, D. L. Trejo-Saavedra, R. F. Rivera-Bustamante, and J. A. Garzón-Tiznado. 2018. Resistance to pepper huasteco yellow vein virus and its heritability in wild genotypes of *Capsicum annuum*. Bot. Sci. 96: 52-62.

Retes-Manjarrez, J. E., S. Hernández-Verdugo, B. Pariaud, C. M. Melgoza-Villagómez, A. Pacheco-Olvera, S. Parra-Terrazas, y J. A. Garzón-Tiznado. 2016. Detección de resistencia al virus huasteco vena amarilla del chile y su heredabilidad en genotipos silvestres de *Capsicum annuum* L. Interciencia 41: 541-547.

Rodríguez-Leyva, E., J. R. Lomeli-Flores, J. M. Valdez-Carrasco, R. W. Jones, and P. A. Stansly. 2012. New records of species and locations of parasitoids of the pepper weevil in Mexico. Southwest. Entomol. 37: 73-83.

Rodríguez-Leyva, E., P. A. Stansly, D. J. Schuster, and E. Bravo-Mosqueda. 2007. Diversity and distribution of parasitoids of *Anthonomus eugenii* (Coleoptera: Curculionidae) from Mexico and prospects for biological control. Fla. Entomol. 90: 693-703.

Sánchez-Peña, P., K. Oyama, J. Núñez-Farfán, J. Fornoni, S. Hernández-Verdugo, J. Márquez-Guzmán, and J. A. Garzón-Tiznado. 2006. Sources of resistance to whitefly (*Bemisia* spp.) in wild populations of *Solanum lycopersicum* var.

cerasiforme (Dunal) spooner GJ Anderson et RK Jansen in northwestern Mexico. Genet. Resour. Crop Ev. 53: 711-719.

Seal, D. R., and K. Bondari. 1999. Evaluation of various cultivars of pepper for resistance against pepper weevil (Coleoptera: Curculionidae). Proc. Fla. State Hort. Soc. 112: 342-345.

Seal, D. R., and C. G. Martin. 2016. Pepper weevil (Coleoptera: Curculionidae) preferences for specific pepper cultivars, plant parts, fruit colors, fruit sizes, and timing. Insects 7: 9.

Smith, C. M. 2005. Plant Resistance to Arthropods: Molecular and Conventional Approaches. Springer, Netherlands.

Soto-Hernández, M., R. W. Jones, and P. Reyes-Castillo. 2013. A key to the Mexican and Central America genera of Anthonomini (Curculionidae, Curculioninae). ZooKeys 260: 31-47.

Stephens, S. G., and H. S. Lee. 1961. Further studies on the feeding and oviposition preferences of the boll weevil (*Anthonomus grandis*). J. Econ. Entomol. 54: 1085-1090.

Vavilov, N. I. 1992. Origin and Geography of Cultivated Plants. Cambridge University Press, United Kingdom.

Wu, P., M. Haseeb, R. Zhang, L. H. B. Kanga, and J. C. Legaspi. 2019. In vitro consumption patterns of pepper weevil, *Anthonomus eugenii* (Coleoptera: Curculionidae) on two commercial pepper cultivars in Florida. Appl. Entomol. Zool. 54: 473-479.

Diversidad de Insectos Entomófagos en Nogal Pecanero con Uso de Insecticidas Biorracionales y Convencionales

Diversity of Entomophagous Insects in Pecans Using Biorational and Conventional Insecticides

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Resumen. El objetivo de esta investigación fue evaluar y comparar la diversidad de insectos entomófagos en huertas de nogal pecanero sin tratar y tratadas con insecticidas biorracionales y convencionales en la Región Lagunera de México. Los insectos se colectaron mensualmente, de abril a septiembre en 2016 y 2017, mediante red entomológica en una huerta sin tratar (testigo), otra tratada con el insecticida spinoteram (manejo biorracional), una tercera tratada con los insecticidas metoxifenozide y benzoato de emamectina en combinación con liberaciones de *Trichogramma pretiosum* (manejo biorracional) y una cuarta asperjada con los insecticidas clorpirfos etil y cipermetrina (manejo convencional). Se utilizaron los estimadores no paramétricos Chao1, Chao2, ACE, ICE, y Michaelis-Menten para estimar la diversidad alfa mediante la construcción de curvas de acumulación de especies. La mayor riqueza fue en la huerta testigo con 37 especies y abundancia de 315 insectos; la riqueza en las dos huertas con manejo biorracional fue de 28 a 29 especies y abundancias de 192 a 315 insectos; mientras que en la huerta con manejo convencional se observó la menor riqueza con 22 especies y abundancia de 108 insectos entomófagos. Las huertas con manejo biorracional mostraron más especies compartidas (diversidad beta) con el testigo (46%), que la huerta con manejo convencional (37%). La diversidad gamma constó de 48 especies (colecta total). Los entomófagos con mayor abundancia fueron los parasitoides *Telenomus* spp. y *Quadrastichus* sp., así como los depredadores *Chrysoperla comanche*, *Lispe* sp. 1 y *Olla v-nigrum*. Estos resultados indican que el manejo biorracional de insectos plaga, mediante el uso de insecticidas de bajo impacto ambiental y selectividad alta, en combinación con liberaciones de *Trichogramma*, puede promover una mayor diversidad y la conservación de insectos benéficos en el agroecosistema del nogal. Considerando el reducido número de huertas estudiadas en el presente estudio, es necesario realizar estudios adicionales sobre la diversidad

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de insectos benéficos que tomen en cuenta las diferencias en condiciones ambientales, incidencia de insectos plaga y manejo agronómico de las huertas de nogal.

Abstract. The objective of this research was to evaluate and compare the diversity of entomophagous insects in untreated and treated pecan orchards with biorational and conventional insecticides in the Comarca Lagunera region of Coahuila and Durango, Mexico. Insects were collected monthly with a sweep net from April to September in 2016 and 2017 in a nontreated orchard (control), two treated with biorational insecticides, and one treated with conventional insecticides. The non-parametric estimators Chao1, Chao2, ACE, ICE, and Michaelis-Menten were used to estimate alpha diversity by species accumulation curves. The greatest species richness was in the untreated orchard with 37 species and abundance of 315 insects. Species richness in the two orchards treated with biorational insecticides was 28 to 29 species, and abundance 192 to 315 insects, while in the orchard treated with conventional insecticides richness was 22 species and abundance of 108 entomophagous insects. Orchards with biorational insecticides showed more species shared (beta diversity) compared with the control orchard (46%), than the orchard with conventional insecticides (37%). The gamma diversity (regional species richness) was of 48 species. The most abundant parasitoids were *Telenomus* sp. 1, and *Quadrastichus* sp., and the predators *Chrysoperla comanche*, *Lispe* sp. 1 and *Olla v-nigrum*. These results indicate that biorational management of pest insects using low environmental impact and high selectivity insecticides in combination with *Trichogramma* releases, can promote greater diversity and conservation of beneficial insects in the pecan agroecosystem. Considering the small number of orchards in this study, additional research on the diversity of beneficial insects, considering differences in environmental conditions, incidence of pest insects and agronomic management of pecan, are necessary.

Introducción

El cultivo de la nuez pecanera (*Carya illinoinensis* (Wangenh K. Koch) es una actividad importante en México y ocupa el segundo lugar en producción después de Estados Unidos de América (Cervantes et al. 2018). En el 2018 se plantaron 135,335 ha de nogal pecanero en México, de las cuales se cosecharon 96,909 ha, con una producción de 159,535 ton y un valor de MX$12,534 millones (SAGARPA-SIAP 2019). La producción de nuez durante 2018 se concentró principalmente en los estados del norte, siendo Chihuahua el principal productor (63.6%), seguido de Sonora (13.6%), Coahuila (10.2%), Durango (5.8%), y Nuevo León (2.7%) (SAGARPA-SIAP 2019). La producción de nuez es afectada por plagas de importancia económica como el gusano barrenador de la nuez (*Acrobasis nuxvorella* Neunzig), gusano barrenador del ruezno (*Cydia caryana* Fitch), y el complejo de pulgones formado por el pulgón amarillo *Monelliopsis pecanis* Bissell, el pulgón amarillo de alas con márgenes negros *Monellia caryella* Fitch y el pulgón negro *Melanocallis caryaefoliae* Davis (Aguirre et al. 1995, Nava y Ramírez 2002). Otras plagas importantes son el salivazo *Clastoptera achatina* Germar (Aguilar 2007) y las chinches manchadoras de almendra como *Nezara viridula* L., *Euchistus servus* Say, *Chlorochroa ligata* Say, *Brochymena* spp., y chinche de patas laminadas *Leptoglossus zonatus* Dallas (Dutcher y Todd 1983, Cottrell et al. 2000). El control de estas plagas se realiza con insecticidas de diferentes grupos toxicológicos,

algunos ejercen un alto impacto ambiental y poseen toxicidad elevada para la fauna benéfica, tales como clorpirifós etil, dimetoato y cipermetrina (Nava et al. 2019).

En el agroecosistema del nogal se encuentran diferentes insectos depredadores y parasitoides (Gunasena y Harris 1988, Romero et al. 2001, Aguirre et al. 2010, Tarango 2014, Torres-Delgado et al. 2018). Sin embargo, se requieren más estudios para evaluar la diversidad de la entomofauna en huertas de este frutal, por lo cual el objetivo del presente trabajo fue medir y comparar la diversidad de especies de insectos entomófagos, asociados a huertas de nogal pecanero con diferente manejo de insecticidas para el control de insectos plaga.

Materiales y Métodos

El estudio se realizó en cuatro huertas comerciales de 4.0 ha y 35 años de edad de la Región Lagunera de Coahuila y Durango: 1) "P.P. Hormiguero", en Matamoros, Coahuila (25°41'19.0"N, 103°20'1.5"O), con manejo biorracional que incluyó dos liberaciones de *Trichogramma pretiosum*, una aplicación de benzoato de emamectina (selectividad alta) y una aplicación de methoxifenozide (selectividad alta) en 2016, así como dos liberaciones de *T. pretiosum* y cuatro aplicaciones de methoxyfenozide en 2017; 2) "Ejido Hormiguero", en Matamoros, Coahuila (25°41'5.0"N, 103° 20' 44.0"O), con manejo convencional donde se realizaron una aplicación de clorpirifós etil y otra de clorpirifós etil + cipermetrina (insecticidas de amplio espectro de control) en 2016, y tres aplicaciones de clorpirifós etil en 2017; 3) "El Refugio", en Gómez Palacio, Durango (25°35'27.8"N, 103° 31' 20.0"O), con manejo biorracional con tres y dos aplicaciones de spinetoram (selectividad moderada) en 2016 y 2017; y 4) "FAZ-UJED", en Gómez Palacio, Durango (25°46'82.0"N, 103° 21' 35.0"O), sin aplicación de insecticidas y ningún otro método de manejo de plagas (testigo absoluto).

Se realizaron seis muestreos con intervalos mensuales durante cada ciclo agrícola entre abril y septiembre de 21016 y 2017. Se utilizó una red entomológica de 40 cm de diámetro, para cada muestra se consideraron 10 árboles al azar por sitio de muestreo, dando 10 golpes de red en la periferia del tercio inferior del árbol a una altura de 3 m. Los insectos colectados se conservaron en alcohol etílico al 70%, y se identificaron y corroboraron a nivel de género o especie con el apoyo de entomólogos especialistas Dr. Enrique Ruiz Cancino y Dra. Juana María Coronado Blanco, de la Universidad Autónoma de Tamaulipas (FIC-UAT) y la Dra. Dulce H. Zetina, del Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA).

La riqueza y abundancia de los insectos entomófagos se compararon entre los diferentes métodos de control de plagas en las cuatro huertas de nogal. En cada huerta se contabilizó el número total de especies (morfoespecies) construyendo curvas de acumulación de especies para estimar la diversidad local (alfa), utilizando los estimadores no paramétricos Chao 1 y Chao 2, ACE, ICE, y Michaelis-Menten (Chao 1984, Walther y Morand 1998, Moreno 2001) mediante el programa EstimateS versión 9.1 (Colwel 2013). El recambio de especies (diversidad beta) entre los diferentes métodos de manejo de plagas se comparó mediante métodos de ordenación y clasificación, construyendo una matriz sintética de especies compartidas y diferentes. La diversidad gamma o riqueza regional (dentro de varias unidades del paisaje) fue la suma de cada uno de los sitios de muestreo (diversidad alfa) y comprendió el listado total de especies de los cuatro predios (Moreno et al. 2011). Los números de especies y sus datos de abundancia fueron transformados

mediante el logaritmo natural de (x + 1) y se sometieron a análisis de varianza mediante un diseño completamente al azar con dos factores, considerando a las localidades (cuatro huertas), y años (dos) como factores y a los meses de muestreo (seis) como repeticiones (Ott 1988). Se efectuaron comparaciones de medias mediante la prueba de diferencia mínima significativa (DMS, $P < 0.05$) (SAS Institute 2002).

Resultados y Discusión

El Cuadro 1 muestra la diversidad alfa y gamma y abundancia de especies entomófagas en las cuatro huertas de nogal estudiadas durante 2016 y 2017. Se colectaron 966 insectos, agrupados en 48 especies, 40 géneros, 25 familias, y 5 órdenes. Estos resultados estiman la diversidad gamma o riqueza regional de especies.

El número o riqueza de especies fue significativamente diferente entre huertas ($P = 0.0018$) y años ($P < 0.0001$), siendo mayor en la huerta sin uso de insecticidas (FAZ-UJED) que en las que fueron tratadas con insecticidas de bajo impacto ambiental (Refugio y P.P. Hormiguero) o insecticidas más tóxicos (Ej. Hormiguero). La riqueza de especies fue mayor en 2016 que en 2017. La abundancia del total de insectos benéficos fue significativamente diferente entre huertas ($P = 0.0006$) y años ($P = 0.0062$), siendo mayor en las huertas FAZ-UJED y P. P. Hormiguero que en las huertas Refugio y Ej. Hormiguero. La densidad de insectos benéficos fue mayor en 2016 que en 2017.

Las densidades de especies depredadoras fueron mayores en las localidades FAZ-UJED y P. P. Hormiguero que en Refugio y Ej. Hormiguero ($P = 0.0001$); además la abundancia de depredadores fue mayor en 2016 que en 2017 ($P = 0.0195$). Las diferencias entre localidades se debieron a las densidades observadas de catarinas (*O. v-nigrum*, *H. axyridis*, e *H. convergens*) ($P = 0.0044$) y crisopas verdes (*C. comanche*, *C. nigricornis*, y *C. carnea*) ($P = 0.0185$). No se detectaron diferencias significativas en la abundancia de escarabajos (*C. vittatus*, *C. fusca*, y una especie de Staphylinidae) ($P = 0.1447$), chinches (*Zelus* sp., *Sinea* sp., *O. insidiosus*, y *Nabis* sp.) ($P = 0.4743$), y moscas depredadoras (*Atomasia* sp., *Condylostylus* sp., *Dolichus* sp., *Enilia* sp., *Lispe* sp. 1, *Lispe* sp. 2, *Tripanurga* sp., *Salpingogaster* sp., *Syrphus* sp. 1, y *Syrphus* sp. 2) ($P = 0.0553$). Las especies de depredadores más abundantes fueron *C. comanche* (13.3% del total de insectos), *Lispe* sp. 1 (6.7%), *O. v-ningrum* (6.4%), *H. convergens* (3.4%), y *O. insidiosus* (1.9%) (Cuadro 1).

Las densidades de especies de parasitoides fueron mayores en las localidades P. P. Hormiguero y FAZ-UJED que en Refugio y Ej. Hormiguero ($P = 0.0308$); también la abundancia de parasitoides fue mayor en 2016 que en 2017 ($P = 0.0330$). Las diferencias indicadas entre localidades se debieron a las densidades de avispas parasíticas (22 especies, Cuadro 1) ($P = 0.0219$). Al contrario, las densidades de moscas parasíticas (*Distichona* sp., *Houghia* sp., y *L. archippivora*) fueron mayores en la huerta Ej. Hormiguero que en las huertas Refugio, P. P. Hormiguero y FAZ-UJED ($P = 0.0116$). Las especies de parasitoides más abundantes fueron *Telenomus* sp. 1 (23.4% del total de insectos), *Quadrastichus* sp. (6.9%), *A. reduvii* (3.5%), Platigastridae sp. 1 (3.3%), y *Pnigalio* sp. 1 (2.9%).

Cuadro 1. Diversidad y Abundancia de Especies de Insectos Entomófagos Colectados en Huertas de Nogal Pecanero con Diferente Manejo de Insecticidas, durante 2016 y 2017, en la Comarca Lagunera, México

Tabla 1. Diversity and Abundance of Species of Entomophagous Insects Collected in Pecan Orchards Under Different Insecticide Management, during 2016 and 2017, in the Comarca Lagunera Region, Mexico

Morfoespecie	FAZ-UJED	Refugio	P.P. Hormiguero	Ej. Hormiguero	N[a]
Olla v-nigrum	29[b]	7	23	3	62
Harmonia axyridis	2	4	2	0	8
Hyppodamia convergens	17	9	12	0	38
Cantharis fusca	1	0	0	0	1
Collops vittatus	2	0	0	0	2
Staphylinidae sp.1	0	1	0	0	1
Zelus sp.	1	2	5	1	9
Sinea sp.	0	0	1	0	1
Orius insidiosus	4	7	4	3	18
Nabis sp.	0	1	1	0	2
Chrysoperla comanche	63	19	31	15	128
Chrysoperla nigricornis	6	3	2	1	12
Chrysoperla carnea	4	1	0	0	5
Atomasia sp.	1	0	0	0	1
Condylostylus sp.	5	3	7	0	15
Dolichopus sp.	3	0	5	1	9
Enilia sp.	1	0	0	0	1
Lispe sp. 1	20	20	17	8	65
Lispe sp. 2	10	5	0	1	16
Tripanurga sp.	7	0	0	0	7
Salpingogaster sp.	7	0	0	0	7
Syrphus sp. 1	9	2	1	1	13
Syrphus sp. 2	1	0	0	0	1
Distichona sp.	0	0	0	5	5
Hougia sp.	2	0	0	0	2
Lespesia archippivora	0	0	1	0	1
Aphidius sp. 1	8	3	3	2	16
Aphidius sp. 2	0	5	2	1	8
Aleiodes sp.	5	0	0	0	5
Chelonus sp.	0	3	0	0	3
Telenomus sp. 1	41	40	109	36	226
Telenomus sp. 2	3	2	9	3	17
Semiotellus sp.	0	5	0	0	5
Lysirinia sp. 1.	1	2	4	2	9
Lysirinia sp. 2	1	0	3	1	5
Quadrastichus sp.	17	8	36	6	67
Pnigalio sp. 1	14	0	12	2	28
Pnigalio sp. 2	5	0	0	0	5
Anastatus reduvii	34	0	0	0	34
Eurytoma sp.	11	1	8	2	22

Diversinervus sp.	0	12	4	2	18
Doliopria sp.	5	0	0	0	5
Eupelmus sp.	2	1	0	0	3
Cynipidae sp. 1	2	5	4	0	11
Platygastridae sp. 1	6	10	5	11	32
Platygastridae sp. 2	1	0	3	0	4
Mymaridae sp. 1	0	9	1	1	11
Mymaridae sp. 2	0	2	0	0	2
N	351	192	315	108	966
S[c]	37	29	28	22	48

[a]Número total de individuos colectados. [b]Valores son número de insectos colectados en 12 fechas, mediante 100 golpes de red por fecha. [c]Número de especies.

El orden Hymenoptera presentó la mayor riqueza y abundancia en las cuatro huertas, observándose esto particularmente en la huerta testigo; de la misma manera, este grupo de parasitoides fue el de mayor contribución para la diversidad regional. Esto coincide con lo propuesto por otros autores, quienes mencionan que este grupo es un bioindicador de condiciones de escaso disturbio (Kevan 1999, Paoletti 1999). El segundo lugar en riqueza le correspondió al orden Diptera dentro de huertas y entre ellas, también presentó esta posición en diversidad regional (diversidad gamma). De los insectos depredadores, el orden Neuroptera fue el más abundante dentro de huertas y entre éstas, específicamente *Chrysoperla comanche*, la cual juega un papel importante en la regulación de las poblaciones de áfidos del nogal (Tarango 2014).

Aunque se cuenta con un listado de especies de parasitoides y depredadores asociadas a nogal (Gunasena and Harris 1988, Romero et al. 2001, Aguirre et al. 2010, Tarango 2014, Torres-Delgado et al. 2018) que pueden estar ejerciendo un control biológico natural, dicha entomofauna no se ha descrito de manera analítica que exprese la riqueza y abundancia de especies (Escalante 2003).

En la Fig. 1 se muestra la riqueza específica observada y la estimada con base en los estimadores no paramétricos Chao1, Chao2, ACE, ICE, y Michaelis-Menten, para los diferentes manejos de plagas en las huertas de nogal pecanero. Los estimadores ACE y Chao1 presentaron el mejor desempeño para las huertas con manejo biorracional, Refugio (91 y 95%) (Fig. 1B) y P. P. Hormiguero (91 y 92%) (Fig. 1C), y sin tratar, FAZ-UJED (87 y 89%) (Fig. 1 D); mientras que para la huerta con uso de insecticidas tóxicos, Ej. Hormiguero (Fig. 1 A), los mejores estimadores de la riqueza de especies fueron Chao1 (83%) y Chao2 (88%).

Los modelos de estimación de la riqueza de especies se utilizan cuando se considera que las muestras son representaciones incompletas de las comunidades (Moreno et al. 2011), tal como ocurrió en la huerta con uso de insecticidas convencionales, donde se colectó el porcentaje más bajo de especies (83 a 88%). Los estimadores son una herramienta útil para saber si se requiere realizar un segundo estudio en una zona, aun cuando se obtenga una asíntota en la curva de acumulación de especies, incluso puede ser importante en términos de costos, para reducir o aumentar la intensidad del muestreo (Escalante 2003).

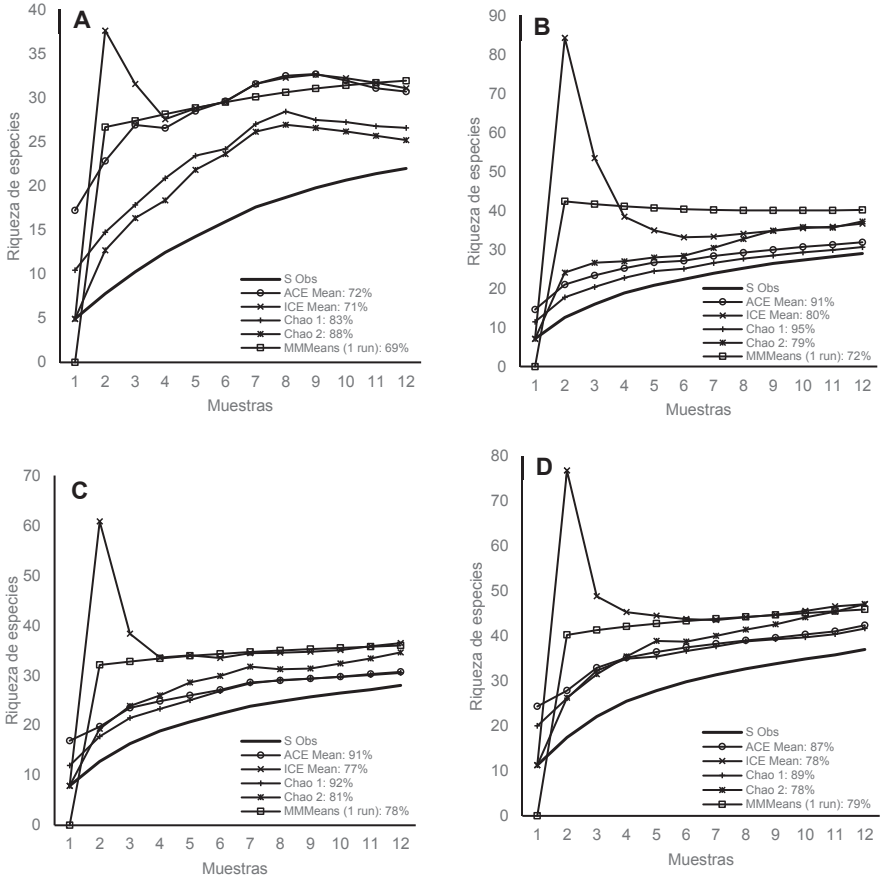

Fig. 1. Curvas de acumulación de especies de insectos entomófagos asociados a nogal obtenidas mediante estimadores no paramétricos Chao1, Chao2, ACE, ICE, y Michaelis-Menten. Huertas con aplicaciones de clorpirifós y cipermetrina (A), spinetoram (B), uso combinado de *T. pretiosum*, methoxyfenozide y benzoato de emamectina (C), y sin tratar (testigo) (D), Comarca Lagunera, México.
Fig. 1. Species accumulation of entotomophagous insects associated to pecans obtained by nonparametric estimators Chao1, Chao2, ACE, ICE, and Michaelis-Menten. Pecan orchards with applications of chlorpyrifos and cypermethrin (A), spinetoram (B), combined use of *T. pretiosum*, methoxyfenozide and emamectin benzoate (C), and nontreated (control) (D), Comarca Lagunera, Mexico.

