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POPULATION DYNAMICS OF *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA: CURCULIONIDAE) ON BLUE AGAVE

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ABSTRACT

The agave weevil, *Scyphophorus acupunctatus* Gyllenhal, is considered the main pest of *Agave tequilana* Weber var. 'Azul' (Asparagales: Asparagaceae) in Mexico. In this study, we investigated the population fluctuations of *S. acupunctatus* for 19 months using 2 sampling methods in blue agave fields in Ahualulco and Amatitán, Jalisco, Mexico. We recorded the direct damage caused by weevils on the sampled agave plants and evaluated the correlation between the number of *S. acupunctatus* captured by pheromone traps and the density of weevils located on plants. In Ahualulco, the highest agave weevil population density (for all developmental stages) was detected in Mar 2008, although the peak of trap captures occurred in May 2009. In Amatitán, the highest agave weevil population density was observed in Apr 2009, and the highest number of insects was captured in May and