En el Cuadro 2 se muestra la diversidad beta, con base en el número de especies compartidas y diferentes de insectos entomófagos en huertas de nogal con diferente manejo de insecticidas. Las huertas sin tratar (FAZ-UJED) y con manejo biorracional (P. P. Hormiguero); así como ambas huertas con manejo biorracional (Refugio y P. P. Hormiguero) presentaron el mayor número de especies comunes

(22 especies); mientras que la huerta con manejo convencional (Ej. Hormiguero) tuvo el menor número de especies comunes (18 especies) con las huertas FAZ-UJED y Refugio. Por lo tanto, las huertas con manejo biorracional mostraron más especies compartidas con el testigo (46%) que la huerta con manejo convencional (37%). Las huertas FAZ-UJED y Refugio presentaron el mayor número de especies diferentes entre sí (24 especies); mientras que las huertas P. P. Hormiguero y Ej. Hormiguero tuvieron menos especies diferentes entre sí (10 especies).

Cuadro 2. Diversidad Beta con Base en el Número de Especies Compartidas y Diferentes de Insectos Entomófagos Colectados en Huertas de Nogal Pecanero con Diferente Manejo de Insecticidas en 2016 y 2017, Comarca Lagunera, México
Table 2. Beta Diversity Base on the Number of Common and Different Species of Entomophagous Insects Collected in Pecan Orchards Under Different Insecticide Management in 2016 and 2017 at the Comarca Lagunera Region, Mexico

Huerta[a]	FAZ-UJED	Refugio	P.P. Hormiguero	Ej. Hormiguero
FAZ-UJED	0	21[b]	22	18
Refugio	24	0	22	18
P.P. Hormiguero	21	13	0	20
Ej. Hormiguero	23	15	10	0

[a]Manejo de insecticidas: FAZ-UJED sin tratar, Refugio con aplicaciones de spinetoram (biorracional), P.P. Hormiguero con aplicaciones de benzoato de emamectina, methoxifenozide y liberaciones de *T. pretiosum* (biorracional), y Ejido Hormiguero con aplicaciones de clorpirifós etil y cipermetrina (convencional).
[b]Valores por arriba de la diagonal con ceros corresponden al número de especies compartidas y por debajo de ella al número de especies diferentes.

Las tendencias que se observaron en los resultados del presente estudio coinciden con lo esperado, en la huerta testigo (FAZ-UJED) se encontró mayor diversidad alfa y abundancia de insectos benéficos, ya que es un sitio no perturbado debido a que no se usan insecticidas. En las huertas con manejo biorracional de plagas, se encontró una riqueza de especies similar, sin embargo, la abundancia fue mayor donde se utilizó la combinación del control biológico mediante liberaciones de *T. pretiosum* y los insecticidas metoxifenozide y benzoato de emamectina (huerta P. P. Hormiguero), de bajo impacto en la fauna benéfica (Nevárez y Tarango 2014), que donde se usó solo spinetoram (huerta Refugio), el cual se indica que es moderadament tóxico para los parasitoides *Trichogramma platneri*, *Macrocentrus instabilis*, y moscas taquínidas. Esta diferencia fue influenciada fuertemente por la mayor abundancia de los parasitoides *Telenomus* spp. y *Quadrasticus* sp., así como del depredador *O. v-nigrum* en la huerta P. P. Hormiguero que en la huerta Refugio. La gran abundancia de *Telenomus* spp. puede deberse a que parasita huevos de especies de chinches comunes en las huertas de nogal, tales como *Euschistus servus* y *Brochymena sulcata* (Tarango y González 2009). La menor riqueza y abundancia de especies de insectos entomófagos en la huerta Ejido Hormiguero parece deberse al uso de los insecticidas de amplio espectro clorpirfós etil y cipermetrina. Estos insecticidas no poseen selectividad a los insectos depredadores y parasitoides más comunes en las huertas de nogal, tales como *O. v-nigrum*, *H. convergens*, *C. carnea*, *C. nigricornis*, *C. rufilabris*, *Aphelinus perpallidus*, *Trichogramma* spp., y moscas taquínidas (Nevárez y Tarango 2014). El uso de

sustancias químicas sintéticas en los agroecosistemas perjudica principalmente a los enemigos naturales, disminuyendo la regulación natural de los insectos plaga (Zalazar y Salvo 2007); además, reducen la fecundidad y capacidad de búsqueda de huéspedes por los parasitoides, así como el porcentaje de parasitismo (García et al. 2008, Liu et al. 2008, Rimoldi et al. 2012). En los sistemas de producción intensivos aumenta la abundancia de insectos fitófagos y disminuye la de entomófagos, lo cual se atribuye a que los enemigos naturales son más susceptibles que sus presas a los insecticidas convencionales (Symington 2003, Gregor et al. 2008).

La suma de la diversidad alfa da como resultado la diversidad gamma, en la cual se puede apreciar la diversidad de un paisaje o una región. En el presente estudio se encontró una riqueza de 48 especies y una estimación de la riqueza del 83 al 95%, con base en las colectas realizadas en solo cuatro huertas de nogal. La inclusión de un mayor número de huertas representativas de cada tipo de práctica agrícola (réplicas para el análisis de datos) puede permitir niveles de desempeño más altos para mejorar el conocimiento de la riqueza de especies existente (Zalazar y Salvo 2007).

El uso de métodos biorracionales para el manejo de plagas, así como de otros componentes tecnológicos coadyuva a conservar la fauna benéfica en las huertas de nogal. Los cultivos perennes, como el nogal, son lugares adecuados para el crecimiento y desarrollo de los ciclos de vida de los insectos, sobre todo en épocas desfavorables (Zumoffen et al. 2010). Lo anterior permitirá preservar y aumentar la diversidad y abundancia de los insectos benéficos en los agroecosistemas, especialmente en las huertas productoras de nuez pecanera.

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Referencias Citadas

Aguilar P., H. 2007. Principales plagas de nogal en el estado de Coahuila. INIFAP-CIRNE. Campo Experimental Saltillo. Sitio Experimental Zaragoza. Manual Técnico 14, Zaragoza, Coahuila, México. http://www.inifapcirne.gob.mx/Biblioteca/Publicaciones/233.pdf

Aguirre, L. A., E. Tucuch, and M. K. Harris. 1995. Oviposition and nut entry behavior of the pecan nut casebearer *Acrobasis nuxvorella*. Southwest. Entomol. 20: 447-451.

Aguirre, L. A., M. Flores, A. Urrutia, E. Cerna, L. P. Guevara, Y. Ochoa, y J. Landeros. 2010. Parasitismo natural de *Cydia caryana* (Fitch, 1856) (Lepidoptera: Tortricidae) y su potencial en control biológico. Folia Entomol. Mex. 49: 9-15.

Cervantes V., M. G., I. Orona C., C. Vázquez V., M. Fortiz H., y J. J. Espinoza A. 2018. Análisis comparativo de huertos de nuez pecanera (*Carya illinoensis* Koch) en la Comarca Lagunera. Revista Mexicana de Ciencias Agrícolas 9: 25-35.

Chao, A. 1984. Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 11: 265-270. http://dns2.asia.edu.tw/~ysho/YSHO-English/1000%20Taiwan%20(Independent)/PDF/Sca%20J%20Sta11,%20265.pdf.

Colwell, R. K. 2013. EstimateS 9.1.0: statistical estimation of species richness and shared species from samples. University of Connecticut. http://viceroy.eeb.uconn.edu/estimates/estimatespages/estimates.php.

Cottrell, T. E., C. E. Yonce, and B. W. Wood. 2000. Seasonal occurrence and vertical distribution of *Euchistus servus* (Say) and *Euchistus tristigmus* (Say) (Hemiptera: Pentatomidae) in pecan orchards. J. Entomol. Sci. 35: 421-431.

Dutcher, J. D., and J. W. Todd. 1983. Hemipteran kernel damage of pecan. Miscellaneous Publications of the Entomological Society of America 13: 1-11.

Escalante, E. T. 2003. ¿Cuántas especies hay? Los estimadores no paramétricos de Chao. Elementos: Ciencia y Cultura 52: 53-56. https://elementos.buap.mx/directus/ storage/uploads/00000002590.pdf.

García, G. F., M. Ramírez G., V. M. Pinto, S. Ramírez A. 2008. Efectos adversos de plaguicidas en *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae). Revista Chapingo Serie Zonas Áridas 7: 177-186.

Gregor, J. D., D. Eza, E. Ogusuku, y M. J. Furlong. 2008. Uso de insecticidas: contexto y consecuencias ecológicas. Revista Peruana de Medicina Experimental y Salud Pública 25: 74-100. http://www.scielo.org.pe/pdf/rins/v25n1/a11v25n1.pdf.

Gunasena, G. H., and M. K. Harris. 1988. Parasites of hickory shuckworm and pecan nut casebearer with five new host-parasite records. Southwest. Entomol. 13: 107-111.

Kevan, P. G. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agric. Ecosyst. Environ. 74: 373-393.

Liu, Y., Y. Lu, K. Wu, K. A. G. Wyckhuys, and F. Xue. 2008. Lethal and sublethal effects of endosulfan on *Apolygus lucorum* (Hemiptera: Miridae). J. Econ. Entomol. 101: 1805-1810.

Moreno, C. E. 2001. Métodos para medir la biodiversidad. M & T Manuales y Tesis SEA. Vol. 1. Primera ed. Zaragoza, España. https://www.researchgate.net/publication/304346666_Metodos_para_medir_la_biodiversidad.

Moreno, C. E., F. Barragán, E. Pineda, y N. P. Pavón. 2011. Reanálisis de la diversidad alfa: alternativas para interpretar y comparar información sobre comunidades ecológicas. Rev. Mex. Biodivers. 82: 1249-1261.

Nava, C. U., y M. Ramírez D. 2002. Manejo integrado de plagas del nogal, pp. 145-176. *In* A. J. Arreola y I. Reyes J. [eds.] Tecnología de Producción en Nogal Pecanero. Libro Técnico No. 3. Campo Experimental La Laguna, CIRNOC, INIFAP, Matamoros, Coahuila, México.

Nava, U., V. Ávila, E. Morales, y J. Maltos. 2019. Estrategia de manejo de insecticidas en nogal pecanero. Revista Pacana 5: 10-21.

Nevárez G., G. y S. H. Tarango R. 2014. Selectividad de plaguicidas, pp. 281-296. *In* S. H. Tarango R. [ed.], Manejo Integrado de Plagas del Nogal Pecanero. Libro Técnico No. 1. Campo Experimental Delicias, Centro de Investigación Regional Norte Centro, INIFAP, Delicias, Chihuahua, México.

Ott, L. 1988. An Introduction to Statistical Methods and Data Analysis. 3rd ed. PWS-Kent Publishing Co., Boston, MA.

Paoletti, M. G. 1999. Using bioindicators based on biodiversity to assess landscape sustainability. Agric. Ecosyst. Environ. 74: 1-18.

Rimoldi, F., M. I. Schneider, and A. E. Ronco. 2012. Short and long-term effects of endosulfan, cypermethrin, spinosad, and methoxyfenozide on adults of *Chrysoperla externa* (Neuroptera: Chrysopidae). J. Econ. Entomol. 105: 1982-1987.

Romero, J. C., J. J. Ellington, y D. B. Richman. 2001. Pecan nut casebearer, *Acrobasis nuxvorella* Neuzing parasites collected in Doña Ana County, NM and El Paso County, TX. Southwest. Entomol. 26: 269-270.

SAGARPA-SIAP (Servicio de información Agroalimentaria y Pesquera). 2019. Anuario Estadístico de la Producción Agrícola. http://www.siap.gob.mx/. Abril 2019.

SAS Institute. 2002. SAS User´s Guide. version 9.1. SAS Institute, Inc., Cary, NC.

Symington, S. A. 2003. Lethal and sublethal effects of pesticides on the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and its parasitoid *Orgilus lepidus* Muesebeck (Hymenoptera: Braconidae). Crop Prot. 22: 513-519.

Tarango R., S. H. 2014. Insectos benéficos, pp. 49-80. *In* S. H. Tarango R. [ed.], Manejo Integrado de Plagas del Nogal Pecanero. Libro Técnico No. 1. Campo Experimental Delicias, Centro de Investigación Regional Norte Centro, INIFAP, Delicias, Chihuahua, México.

Tarango R., S. H., and A. González H. 2009. Especies, fluctuación poblacional y enemigos naturales de chinches (Hemiptera: Pentatomidae, Coreidae, Largidae) asociadas a nogal pecanero. Southwest. Entomol. 34: 305-318.

Torres-Delgado, M. G., E. Ruíz-Cancino, V. Ávila-Rodríguez, U. Nava-Camberos, J. M. Coronado-Blanco, O. G. Alvarado-Gómez, and E. Morales-Olais. 2018. Parasitoids of the pecan casebearer, *Acrobasis nuxvorella* Neuzing in the Comarca Lagunera, Mexico. Southwest. Entomol. 43: 175-181.

Walther, B. A., and S. Morand. 1998. Comparative performance of species richness estimation methods. Parasitology 116: 395-405.

Zalazar, L., y A. Salvo. 2007. Entomofauna asociada a cultivos hortícolas orgánicos y convencionales en Córdoba, Argentina. Neotrop. Entomol. 36: 765-773.

Zumoffen L., L. Salto, y M. Signorini. 2010. Alfalfa (*Medicago sativa* L.) como reservorio de insectos entomófagos. Revista FAVE – Ciencias Agrarias 9: 73-82.

Current Advances in Biology, Distribution, and Management of *Melanaphis sacchari* (Zehntner)[1] in México and United States of America

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Abstract. The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is a major insect pest of sorghum (*Sorghum bicolor* (L.) Moench) in Mexico and United States of America. This review reports current advances for the biology of the aphid, crop damage, and sampling strategies to detect the pest. We focused on management through cultural control including resistant varieties, biological control with native parasitoids and predators and use of entomopathogenic fungi, legal control, and chemical control. Life stages of the aphid are nymph, nymphal alate, super nymph, and adult. Females have parthenogenetic reproduction and under adverse conditions, viviparous reproduction. Damage to the sorghum crop by feeding nymphs and adults causes stress to the plants and yield loss. To detect the pest, leaves should be sampled each week or yellow traps used with soapy water to monitor arrival of winged adults. Cultural practices recommended are destroying foci of infection and using established official planting dates and appropriate fertilization. Currently, the main control strategy is foliar application of insecticides. However, numerous species of predators, parasitoids, and entomopathogenic fungi affect sugarcane aphids in the USA and Mexico. The organisms could sustain control of the aphid, but few have been evaluated in the field. Studies of sorghum varieties in areas of México and the USA allowed identification of genotypes with resistance to sugarcane aphids. Considering sampling strategies, biology, and life cycle studies, search for an organism with potential for biological control of *M. sacchari*, and identification of sorghum genotypes with resistance to damage, it is possible to develop an integrated management strategy against sugarcane aphid. For future research, it is important to develop bioassays for monitoring resistance to insecticide, study interaction between factors that favor natural and induced control with natural enemies, and promote planting of sorghum genotypes with resistance.

Resumen. El pulgón amarillo del sorgo, *M. sacchari* (Zehntner) es la principal plaga de este cultivo en México y Estados Unidos de América. El ciclo de vida de este áfido consta de cuatro estadios ninfales, ninfas alatoides, superninfas, y formas adultas. Las hembras presentan reproducción partenogenética y bajo condiciones

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adversas reproducción vivípara. El daño es causado por la alimentación de ninfas y adultos, provocando estrés en plantas y pérdidas de producción. Para su detección, es importante realizar muestreos semanales en hojas recolectadas sobre la periferia del cultivo o usar bandejas amarillas con agua jabonosa para determinar la llegada de adultos alados. El control cultural se basa en la destrucción de focos de infestación, el establecimiento de fechas oficiales de siembra y un programa de fertilización adecuado. Actualmente, la principal estrategia de manejo es la aplicación de insecticidas químicos. Sin embargo, diversas especies de depredadores, parasitoides y hongos entomopatógenos (HE) afectan a *M. sacchari* en México y EUA. Estos organismos pueden ofrecer un control sostenible de la plaga; sin embargo, su eficiencia en condiciones de campo ha sido evaluada parcialmente. Los estudios de resistencia varietal realizados en áreas de México y EUA han permitido la identificación de genotipos de sorgo con tolerancia al PAS. Considerando las estrategias de muestreo, los estudios sobre biología y ciclo de vida, la búsqueda de un organismo con potencial para el control biológico de *M. sacchari* y la identificación de genotipos de sorgo con tolerancia al daño, es posible desarrollar una estrategia de manejo integrado para *M. sacchari*. Entre las investigaciones futuras, es importante desarrollar bioensayos para el monitoreo de la resistencia a los insecticidas, realizar estudios que contemplan la interacción entre los factores que favorecen el control natural e inducido por enemigos naturales y fomentar la siembra de genotipos de sorgo con tolerancia al daño.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) after winter wheat, *Triticum aestivum* (L.); rice, *Oryza sativa* (L.); maize, *Zea mays* (L.); and oat, *Avena sativa* (L.), is the fifth most important cereal in the world (Pérez et al. 2010). Since 2013, an invasive aphid species caused significant economic damage to sorghum cultivars in the United States of America and Mexico (Villanueva et al. 2014, Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Medina et al. 2017). The aphid is a major insect pest of sorghum in both countries, because despite control, it can affect crops at all stages of development, cause yield loss as much as 100%, and affect the quality of harvested grain (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Medina et al. 2017). Taxonomic identification of the aphid is not clear; Peña-Martínez et al. (2016a) indicated the species related to sorghum and sugarcane (*Saccharum officinarum* L.) was a group formed by *Melanaphis sacchari* (Zehntner 1987) and *M. sorghi* (Theobal 1904). Both species are morphologically and genetically similar (Nibouche et al. 2014). Considering the taxonomic controversy and inadequate biological information, Pena-Martínez et al. (2015, 2016a) considered the aphid the "*M. sacchari/sorghi* complex". In this document, we used the name sugarcane aphid, *M. sacchari*. Since 2013, several studied the biology and management of the aphid in Mexico and the USA. This reviews current advances in distribution of sugarcane aphid in México and the USA, aspects of biology, and management with cultural strategies including sorghum genotypes with resistance to damage, use of biological control agents, and application of insecticide.

Geographical Distribution in Mexico and USA. Sugarcane aphid is native to Asia and Africa (Singh et al. 2004, Peña-Martínez et al. 2017), with distribution in tropical and subtropical regions of the world (Rodriguez-del-Bosque and Terán 2015, CABI 2019). In America, the aphid was first reported in Florida in 1977 and in Louisiana in 1999 (Denmark 1988, White et al. 2001). *M. sacchari* remained a

sporadic pest of sugarcane and was reported as a pest of sorghum in the USA in 2013. It was reported in 17 states including Arkansas, Florida, Georgia, Illinois, Kansas, Louisiana, Mississippi, Oklahoma, South Carolina, Tennessee, Texas, and Virginia (Villanueva et al. 2014, Bowling et al. 2016, Medina et al. 2017). In 2013, a significant outbreak of *M. sacchari* occurred at northern Tamaulipas, Mexico and caused loss as much as 100% (Maya and Rodríguez-del-Bosque 2014). Its range from 2015 to 2018 expanded to more than 90% of sorghum production areas in Mexico, being a pest of economic importance in the states of Campeche, Chiapas, Chihuahua, Coahuila, Colima, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Querétaro, Quintana Roo, San Luis Potosí, Sinaloa, Sonora, Tabasco, Tamaulipas, Veracruz, and Yucatán (Rodríguez del Bosque and Terán 2015, Moreno-Hernández et al. 2016, Salas-Marina et al. 2017, SENASICA 2019).

Taxonomic Classification. Blackman and Eastop, 2000; Center for Invasive Species and Ecosystem Health (2017) and Peña-Martínez et al. (2017) classified *M. sacchari* as: kingdom Animalia, phylum Arthropoda, subphylum Atelocerata, class Insecta, infraclass Neoptera, subclass Pterygota, order Hemiptera, infraorder Aphidomorpha, suborder Sternorrhyncha, superfamily Aphidoidea, family Aphididae, subfamily Aphidinae, tribe Aphidini, subtribe Rhopalosiphina, genera *Melanaphis* Van der Goot 1917, species *M. sacchari* (Zehntner 1897).

Biology and Life Cycle. According to Peña-Martínez et al. (2016b, 2017, and 2018) Reay-Jones and J. Greene, (2017) post-embryonic development of the sugarcane aphid consists of four juvenile stages (N_1, N_2, N_3, and N_4), alate nymph, super nymph, and adult (viviparous aptera or winged female and winged male). Most reproduction of the sugarcane aphid is parthenogenetic or asexual. Apterous females reproduce, and winged individuals disperse to new sorghum crops. Oviparous females also observed indicated the population reproduced sexually. In many regions of the world, sugarcane aphids have parthenogenetic reproduction, but also can have viviparous reproduction, with the life cycle described as either anholocyclic or holocyclic. Peña-Martínez (2016ab, 2017) described the morphology of each stage of development of the species, including the egg.

Temperature directly influences development of sugarcane aphids. Peña-Martinez et al. (2018) indicated that development of stages N_1 to N_4 required 69.7 to 0.7 days at 5 to 40°C. The reproductive period was 19 to 3 days at 10 to 35°C. Average fecundity was 147 female nymphs at 10 to 30°C. The minimum temperature for development of sugarcane aphids was 3.5°C, and the maximum temperature threshold was 40°C. The average time per generation (from birth to adult) was 34.0 to 8.6 days at 10 to 35°C.

Host Range. Sugarcane aphids are associated with two genera of Araceas and 23 of Poaceae (Denmark 1988, Peña-Martínez et al. 2015, Bowling et al. 2016), and since 2013 was an invasive pest threatening sorghum production in the USA and Mexico (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016). The species of aphid was observed in sugarcane and forage sorghum (*S. vulgare* Pers.) for more than a century, without considerable damage (White et al. 2001, Akbar et al. 2010, Moreno-Hernández et al. 2016). Genetic diversity of *M. sacchari* usually is low, and aphids on sugarcane and sorghum in the USA and Mexico seem to be genetically identical (Ibarra et al. 2016, Harris-Schultz et al. 2017, Medina et al. 2017). However, a new haplotype (Nibouche et al. 2014) was thought to be responsible for eruptive population dynamics of *M. sacchari* on sorghum.

Alternative hosts of sugarcane aphids include Johnsongrass (*Sorghum halepense* (L.) Pers.), widely distributed in agricultural areas of Mexico, cane or milpilla (*S. bicolor* (L.) Moench), and sorghum-sudan hybrids (Maya and Rodríguez-del-Bosque 2014, Rodríguez-del-Bosque and Terán 2015, Moreno-Hernández et al. 2016, Quijano-Carranza et al. 2017). Sugarcane aphids also were observed on but do not reproduce on maize and cotton (*Gossypium hirsutum* L.) (Bowling et al. 2016). Among other possible hosts of sugarcane aphids in Mexico are economically important crops such as oat, rice, barley, winter wheat, and pearl millet (*Pennisetum glaucum* (L.) R. Br.), but to date no evidence has been found of establishment in the crops.

Damage and Crop Loss. Sugarcane aphids infest sorghum at all stages of development (Bowling et al. 2016); however, damage depends of the number of aphids, duration of infestation, and resistance of sorghum genotypes (Singh et al. 2004, Moreno-Hernández et al. 2016). Infestation begins with aphid colonies on lower leaves; when abundant, the aphids colonize the upper leaves, stem, and even the panicle (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016). Damage is caused by nymphs and adults, that feed on sap in the phloem and xylem of the leaves. Feeding symptoms include yellow, red, purple, and brown lesions on foliage. Plants have less photosynthetic efficiency and more stress, chlorosis, signs of necrosis, premature death, and delayed development, flowering, and maturity (Singh et al. 2004, Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016). Infestation during early plant stages can result in no panicle or inadequate filling of grain.

Sugarcane aphids also vector millet red leaf virus, sugarcane yellow leaf virus, and sugarcane mosaic virus (Denmark 1988, Paray et al. 2011). Among indirect damage, the aphids secrete sticky honeydew on which black mold fungus grows; honeydew also affects the harvesting process, causing yield loss (Kerns et al. 2015). Honeydew in forage can affect cutting and packaging the crop for livestock feed, as well as quality (Bowling et al. 2016). Depending on control measures, yield loss can be 20 to 100% (van den Berg 2002, Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Quijano-Carranza et al. 2017) and also affect maltose content in grain from infested crops, even when chemical control is applied (van den Berg et al. 2003).

Management Strategies for Control of Sugarcane Aphid

Sampling Protocol. According to Bowling et al. (2015, 2016), to make management decisions, it is necessary to inspect foliage weekly after emergence of the crop. Sampling protocol involves inspections along edges to 10 m into a crop. At each sampling site, 15-20 plants should be randomly selected in 15 linear meters. To determine the average number of aphids in a field, sample leaves in both the upper and lower canopies and calculate the average number of aphids using a scale by Bowling et al. (2015). Abundance in the field is determined by the equation:

Field average = (total of all estimates / total number of leaves examined)

If average infestation is more than 50 aphids per leaf, control including insecticide should be applied. If infestation is minor, it is necessary to sample twice per week. Quijano-Carranza et al. (2017) indicated it was important to verify the presence of natural enemies associated with sugarcane aphids; if predators and parasitoids are scarce, entomopathogenic fungi, agricultural soap, or non-toxic products can be applied.

In México, Silva-Martínez et al. (2019) recommended sampling 39 leaves for aphids to estimate infestation with 80% reliability. Leaves were collected from the

upper canopy of forage sorghum, and the size of the sample was determined by a mathematical model.

Cultural Control. As prevention to manage sugarcane aphids, destruction of any source of infection before planting the sorghum crop is recommended to remove residues from previous harvest, volunteer sorghum plants, and Johnson grass, and avoid outbreak from previous harvest (Rodríguez-del-Bosque and Teran 2015, Bowling et al. 2016). These actions help to obtain new fields free from sugarcane aphids and shelter during early stages of crop development (Moreno-Hernández et al. 2016, Quijano-Carranza et al. 2017).

According to Quijano-Carranza et al. (2017) if the crop is sown by conventional tillage, fallow should be used immediately after harvest to eliminate residue. In crops established by conservation tillage, it is advisable to apply herbicide to prevent proliferation of volunteer sorghum plants. Burning crop residue is not recommended, because regrowth of sorghum quickly creates shelters for sugarcane aphids (Quijano-Carranza et al. 2017). At Sinaloa, vegetable farmers use Sudan grass (*S. sudanense*) as a living barrier in production plots; this regulates grass and keeps production units free of sugarcane aphids (Orduño-Cota et al. 2016). Using yellow trays with soapy water to capture dispersing aphids can be effective for monitoring arrival of winged adults to sorghum crops (Marín-Jarillo 2016). Fertilization of sorghum also is important. Application of excessive nitrogen promotes more free amino acids that attract insects that infest sorghum (Tanzubil 2014). Archer et al. (1982) found greater abundance and increased reproduction of greenbug, *Schizaphis graminum* (Rondani), in relation to increased nitrogen applied to soil. For this reason, it is important to use adequate crop nutrition based on soil analysis, use of slow-release nitrogen, and avoid excessive fertilization with nitrogen (Moreno-Hernández et al. 2016).

Rodriguez-del-Bosque and Terán (2018) evaluated effects of rain, wind, wind gusts, and soil moisture on abundance of sugarcane aphids in sorghum. Abundance of the aphid in sorghum crops at northern Tamaulipas decreased from three to 38% with rainfall of 18 to 51 mm hour^{-1}. However, simulations of rain and wind in combination with gusts of more than 70 km hr^{-1} indicated biotic factors might be more effective in naturally reducing abundant sugarcane aphids on the bottom of leaves. Survival of aphids that fall to the ground because of weather factors varies in soils with humidity of 12% (field capacity) and in flooded soil where mortality was greater. Abundance is influenced by temperature and rainfall. Sugarcane aphid abundance increases rapidly during warm, dry weather (Singh et al. 2004) common in many sorghum-producing regions of North America. Wind-assisted movement probably influences local and long-distance dispersal of alate sugarcane aphids. Once established, a colony can increase exponentially during sorghum crop development.

Legal and Regulatory Control. At Guanajuato, México, the Ministry of Agri-Food and Rural Development (SDAyR) together with the National Service for Health, Safety and Agri-Food Quality (SENASICA) since 2017 authorized establishment of official dates for sowing sorghum. The period is 15 April to 15 June for irrigated sorghum and 15 April to 5 July for dryland. The management practice was established based on the Official Mexican Standard NOM-081-FITO-2001 that indicated regulatory control for management and elimination of refuges by establishment, or reorganizing dates of planting, harvesting, and destruction of crop residue (SDAyR 2018).

Plant Resistance. Host resistance was successfully developed to alleviate the impact of sugarcane aphids on sorghum and proven to be effective at suppression

in the field (Smith et al. 1994, Kiani and Szczepaniec 2018, Szczepaniec 2018). Resistance of a genotype to damage, by pests is characterized by antixenosis (infestation on a genotype is regulated by characters of the plant and adverse response of a pest to the factors), antibiosis (when the pest prefers a host plant that causes adverse effects such as abnormal growth, reduced fertility, smaller size, and increased mortality of the pest), and tolerance (the genotype has normal development and yield despite damage that would cause loss to a susceptible plant) (Horber et al. 1980, Smith et al. 1994, Armstrong et al. 2015). Studies determined response of sorghum genotypes (parental lines, hybrids, and commercial varieties) to natural infestation by sugarcane aphids in agricultural regions of the USA and Mexico (Armstrong et al. 2015, Mbulwe et al. 2016, Pecina-Quintero et al. 2016, Perales-Rosas et al. 2017, Rodriguez-del-Bosque and Terán 2018). In Texas, Armstrong et al. (2015) reported high to moderate resistance to *M. sacchari* in genotypes B11070, AB11055-WF1-CS1/RTx436, AB11055-WF1-CS1/RTx437SC110, SC170, Ent62/ SADC, (Macia/TAM428)-LL9, (SV1*Sima/IS23250)-LG15, and Tam428. Mbulwe et al. (2016) and Hayes et al. (2019) developed lines Tx3408 (ATx3408, Reg. No. GP-838, PI 675360; BTx3408, Reg. No. GP-839, PI 675361), Tx3409 (ATx3409, Reg. No. GP- 840, PI 675362; BTx3409, Reg. No. GP-841, PI 675363), R.LBK1 (Reg. No. GP-865, PI 687244), and R.LBK2 (Reg. No. GP-866, PI 687245) very resistant to sugarcane aphids. Peterson et al. (2018) created germplasm lines RTx3410 to RTx3428 evaluated in Texas and African countries that resisted damage by *M. sacchari*. Limaje et al. (2018) observed tolerance in pollinating lines R.11143 and R.11259 developed by the USDA-ARS at Lubbock, TX. Resistance was attributed to antibiosis because of fewer nymphs and less intrinsic rate of increase, reproductive period, and net reproduction compared with other sorghum lines. Tetreault et al. (2019) compared response between a resistant (RTx2783) and susceptible line (A / BCK60). The susceptible genotype had more gene expression related to cell wall modification, photosynthesis, and phytohormone biosynthesis, while genes associated with sugar and starch content were suppressed in the resistant line. Based on an electrical penetration graph test, aphids spent less time ingesting phloem on resistant than susceptible plants. The resistant line had antixenosis and antibiosis.

Evaluation in various agricultural regions of México identified diversity of commercial hybrids with resistance to sugarcane aphids. Gastélum-Luque et al. (2015) indicated fewest aphids on leaves of DKS 26, DKS 2805, Gaylan GW 9320, Perla 101, Gavatero 203, Sinaloense 202, Pioneer 85P15, and Asgrow AZ 400. Grain of DKS 26, DKS 2805, Gaylan GW 9320, Asgrow AZ 400, and Pioneer 85P15 was less damaged (less than 26%). In the Bajio region at Guanajuato, Pecina-Quintero et al. (2016) reported 10 hybrids of intermediate and late vegetative stage with high yield and tolerance to natural infestations by sugarcane aphids evaluated four times at sowing (Table 1). Yield of all genotypes was reduced at each planting date. No tolerance was observed in early stage vegetative hybrids. Perales-Rosas et al. (2017) observed few *M. sacchari* on commercial hybrids P15, Ambar, and DKS-46 evaluated under natural conditions at Morelos. North of Tamaulipas, the main sorghum-producing State of Mexico, Rodriguez-del-Bosque and Terán (2018) observed non-preference and antibiosis in commercial hybrids such as Mezquite that is resistant to sugarcane aphids. G-Star 7208, Supremo 1672, Asgrow Pulsar, QL50, WS 2206, and Pioneer 83P56 also had resistance to *M. sacchari*.

Biological Control. Among the main species of natural enemies associated with sugarcane aphids are *Hippodamia convergens* (Guerin), *Harmonia axyridis*

Table 1. Commercial Hybrids of Sorghum with Tolerance to Sugarcane Aphid in the Bajio Region, Guanajuato, Mexico[a]

Commercial hybrid	Seed company	Vegetative cycle[b]
Apache	ConLee Seeds	Late
San Jeronimo	Cincinnati Seeds	Late
BRS 70	DeKalb	Late
DKS 48	DeKalb	Late
Aguila Real	Seeds Producers Azteca	Late
M-550	Majestic Seeds	Intermediate
Mezquite	Avante Seeds	Intermediate
SG – 673	Sun Rey Seeds	Late
GSTAR 7205	G-Star	Intermediate
NUS 500	Nuseed	Intermediate

[a]Evaluated at Celaya, Guanajuato, during the spring-summer cycle of 2016.
[b]In early cycle hybrids, no resistance was observed.

(Pallas), *Cycloneda sanguinea* (L.), *Diomus terminatus* (Say), *D. roseicollis* (Mulsant), *Scymnus loewii* (Mulsant), *Coleomegilla maculata* (DeGeer), *Coccinella septempunctata* (L.), *Brachiacantha decora* (Casey), *Hyperaspis wickhami* (Casey), *Olla-v-nigrum* (Mulsant), *Chilocorus cacti* (L.), and *Ch. stigma* (Say) (Bowling et al. 2016; Cortez-Mondaca et al. 2016; Lomeli-Flores et al. 2016; Marín and Herrera 2016; Rodríguez-Velez et al. 2016; Jaimes-Orduña et al. 2018, 2020). The species affect sugarcane aphids when abundant in sorghum crops (Rodriguez-del-Bosque and Terán 2018). Species of the family Chrysopidae including *Ceraeochrysa* sp. nr. *cincta* (Schneider), *C. caligata* (Banks), *C. cubana* (Hagen), *C. valida* (Banks), *Chrysopa quadripunctata* (Burmeister), *Chrysoperla carnea s. lat.* (Stephens), *Ch. externa* (Hagen), *Ch. comanche* (Banks), *Ch. plorabunda* (Fitch), and *Ch. rufilabris* (Burmeister) also affect aphids (Bowling et al. 2016, Cortez-Mondaca et al. 2016, Lomelí-Flores et al. 2016, Marín and Herrera 2016, Salas-Marina et al. 2017). *Micromus subanticus* (Walker) and *M. posticus* (Walker) are the only species of Hemerobiidae reported as predators of *M. sacchari* (Bowling et al. 2016, Almaraz-Valle et al. 2020). Other predators are flies, *Allograpta obliqua* (Say), *A. exótica* (Wiedemann), *Pseudodoros clavatus* (Fabricius), *Eupeodes americanus* (Wiedemann), *Palpada vinetorum* (Fabricius), *Toxomerus politus* (Say), *T. marginatus* (Say) (Bowling et al. 2016, Cortez-Mondaca et al. 2016, Marín and Herrera 2016) and minute pirate bug, *Orius insidiosus* (Say) that is the only hemipteran predator affecting *M. sacchari* (Colares et al. 2015a, Bowling et al. 2016).

Studies in laboratories in the USA indicated *H. convergens*, *C. maculata*, *Ch. carnea*, *O. insidiosus*, and *D. terminatus* had potential to be used in a biological control program against sugarcane aphids (Akbar et al. 2009, Colares et al. 2015a). Under natural conditions, Rodriguez-del-Bosque and Terán (2018) observed at northern Tamaulipas, México, that a specimen of Coccinellidae consumed 11 sugarcane aphids per hour; however, natural control of the pest occurs after the crop is damaged. In the Bajio region, Lomeli-Flores et al. (2016) investigated the possibility of using biological control by releasing 10,000 eggs of *C. carnea* per hectare five times during the vegetative stage of the crop. Releases of *C. carnea* failed to reduce or control sugarcane aphids in experimental plots. At Sinaloa, México, Cortez-Mondaca et al. (2016) observed eight Chrysopidae species associated with *M. sacchari.* Most frequent and abundant in sugarcane aphid

colonies was *C. cubana* they recommended as a biological control agent, but the species is not commercially available.

Among parasitoids in the USA, Bowling et al. (2016) documented *Aphelinus* sp. *varipes* group (Foerster) and *Lysiphlebus testaceipes* (Cresson) in fields, while *Syrphophagus aphidivorus* (Mayr) was a hyperparasitoid. In México; García-González et al. (2018) reported in the Laguna region *A. varipes* and *L. testaceipes* as parasitoids of sugarcane aphid, while *S. aphidiovorus*, *Pachyneuron aphidis* (Bouche), and *Alloxysta* sp. were hyperparasitoids. Natural parasitism by the specimens in the region was 0.4 to 12.8%. In the Bajio region, Salas-Araiza et al. (2017) reported low emergence of adult wasps from parasitized aphids collected in the field. The genera were *Praon* spp., *Binodoxys* spp., *Diaretiella* spp., and *Aphelinus* spp, while Jaimes-Orduña et al. (2020) reported *Aphidius* sp. and *Pachyneuron* sp. at Nuevo Leon. In some regions, symbiotic organisms in sugarcane aphid populations confer protection against local strains of *L. testaceipes*, and thus could impede biological control where the wasp is a key mortality factor (Colares et al. 2015b, Peña-Martinez et al. 2017).

Entomopathogenic fungi kill many aphids (Steinkraus 2006, Manfrino et al. 2014). The first reports of entomopathogenic fungi associated with sugarcane aphids indicated *Lecanicillium* (=*Verticillium*) *lecanii* (Zimm.) Viegas (Hypocreales: Clavicipitaceae) caused epizootics in sugarcane aphids in Florida (Hall 1987). Currently, Haar et al. (2018) observed the species causing epizootics in the states of Texas and Georgia. In México, *L. longisporum* (*Zare and Gams*), *B. bassiana* (Bals.-Criv.) Vuili, and *I. javanica* (Friedrichs and Bally) (Hypocreales: Clavicipitaceae) infected *M. sacchari* at Colima, Guanajuato, and Tamaulipas (Berlanga-Padilla et al. 2016; Zambrano-Gutiérrez et al. 2019). In all states, most infection was by *L. longisporum* causing 30.0 to 90.0% (Berlanga-Padilla et al. 2016; Zambrano-Gutiérrez et al. 2019). At Guanajuato, *Conidiobolus thromboides* (Drechsler) (Entomophthorales: Entomophthoraceae) was widely distributed during 2016, infecting 2.0 to 98.0% (Alatorre-Rosas et al. 2016). *C. thromboides* disperses by spores expelled under pressure from the cadaver of an aphid or by dispersal of sick, winged adults. This favored epizootic levels in *M. sacchari* in most sorghum-growing areas of the state. Research on entomopathogenic fungi should continue because fungal isolates vary in response to temperature, which complicates use of the living organisms in the field. Effects of environmental conditions, specificity of the host, and application techniques are important when selecting isolates as biological control agents against sugarcane aphids.

Chemical Control. In both countries, the first practice for management of sugarcane aphids is treating seed with clothianidin, thiamethoxam, or a mixture of imidacloprid with bifenthrin to help control early infestation; these insecticides protect the crop for 30 days (Bowling et al. 2016, Bujanos-Muñiz et al. 2016). Based on evaluation in the field, insecticides with efficacy greater than 90% were afidopyropen, fluopyradifurone, imidacloprid, metamidofos, pymetrozine, spirotetramat, sulfoxaflor, and thiamethoxam applied to foliage (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Bujanos-Muñiz et al. 2016, Tejeda-Reyes et al. 2017, Perales-Rosas et al. 2017). Enough water to coat the crop is necessary for effective chemical control, with 200 liters ha^{-1} recommended for ground application and 50 liters ha^{-1} for application by air (Rodriguez-del-Bosque and Terán 2018). Other important factors are pH of water (5.5 to 7.0), calibration of equipment, not applying with wind greater than 10 km per hour, proper functioning of nozzles, and considering use of mineral agricultural oil to prevent evapotranspiration and drag of the product (Quijano-

Carranza et al. 2017, Tejeda-Reyes et al. 2017, Rodriguez del Bosque and Terán-Vargas 2018).

Future Research

Application of insecticide is the main strategy for management of sugarcane aphids. However, because of selection pressure, resistance to insecticides to control *M. sacchari* should be monitored. Considering low yield and profitability of sorghum, it is important to design a regional strategy for integrated management using natural enemies and sorghum with resistance to sugarcane aphids. Varieties, hybrids, and parental lines with resistance to *M. sacchari* have been identified and must be promoted for planting in the main sorghum-producing regions in Mexico and the USA. Several species of parasitoids and predators help regulate *M. sacchari*, but because of rapid reproductive rate of the aphid, predators and parasitoids alone are not sufficient. Selecting species with high reproductive rates and capacity for parasitism or predation under field conditions is necessary. Molecular studies are important for determining symbionts associated with sugarcane aphids; the organisms help aphids evade infection by pathogens or attack by parasitoids. Several species of entomopathogenic fungi naturally infect *M. sacchari*. In Mexico, species formulated as micoinsecticides were applied in experimental plots by flood, but their effectiveness and persistence in sugarcane aphid populations have not been demonstrated. It is important to consider interactions of all factors that favor disease development, and transmission of pathogens in sugarcane aphid populations and development of epizootics. Considering predators, parasitoids, and entomopathogenic fungi are living organisms, it is important to select entomophagous insects and pathogens that quickly kill, persist in the environment or host plant, and regulate the pest insect. Determining interaction of environmental factors, biotic and abiotic, and timing application are necessary to favor control by biological agents.

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References Cited

Akbar, W., C. Carlton, and T. E. Reagan. 2009. Life cycle and larval morphology of *Diomus terminatus* (Coleoptera: Coccinellidae) and its potential as a biological control agent of *Melanaphis sacchari* (Hemiptera: Aphididae). Ann. Entomol. Soc. Am. 102: 96-103.

Akbar, W., A. T. Showler, T. E. Reagan, and W. H. White. 2010. Categorizing sugarcane cultivar resistance to the sugarcane aphid and yellow sugarcane aphid (Hemiptera: Aphididae). J. Econ. Entomol. 103: 1431-1437.

Alatorre-Rosas, R., J. Zambrano-Gutiérrez, F. Tamayo-Mejía, and O. D. Castillo-Ramírez. 2016. Microbial insecticides an alternative for regulation of populations of *Melanaphis sacchari* in Guanajuato, pp. 54-74. *In* R. Yáñez-López [ed.], Advances in Research on the Integrated Management of Sugarcane Aphid. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. ISBN: 978-607-96123-2-0.

Almaraz-Valle, V. M., J. R. Lomeli-Flores, E. Rodríguez-Leyva, J. M. Vázquez-Navarro, and J. M. Vanegas-Rico. 2020. Two new species of brown lacewings as predators of *Melanaphis sacchari* in Central Mexico. J. Agric. Urban Entomol. 36: 64-69. https://doi.org/10.3954/1523-5475-36.1.64

Archer, T. L., A. B. Onken, R. L. Matheson, and E. D. Bynum Jr. 1982. Nitrogen fertilizer influence on greenbug (Homoptera: Aphididae) dynamics and damage to sorghum. J. Econ. Entomol. 75: 695-698.

Armstrong, J. S., W. L. Rooney, G. C. Peterson, R. T. Villanueva, M. J. Brewer, and D. Ortiz-Sekula. 2015. Sugarcane aphid (Hemiptera: Aphididae): host range and sorghum resistance including cross-resistance from greenbug sources. J. Econ. Entomol. 108: 576-582.

Berlanga-Padilla, A. M., M. A. Ayala-Zermeño, A. Gallou, M. G. Serna-Domínguez, R. Montesinos-Matías, and H. C. Arredondo-Bernal. 2016. Identificación de *Lecanicillium longisporum* asociado a *Melanaphis sacchari* (Hemiptera: Aphididae), en sorgo. Revista Mexicana de Micologia 44: 51-54.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the World Crops. An Identification and Information Guide. 2nd ed. John Wiley Sons.

Bowling, R., M. Brewer, A. Kutson, S. Biles, M. Way, and D. Sekula-Ortiz. 2015. Monitoring yellow aphid in sorghum in South, Central, and West Texas. Texas A&M Agrilife Ext. Serv. agrilife.org/ccag/files/2016/04/NTO043S.pdf.

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliott, G. D. Buntin, M. O. Way, T. A. Royer, S. Biles, and E. Maxson. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. J Integrated Pest Management 7: 1-13. http://dx.doi.org/10.1093/jipm/pmw011.

CABI. 2019. Invasive Species Compendium. *Melanaphis sacchari* (yellow sugarcane aphid). Consulted online 20 January 2019.

Center for Invasive Species and Ecosystem Health. 2017. Invasive and Exotic Species Profiles and State, Regional and National Lists. https://www.invasive.org/species.cfm. Consulted online 20 November 2017.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2105a. Indigenous aphid predators show high levels of preadaptation to a novel prey, *Melanaphis sacchari* (Hemiptera: Aphididae). J. Econ. Entomol. 108: 2546-2555.

Colares, F., J. P. Michaud, C. L. Bain, and J. B. Torres. 2015b. Recruitment of aphidophagus arthropods to sorghum plants infested with *Melanaphis sacchari* and *Schizaphis graminium* (Hemiptera: Aphididae). Biological Control 90: 16-24.

Cortez-Mondaca, E., M. López-Buitimea, J. I. López-Arroyo, F. J. Orduño-Cota, and G. Herrera-Rodríguez. 2016. Chrysopidae species associated with the sugarcane aphid in northern Sinaloa, Mexico. Southwest. Entomol. 41: 540-546.

Denmark, H. A. 1988. Sugarcane aphids in Florida (Homoptera: Aphididiae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Entomology Circular 302.

García-González, F., M. Ramírez-Delgado, H. Cortez-Madrigal, M. de L. Ramírez-Ahuja. 2018. New reports of parasitoids and hyperparasitoids of *Melanaphis sacchari* (Zehntner) in México. Southwest Entomol. 43: 787-790.

Gastélum-Luque, R., T. P. Godoy-Angulo, M. López-Meza, J. F. Inzunza-Castro, y M. G. Yañez-Juárez. 2015. Respuesta de variedades e híbridos de sorgo para grano al pulgón amarillo *Melanaphis sacchari* (Zehntner) en el Valle de

Culiacán, Sinaloa. Informe Técnico. Universidad Autónoma de Sinaloa-Facultad de Agronomía.
Haar, P. J., R. Bowling, W. A. Gardner, and G. D. Buntin. 2018. Epizootics of the entomopathogenic fungus *Lecanicillium lecanii* (Hypocreales: Clavicipitaceae) in sugarcane aphid (Hemiptera: Aphididae) populations infesting grain sorghum in Georgia and Texas. J. Entomol. Sci. 53: 104-106.
Hall, D. G. 1987. The sugarcane aphid, *Melanaphis sacchari* (Zehntner), in Florida sugarcane. Journal of American Society of Sugarcane Technologists 7: 26-29.
Harris-Shultz, K., X. Ni, P. A. Wadl, X. Wang, H. Wang, F. Huang, K. Flanders, N. Seiter, D. Kerns, R. Meagher, Q. Xue, D. Reisig, D. Buntin, H. E. Cuevas, M. J. Brewer, and X. Yang. 2017. Microsatellite markers reveal a predominant sugarcane aphid (Homoptera: Aphididae) clone is found on sorghum in seven states and one territory of the USA. Crop Sci. 57: 2064-2072.
Hayes, C. M., J. S. Armstrong, A. Limaje, Y. E. Emendack, S. Bean, J. Wilson, and Z. Xin. 2019. Registration of R.LBK1 and R.LBK2 sorghum germplasm with resistance to the sugarcane aphid [*Melanaphis sacchari* (Zehntner)]. J. Plant Registrations 13: 91-95.
Ibarra, J. E., R. García-Suárez, J. Luévano-Borroel, y R. Peña-Martínez. 2016. Identificación molecular de biotipos del pulgón amarillo del sorgo, *Melanaphis sacchari*, en el estado de Guanajuato. 2016 c, pp. 11-24. *En* R. Yañez-López [ed.], Memoria del Simposio Avances en la Investigación del Manejo Integrado del Pulgón Amarillo del Sorgo en Guanajuato. ISBN: 978-607-96123-2-0.
Kerns, D. L., S. Brown, J. Beuzelin, and K. M. Guidry. 2015. Sugarcane aphid: a new invasive pest of sorghum. Louisiana Agriculture 58: 12-14.
Kiani, M., and A. Szczepaniec. 2018. Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. BMC Genomics 19: 1-18.
Limaje, A., C. Hayes, J. S. Armstrong, W. Hoback, A. Zarrabi, S. Paudyal, and J. Burke. 2018. Antibiosis and tolerance discovered in USDA-ARS sorghums resistant to the sugarcane aphid (Hemiptera: Aphididae). J. Entomol. Sci. 53: 230-241.
Lomelí-Flores, J. R., E. Rodríguez-Leyva, H. González-Hernández, and J. M. Vanegas-Rico. 2016. Evaluations and recommendations for the extensive use of predators in the management of sugarcane aphid, pp. 54-74. *In* R. Yáñez-López [ed.], Advances in Research on the Integrated Management of Sugarcane Aphid. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. ISBN: 978-607-96123-2-0.
Manfrino, R. G., L. Zumoffen, C. E. Salto, and L. C. C. Lopez. 2014. Natural occurrence of entomophthoroid fungi of aphid pests on *Medicago sativa* L. in Argentina. Revista Argentina de Microbiología 46: 49-52. https://doi.org/10.1016/S0325-7541(14)70048-3
Marín-Jarillo, A., and C. C. Herrera. 2016. Natural enemies associated with sugarcane aphid *Melanaphis sacchari* (Zehntner) Hemiptera: Aphididae) and its population fluctuation in the Bajio, pp. 42-52. *In* R. Yáñez-López [ed.], Advances in Research on the Integrated Management of Sugarcane Aphid. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. ISBN: 978-607-96123-2-0.
Maya, H. V., and L. Rodríguez del Bosque. 2014. Sugarcane aphid: new pest of sorghum crop in Tamaulipas. Drop-down for producers. MX-0-31034-45-03-13-12-30. INIFAP-CIRNE-Campo Experimental Rio Bravo.

Mbulwe, L., G. C. Peterson, J. S. Armstrong, and W. L. Rooney. 2016. Registration of sorghum germplasm Tx3408 and Tx3409 with tolerance to sugarcane aphid [*Melanaphis sacchari* (Zehntner)]. Journal of Plant Registrations 10: 51-56.

Medina, F. R., J. S. Armstrong, and K. Harrison. 2017. Genetic population structure of sugarcane aphid, *Melanaphis sacchari*, in sorghum, sugarcane, and Johnson grass in the continental USA. Entomol. Exp Appl. 162: 358-365.

Moreno-Hernández. J. M., E. Cortez-Mondaca, T. Moreno-Gallegos, J. A. López-Guzmán, and J. Márquez-Pérez. 2016. Production of seasonal sorghum in Sinaloa against sugarcane aphid *Melanaphis sacchari* Zehntner. INIFAP-CIRNO. Campo Experimental Valle de Culiacán. Folleto Técnico 62. ISBN 978-607-37-0618-6.

Nibouche, S., B. Fartek, S. Mississipi, H. Delatte, B. Reynaud, and L. Costet. 2014. Low genetic diversity in *Melanaphis sacchari* aphid populations at the worldwide scale. Plos One 9: e106067.

Paray, N. B., M. H. R. Khoodoo, A. S. Saumtally, and S. Ganeshan. 2011. Vector-virus relationship for *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) transmitting sugarcane yellow leaf luteovirus in Mauritius. Sugar Technology 13: 77-80. https://doi.org/10.1007/s12355-010-0058-9

Pecina-Quintero, V., C. Herrera-Corredor, R. Bújanos-Muñiz, and M. Hernández-Martínez. 2016. Evaluation of the agronomic behavior and resistance of commercial hybrids of sorghum to sugarcane aphid *Melanaphis sacchari* (Zehntner) at different planting dates in Guanajuato. *In*: Yañez-Lopez, R. (Ed). Advances in research on the integrated management of sugarcane aphid. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. 130-137. ISBN: 978-607-96123-2-0.

Peña-Martínez, R., A. L. Muñoz-Viveros, Ma. G. Ramos-Espinosa, and R. Terrón-Sierra. 2015. List of host plants of the *Melanaphis sacchari/sorghi* complex (Hemiptera: Aphididiae), international and potential registrations in Mexico. Entomología Mexicana 2: 582-587.

Peña-Martínez, R., A. L. Muñoz-Viveros, R. Bújanos-Muñiz, J. Luévano-Borroel, F. Tamayo-Mejía, and E. Cortez-Mondaca. 2016a. Sexual forms of sorghum aphid complex *Melanaphis sacchari/sorghi* in Mexico. Southwest. Entomol. 41: 127-132.

Peña-Martínez, R., A. L. Muñoz-Viveros, A. Marín-Jarillo, R. Bújanos-Muñiz, J. Luévano-Borroel, and J. Ibarra-Rendón. 2016b. Biology and morphology of sugarcane aphid, *Melanaphis sacchari/sorghi* complex (Hemiptera: Aphididae) in the state of Guanajuato, Mexico, pp. 26-40. *In* R. Yáñez-López [ed.], Advances in Research on the Integrated Management of Sugarcane Aphid. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. ISBN: 978-607-96123-2-0.

Peña-Martínez, R., A. L. Muñoz-Viveros, A. Marín-Jarillo, R. Bújanos-Muñiz, F. Tamayo-Mejía, J. Luévano-Borroel, L. Sánchez-Segura, and J. Ibarra-Rendón. 2017. Illustrated guide for identification of aphids (Hemiptera: Aphididae) of cereals in Mexico. Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México. ISBN: 978-607-96123-3-7.

Peña-Martínez, R., J. R. Lomelí-Flores, R. Bújanos-Muñiz, A. L. Muñoz-Viveros, J. M. Vanegas-Rico, R. Salas-Monzón, O. E. Hernández-Torres, A. Marín-Jarillo, y J. E. Ibarra-Rendón. 2018. Pulgón amarillo del sorgo (PAS), *Melanaphis sacchari* (Zehntner, 1897), interrogantes biológicas y tablas de vida.

Fundacion Guanajuato Produce, A. C. Celaya, Guanajuato, México ISBN: 978-607-96123-8-2.
Perales-Rosas, D., D. Guillén-Sánchez, V. López-Martínez, M. Andrade-Rodríguez, I. Alia-Tejacal, R. Hernández-Pérez, and P. Juárez-López. 2017. Performance of *Sorghum bicolor* (L.) Moench hybrids against *Melanaphis sacchari/sorghi* in Morelos, México. Southwest. Entomol. 42: 815-820.
Pérez, A., O. Saucedo, J. Iglesias, H. B. Wencomo, F. Reyes, G. Oquendo, and I. Milián. 2010. Characterization and potentialities of sorghum grain (*Sorghum bicolor* L. Moench). Pastos y Forrajes 33: 1-14.
Peterson, G. C., J. S. Armstrong, B. B. Pendleton, M. Stelter, and M. J. Brewer. 2018. Registration of RTx3410 through RTx3428 sorghum germplasm resistant to sugarcane aphid [*Melanaphis sacchari* (Zehntner)]. J. Plant Registrations 12: 391-398.
Quijano-Carranza, J. A., V. Pecina-Quintero, R. Bújanos-Muñiz, A. Marín-Jarillo, and R. Yáñez-López. 2017. Guide 2017 for the management of sugarcane aphid. Folleto para Productores N°1. Fundacion Produce Guanajuato, A. C. Celaya, Guanajuato, México.
Reay-Jones, F., and J. Greene. 2017. Sugarcane aphid (*Melanaphis sacchari*) – a new pest of sorghum. Clemson University Cooperative Extension Service.
Rodríguez-del-Bosque, L. A., and A. Terán. 2015. *Melanaphis sacchari* (Hemíptera: Aphididae): a new sorghum insect pest in Mexico. Southwest. Entomol. 40: 433-434.
Rodríguez-del-Bosque, L. A., and V. A. P. Terán. 2018. Manejo integrado del pulgón amarillo del sorgo en Tamaulipas. Folleto Técnico MX-0-310304-45-03-13-09-72. INIFAP-CIRNE-Campo Experimental Rio Bravo. ISBN: 978-607-37-0976-7.
Rodríguez-Vélez, J. M., B. Rodríguez-Vélez, M. A. Sarmiento-Cordero, M. Palomares-Pérez, and H. C. Arredondo-Bernal. 2016. Species of Coccinellidae (Coleoptera: Cucujoidea) associated with *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae) in Tamaulipas, Mexico. Entomol. News 126: 97-105. https://doi.org/10.3157/021.126.0205
Salas-Marina, M. A., V. Hernández-García, C. Ríos-Velasco, N. Lule-Chávez, J. A. Castillo-Mauricio, and M. H. Chávez-Hernández. 2017. *Melanaphis sacchari*, a new pest of *Sorghum halepense* at Chiapas, México, and its predators. Southwest. Entomol. 42: 401-404.
SDAyR (Secretaría de Desarrollo Agroalimentario and Rural). 2018. http://boletines.guanajuato.gob.mx/2016/03/04/sdayr-sagarpa-y-senasica. danaconocer-accionescontrapulgon-amarillo-del-sorgo Consulted 11 January 2018.
SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria). 2019. Pulgón amarillo del sorgo. https://www.gob.mx/senasica/documentos/pulgon-amarillo-del-sorgo-110905 Consulted 20 January 2019.
Singh, B. U., P. G. Padmaja, and N. Seetharama. 2004. Biology and management of the sugarcane aphis, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Prot. 23: 739-755.
Smith, C. M., Z. R. Khan, and M. D. Pathak. 1994. Techniques for Evaluating Insect Resistance in Crop Plants. CRC Press, Boca Raton, FL.
Steinkraus, D. C. 2006. Factors affecting transmission of fungal pathogens of aphids. Journal of Invertebrate Pathology 92: 125-131.

Szczepaniec, A. 2018. Interactive effects of crop variety, insecticide seed treatment, and planting date on population dynamics of sugarcane aphid (*Melanaphis sacchari*) and their predators in late-colonization sorghum. Crop Prot. 109: 72-79.

Tanzubil, P. B. 2014. Effect of variety and nitrogen fertilization on insect pest incidence in sorghum in the Sudan Savanna of Ghana. J. Entomol. Zool. Studies 2: 12-15.

Tejeda-Reyes, M. A., J. F. Diaz-Nájera, J. C. Rodríguez-Maciel, M. Vargas-Hernández, J. F. Solís-Aguilar, S Ayvar-Serna, and J. A. Flores-Yáñez. 2017. Evaluation in field of insecticides on *Melanaphis sacchari* (Zehntner) in sorghum. Southwest. Entomol. 42: 545-550.

Tetreault, H. M., S. Grover, E. D. Scully, T. Gries, N. A. Palmer, G. Sarath, J. Louis, and S. E. Sattler. 2019. Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). Front. Plant Sci. 10: 1-19. https://doi.org/10.3389/fpls.2019.00145

van den Berg, J. 2002. Status of resistance of sorghum hybrids to the aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae). South African Journal of Plant and Soil 19: 151-155.

van den Berg, J., A. J. Preotorius, and M. van Loggerenberg. 2003. Effect of leaf feeding by *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), on sorghum grain quality. South African Journal of Plant and Soil 20: 41-43.

Villanueva, R. T., M. Brewer, M. O. Way, S. Biles, D. Sekula, E. Bynum, J. Swart, C. Crumley, A. Knutson, P. Porter, R. Parker, G. Odvody, C. Allen, D. Ragsdale, W. Rooney, G. Peterson, D. Kerns, T. Royer, and S. Armstrong. 2014. Sugarcane aphid: a new pest of sorghum. Texas A&M Agrilife Extension. Ento-035. Weslaco, TX.

White, W., R. Reagan, and D. Hall. 2001. *Melanaphis sacchari* (Homoptera: Aphididae), a sugarcane pest new to Louisiana. Fla. Entomol. 84: 435-436.

Zambrano-Gutiérrez, J., R. Alatorre-Rosas, M. G. Carrillo-Benítez, J. R. Lomelí-Flores, R. A. Guzmán-Plazola, A. Azuara-Domínguez, and A. P. Terán-Vargas. 2019. Species diversity of entomopathogenic fungi infecting the sugarcane aphid *Melanaphis sacchari*, a recently introduced pest in Mexico. Advances in Microbiology 9: 38-55. https://doi.org/10.4236/aim.2019.91004

SCIENTIFIC NOTE

First Record of *Melanaphis sacchari*[1] in Paraguay

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Ruth Fabiola Scholz Drodowski[4]

Abstract. We report the first record of *Melanaphis sacchari* (Zehntner 1897) (Hemíptera: Aphididae) attacking the sorghum (*Sorghum bicolor* L. Moench) crop in Paraguay.

Melanaphi sacchari (Zehntner 1897) (Hemiptera: Aphididae) is one of the main pests of sorghum (*Sorghum bicolor* (L.) Moench) in the Americas (Zapata et al. 2016). In 2018, it was classified as a "super clone" because of its specificity with cultivation of sorghum (Nibouche et al. 2018). The aphid and can affect all stages of development of sorghum, causing severe damage by feeding on sap of the plant, causing chlorosis and later necrosis of leaves by direct damage, while indirect damage is caused by development of sooty mold on honeydew excreted by aphids on foliage, which decreases photosynthetic activity (Bowling et al. 2016, Peña-Martinez et al. 2018). The damage decreases yield potential, and some authors reported losses of 100% if management was not used (Rodríguez-del-Bosque and Terán 2015, Bowling et al. 2016, Knutson et al. 2016).

M. sacchari goes through four nymphal stages before reaching the adult stage (Rodríguez-del-Bosque and Terán 2015). Most of the time, reproduction of the species is viviparous parthenogenesis, but in some regions where winter is prolonged, an oviparous cycle can occur. A fertilized female lays eggs from which emerge the fundatrix, which are the result of genetic exchange of parents that initiate new viviparous parthenogenetic lines (Peña-Martinez et al. 2018). It is a cosmopolitan aphid, with moderate to good adaptability. *M. sacchari* can withstand extreme cold and warm temperatures, but the optimum temperature is 20 to 25ºC, with relative humidity of 50% (Kiani and Szczepaniec 2018, Peña-Martinez et al. 2018).

This note reports for the first time the presence of *M. sacchari* in Paraguayan territory, where since February 2020 in the municipalities of Capitan Meza (26° 54' 31.3" S y 55° 14' 27.7" W) and Edelira (26° 42' 51.1" S y 55°18'09.9" W) in the northeast of the department of Itapúa, Paraguay, *M. sacchari* were observed attacking the sorghum crop. The aphid populations consisted of different stages of nymphs and winged and wingless adults (Fig. 1).

Taxonomic keys proposed by Peña-Martínez et al. (2017) were used for identification. The individuals collected were pale to bright yellow color and 1.0 to 2.0

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mm long (Fig. 1A). All individuals had a sinuous forehead and antennae with five or six segments. The terminal process of the antenna was approximately four times larger than the base. Short, dark cornicles were observed. The leg tarsi were dark colored. Thick wing veins were evident in winged individuals, with the midline vein divided twice, characteristic of the species *M. sacchari* (Fig. 1B).

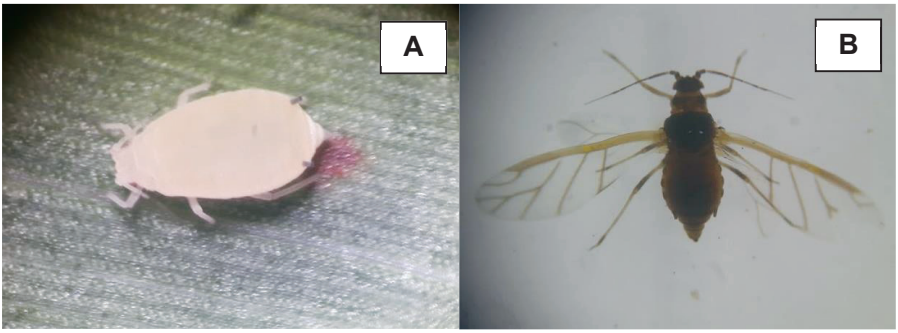

Fig. 1. Female *Melanaphis sacchari* wingless adult (A) and winged adult (B).

References Cited

Bowling, R. D., M. J. Brewer, D. L. Kerns, J. Gordy, N. Seiter, N. E. Elliott, and E. Maxson. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. J. Integr. Pest Manage. https://doi.org/10.1093/jipm/pmw011.

Kiani, M., and A. Szczepaniec. 2018. Effects of sugarcane aphid herbivory on transcriptional responses of resistant and susceptible sorghum. BMC Genomics. https://doi.org/10.1186/s12864-018-5095-x.

Knutson, A., R. Bowling, M. J. Brewer, E. Bynum, and P. Porter. 2016. The sugarcane aphid: management guidelines for grain and forage sorghum in Texas. Texas A&M AgriLife Extension and Research, Texas A&M University. http://ccag. tamu. edu/sorghum-insect-pests/.

Nibouche, S., L. Costet, J. R. Holt, A. Jacobson, A. Pekarcik, J. Sadeyen, J. S. Armstrong, G. C. Peterson, N. McLaren, and R. F. Medina. 2018. Invasion of sorghum in the Americas by a new sugarcane aphid (*Melanaphis sacchari*) superclone. PLoS ONE 13: e0196124. https://doi.org/10.1371/journal.pone. 0196124.

Peña-Martinez, R., R. Lomeli-Flores, R. Bujanos, A. L. Muñoz-Viveros, J. M. Vanegas-Rico, R Salas-Monzón, y J. Ibarra-Rendón. 2018. Pulgón amarillo del sorgo, (PAS), *Melanaphis sacchari* (Zehntner, 1897), interrogantes biológicas y tablas de vida. R. Yáñez-López [ed.], Nuevo León, México.

Peña-Martínez, R., A. L. Muñoz-Viveros, A. Marín-Jarillo, R. Bujanos-Muñiz, F. Tamayo-Mejía, I. J. Luévano-Borroe, L. Sánchez-Segura, y J. Ibarra-Rendón. 2017. Guía ilustrada para la identificación de los pulgones (Hemiptera: Aphididae) de cereales en México. ISBN: 978-607-96123-3-7.

Rodríguez-del-Bosque, L. A., and A. P. Terán. 2015. *Melanaphis sacchari* (Hemiptera: Aphididae): a new sorghum insect pest in Mexico. Southwest. Entomol. https://doi.org/10.3958/059.040.0217.

Zapata, S. D., R. Villanueva, D. Sekula, G. Esparza-Diaz, K. Duke, and M. Mutaleb. 2016. The economic impact of the sugarcane aphid on sorghum production. 2016 Southern Agricultural Economics Association Annual Meeting, 6-9 February 2016, San Antonio, TX.

SCIENTIFIC NOTE

Detection of *Bartonella henselae* and *Rickettsia felis* in Fleas from Client-Owned Pets in Oklahoma

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Abstract. *Bartonella* sp. and *Rickettsia* sp. DNA were detected in cat fleas, *Ctenocephalides felis* (Bouché), collected from urban, client-owned cats (*Felis catus* L.) and dogs (*Canis lupus familiaris* L.) at Tulsa, OK, during 2017. *Bartonella henselae* (Regnery et al. 1992) was detected in four of six fleas (67%) from one cat, while *Rickettsia felis* (Bouyer et al. 2001) was detected in eight fleas from five animals.

Flea-borne pathogens, including *Bartonella henselae* (Regnery et al. 1992) and *Rickettsia felis* (Bouyer et al. 2001) affect humans and companion animals worldwide. Infections with *B. henselae,* commonly known as cat-scratch disease, are usually associated with infected cats and the cat flea, *Ctenocephalides felis* (Bouché) (Cheslock and Embers 2019). *Rickettsia felis,* an intracellular bacterium in *C. felis*, produced adverse effects in humans in central Africa but has never been isolated from humans in the United States (Brown and Macaluso 2016). Both pathogens are not notifiable conditions in the United States, so regional epidemiology is limited (Brown and Macaluso 2016, Nelson et al. 2016) as are flea-focused studies. The purpose of this study was to determine whether the pathogens were in client-owned dogs and cats at Tulsa, OK.

We collected fleas from client-owned cats and dogs in the Tulsa metropolitan area between May and August 2017. The study was designed as a one-semester undergraduate research project. The clinic was selected because it serves a variety of socioeconomic backgrounds and rural and urban pet ownership practices. The collection protocol was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. After examination of the animals upon arrival, the owner was informed about the project if fleas were encountered, and approval was requested for removal of the fleas for research purposes. The species of host animal and zip code were documented. Fleas were put into 1.5-ml vials containing 70% EtOH until identified using established keys (CDC 2006).

Individual fleas were processed and tested for pathogen DNA using published polymerase chain reaction (PCR) protocols (Noden et al. 2017). To limit contamination, all DNA extractions used site-specific reagents in a different laboratory than where PCR assays were done. Fleas were evaluated for the citrate synthase (*gltA*) gene for *Bartonella* sp. (primer pair BhCS781.p / BhCS1137.n; (Norman et al. 1995)) and *Rickettsia* sp. (primer pair CS-78 / CS-323; (Labruna et al.

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2004)). All positive amplicons were bidirectionally sequenced at the Oklahoma State University Core Facility. Each resulting sequence was verified using BioEdit 7.2 (Ibis Therapeutics, https://bioedit.software.informer.com/7.2/) and aligned bidirectional sequences to create individual consensus sequences using Clustal Omega (EMBL-EBI, https://www.ebi.ac.uk/Tools/msa/clustalo/). Resulting sequences were compared with Genbank submissions using default conditions on NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) where the greatest percent sequence identity was used to determine similarity of species.

Forty *C. felis* were collected, with 22 (55%) from seven cats and 18 (45%) from six dogs from 13 houses in the Tulsa metropolitan area (six zip codes). Of the fleas collected, 26 (65%) were female.

Evaluation of the 40 fleas detected 12 (30%) positives (four *Bartonella* sp. and eight *Rickettsia* sp.), with flea prevalence of 10% (*Bartonella*) and 20% (*Rickettsia*) from six (46%) animals. No co-infected fleas were detected. Consensus sequences demonstrating 100% identity were determined with the 305-bp portion of the *gltA* gene of a *B. henselae* isolate (GenBank accession no. MN107415) and the 353-bp portion of the gltA gene of a *R. felis* isolate (GenBank accession no. MG952935). All four fleas infected with *B. henselae* were from one cat/house (four of six (67%) fleas infected, three of four (75%) females). The eight fleas infected with *R. felis* were from five animals (two dogs and three cats) from five houses in four zip codes, with most being female fleas (five of eight - 62.5%). The cat with the *B. henselae*-infected fleas and two (40%) animals with *R. felis*-infected fleas were from a high socio-economic neighborhood in south-central Tulsa.

Fleas infected with *B. henselae* and *R. felis* were identified from client-owned dogs and cats at Tulsa, OK. The west-south central region of the United States, in which Oklahoma is located, has the most incidence of cat scratch fever (Nelson et al. 2016), yet little is known of the ecology of the pathogen at the community level. As a zoonotic pathogen, it is especially dangerous for immuno-compromised people, but the greatest incidence occurs in children and the elderly (Nelson et al. 2016). While *B. henselae* is named after Diane Hensel who worked at an Oklahoma City laboratory (etymologia 2008), this is the first published record of *B. henselae* in fleas in Oklahoma. Detection of the pathogen in a relatively small random sample of fleas collected at a centrally-located veterinary clinic in one of the largest metropolitan areas in the state was surprising and led to questions regarding the general prevalence of the pathogen in fleas in the region. While *B. henselae* has never been documented in fleas in Oklahoma, *Bartonella* spp. were detected in *Oropsylla hisuta* (Baker) fleas collected in 2004 from prairie dogs (*Cynomys* spp.) near Oklahoma City (Reeves et al. 2007).

While potentially interesting as a pathogen of public health importance, Noden et al. already reported *R. felis* in fleas on Oklahoma-based pets in 2017. The potential presence of the two human pathogens in a large metropolitan area in Oklahoma necessitates the need for more focus on flea-borne pathogens in this region of the US.

Acknowledgment

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References Cited

Brown, L., and K. Macaluso. 2016. *Rickettsia felis*, an emerging flea-borne rickettsiosis. Curr Trop Med Rep 3: 27-39.

CDC (Centers for Disease Control and Prevention). 2006. Pictorial key to arthropods, reptiles, birds, and mammals of public health significance. http://www.cdc.gov/nceh/ehs/publications/pictorial_keys.htm. Accessed 22 August 2020.

Cheslock, M. A, and M. E. Embers. 2019. Human bartonellosis: an underappreciated public health problem? Trop Med Infect Dis 4: 69.

etymologia: *Bartonella henselae*. 2008. Emerg. Infect. Dis. 14: 980.

Labruna, M. B., T. Whitworth, M. C. Horta, D. H. Bouyer, J. W. McBride, A. Pinter, V. Popov, S. M. Gennari, and D. H. Walker. 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol 42: 90-98.

Nelson, C. A., S. Saha, and P. S. Mead. 2016. Cat-scratch disease in the United States, 2005-2013. Emerg. Infect. Dis. 22: 1741-1746.

Noden, B. H., S. Davidson, J. L. Smith, and F. Williams. 2017. First detection of *Rickettsia typhi* and *Rickettsia felis* in fleas collected from client-owned companion animals in the southern Great Plains. J. Med. Entomol. 54: 1093-1097.

Norman, A. F., R. Regnery, P. Jameson, C. Greene, and D. C. Krause. 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J. Clin. Microbiol. 33: 1797-1803.

Reeves, W. K., T. E. Rogers, and G. A. Dasch. 2007. *Bartonella* and *Rickettsia* from fleas (Siphonaptera: Ceratophyllidae) of prairie dogs (*Cynomys* spp.) from the western United States. J. Parasitol. 93: 953-955.

SCIENTIFIC NOTE

Importation and Preliminary Evaluation of the Stem Boring Moth *Buakea kaeuae*[1] as a Potential Biological Control Agent of Invasive Guineagrass, *Megathyrsus maximus*[2]

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Abstract. Guineagrass, *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs, is an African grass that is invasive in Florida, Hawaii, southern Texas, and northeastern Mexico. *Buakea kaeuae* Moyal et al., a stem-boring African moth found only on *M. maximus*, was imported from Kenya and evaluated for its ability to feed and develop on Guineagrass naturalized in southern Texas. Neither larvae nor adults developed or reproduced on Texas Guineagrass.

Resumen. El zacate Guinea (*Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs*),* originario de África, es considerado invasivo en Florida, Hawaii, sur de Texas, y noreste de México. *Buakea kaeuae* Moyal et al. es una palomilla especifica de *M. maximus* que ataca a los tallos de esta maleza. Esta palomilla fue importada de Kenia y ha sido evaluada por su habilidad para alimentarse y desarrollar su ciclo biológico solo en plantas de zacate guinea, naturalizadas en el sur de Texas. Ni las larvas ni los adultos de *B. kaeuae,* se desarrollan y reproducen en especies de zacate Guinea de Texas.

Guineagrass, *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs (formerly known as *Panicum maximum* Jacq. and *Urochloa maxima* (Jacq.) R. Webster), is an invasive grass in Florida (Overholt and Franck 2017), Hawaii (Daehler and Carino 1998), southern Texas (Lonard and Judd 2002), and northeastern Mexico (CONABIO 2020). This invasive African grass occurs in two distinct forms: giant and small with the latter invasive in South Texas, and the target of the biological control program (Mercadier et al. 2009, Rhodes et al. in litt.). The giant form has been found along the Rio Grande at Brownsville, TX (Soti et al. 2020). *Megathyrsus maximus* is native to much of Africa (CABI 2020). Giant Guineagrass is more common in lowland wet areas, along rivers and creeks. The small form grows in hot, dry upland habitats common in northern Kenya (very similar to South Texas). The small form may be a hybrid between *Megathyrsus maximus and M. infestus* (*Panicum infestum*) (Bon et al. 2011).

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Small Guineagrass was introduced to South Texas as a forage grass and subsequently spread throughout the region. It out-competes other more valuable forage grasses and creates a large fuel load for wildfires that damage native brush species and rangeland ecology of South Texas. Guineagrass and other exotic grasses affect wildlife including northern bobwhite, *Colinus virginianus* L., and scaled quail, *Callipepla squamata* Vigors, because these grasses are not hosts of the native insect fauna. Juvenile quail feed mainly on insects and therefore lack sufficient food for development in exotic grass pastures. Also, exotic grasses tend to reduce the density and diversity of forbs whose seeds may be eaten by quail (Kuvlesky et al. 2002). In Mexico and along the border with Texas, Guineagrass interacts with the southern cattle fever tick, *Rhipicephalus microplus* Canestrini, by reducing populations of tick-feeding beetles and ants and by creating a cool, humid environment that promotes survival of ticks (Esteve-Gassent et al. 2014). Across South Texas, Guineagrass is very invasive in wildlife preserves, citrus (Citrus spp. L.) orchards, sugarcane (*Saccharum officinarum* L.) fields, urban landscapes, and along highway right-of-ways. Because this invasive grass is so widespread, mechanical and/or chemical controls are ineffective (Rhodes et al. in litt.). Biological control may be the best option of sustainable management of this widespread exotic weed.

Moyal et al. (2011) identified a new genus and species of specialist stem-boring moths on *M. maximus*, *Buakea kaeuae* Moyal et al., which is specific to small Guineagrass of south-central Kenya. This specialist moth lays eggs on stems of Guineagrass, and the larvae bore into the crown of the grass. Because this insect is very damaging and already known to be specific to small Guineagrass, it was imported for evaluation as a potential biological control agent.

Three shipments of *B. kaeuae* were imported to the U.S. from Kenya in 2016. The insects were originally collected from small Guineagrass at Machakos, Kenya, and identified at the International Centre for Insect Physiology and Ecology, Nairobi, Kenya, by B. Le Ru. At the USDA Arthropod Quarantine Facility at Moore Airbase, Edinburg, TX, several methods were evaluated for rearing the Guineagrass moth. Newly hatched larvae were placed on cut stems or on two different artificial diets (European corn borer and southwestern corn borer diets – Frontier Scientific Services, DE). No larva fed on artificial diet. Larvae fed and stayed alive for more than 3 months using the cut stems of small Guineagrass. Larvae also fed on stems of giant Guineagrass but died within 6 weeks. Larvae were also placed on stems and leaf axils of live plants in both greenhouse and environmental growth chambers. No feeding was observed, and none of the 200 plus larvae survived on the Texas Guineagrass. We also received Guineagrass moth pupae from Kenya. Moths were kept in cages with small or giant Guineagrass for mating and observed daily. No eggs were observed from any adult moth.

In summary, all stages of the Guineagrass moth did not find Texas Guineagrass or the rearing system suitable. This probably indicated that Texas Guineagrass is genetically or phytochemically different from small Guineagrass at Machakos, Kenya. Stem-boring moths are generally difficult to rear, and this also might have contributed to the lack of rearing success.

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References Cited

Bon, M. C., J. Goolsby, G. Mercadier, T. Le Bourgeois, P. Poilecot, M. Jeanneau, and A. Kirk. 2011. What do chloroplast sequences tell us about the identity of Guinea grass, an invasive Poaceae in the southern United States?, p. 322. *In* Proceedings of the XIII International Symposium on Biological Control of Weeds.

CABI. 2020. *Megathyrsus maximus* [original text by J. Rojas-Sandoval and P. Acevedo-Rodriguez]. *In* Invasive Species Compendium. CAB International, Wallingford, UK. https://www.cabi.org/isc/datasheet/38666

CONABIO (La Comisión Nacional para el Conocimiento y Uso de la Biodiversidad). 2020. *Panicum maximum* Jacq. [original text by H. Vibrans]. http://www.conabio.gob.mx/malezasdemexico/poaceae/panicum-maximum/fichas/ficha.htm

Daehler, C. C., and D. A. Carino. 1998. Recent replacement of native pili grass (*Heteropogon contortus*) by invasive African grasses in the Hawaiian Islands. Pacific Science 52: 220-227.

Esteve-Gassent, M. D., A. A. Pérez de León, D. Romero-Salas, T. P. Feria-Arroyo, R. Patino, I. Castro-Arellano, G. Gordillo-Pérez, A. Auclair, J. Goolsby, R. I. Rodriguez-Vivas, and J. G. Estrada-Franco. 2014. Pathogenic landscape of transboundary zoonotic diseases in the Mexico-US border along the Rio Grande. Frontiers Public Health 2: 1-23.

Kuvlesky, W. P. Jr., T. E. Fulbright, and R. Engel-Wilson. 2002. The impact of invasive exotic grasses on quail in the southwestern United States. National Quail Symposium Proceedings 5: 118-128.

Lonard, R. I., and F. W. Judd. 2002. Riparian vegetation of the lower Rio Grande. Southwest. Nat. 47: 420-432.

Mercadier, G., J. A. Goolsby, W. A. Jones, and J. L. Tamesse. 2009. Results of preliminary survey in Cameroon, Central Africa, for potential natural enemies of *Panicum maximum* Jacq. (Poales: Poaceae), Guineagrass. Subtropical Plant Science 61: 31-36.

Moyal, P., B. Le Rü, D. Conlong, D. Cugala, B. Defabachew, T. Matama-Kauma, B. Pallangyo, and J. Van Den Berg. 2011. *Buakea* (Lepidoptera: Noctuidae), a new genus of African stem borers: morphological, ecological and molecular data. African Entomology 19: 23-35.

Overholt, W. A., and A. R. Franck. 2017. The invasive legacy of forage grass introductions into Florida. Natural Areas Journal 37: 254-265.

Soti, P., J. A. Goolsby, and A. E. Racelis. 2020. Agricultural and environmental weeds of South Texas and their management. Subtropical Agriculture and Environments 71: 1-11.

SCIENTIFIC NOTE

First Report of *Billaea* (*Paratheresia*) *claripalpis* Wulp[1] as a Parasitoid of *Diatraea considerata* Heinrich[2] at Nayarit, Mexico

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Abstract. The stalk borer *Diatraea considerata* Heinrich, 1931 is a major pest of sugarcane (*Saccharum officinarum* L.) in Mexico, causing losses of 50% of yield. During the larval stage, the species naturally is attacked by parasitoid flies (Diptera: Tachinidae). In this study, *Billaea* (*Paratheresia*) *claripalpis* (Wulp, 1896) was determined as a solitary endoparasitoid of *D. considerata* in sugarcane, with 0.83% natural parasitism. This is the first record of *B. claripalpis* at Nayarit. The parasitoid should be distributed and used in an augmentative strategy for biological control of the stalkborer in Mexico.

The larval stage known as a stalk borer (Lepidoptera: Crambidae) is the major pest of sugarcane (*Saccharum officinarum* L.) in Mexico (Rodríguez-del Bosque et al. 2014). In the states of Nayarit and Sinaloa, *Diatraea considerata* Heinrich is the main species that can destroy 2 to 10 tons of yield potential (Flores-Cáceres 2007), with annual loss of as much as 10 million U.S. dollars (30,000 ha) in the State of Sinaloa (Vejar-Cota et al. 2009). For control, insecticides such as endosulfan and methyl parathion are applied (Astola-Mariscal 2011). However, because of the cryptic habits of the insect, the active ingredients are not effective (Rodríguez-del Bosque et al. 2012). Effective alternatives are needed for its control, and dipteran parasitoids used as natural enemies is a viable biological alternative. Parasitoid "haired" flies of the Tachinidae family are most important for classical biological control (Van Driesche 2007). Most larvae attack as a solitary or gregarious endoparasite (Smith et al. 1993). Ovolarviparous females of *B. claripalpis* (Dexiinae: Dexiini) that contain fully developed first instars deposit eggs near the gallery of a host, where soon after, the larva emerges and finds a host to parasitize (O'Hara 2008).

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For collection of stalk borers from August to December 2019 and January to March 2020, sampling was directed toward sugarcane stalks with "dead heart" symptoms, or toward grinding stalks perforated by the insect, in plots at three sugarcane ejidos at the municipalities of Tepic and Xalisco. Twenty stalks per plot were examined, using the “five gold” method (Astola-Mariscal 2011). Each dead heart or stalk was dissected for stalk borer larvae and put into a plastic Petri dish 60 mm in diameter by 14 mm tall, labeled, and covered with a lid with small holes to facilitate ventilation during transportation to the Laboratory of Agricultural Parasitology CEMIC 03, at the Universidad Autónoma de Nayarit (UAN). In the laboratory, the larvae were put individually into 60-mm plastic Petri dishes labeled with the collection code, to which a diet of small slices of young maize (*Zea mays* L.) was provided for food sterilized with 0.5% sodium hypochlorite. The larvae were incubated at room temperature, with food replaced every 3rd day, until emergence of adult stalk borers and possible parasitoids (Lastra and Gómez 2006). For determination of genera of *Diatraea* larvae, keys by Gilligan and Passoa (2014) were used, while for determination of species in the adult state, keys by Solis and Metz (2016) were used. The emerged tachinids for later identification were kept in 70% alcohol in 2-ml centrifuge tubes. The species were determined by using keys of Thompson (1963).

The four specimens *B. claripalipis* that emerged, males and female (Fig. 1), were obtained from *D. considerata* larvae, being a new record for the State of Nayarit. Percentage of parasitism was low; of 480 larvae collected only four (0.83%) were parasitized. According to Thompson (1963), adults of *B. claripalpis* having a medium-sized body 6.0 to 9.0 mm long, grayish color with antennae and dark legs, orange palpi, thorax with three broad shiny black stripes, gray pollinose scutellum with anterior angles, blackish center of the base and light spot near the apex; gray pollinose pleura, mesopleura with a blackish border, male abdomen with black lateral bands and a fainter central vitta, female with no distinct bands, and red tip of the male abdomen and most of the last two segments of the female. The female differed from the male in having the 1st and 2nd antennal joints and base of the 3rd, red, palpi markedly clavate, with the abdomen rounded, not pointed, and mostly reddish.

Specimens Examined. *Billaea* (*Paratheresia*) *claripalpis*, Mexico, Nayarit, Tepic. ejido Francisco I. Madero (Puga): ♂ (4-035) 18-I-2020, 21°61’26’’ N; 104°84’79’’ W, 680 m. ♀ (4-005) 18-IX-2019, property “El Manguito” from Puga to Atonalisco highway, 21°37’12” N; 104° 50’02’’ W, 575 m.; Ejido Xalisco: ♂ (1-001) 13-IX-2019, on the way to “El Tacote” 21°42’04’’ N; 104°41’38’’ W, 1,052 m.; ejido Pantanal: ♂ (2-004) 22-X-2019, highway Xalisco to Pantanal, 21°25’35’’ N; 104°52’16’’ W, 947 m. Rafael Robles Pérez (collector). All specimens collected were deposited in the Insect collection of the Laboratory of Agricultural Parasitology CEMIC 03 UAN.

The first record of *B. claripalpis* in Mexico was identification by Van Der Wulp in 1896, with the name *Sarcophaga claripalpis*, based on five specimens collected (one male and four females) at Chilpancingo, Guerrero, and northern Yucatán (O'Hara and Wood 2004). Osborn later reported its presence in 1922 at the El Potrero sugar mill (at Atoyac, Veracruz), collecting *Paratheresia* specimens and sending to Van Zwaluwenburg at Los Mochis, Sinaloa with the goal to establish them (Flores-Cáceres 2007). In a review of tachinid specimens from the insect collection of the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM), Zetina et al. (2018), one female and two males of *B. claripalpis* were collected between 1977 and 1978 at the town of Cañón de Lobos (Yautepec, Morelos), a female was collected at Zaquiapan National Park (State of Mexico) in 1980, and two

males at San Ángel Zurumcapio (Ziracuaretiro, Michoacán) in 1983, but without specifying any parasitism of stalk borers. Astola-Mariscal (2011) reported *B. claripalpis* as a natural enemy of *Diatraea* sp. in sugarcane in southern Tamaulipas. Vejar-Cota (2004) recorded the species as a parasitoid of *D. considerata*, *D. grandiosella* Dyar 1911, and *Eoreuma loftini* (Dyar, 1917) in sugarcane areas at the municipalities of Navolato, El Dorado, and Los Mochis (Sinaloa).

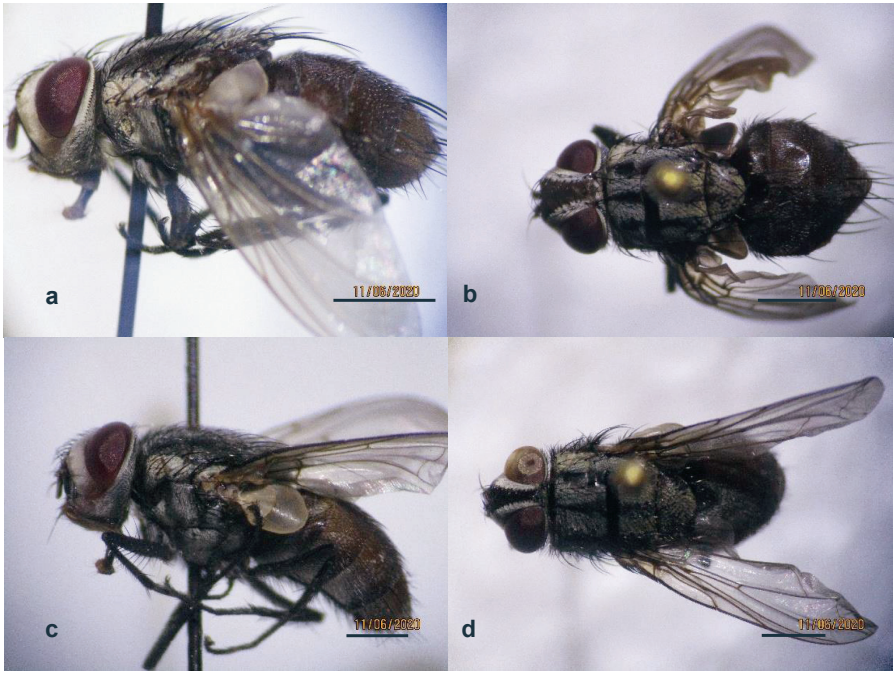

Fig. 1. *Billaea* (*Paratheresia*) *claripalpis* adults, ♂ a) left lateral view, b) dorsal view. ♀ c) left lateral view, d) dorsal view, 2-mm scale. ©Lino E. Romero-Ramos.

References Cited

Astola-Mariscal, S. Z. 2011. Ficha técnica barrenador de la caña de azúcar. SAGARPA, SIPOVE (Sistema Potosino de Vigilancia Epidemiológica), San Luís Potosí, México.

Flores-Cáceres, S. 2007. Las plagas de la caña de azúcar en México. Servicios Gráficos OREL, Córdova, Veracruz, México.

Gilligan, T. M., and S. C. Passoa. 2014. Crambidae-*Diatraea considerata* Heinrich. Larval diagnosis. LepIntercep. An identification resource for intercepted Lepidoptera larvae. Identification Technology Program (ITP), Fort Collins, CO. United States Department of Agriculture (USDA). Colorado State University.

Lastra, B. L. A., y L. L. A. Gómez. 2006. La cría de *Diatraea saccharalis* (F.) para la producción masiva de sus enemigos naturales. Cali, Cenicaña (Serie Técnica 36).

O'Hara, J. E. 2008. Tachinid flies (Diptera: Tachinidae), pp. 3675-3686. *In* J. L. Capinera [ed.], Encyclopedia of Entomology. 2nd ed. Springer, Dordrecht, Netherlands.

O'Hara, J. E., and D. M. Wood. 2004. Catalogue of the Tachinidae (Diptera) of America north of Mexico. Memoirs on Entomology International 18.

Rodríguez-del-Bosque, L. Á., R. Loredo-Pérez, H. Mata-Vázquez, y J. Ávila-Valdez. 2012. Manejo integrado de barrenadores en caña de azúcar en el sur de Tamaulipas. SAGARPA, INIFAP. Centro de Investigación Regional del Noroeste, Campo Experimental Río Bravo, Río Bravo, Tamaulipas. Folleto Técnico MX-0-310384-04-03-13-09-53.

Rodríguez-Del Bosque, L. Á., G. Vejar-Cota, I. Vásquez-López, J. A. Villanueva-Jiménez, J. López-Collado, R. Alatorre-Rosas, y F. Hernández-Rosas. 2014. Capítulo X Plagas, pp. 147-186. *In* H. Mata-Vázquez, L. A. Rodríguez-del Bosque, V. H. Rodríguez-Morelos, y E. Vázquez-García. [eds.], Manejo Integral de Caña de Azúcar. SAGARPA, INIFAP y Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México.

Smith, Jr., J. W., R. N. Widenmann, and W. A. Overholt. 1993. Parasites of lepidopteran stemborers of tropical gramineous plants. ICIPE Science Press, Nairobi, Kenya.

Solis, M. A., and M. A. Metz. 2016. An illustrated guide to the identification of the known species of *Diatraea* Guilding (Lepidoptera: Crambidae: Crambinae) based on genitalia. ZooKeys 565: 73-121.

Thompson, W. R. 1963. The tachinids of Trinidad. II. Echinomyiines, Dexiines, and allies. Can. J. Zool. 41: 335-576.

Van Driesche, R. G., M. S. Hoddle, and T. D. Center. 2007. Control de plagas y malezas por enemigos naturales. Translated by E. Ruiz-Cancino, J. Coronado-Blanco, and J. M. Álvarez. US Department of Agriculture (USDA).

Vejar-Cota, G. 2004. Control biológico del gusano barrenador del tallo (Lepidoptera: Crambidae), de la caña de azúcar en Sinaloa, pp. 70-84. *En* L. A. Rodríguez-del-Bosque, G. Vejar-Cota, y E. Cortéz [eds.], Taller Internacional sobre Barrenadores del Tallo de Caña de azúcar. Sociedad Mexicana de Control Biológico, Los Mochis, Sinaloa, México.

Vejar-Cota, G., L. A. Rodríguez-del Bosque, and D. Sahagún. 2009. Economic and ecological impacts of hand removing dead hearts caused by *Diatraea considerata* (Lepidoptera: Crambidae) on sugarcane in Mexico. Southwest. Entomol. 33: 157-159.

Zetina, D. H., J. Romero-Nápoles, A. Contreras-Ramos, and J. L. Carrillo-Sánchez. 2018. Checklist of Tachinidae (Insecta: Diptera) in Mexico. Trans. Am. Entomol. Soc. 1-88.

New Host Data and Distribution for an Ectoparasitic Larva of *Leptus*[1] at Tamaulipas, Mexico

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Abstract. This is the first record of parasitic mite *Leptus* sp. (Trombidiformes, Erythraeidae) parasitizing adult males of click beetles *Monocrepidius flavangulus* Candeze 1859 and *Glyphonyx flohri* Champion 1896 (Coleoptera: Elateridae), as well as the first record of *Leptus* species in the municipalities of Ocampo and Tula. It also is the first record for the Coleoptera species in this study in the State of Tamaulipas, Mexico.

Members of the family Erythraeidae Robineau-Desvoidy, 1828 have potential for use in biological control of insect pests (Welbourn 1983). Mites in the large and cosmopolitan genus *Leptus* Latreille 1796 are parasites in the larval stage, feeding on a wide range of arthropods (Welbourn 1983, Muñoz-Cárdenas et al. 2015). The larvae pierce the cuticle of the host and ingest hemolymph and interstitial fluids (Âbro 1988). After engorging, larvae drop from the host and live on the soil until becoming nymphs and adults that are free-living; deutonymphs can be predatory (Welbourn 1983).

Genus *Leptus* with about 260 species described from the larval stage (Basini-Silva et al. 2020) frequently are ectoparasites, especially on Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera (Felska et al. 2018). Fifty-four larval *Leptus* species were described from the America Continent and of those, 38 species were collected from arthropods associated with Ixodida (Argasidae), Sarcoptiformes (Oribatida), Araneae, Opiliones, Hemiptera, and Hymenoptera (Haitlinger 1991; Makol and Wohltmann 2012; Salvatierra and Almeida 2017; Šundić et al. 2017, Haitlinger et al. 2017, 2020; Jacinavicius et al. 2019; Saboori et al. 2020).

In Mexico, six species of mites of the genus *Leptus* have been recorded, mostly associated with arachnids (Hoffmann and López-Campos 2000, Montiel-Parra and Pérez-Ortiz 2009). In the State of Tamaulipas, *Leptus* was recorded only in foliage of piquin chili, *Capsicum annuum* cv. *glabriusculum*, at Ciudad Victoria (Monjarás-Barrera et al. 2019). However, there are a few studies of mite diversity at Tamaulipas and Mexico (Ruíz-Cancino and Coronado 2002). Most relationships of

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[1]*Leptus* (Prostigmata: Erythraeidae)

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Leptus species with hosts are poorly known. Most host-parasite relationships are for unidentified *Leptus* species (Pereira et al. 2012).

The click beetle *Monocrepidius flavangulus* previously was recorded in Belize, Guatemala, Honduras, Nicaragua, and Mexico (Champion 1896, Johnson 2010). In Mexico, it is in the states of Oaxaca (without locality data) and Veracruz (Atoyac, Córdoba, Orizaba, Playa Vicente, Tuxtla, and Xalapa) (Champion 1896). In this study, two other states were included in the distribution of the species: Chiapas (Cintalapa) and San Luis Potosí (El Naranjo) (data from specimens in the Coleoptera TAMU-ENTO collection at Texas A&M University, United States of America, according to EOML). This is the first record for the click beetle in the State of Tamaulipas (Tula). *Glyphonyx flohri* was recorded in the State of Veracruz (Tezonapa) in Mexico (Champion 1896), but this is the first record in the State of Tamaulipas (Ocampo) and is the first record of the mite in the municipalities of Ocampo and Tula, State of Tamaulipas. Distribution and host insect records were expanded by this record.

New data on new host and locality records are presented, and the work records the first occurrence of larvae of the genus *Leptus* parasitizing adult click beetles *Monocrepidius flavangulus* Candeze, 1859, and *Glyphonyx flohri* Champion 1896. This is the first record of *Leptus* in the municipalities of Ocampo and Tula, State of Tamaulipas.

Material (insects with mites) was collected from 2016 to 2017 in two Malaise traps: Ocampo (Ejido Bejuco, 22.925459 N, 99.516195 W, 940 m above sea level, in low deciduous tropical forest) and Tula (Ejido Gallitos, 22.887973 N, 99.551983 W, 979 m above sea level, low sub-deciduous tropical forest). The collection jar was changed every 15 days.

Mites were clarified with Nesbit liquid and mounted in Hoyer's medium (Walter and Krantz 2009). Keys by Southcott (1992) and Saboori et al. (2020) were used to identify mites. Slide-mounted specimens were examined using phase contrast and differential interference contrast. Measurements and illustrations were made with Zeiss Axioscope Plus2. Keys by Champion (1896), Blackwelder (1944), Goldbach (1994), Aguirre-Tapiero (2009), Johnson (2010, 2012), and Kundrata et al. (2019) were used to identify beetles. The biological material was deposited in the Insect Museum of the Facultad de Ingeniería y Ciencias, Universidad Autonóma de Tamaulipas (MIFA-UAT), at Ciudad Victoria, Tam., Mexico.

Three *Monocrepidius flavangulus* Candeze 1859 males were parasitized by eight *Leptus* larvae (three, three, and two each) (Fig. 1A) in a sample from 16-31 July 2016 in a low sub-deciduous forest at the Municipality of Tula, Ejido Gallitos. Two other *Leptus* larvae were on males of *Glyphonyx flohri* Champion, 1896 (Fig. 1B) in the Municipality of Ocampo, Ejido El Bejuco, in a sample from 26 September-9 October.

Leptus larvae (Fig. 1 C, D) are associated mostly with insects and arachnids (Southcott 1992). *Leptus* larvae were obtained only from 13 families of Coleoptera. For Elateridae, there are only two records worldwide, one for Asia (*L. japonicus* Kawashima, Japan) and one for Africa (*L. soddagus* Haitlinger, Tanzania) (Haitlinger 1994). This is the first record of click beetles *M. flavangulus* and *G. flohri* associated with *Leptus* in Mexico, in the municipalities of Ocampo and Tula in the State of Tamaulipas.

Little is known of the associations of mites with beetles. The paper revealed an unrecorded association between *Leptus* larvae on their hosts. *Leptus* larvae distributed over ventral segments (Fig. 1 A, B) and body of its host and the location

of the larva described here are consistent with observations that *Leptus* larvae prefer attachment to areas of the heavily sclerotized integument (Southcott 1992).

This article presents an unrecorded association between click beetles and a *Leptus* mite in Mexico. Similar interactions are likely with other organisms at the study areas.

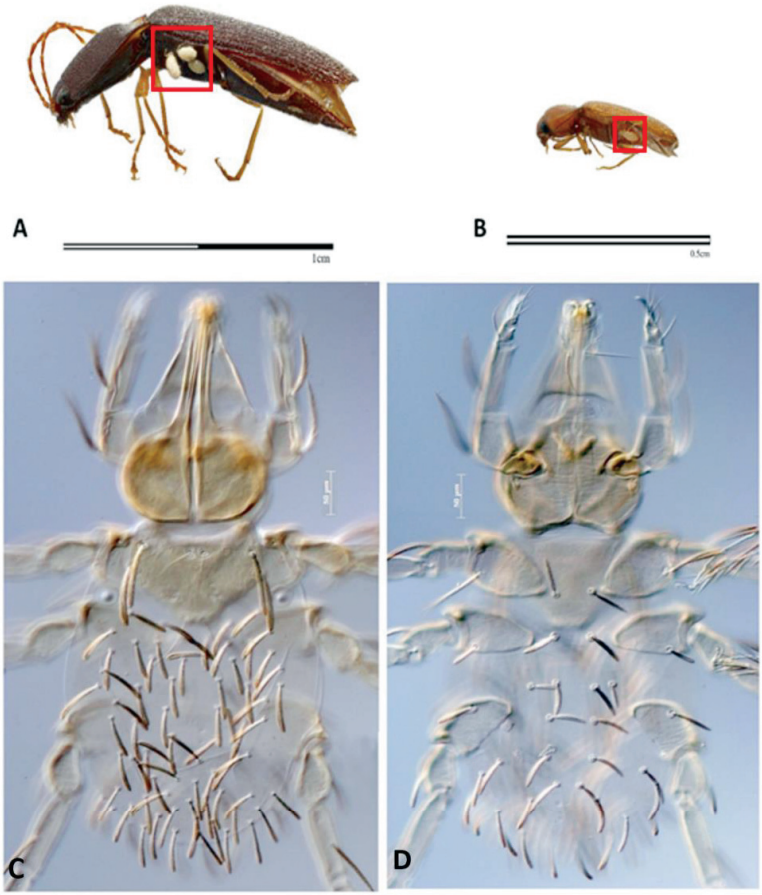

Fig. 1. A) *Monocrepidius flavangulus,* B) *Glyphonyx flohri* parasitized by *Leptus* larva (squares); *Leptus* larva C) dorsal view and D) ventral view.

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References Cited

Âbro, A. 1988. The mode of attachment of mite (*Leptus* spp.) to harvestmen (Opiliones). Journal of Natural History 22: 123-130.

Aguirre-Tapiero, P. 2009. Clave de Identificación de géneros conocidos y esperados de Elateridae Leach (Coleoptera: Elateroidea) en Colombia. Boletín del Museo de Entomología de la Universidad del Valle 10: 25-35.

Bassini-Silva, R., F. D. C. Jacinavicius, R. S. Bouzan, L. F. M. Iniesta, E. Campos-De-Oliveira, C. Welbourn, M. Šundić, R. Ochoa, A. D. Brescovit, and D. M. Barros-Battesti. 2020. A new species of *Leptus* (*Leptus*) (Trombidiformes: Erythraeidae) and new records of *Leptus* (*Leptus*) *haitlingeri* Jacinavicius, Bassini-Silva & Welbourn, 2019 for Brazil. Int. J. Acarol. 46: 213-221

Blackwelder, R. H. 1944. Checklist of the Coleopterous Insects of Mexico, Central America, the West Indies, and South America, Part 2. Bull. U.S. Natl. Mus. 185: 280-303.

Champion, G. C. 1896. Elateridae, pp. 258-584. *In* F. D. Godman and O. Salvin [eds.], Biologia Centrali-Americana. Insecta: Coleoptera. Serricornia, Vol. 3, Part 1. Taylor and Francis, London.

Felska, M., A. Wohltmann, and J. Mąkol. 2018. A synopsis of host-parasite associations between Trombidioidea (Trombidiformes: Prostigmata, Parasitengona) and arthropod hosts. Syst. Appl. Acarol. 2: 1375.

Goldbach, R. 1994. Elateridae (Col.) de la Argentina, Historia, Catálogo actualizado hasta 1991 inclusive y clave de subfamilias y de géneros de Centro y Sudamérica. Opera Lilloana 41: 1-48.

Haitlinger, R. 1991. Six new species of *Leptus* Latreille, 1796 (Acari, Prostigmata, Erythraeidae) from Neotropical Region. Zesz. Nauk. Akad. Roln. Wroc., Zootechnika 35: 265-272.

Haitlinger, R. 1994. Two new species of *Leptus* Latreille, 1796 (Acari, Prostigmata, Erythraeidae) associated with Tenebrionidae (Insecta, Coleoptera). Israel J. Entomol. 28: 139-149

Haitlinger, R., M. Šundić, and V.T. Pompermaier. 2017. Two new larval *Leptus* Latreille (Trombidiformes: Erythraeidae) from Brazil. Syst. Appl. Acarol. 22: 874-884.

Haitlinger, R., M. Šundiċ, L. Ázara, and L. F. O. Bernardi. 2020. A new species of larval *Leptus* (*Leptus*) (Trombidiformes: Erythraeidae) from Brazil with a list of host-parasite associations between *Leptus* and arthropods in America. Biologia. Doi: 10.2478/s11756-020-00453-7

Hoffmann, A., and M. G. López-Campos. 2000. Biodiversidad de los ácaros de México. Primera edición. CONABIO, México.

Jacinavicius, F., R. Bassini-Silva, J. F. Soares, F. Virginio, W. C. Welbourn, and D. M. Barros-Battesti. 2019. Description of *Leptus* (*Leptus*) *haitlingeri* n. sp. (Trombidiformes: Erythraeidae) parasitizing horse-flies (Diptera: Tabanidae), and a key to the larvae of *Leptus* spp. in Brazil. Syst. Parasitol. 96: 723-734.

Johnson, P. J. 2010. Checklist of the Elateridae of Mexico. South Dakota State University, Brookings.

Johnson, P. J. 2012. A new genus of *Conoderini*, with new generic classifications for *Ctenicera sleeperi* Becker and *Ctenicera pilatei* (Champion), and a new species from Jamaica (Coleoptera: Elateridae). Coleopt. Bull. 49: 59-71.

Kundrata, R., M. Kubaczkova, A. S. Prosvirov, H. B. Douglas, A. Fojtikova, C. Costa, Y. Bousquet, M. A. Alonso-Zarazaga, and P. Bouchard. 2019. World

catalogue of the genus-group names in Elateridae (Insecta, Coleoptera). Part I. Agrypninae, Campyloxeninae Hemiopinae, Lissominae, Oestodinae, Parablacinae, Physodactylinae, Pityobiinae, Subprotelaterinae, Tetralobinae. Zookeys 839: 83-154.

Mąkol, J., and A. Wohltmann. 2012. An annotated checklist of terrestrial Parasitengona (Actinotrichida: Prostigmata) of the world, excluding Trombiculidae and Walchiidae. Ann. Zool. 62: 359-562.

Monjarás-Barrera, J. I., J. C. Chacón-Hernández, G. L. Da Silva, L. Johann, O. Santos-Da Silva, J. Landeros-Flores, V. Vanoye-Eligio, F. Reyes-Zepeda, and N. Juarez-Ferla. 2019. Mites associated to chile piquín (*Capsicum annuum* L. var. *glabriusculum*) in two protect natural areas in northeastern México. Systematic & Applied Acarology 24: 2537-2551.

Montiel-Parra, G., y T. M. Pérez-Ortiz. 2009. Nuevos registros de ácaros de las familias Trombidiidae y Erythraeidae parásitos de arácnidos en México. 2009. Entomología Mexicana 35-40.

Muñoz-Cárdenas, K., L. S. Fuentes-Quintero, D. Rueda-Ramírez, C. D. Rodríguez, and R. F. Cantor. 2015. The Erythraeoidea (Trombidiformes: Prostigmata) as biological control agents, with special reference to the genus *Balaustium*. *In* D. Carrillo, G. J. de Moraes, and J. E. Peña [eds.], Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms, Progress in Biological Control. Springer International Publishing, Basel 19: 207-239.

Pereira, A. I., M. A. Fadini, T. G. Pikart, J. C. Zanuncio, and J. E. Serrão. 2012. New hosts and parasitism notes for the mite *Leptus* (Acari: Erythraeidae) in fragments of the Atlantic Forest, Brazil. Braz. J. Biol. 72: 611-616.

Ruíz-Cancino, E., and J. M. Coronado. 2002. Artrópodos terrestres de los estados de Tamaulipas y Nuevo León, México. Ciudad Victoria: Serie Publicaciones Científicas, CIDAFF-UAT, No. 4. Ciudad Victoria, Tamaulipas.

Saboori, A., M. Hakimitabar, N. Khademi, H. Masoumi, and A. R. Katouzian. 2020. *Leptus* Latreille (Trombidiformes: Erythraeidae) of the world: revised classification and keys. Persian J. Acarol. 9: 1-57.

Salvatierra, L., and M. Q. Almeida. 2017. First record of a *Leptus* mite (Trombidiformes, Erythraeidae) associated with a neotropical trapdoor spider (Araneae: Mygalomorphae: Actinopodidae). Cogent Biology 3: 1295823.

Southcott, R. V. 1992. Revision of the larvae of *Leptus* Latreille (Acarina, Erythraeidae) of Europe and North America, with descriptions of post-larval instars. Zool. J. Linn. Soc. 105: 1-153.

Šundić, M., R. Haitlinger, and V. T. Pompermaier. 2017. A new species of larval *Leptus* Latreille from Brazil (Acari, Prostigmata, Erythraeidae). Spixiana 40: 89-93.

Walter, D. E., and G. W. Krantz. 2009. Collection, rearing and preparing specimens, pp. 83-96. *In* G. W. Krantz and D. E. Walter [eds.], A Manual of Acarology. Texas Tech University Press. Lubbock.

Welbourn, W. C. 1983. Potential use of trombidioid and erythraeoid mites as biological control agents of insect pests, pp. 103-140. *In* M. A. Ho, G. L. Cunninham, and L. Knutson [eds.], Biological Control of Pests by Mites. Proceedings of a Conference Help, 5-7 April 1982, University of California, Berkeley.

SCIENTIFIC NOTE

First Report of *Spodoptera ornithogalli*[1] in *Agave salmiana*

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Abstract. The agave pulquero (*Agave salmiana* Otto) has ecological, economic, and social value in several regions of México, especially Hidalgo State. The current importance of the plant requires developing more agricultural practices for effect production of the crop. From September to October 2019 at the municipality of Alfajayucan, Hidalgo, México, unusual damage was detected on *A. salmiana* plants and suckers with symptoms of severe defoliation by lepidopteran larvae, later identified as *Spodoptera ornitogalli* (Guenée). The species damaged 23% of plants with severity as much as 100% on the agave pulquero crop. This is the first report of *A. salmiana* foliage damaged by *S. ornithogalli*, which should be considered a potential pest of the agave crop.

Since pre-hispanic times species of *Agave* have been used to provide different products and by-products with nutritional, medical, industrial, and social importance. Several *Agave* species are important for social, environmental, and economic development in different regions of the country (SEMARNAT 2020). In central Mexico, specifically Hidalgo State, the *Agave salmiana* Otto *ex* Salm-Dyck variety Xhaminí (Asparagaceae), is valued for use in barbecued meat prepared using leaves (pencas), providing pulque to drink, soil conservation, and to exploit red and white worms used as human food (Ramos-Elorduy 2006, García et al. 2010, Álvarez-Duarte et al. 2018). The agave crop is essential for development in Hidalgo State and attention must be paid to management of the crop for proper exploitation.

During 2019, an *A. salmiana* commercial plantation with 288 plants of the Xhaminí variety was managed agronomically by manual weed control, fertilization with both organic and chemical fertilizers, drip irrigation, as well as monitoring and control of pests and diseases. Plants were planted in 2015 within the experimental field facilities of Colegio de Bachilleres, Tecnológico Agropecuario (CBTA 179) (20º 25' 45.84" N y 99º 22' 39.95" W; 1,880 m above sea level), at Zozea, Alfajayucan, Hidalgo State, México.

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During September and October 2019, maguey plants and suckers were observed with damaged leaves with defoliation symptoms caused by lepidopteran larvae. The larvae were taken to a laboratory to be identified and photographed. Identification of the lepidopteran was corroborated at the Entomology Laboratory of Centro Nacional de Referencia Fitosanitaria, Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria, Secretaría de Agricultura y Desarrollo Rural (National Center of Phytosanitary Reference of National Service of Health, Safety and Agro-Food Quality, Agriculture Ministry and Rural Development). All larval specimens, were identified as *Spodoptera* (=*Prodenia*) *ornithogalli* (Guenée) (Lepidoptera: Noctuidae), commonly called "yellow-striped armyworm" in English or "gusano cuerudo" or “gusano soldado de bandas amarillas” in Spanish (EPPO 2015, Sermeño-Chicas et al. 2017). *S. ornithogalli* on the American continent is distributed from Canada to Brazil, attacking different plant species mostly horticultural crops such as tomatoes (*Solanum lycopersicum* L), peppers (*Capsicum annuum* L), beans (*Phaseolus vulgaris* L), and some Cucurbitacea species as well as cabbage (*Brassica oleracea* L), tobacco (*Nicotiana tabacum* L), soybeans (*Glycine max* L), maize (*Zea mays* L, alfalfa (*Medicago sativa* L), and sunflower (*Helianthus annuus* L), and seriously affecting the growth stage including fruit and foliage of the crop (EPPO 2015). The life cycle of *S. ornithogalli* is 33 days, with six larval instars. Adults have average longevity of 13 days for females and 11 days by males in laboratory conditions. The eggs are spherical, radially striated, and white, and laid in groups covered by a fine layer of grayish scales between the eggs and over the egg mass (Fernández et al. 2004).

The larvae are grayish-pink with two dark triangular spots in the dorsal area of each segment of the body, three longitudinal dorsal lines are yellow-orange (Fig. 1c), with external white spots and an inverted “Y” on the head (Fig. 1d). Females can lay more than 2,000 eggs that hatch in 5-7 days (Caballero et al. 1994, Urretabizkaya et al. 2010). The prepupa is smaller and darker. The pupa is brown and darkens as it matures. There are no reports of *S. ornithogalli* damaging agave pulquero or being a pest of tequilero. Halffter (1957) is the only author to mention *S. ornithogali* as a pest of *Agave sisalana* Perrine *ex* Engelm. There were no previous reports of *S. ornithogalli* attacking *A. salmiana.* Therefore, this note is the first formal report of the species feeding and behaving as a pest on *A. salmiana*. In large plants of *A. salmiana* variety Xhaminí, damage by *S. ornithogalli* caused superficial and irregular bites on the leaf, and larvae were observed feeding on different places on the same leaf (Fig. 1b), while complete defoliation was observed of suckers (Fig. 1a). In the total plantation, the incidence of the insect was 23% and severity to the plants was 10 to 100%. The most severe damage in the field was defoliation of 100% of a shoot about 15-cm high with six leaves. In the laboratory, *S. ornithogalli* larva was provided a shoot with the same characteristics mentioned, which was consumed in 7 days. Because of the kind of damage caused by *S. ornithogalli* larvae, the symptoms when present at the edges of a leaf could be confused with rodent bites and also with damage by adults of the agave funerary beetle, *Acanthoderes funeraria* (Bates), when damage occurs in the central base of the leaf (Olivares-Orozco et al. 2017, Sánchez-Jiménez et al. 2019). However, damage caused by *S. ornithogalli* larva is slightly deeper and more severe.

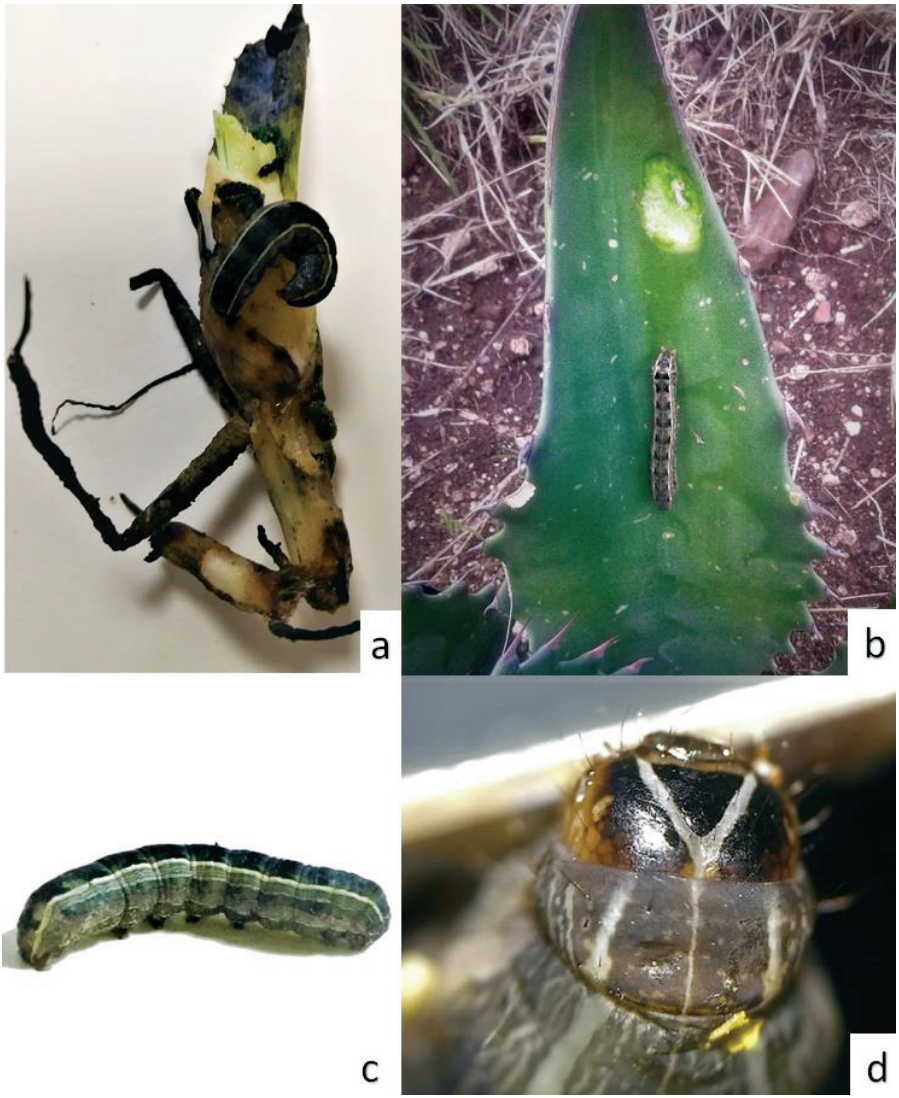

Fig. 1. a) Shoot of *Agave salmiana* defoliated by larva of *Spodoptera ornithogalli*; b) damage by *S. ornithogalli* on *A. salmiana* leaf in the field where irregular wounds were observed; c) dorsal view of *S. ornithogalli* larva with two dorsal longitudinal yellow bands; d) *S. ornithogalli* head with external white spots and a “Y” mark on front area.

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References Cited

Álvarez-Duarte, M. C., E. García-Moya, J. Suárez-Espinosa, M. Luna-Cavazos, y M. Rodríguez-Acosta. 2018. Conocimiento tradicional, cultivo y aprovechamiento del maguey pulquero en los municipios de Puebla y Tlaxcala. Polibotánica 45: 205-222.

Caballero, R., D. H. Habeck, y K. L. Andrews. 1994. Clave ilustrada para larvas de Noctúidos de importancia económica de El Salvador, Honduras y Nicaragua. Ceiba 35*:* 225-237.

EPPO (European and Mediterranean Plant Protection Organization). 2015. Mini hoja de datos sobre *Spodoptera ornithogalli* estudio de tomate. https://gd.eppo.int/taxon/PRODOR/documents.

Fernández, L. S., C. Fernández, y J. E. Mejía. 2004. Ciclo de vida de *Spodoptera ornithogalli* (Guenée) en el cultivo del algodonero en el Valle Medio del Sinú. Temas Agrarios 9: 30-36.

García, J., S. Méndez, y D. Talavera. 2010. El género *Agave* spp. en México: Principales usos de importancia socioeconómica y agroecológica. Revista Salud Pública y Nutrición 5: 109-121.

Halffter, G. 1957. Plagas que afectan a las distintas sspecies de agave cultivadas en México. No. 633.5 H35. Secretaría de Agricultura y Ganadería, México.

Olivares-Orozco, E., S. E. Ramírez-Sánchez, A. Jiménez-Aguilar, O. Guerrero-Andrade, D. Montiel-Salero, y J. G. Rodríguez-Diego. 2017. Notificación de *Acanthoderes funeraria* (Bates) sobre dos tipos de *Agave salmiana* (Otto ex Salm-Dyck) en Hidalgo, México. Rev. Protección Veg. 32: 1-5.

Ramos-Elorduy, J. 2006. Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. J. Ethnobiol. Ethnomed. 2: 1-10.

Sánchez-Jiménez, E., D. I. Orbe-Díaz, Y. Romero, y J. Toribio-Jiménez. 2019. Primer reporte del escarabajo *Acanthoderes funeraria* (Bates) (Coleoptera: Cerambycidae) en el agave silvestre *Agave cupreata* (Trel. & Berger) en Guerrero, México. Acta Zoológica Mexicana (nueva serie) 35: 1-4.

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2020. Agaves, maravillosas y magnánimas plantas. https://www.gob.mx/semarnat/articulos/agaves-maravillosas-y-magnanimas-plantas

Sermeño-Chicas, J. M., F. A. Parada-Berrios, E. A. Rodríguez-Urrutia, D. Pérez, A. L. Joyce, y J. R. Quintanilla-Quintanilla. 2017. Gusano cuerudo *Spodoptera ornithogalli* (Guenée 1852) (Lepidoptera: Noctuidae) en cacao Theobroma cacao L. en El Salvador. Revista Minerva 1: 62-72.

Urretabizkaya, N., A. Vasicek, y E. Saini. 2010. Insectos perjudiciales de importancia Agronómica. 1. Lepidópteros. Instituto Nacional de Tecnología Agropecuaria. Ministerio de Agricultura, Ganadería y Pesca, Presidencia de la Nación. Buenos Aires, Argentina.

SCIENTIFIC NOTE

First Report of *Centruroides balsasensis*[1] in Northern Mexico

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In Mexico, the family Buthidea is represented by three genera: *Centruroides* Marx 1890; *Chaneke* Francke, Teruel and Santibañez-Lopez 2014; and *Tityopsis* Armas 1974 (de Armas and Martin-Frias 1998, Francke et al. 2014). The *Centruroides* is the most important genus because of its impact on human health. The number of scorpion stings by *Centruroides* spp. is by far the most important in the world (Lourenço 2016). In the country, an average of 284,932 cases are reported every year, with 80% of fatal outcomes in children (<5 years) and elderly people (>65 years) (Secretaria de Salud 2013).

The genus *Centruroides* is widely distributed in Mexico. The Mexico Balsas River Basin biogeographic province at the Neotropical West/Central/South region of the country (including Guerrero, Jalisco, State of México, Michoacán, Morelos, Oaxaca, Puebla, and Tlaxcala states), has the greater species richness and/or endemism (39 species) (Ponce-Saavedra et al. 2015). In northern México (Nearctic region), only eight species have been reported (Sissom and Hendrixson 2005). Diversity and geographic delimitation of species have been continuously updated (e.g., Francke et al. 2014).

Recent collection at Ciudad Juarez, Chihuahua included *C. balsasensis* Ponce-Saavedra and Francke (2004), a very toxic endemic species from the Balsas River Basin province. Ciudad Juárez is at 1,120 m above sea level in the border region of the Chihuahua Desert near El Paso, TX (Fig. 1). Mean annual temperature is 16.7°C, ranging from 5.3ºC in winter to 27.3ºC in summer. Annual rainfall is 40-50 mm. To our knowledge, this is the first record of *C. balsasensis* in northern Mexico.

In March and April 2019, two specimens were collected during daytime as part of direct sampling (31°38 ′31″ N, 106°26 ′34.4″ W). Organisms were found under large pieces of construction debris and cardboard in wasteland. Scorpions were transported and identified in the Entomological and Bioassay Research Unit of Chihuahua by using available taxonomic keys (Stahnke and Calos 1977, Ponce and Francke 2013, Ponce-Saavedra et al. 2016). The specimens were studied using a

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stereomicroscope (ZEISS Stemi 305). Photos were taken using pixel fox image package with digital microscope camera model AB00484 and pixel fox software. One scorpion was preserved in 70% ethanol and deposited at the research unit as part of the archive of arthropod fauna of Chihuahua State. One gravid organism was kept alive for research purposes.

Examination in a laboratory revealed the specimens matched with main diagnostic characters of *C. balsasenesi*. Two females (one gravid) of *C. balsasensis* were collected. They were scorpions of medium adult size (3.7 and 4 cm), reddish-yellow, paler ventrally, and with two dorsal longitudinal blackish stripes on the mesosoma (Fig. 1A). The carapace has four parallel fuscous longitudinal stripes; external stripes extend from the sides of the ocular tubercle through the lateral eyes, the median stripes are faded in the anterior margin of the carapace (Fig. 1B). Pectinal tooth count was 24 (Fig. 1C).

Fig. 1. Adult female of *Centruroides balsasensis* collected in Cd, Juarez, Chihuahua, Mexico A) carapace, B) dorsal view, C) pectinal tooths.

Centruroides balsasensis is common in the Balsas Basin province (Ponce-Saavedra and Francke 2004). The province is relatively dry, and tropical deciduous forest is the dominant vegetation type with much plant and animal endemism (Rzedowski 1983). The indigenous presence of *C. balsasensis* at Chihuahua is difficult to explain from an ecological/biogeographic perspective. Scorpions also are not typically very mobile. Ciudad Juarez is the northern largest and busiest border crossing of Mexico and the United States, experiencing massive local and non-local human transit. Consequently, *C. balsasensis* was presumed to have been accidentally introduced into Chihuahua.

The updated scorpion list reported for Chihuahua now includes a total of 12 species (Family Buthidae: *Centruroides balsasensis* Ponce and Francke 2004, *Centruroides vittatus* Say 1821; Family Chactidae: *Diplocentrus whitei* Gervais 1844; Family Vaejovidae: *Chihuahuanus crassimanus* Pocock 1808, *Maaykuyak waueri* Gertsch & Soleglad 1972, *Paruroctonus gracilior* Hoffmann 1931, *Paruroctonus utahensis* William 1968, *Pseudouroctonus chicano* Gertsch and Soleglad 1972, *Vaejovis coahuilae* Williams 1968, *Vaejovis decipiens* Hoffmann 1931, *Vaejovis intermedius* Borelli 1915, *Vaejovis vaquero* Gertsch & Soleglad 1972). However, the diversity of Chihuahua is not well understood, probably because acute cases of scorpionism are not common. The confirmed presence of *C. balsasensis* and current presence of *C. vittatus* could be a significant public health concern for the State and the US-Mexico border region because both species are very toxic. Constant scorpion surveillance in Chihuahua and other northern states of Mexico is very encouraged.

Acknowledgment

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References Cited

De Armas, L. F., y E. Martín-Frías. 1998. Presencia del género *Tityopsis* en México y descripción de una especie nueva (Scorpiones: Buthidae). An. Esc. Nac. Ciencias Biol. 43: 45-49.

Francke, O. F., R. Teruel, y C. E. Santibañez-Lopez. 2014. A new genus and a new species of scorpion (Scorpiones: Buthidae) from southeastern Mexico. J. Arachnology 42: 220-232.

Lourenço, W. R. 2016. Scorpion incidents, misidentification cases and possible implications for the final interpretation of results. Journal of Venomous Animals and Toxins including Tropical Diseases 22: 1-25.

Ponce-Saavedra, J., y O. F. Francke. 2004. Una nueva especie de alacrán del género *Centruroides* Marx (1890) (Scorpiones, Buthidae) de la depresión del Balsas, México. Acta Zool. Mex. 20: 221-232.

Ponce-Saavedra, J., y O. F. Francke. 2013. Clave para la identificación de especies de alacranes del género *Centruroides* Marx 1890 (Scorpiones: Buthidae) en el Centro Occidente de México. Biológicas 15: 52-62.

Ponce-Saavedra, J., I. Martinez-Rodriguez, y A. F. Quijano-Ravell. 2015. Alacranes de importancia médica en la depresión del Balsas. Entomología Mexicana 2: 66-70.

Ponce-Saavedra, J., O. F. Francke, A. F. Quijano-Ravell, y R. Cortés-Santillán. 2016. Alacranes (Arachnida: Scorpiones) de importancia para la salud pública de México. Folia Entomológica Mexicana 2: 45-70.

Rzedowski, J. 1983. Vegetación de México. 2nd edition. Editorial Limusa, México.

Secretaria De Salud. 2013. Programa de Acción Específico Prevención y Control de La Intoxicación Por Picadura de Alacrán, 1-70. http://www.cenaprece.salud.gob.mx/descargas/pdf/PAE_PrevencionControlIntoxicacionPicaduraAlacran2013_2018.pdf

Sissom, W. D., y B. Hendrixson. 2005. Scorpion biodiversity and patterns of endemism in northern Mexico, pp. 122-137. *In* J. L. E Cartron, G. Ceballos, and R. S. Felger [eds.], Biodiversity, Ecosystems and Conservation in Northern Mexico. Oxford University Press, UK.

Stahnke, H. L., y M. Calos. 1977. A key to the species of the genus *Centruroides* Marx (Scorpionida: Buthidae). Entomol. News 88: 111-120.

SCIENTIFIC NOTE

Damage by *Optatus palmaris* Pascoe[1] on *Annona* L. (Annonaceae) in the State of Guerrero, Mexico

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Abstract. Farmers who grow ilama (*Annona diversifolia* (L.) Saff.) in small orchards and backyards have had pest problems during the past 5 years. During a tour in towns at seven municipalities, a weevil (*Optatus palmaris* Pascoe) was observed as the principal cause of damage to the fruits of ilama in the northern region of the State of Guerrero. Damage by the weevil ranged from 5 to 100% (total fruit damage). Fruits damaged and oviposited in by *O. palmaris* do not reach physiological maturity and fall to the ground. Adults of *O. palmaris* when feeding on the exocarp of ilama cause necrosis and loss of quality of fruit for commercialization and economic loss of income for the farmer.

Species of fruit with high commercial value in Mexico, such as avocado (*Persea americana* Mill., Lauraceae), Nanche rojo de monte (*Malpighia mexicana* A. Juss., Malpighiaceae), guava (*Psidium guajava* Mill., Myrtaceae), ilama (*Annona diversifolia* (L.) Saff., Annonaceae), nopal (*Opuntia ficus-indica* (L.) Mil., Cactaceae), and tejocote (*Crataegus mexicanus* DC, Rosaceae) are affected by weevils (Castañeda-Vildózola et al. 2009, Bautista-Martínez et al. 2018, Mancera-Silva et al. 2018, Jones et al. 2019). Fruit from tropical trees of the Annonaceae family are rich in calories, vitamins, and minerals, and are sold in local markets by farmers who produce them. The best known and most cultivated Annonas are soursop (*Annona muricata* L.), saramuyo (*Annona squamosa* L.), and custard apple (*Annona cherimolla* Mill.). In Mexico, the area cultivated with of Annonaceae is small, and the fruits are consumed locally. Little-known species of Annonaceae with genetic potential are Amazonian anon (*Rollinia mucosa* Jacq.), custard apple or ox heart (*Annona reticulata* L.), blackhead or guanacona (*Annona purpurea* Moc. & Sessé ex Dunal), and ilama or ilamazapotl (*A. diversifolia*). Ilama, native to Mexico and Central America, is considered a phytogenetic resource threatened by logging of tropical deciduous forests. The quality fruit has excellent flavor and attractive pulp color, although in the last 5 years it has been affected by the fruit weevil.

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The weevil belongs to the Curculionidae family that has 62,000 described species (Oberprieler et al. 2007). Weevils use the fruit of Annonaceae as a source of food for their developing larvae. Therefore, it is considered a pest of agricultural importance because of its specificity for Annonaceae (Castañeda-Vildózola et al. 2009, Jones et al. 2019).

The genus *Optatus* Pascoe has three species in the Optatini tribe (Wibmer and O'Brien 1982). *Optatus inornatus* Champion was reported in Guatemala (Champion 1907); *Optatus fasciculosus* Champion is in Mexico, Guatemala, and Costa Rica (Champion 1907); and *Optatus palmaris* (Pascoe) is in Mexico and Honduras (O'Brien and Wibmer 1982). *O. palmaris* is 6.5 to 9.3 mm long, with distinctive morphological characteristics. The general color of the body is opaque black, except the back of the pronotum has red lines. In lateral view, structures of the thorax and ventricles are red (Fig. 1A) (Barbosa-Leitão 2007). Maldonado et al. (2014) mentioned that the life cycle of *O. palmaris* was 216 days.

Larvae feed on the pulp and seeds of soursop (*A. muricata*) for a period of 73 days. Close to pupation, they leave the fruits to pupate underground for 22 days. Adults have diurnal and nocturnal habits. Damage attributed to *O. palmaris* was quantified between 68 and 79%. Determining the range of expansion for insects of agricultural interest is of utmost importance for evaluating the negative impact of pests that infest agroecosystems. Therefore, the objective was to identify the presence of *O. palmaris* and its association with ilama fruits in the northern region of the State of Guerrero.

In September 2015 to 2020, fruiting ilama trees were visited and reviewed by farmers in the northern region of the State of Guerrero, to verify the presence of *O. palmaris* in fruits and estimate the number of fruit damaged by the weevils. During field and backyard work each year, the presence of and degree of damage by *O. palmaris* to ilama fruit were observed. Of the 100 trees sampled, weevils were found on 88 plants with fruit, three plants isolated in the field, and with an average of 12 fruits. No weevils were found on nine trees in a backyard, and no *O. palmaris* was observed because the farmer applied chlorpyrifos insecticide. Five specimens per tree were manually captured for transfer to a laboratory for identification. The insects were preserved in alcohol (70%) for specific determination with the aid of a stereoscopic microscope and taxonomic key using external morphological characters proposed by Champion (1907). All specimens were kept in the Faculty of Agricultural and Environmental Sciences, Autonomous University of Guerrero, Mexico.

Fruit of *A*. was infested by *O. palmaris*. The species of curculionid was reported in the states of Guanajuato, Michoacán, Nayarit, Oaxaca, and Mexico (Castañeda-Vildózola et al. 2009). Therefore, the recent record partially clarifies the current knowledge about the distribution of *O. palmaris* in Mexico.

The *O. palmaris* weevil observed in fruits of the Annonaceae, caused diverse degrees of damage (Fig. 1C, D, F, and H) in the municipalities of Apaxtla de Castrejon, Cocula, Huitzuco de los Figueroa, Iguala de la Independencia, Taxco de Alarcon, Teloloapan, and Tepecoacuilco de Trujano oin the northern region of the State of Guerrero. In previous studies, the weevil was reported to damage the flowers (Castañeda-Vildozola et al. 2009), peduncle, and exocarp, and pierce fruits to deposit eggs. Larvae emerge inside fruits and feed on the pulp and seed. Ilama fruits showed necrotic exocarp variation because of mechanical damage caused by the weevil (Fig. 1C, D, G, H and I). Incidence of *O. palmaris* ranged from two to 15 insects per fruit. The length of the weevil ranged from 8 to 13 mm.

Fruit damaged by the weevil ranged from 5 to 100% (total fruit damage). Fruits more than 70% damaged did not reach physiological maturity, and less than 69% damaged shortened the shelf life and caused loss of physical quality. Fruits with 10% damage by the weevils reached physiological maturity, retained morphological quality, and were accepted for commercialization in local markets, although consumers preferred fruits of better physical quality.

O. palmaris can be considered the main plague of Annonaceae cultivated in the northern region of the State of Guerrero. It is important to study its control or design local strategies for integrated pest management, because the insect follows the natural distribution pattern of its hosts and is a threat to the Annonaceae in the State of Guerrero, Mexico.

Fig. 1. Fruits of *Annona diversifolia* (L.) Saff. damaged by *Optatus palmaris* Pascoe. A = lateral view of *O. palmaris*. B = nondamaged fruit. C and F = adults feeding on fruits (5% damage). D = adult piercing fruit (15% damage). E = damaged fruit pulp. G = 40% damage, H = 70% damage, I = fruit totally damaged (100%) by adults of *O. palmaris*.

References Cited

Barbosa, L. M. L. 2007. Sistemática filogenética da tribo Neotropical Optatini Champion, 1907 (Coleoptera, *Curculionidae*, Baridinae). Tese (Doutorado), Instituto Nacional de Pesquisas da Amazônia, Universidade Federal do Amazonas, Manaus, Amazonas.

Bautista-Martínez, N., C. P. Illescas-Riquelme, and R. W. Jones. 2018. *Cylindrocopturus* (Coleoptera: Curculionidae: Conoderinae) species associated with *Opuntia* (Caryophyllales: Cactaceae) species. Fla. Entomol. 99: 126-127.

Castañeda-Vildózola, A., C. Nava-Díaz, L. M. Hernández-Fuentes, J. Valdez-Carrasco, and B. Treviño-Colunga. 2009. New host record and geographical distribution of *Optatus palmaris* Pascoe 1889 (Coleoptera: Curculionidae) in México. Acta Zoológica Mexicana 25: 663-666.

Champion, G. C. 1907. Biologia Centrali-americana, Insecta, Coleoptera, *Rhynchophora.* Vol. 4, Part 6. London, UK.

Jones, R. W., C. Illescas-Riquelme, V. López-Martínez, N. Bautista-Martínez, and C. W. O'Brien. 2019. Emergent and possible invasive pest species of weevils in Mexico. Fla. Entomol. 102: 480-485.

Mancera-Silva, A. V, N. Bautista-Martínez, C. P. Illescas-Riquelme, J. M. Valdez-Carrasco, and A. Castañeda-Vildózola. 2018. *Conotrachelus* species of agricultural and quarantine importance for Mexico. Southwest. Entomol. 43: 45-55.

O'Brien, C. W., and G. J. Wibmer. 1982. Annotated checklist of the weevils (Curculionidae sensu lato) of North America, Central America, and the West Indies (Coleoptera: *Curculionoidea*). Mem. Am. Entomol. Institute 34: 1-382.

Vidal-Hernández, L., H. López-Moctezuma, N. A. Vidal-Martínez, R. Ruiz-Bello, D. G. Castillo-Rocha, and R. Chiquito-Contreras. 2014. La situación de las anonáceas en México: principales plagas, enfermedades y su control. Revista Brasileira de Fruticultura 36: 44-53.

SCIENTIFIC NOTE

Phytophagous Scarab Beetles[1] Associated with Fruit Trees at Oaxaca, Mexico

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Knowledge of the diversity and distribution of phytophagous scarab beetles (Coleoptera: Scarabaeidae "Pleurosticti") in Mexico has increased significantly, but documented information about feeding habits is scarce, generalized, and sometimes imprecise. Morón (1996) studied the habits, abundance, and phenology of 15 species of Melolonthinae and Rutelinae associated with tulip flowers (*Hibiscus rosa-sinensis*, Malvaceae), which is one of the most specifically studied cases in Mexico.

Subsequently, Morón et al. (1997) established that, generally, most adult phytophagous Scarabaeidae feed on the floral structure and sugar content of various plants, while others also include foliage in their diet. They also documented that larvae and adults of the genus *Phyllophaga* Harris are associated with 57 plant families, native or introduced in Mexico and Central America, which include Lauraceae and Rosaceae. Some species of *Anomala* and *Phyllophaga* mainly in the larval stage are of agricultural importance, although adults usually also cause damage. The species are part of the white grub complex associated with various crops, among the main ones are maize (*Zea mays* L.), beans (Fabaceae), sugarcane (*Saccharum officinarum* L.), and sorghum (*Sorghum bicolor* (L.) Moench).

Some studies on association of *Phyllophaga* spp. with avocado (*Persea americana* Mill.) are: Zapata et al. (2018) pointed out that *P. obsoleta* damaged leaves, flowers, and fruits. Londoño et al. (2014) documented that *P. obsoleta* and *P. menetriesi* were mainly responsible for serious damage to fruit. Hernández-Cruz et al (2016) documented female *P. lenis* after copulation defoliating shoots of huizache (*Acacia farnesiana*, Fabaceae). However, until now association of *P. lenis* with avocado has not been documented. Little information exists on preference for *Anomala* spp. host plants; Londoño et al. (2014) reported *A. cincta* and *A. undulata* damaging fruit and causing losses in avocado production; Sisne et al. (2013) reported *A. calceata* in papaya (*Carica papaya* L.). *A. inconstans* has not been documented associated with loquat (*Eriobotrya japonica* (Thunb.) Lindl.

Persea americana Mill. (Lauraceae) whose fruit is commonly known as avocado, is a plant native to Mexico and Central America, fully adapted and cultivated in mountain areas over 1,000 m above sea level, whose economic importance increased significantly in recent years (CEDRSSA 2017). Loquat is a Rosaceae of Asian origin (South China), cultivated mostly in Japan. In tropical regions it is cultivated over 1,000 m above sea level (León 2000). The objective of this document was to report two unpublished cases of two scarab beetle species (Scarabaeidae:

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Melolonthinae and Rutelinae) associated with damage to avocado and loquat at Ocotlán de Morelos, Central Valleys of Oaxaca, Mexico.

In May 2019, approximately 120 specimens of two species of beetles were observed damaging avocado plants and loquat shrubs in an orchard in the municipality of Ocotlán de Morelos, Oaxaca, Mexico, between coordinates 16° 47′ 29″ N and 96° 40′ 30″ W, at an altitude of 1,518 m above sea level. A representative sample of eight specimens of each species was collected, which were preserved in 70% alcohol. Later, the genitalia were removed and mounted on entomological pins (Morón and Terrón 1988). Beetles were identified taxonomically with keys and descriptions by Bates (1888), Morón (1986), and Morón et al. (1997). The images of the males and their genitalia were taken with a Canon Rebel T-6 camera mounted on a Zeiss Stemi 508 stereo-microscope. Images were arranged by Adobe Photoshop CC 2017. The specimens were deposited in the private collections of the first and second authors.

The collected beetles correspond to two species of Scarabaeidae "Pleurosticti": *Anomala inconstans* (Burmeister, 1844) (Fig. 1a, b, c) and *Phyllophaga lenis* (Horn, 1887) (Fig. 1 e, f, g). The specimens of *A. inconstans* were observed feeding on loquat flowers (Fig. 1d). *P. lenis* was observed feeding on avocado leaves (Fig. 1h).

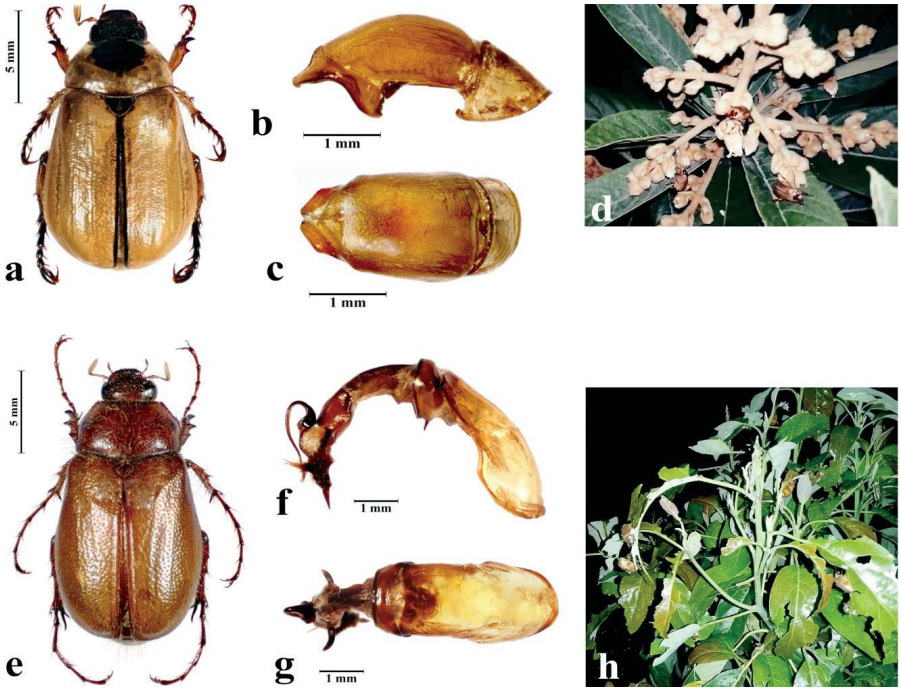

Fig. 1. Adult scarab beetles and host plants at Ocotlan, Oaxaca, Mexico. a) Dorsal habitus of *Anomala inconstans* (Burmeister, 1844) ♂. b and c) Aedeagus in lateral and dorsal views. d) Specimens of *A. inconstans* damaging a loquat inflorescence. e) Dorsal habitus of *Phyllophaga lenis* (Horn, 1887) ♂. f and g) Aedeagus in lateral and dorsal views. h) Specimens of *P. lenis* damaging avocado leaves.

According to what was observed in this research and reported by Pathania et al. (2016), beetles exhibit a different preference for a host plant in a particular locality and select a plant for food according to availability. Oliveira and Frizzas (2019) suggested the preference of beetles for areas with tall plants (trees and shrubs) was due not only to a strategy for a better dispersal of pheromones to locate a mate, but also to increase survival of their offspring, because the presence of vegetation implies roots that signify a source of feeding for rhizophagous species.

Both *Anomala inconstans* and *Phyllophaga lenis* can become important pests in crops such as maize, to cite an example (Saunders et al. 1998, Cano 2007). Therefore, it is important to continue monitoring activity of the beetles in cultivation of loquat and avocado at Ocotlán, Oaxaca, to assess and estimate the damage threshold, and determine if the beetles are causing significant loss in production of fruit trees in the area. In addition, prevention and/or control can be proposed, if necessary. However, it is suggested to continue documenting the beetle-host plant association and food preferences of the adult because accumulation of information will enable establishing behavior patterns of rhizophytophagous Scarabaeidae that can ultimately be used to propose integrated pest management strategies as suggested in Oliviera and Frizzas (2017), for example.

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References Cited

Bates, H. W. 1888-1889. Biología Centrali Americana. Insecta Coleoptera. Vol. 2 (Part 2): 161-415. https://doi.org/10.5962/bhl.title.730

Cano, E. B. 2007. Taxonomía, daño al cultivo y distribución de las especies del complejo “gallina ciega”, (Coleoptera: Scarabaeidae), que atacan el maíz (*Zea maíz* L.) en Guatemala. Revista de la Universidad del Valle de Guatemala 16: 85-98.

CEDRSSA (Centro de Estudios para el Desarrollo Rural Sustentable y Soberanía Alimentaria). 2017. Caso de exportación: El aguacate. Cámara de Diputados LXIII Legislatura. Palacio Legislativo de San Lázaro, Ciudad de México. http://www.cedrssa.gob.mx/files/b/13/54Exportaci%C3%B3n%20aguacate.pdf

Hernández-Cruz, J., M. A. Morón, J. Ruiz-Vega, J. A. Sánchez-García, L. Martínez-Martínez and R. Pérez. 2016. Descripción de la larva de *Phyllophaga lenis* (Coleoptera: Melolonthidae) en Santa Cruz Xoxocotlán, Oaxaca, México, con notas sobre su biología. Acta Zoológica Mexicana (NS) 32: 55-61.

León, J. 2000. Botánica de los cultivos tropicales. Tercera edición. Editorial Agroamérica. Instituto Interamericano de Cooperación para la Agricultura. San José, Costa Rica. ISBN 92-9039-395 5

Londoño, M. E., T. Kondo, A. Carabali, E. Varon, and A. Caicedo. 2014. Insectos y ácaros, pp. 228-283. *In* Bernal y Díaz [eds.], Actualización Tecnológica y Buenas Prácticas Agrícolas (BPA) en el Cultivo de Aguacate. CORPOICA, Rionegro, Antioquia, Colombia.

Morón, M. A. 1986. El género *Phyllophaga* en México. Morfología, sistemática y distribución supraespecífica (Insecta: Coleoptera). Publicación 20. Instituto de Ecología, DF., México.

Morón, M. A. 1996. Coleoptera Melolonthidae asociados con las flores de *Hibiscus rosa-sinensis* L. (Malvaceae) en la región de Xalapa, Veracruz, México. Giornale Italiano di Entomologia 8: 111-123.

Morón, M. A. 1997. White grubs (Coleoptera: Melolonthidae: *Phyllophaga* Harris) in Mexico y Central America. A brief review. Trends in Entomology 8: 117-128.

Morón, M. A., and R. Terrón. 1988. Entomología Práctica. Publicación 22. Instituto de Ecología. A. C. México DF. ISBN-968-7213-08-6

Morón, M. A., B. Ratcliffe, and C. Deloya. 1997. Atlas de los Escarabajos de México. Coleoptera: Lamellicornia. Familia Melolonthidae. Vol. I. Sociedad Mexicana de Entomología, Veracruz, México. ISBN 9680-7801-00-X

Oliveira, C. M., and M. R. Frizzas. 2019. How climate influences the biology and behaviour of *Phyllophaga capillata* (Coleoptera: Melolonthidae) in the Brazilian Cerrado. Austral Entomology. 58: 336-345. https://doi.org/10.1111/ aen.12309

Pathania, M., R. S. Chandel, K. S. Verma, and P. M. Kumar. 2016. Seasonal life cycle of *Holotrichia longipennis* (Blanchard) (Coleoptera: Scarabaeidae: Melolonthinae): a serious foliage and root feeding pest in India. Phytoparasitica 44: 615-629. https://doi.org//10.1007/s12600-016-0557-7

Saunders, J. L., K. S. Coto, and A. B. S. King. 1998. Plagas invertebradas de cultivos anuales alimenticios en América Central. 2nd ed. Centro Agronómico Tropical de Investigación y Enseñanza (CATIE). Turrialba, Costa Rica.

Sisne, M. L., I. A. Rodríguez, H. Grillo, J. C. Nápoles, R. E. Izquierdo, and D. Danay-Rodríguez. 2013. Especies de la familia Scarabaeidae asociadas a la fruta bomba (*Carica papaya* L.) en Ciego de Ávila.

Zapata, J. E., J. D. Tobón, H. I. Patiño, E. H. Palacios, C. A. Mejía, H. D. Marín, C. A. Icaraz, and E. Alcaraz. 2018. El cultivo de aguacate (*Persea americana*) en el Occidente de Antioquia. Servicio Nacional de Aprendizaje (SENA). Centro Tecnológico, Turístico y Agroindustrial del Occidente Antioqueño. Santa Fe de Antioquia. Colombia. Primera edición. ISBN: 978-958-15-0388-9.

SCIENTIFIC NOTE

Ocurrencia de *Platynota* sp.[1] Causando Pudrición de Frutos de Pimiento Morrón en Sinaloa, México

Platynota sp.[1] Causing Fruit Rot on Bell Peppers in Sinaloa, Mexico

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Resumen. Se observaron daños por alimentación en el pedúnculo, orificios en el cáliz, pudrición, y absición de frutos de pimiento morrón ocasionados por larvas de una palomilla hasta entonces desconocida del género *Platynota* en plantas cultivadas en invernadero en el ciclo agrícola de otoño-invierno de 2018-2019 en el Valle de Culiacán, Sinaloa, México. Este insecto es un nuevo riesgo potencial para la producción de pimientos en el Valle de Culiacán y otras zonas productoras de México.

Abstract. Feeding damage to the peduncle, holes in the calyx, rotting, and abscessing of bell pepper fruits caused by larvae of a previously unknown moth of the *Platynota* genus were observed in plants grown in greenhouse in the autumn-winter 2018-2019 agricultural cycle in the Valley of Culiacan, Sinaloa, Mexico. This insect is a new potential risk for the production of peppers in the Culiacan Valley and other producing areas of Mexico.

Durante el ciclo agrícola 2018-2019 productores de pimiento del Valle de Culiacán Sinaloa, notificaron daños de frutos de pimiento morrón cultivados en condiciones de invernadero. Los daños observados fueron por alimentación en el pedúnculo, orificios en el cáliz, pudrición, y absición de frutos. Al inicio, estos daños se confundieron con los que ocasiona el picudo del chile *Anthonomus eugenii* (Cartwright et al. 1990), y con los ocasionados por lepidópteros reportados atacando diferentes tipologías de frutos de chile como *Helicoverpa* sp. y *Spodoptera* sp. (Osorio et al. 2008, Lozano-Gutiérrez et al. 2018, Montezano et al. 2018). Sin embargo, debido a las bajas poblaciones de picudo del chile, a las diferencias en los orificios de los frutos, al tamaño y forma de los estados inmaduros, pupas y palomillas encontradas dentro y fuera de los frutos, se concluyó que los insectos observados

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tampoco pertenecían a estos géneros de insectos, y que esta palomilla desconocida era la responsable de estos daños.

Con base en los caracteres morfológicos (Atkins et al. 1957), se determinó que la palomilla causante de estos daños fue *Platynota* sp., Tortricidae: Lepidoptera. Miembros de este género se han descrito como plagas de pimiento morrón en México, Estados Unidos de América, y España (Atkins et al. 1957, Hejazi y Granett 1986, Groenen y Baixeras 2013). En México, se ha descrito daños por *Platynota* sp. en pimiento morrón en los estados de Baja California, Sonora, y Sinaloa (Atkins et al. 1957).

Platynota son insectos polífagos con cinco instares larvales (Atkins et al. 1957), conocidos como gusanos "enrolladores de las hojas" y "telarañeros" debido a la habilidad que tienen las larvas de enrollar las hojas de sus hospederos y dejar filamentos parecidos a telarañas (Bautista-Martínez et al. 2014). Se han descrito daños por *Platynota* en más de 20 familias de plantas, incluyendo a la familia

Fig. 1. Daños externos en los frutos de pimiento morrón por larvas de *Platynota* sp. y diferentes estados biológicos del insecto en el interior de los frutos. A) Ovipostura de *Platynota* sp., orificio de emergencia y daño en el pedúnculo ocasionados por los hábitos de las larvas. B) Larva de *Platynota* sp. alimentándose del cáliz del fruto. C) Larva. D) Pupa. E) Adulto.

Fig 1. External damage on bell pepper fruits by larvae of *Platynota* sp. and different biological stages of the insect inside the fruits. A) Oviposition of *Platynota* sp., emergence hole and damage on the peduncule caused by larvae habits. B) Larvae of *Platynota* sp. feeding on the calix of the fruit. C) Larvae. D) Pupae. E) Adult.

Solanacea (Atkins et al. 1957, Groenen y Baixeras 2013, Bautista-Martínez et al. 2014). Se ha reportado que la alimentación de larvas no se limita al follaje de las plantas, siendo posible encontrarlas en los frutos de estas familias. Sin embargo, desde el reporte de Atkins et al. (1957), en los años 50, no se ha reportado nuevamente este insecto.

Se colectaron frutos de pimientos dañados en nueve agrícolas con producción de pimiento morrón en invernaderos ubicados en las localidades de Costa Rica (24°36'05"N 107°23'53"O), Villa Juárez (24°32'59"M 107°32'52"), y La Palma (24°48'54"N 107°38'46"O) del Valle de Culiacán, Sinaloa, México. Estas áreas son las más representativas de la región donde se concentra más del 90% de la producción de pimiento morrón cultivado en condiciones de invernadero (Velarde-Félix et al. 2018). Se recolectaron 90 frutos con evidencia de daño por alimentación en el pedúnculo, orificios en el cáliz, y pudrición de frutos en el ciclo otoño-invierno del 2018-2019, los especímenes recuperados del interior de estos fueron preservados de acuerdo con su etapa de desarrollo (Fig. 1). Para la identificación de *Platynota* se usaron las claves en Bautista-Martínez et al. (2014).

Las larvas de *Platynota* son delgadas, de color claro de 1.6 a 2.4 cm. Las larvas L3 y L4 tienen dos líneas longitudinales a lo largo del cuerpo y la cabeza, la placa torácica, y en la placa anal con una estructura tipo peine con siete setas. Los adultos muestran dimorfismo sexual. La hembra es de color marrón rojizo, mientras que el macho marrón oscuro. Las alas anteriores tienen líneas transversales de color oscuro y los palpos largos como la cabeza y el tórax juntos (Fig. 2). El porcentaje promedio del total de frutos recolectados en las tres regiones fue del 96.7%.

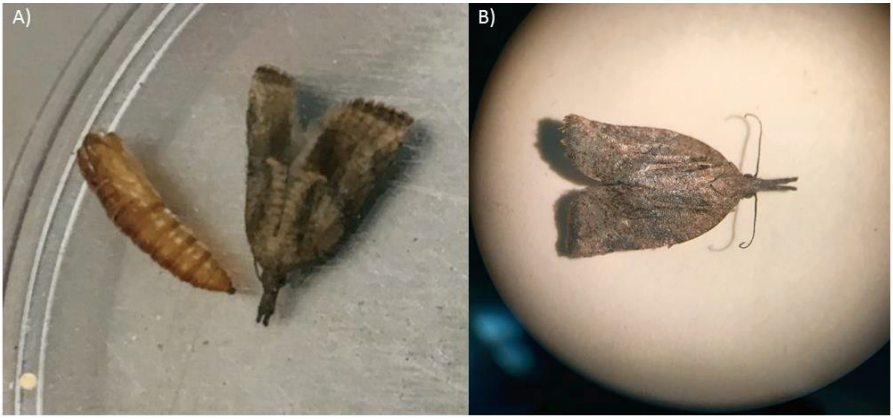

Fig. 2. Adultos de *Platynota* sp. idetnficados con un microscopio estereoscópico. A) Hembra. B) Macho.
Fig. 2. *Platynota* sp. adults identified with a stereoscopic microscope. A) Female. B) Male.

Los resultados de este estudio indican que *Platynota* sp. está presente en las regiones más representativas de este cultivo en el Valle de Culiacán y el estado de Sinaloa. Por tanto, *Platynota* sp. representa un riesgo potencial para la producción de pimiento morrón en esta región y otras que tengan contacto comercial con esta.

Referencias Citadas

Atkins, J. E. L., J. M. H. Frost, L. D. Anderson, and A. S. Deal. 1957. The "omnivorous leaf roller," *Platynota stultana* Wlshm., on cotton in California: nomenclature, life history, and bionomics (Lepidoptera, Tortricidae). Ann. Entomol. Soc. Am. 50: 251-259.

Bautista-Martínez, N., H. Vargas-Madríz, S. Ramírez-Alarcón, and R. Pérez-Pacheco. 2014. First report of the *Platynota* n. sp. (Lepidoptera: Tortricidae) genus in prickly pear (*Opuntia* spp.) in the municipality of Villa Milpa Alta, Mexico DF, Mexico. Southwest. Entomol. 39: 379-381.

Cartwright, B., T. G. Teague, L. D. Chandler, J. V. Edelson, and G. Bentsen. 1990. An action threshold for management of the pepper weevil (Coleoptera: Curculionidae) on bell peppers. J. Econ. Entomol. 83: 2003-2007.

FAO (Food and Agriculture Organization). 2020. Statistical Database. http://www.fao.org/faostat/es/#data/QC. Consulta Julio 2020.

Groenen, F., and J. Baixeras. 2013. The "omnivorous leafroller", *Platynota stultana* Walsingham, 1884 (Tortricidae: Sparganothini), a new moth for Europe. Nota Lepidopterologica 36: 53-55.

Hejazi, M. J., and J. Granett. 1986. Effects of low concentrations of two benzoylphenylureas on *Platynota stultana* (Lepidoptera: Tortricidae) in the greenhouse. J. Econ. Entomol. 79: 759-763.

Lozano-Gutiérrez, J., J. M. Salas-Montes, M. P. España-Luna, A. Lara-Herrera, J. Balleza-Cadengo, y C. A. Martínez-Contreras. 2018. Entomofauna asociada a seis tipos de chile (*Capsicum annuum* L.) en Morelos, Zacatecas, México. Entomología Mexicana 5: 95-99.

Montezano, D. G., A. Specht, D. R. Sosa-Gómez, V. F. Roque-Specht, J. C. Sousa-Silva, S. V. Paula-Moraes, J. A. Peterson, and T. E. Hunt. 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr. Entomol. 26: 286-300.

Osorio, A., A. M. Martínez, M. I. Schneider, O. Díaz, J. L. Corrales, M. C. Avilés, G. Smagghe, and S. Pineda. 2008. Monitoring of beet armyworm resistance to spinosad and methoxyfenozide in Mexico. Pest Manag. Sci. 64: 1001-1007.

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2020. Anuario Estadístico de la Producción Agrícola. https://nube.siap.gob.mx/cierreagricola/. Consulta Julio 2020.

Velarde-Félix, S., J. A. Garzón-Tiznado, S. Hernández-Verdugo, C. A. López-Orona, and J. E. Retes-Manjarrez. 2018. Occurrence of *Fusarium oxysporum* causing wilt on pepper in Mexico. Can. J. Plant Pathol. 40: 238-247.

SCIENTIFIC NOTE

Primer Reporte de *Argyrotaenia montezumae*[1] y *Templemania millistriata*[1] en Tejocote (*Crataegus mexicana*) en Chiautzingo, Puebla, México

First Report of *Argyrotaenia montezumae*[1] y *Templemania millistriata*[1] in Hawthorn (*Crataegus mexicana*) in Chiautzingo, Puebla, Mexico

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Resumen. Entre noviembre 2017 y febrero 2018 se observaron hojas de tejocote (*Crataegus mexicana* Moc. & Sessé ex. DC) con enrollamientos y larvas de *Argyrotaenia montezumae* y *Templemania millistriata* (Lepidoptera: Tortricidae) en Chiautzingo, Puebla. Este es el primer reporte de estas especies en México afectando *C. mexicana*.

Abstract. Tejocote (Crataegus mexicana Moc. & Sessé ex. DC) with leaf curling and larvae of *Argyrotaenia montezumae* and *Templemania millistriata* (Lepidoptera: Tortricidae) were observed in Chiautzingo, Puebla, between November 2017 and February 2018. This is the first report of these species in Mexico affecting *C. mexicana*.

En México, *Crataegus* spp., conocido como tejocote, es un frutal para elaborar diferentes dulces, mermeladas y bebidas, además tiene propiedades medicinales, y es de utilidad en la industria alimenticia debido a su alto contenido en pectinas (Higareda et al. 1995, Edwards et al. 2012, Muñiz-Reyes et al. 2014). En el estado de Puebla en 2018 se sembraron 884 ha de tejocote, con una producción de más de 5 mil toneladas y con un valor económico anual de más de MX$19 millones de pesos (SIAP 2020). El manejo fitosanitario de este cultivo es crucial para la obtención de una producción de calidad, donde destacan la presencia de la mosca de la fruta (*Rhagoletis pomonella*), el picudo del fruto (*Conotrachelus crataegi*), y escamas (*Toumeyella crataegi*), como los problemas de mayor importancia (Muñiz-Merino et al. 2012, Rull et al. 2014, Muñiz-Reyes et al. 2014, Kondo and González 2018).

Durante los meses de noviembre 2017 y febrero 2018 se observaron en huertas comerciales de tejocote en el municipio de Chiautzingo, Puebla (19° 11.406' N, 98° 30.880' W), signos de enrollamiento en brotes foliares (Fig. 1a) y al examinarlos se encontraban larvas de color verde claro que sujetaban con seda estas hojas (Fig. 1b). Se recolectaron 200 hojas con daños de las cuales se obtuvieron 41 larvas de diferentes instares. Los adultos se identificaron como

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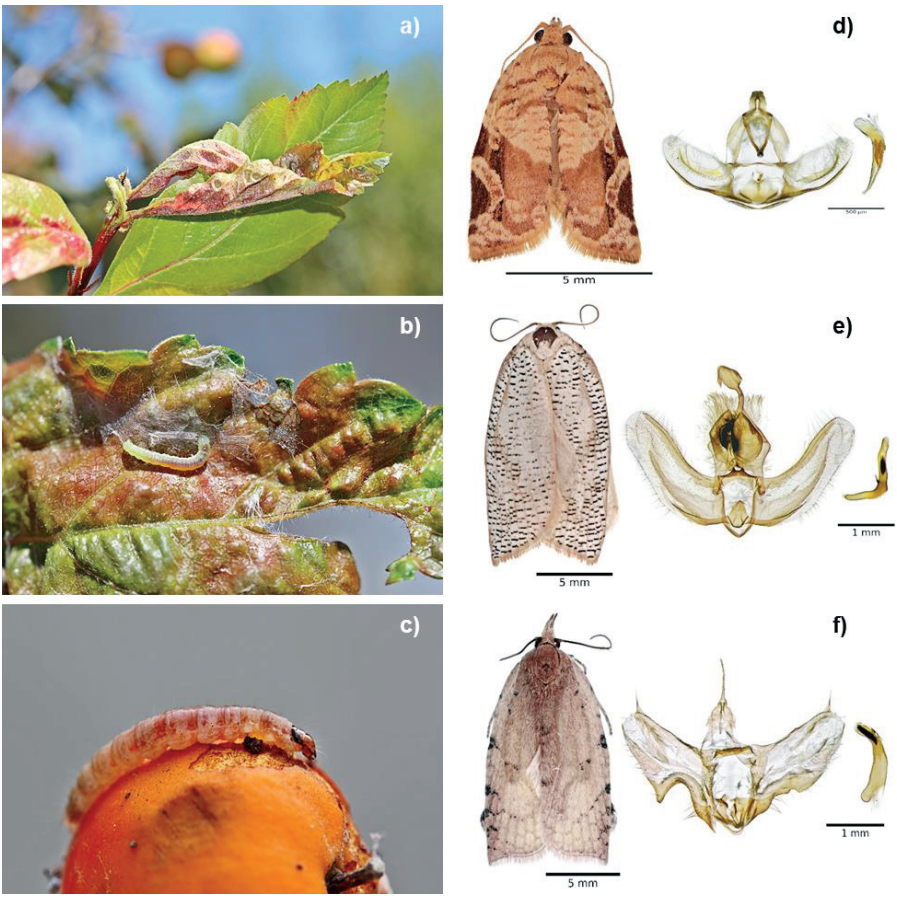

Fig. 1. a) Daño en hojas, b) larva de color verde claro en hojas sujetadas por seda, c) daño en epicarpio de frutos de tejocote por larva, d) Genitalia de macho de *Argyrotaenia montezumae*, e) Genitalia de macho de *Templemania millistriata*, y f) Tortrícido no identificado.
Fig. 1. a) Leaf damage, b) pale green larva on leaf tight by silk threads, c) hawthorn's skin damage by a larva, d) *Argyrotaenia montezumae* male genitalia, e) *Templemania millistriata* male genitalia, and f) unidentified tortricid.

Argyrotaenia montezumae (Walsingham) (Fig. 1d), *Templemania millistriata* (Walsingham) (Fig. 1e), y un tercer espécimen de Tortricidae no determinado (Fig. 1f).

Argyrotaenia montezumae ha sido reportada en México en *Eupatorium* sp. (Asteraceae), *Lilium longiflorum* var.eximium (Liliaceae), y *Parthenium hysterophorus* L. (Asteraceae) (Brown et al. 2008). Se han capturado adultos de *A. montezumae* en trampas cebadas con feromona sexual sintética para la captura de *Argyrotaenia citrana, Choristoneura rosaceana*, y *Archips argyrospilus* (Aguilera-Peña 2006).

Argyrotaenia montezumae también se ha reportado atacando zarzamora (*Rubus* sp.) en Michoacán (López et al. 2014).

Templemania millistriata ha sido reportado en Veracruz (Razowski and Becker 2011), pero este es el primer reporte de esta especie afectando árboles frutales. Las larvas de *A. montezumae,* y *T. millistriata* se alimentan de hojas nuevas de tejocote, las cuales enrollan y unen por medio de seda (Fig. 1a,b), con la finalidad de alimentarse, protegerse y posteriormente pupar, comportamiento similar a lo reportado para otros tortrícidos (Valera-Fuentes et al. 2009, Bautista-Martínez et al. 2011). En dos ocasiones se colectaron larvas entre frutos de tejocote, los cuales estaban unidos con seda. Bajo condiciones de laboratorio, cuando las larvas colectadas se alimentaban con tejocotes, éstas solo se alimentan superficialmente del epicarpio (Fig. 1c). También se observó que regularmente pupan en el sitio de la hoja donde se refugian, y los espacios de los frutos que unen.

Referencias Citadas

Aguilera-Peña, M. M. 2006. Implementación en México del acuerdo medidas sanitarias y fitosanitarias de la Organización Mundial del Comercio estudio de caso: plagas cuarentenarias de frutales. Tesis Doctorado en Ciencias, Colegio de Postgraduados, Montecillo, Texcoco, Edo. De México.

Bautista-Martínez, N., C. Chavarín Palacio, y E. López-Bautista. 2011. Primer reporte del enrollador de bandas oblicuas, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), en manzano en el ejido Vista Hermosa, Ciudad Cuauhtémoc, Chihuahua, México. Acta Zool. Mex. 27: 819-824.

Brown, J. W., G. Robinson, and J. A. Powell. 2008. Food plant database of the leafrollers of the world (Lepidoptera: Tortricidae) (Version 1.0). http://www.tortricid.net/foodplants.asp. Accessed 7 May 2020.

Edwards, J. E., P. N. Brown, N. Talent, T. A. Dickinson, and P. R. Shipley. 2012. A review of the chemistry of the genus *Crataegus*. Phytochemistry 79: 5-26.

Higareda, A., E. A. O. Prado, M. J. A. Salazar, R. G. E. Ramos, y D. M. Rosales. 1995. El concentrado comercial de pectina de la pulpa de tejocote. Rev. Chapingo Ser. Horticultura 4: 155-157.

Kondo T., and H. González. 2018. Description of a new species of *Toumeyella* Cockerell (Hemiptera: Coccomorpha: Coccidae) on *Crataegus mexicana* Moc. And Sessé ex DC. (Rosaceae). Insecta Mundi 634: 1-9.

López, I., S. Pineda, J. I. Figueroa, J. A. Sánchez, A. M. Martínez, R. N. Williams, and A. Rebollar-Alviter. 2014. Identification, parasitoids, and population dynamics of a blackberry leafroller (Lepidoptera: Tortricidae) from Michoacán, Mexico. Southwest. Entomol. 39: 503-510.

Muñiz-Merino, M., J. Cibrián-Tovar, y R. Nieto-Ángel. 2012. Fuentes de atracción y preferencia de oviposición de *Conotrachelus crataegi* Walsh (Coleoptera: Curculionidae) en *Crataegus* spp. (Rosaceae: Maloideae). Rev. Chapingo Ser. Horticultura 18: 21-37.

Muñiz-Reyes, E., A. W. Guzmán-Franco, J. Sánchez-Escudero, and R. Nieto-Ángel. 2014. Occurrence of entomopathogenic fungi in tejocote (*Crataegus mexicana*) orchard soils and their pathogenicity against *Rhagoletis pomonella*. J. Appl. Microbiol. 117: 1450-1462.

Razowski, J., and V. O. Becker. 2011. Systematic and faunistic data on Neotropical Tortricidae: Phricanthini, Tortricini, Atteriini, Polyorthini, Chlidanotini (Lepidoptera: Tortricidae). SHILAP Rev. Lepidop. 39: 161-181.

Rull, J., M. Aluja, J. Feder, and S. Berlocher. 2014. Distribution and host range of hawthorn-infesting *Rhagoletis* (Diptera: Tephritidae) in Mexico. Ann. Entomol. Soc. Am. 99: 662-672.

SIAP (Servicio de Información Agroalimentaria y Pesquera). 2020. Producción de Tejocote por Estado. http://www.siap.gob.mx/.

Varela-Fuentes, S., J. W. Brown, y G. Silva-Aguirre. 2009. Registro de *Platynota rostrana* (Walker, 1863) (Lepidoptera: Tortricidae) en cítricos de México. Acta Zool. Mex. 25: 651-654.

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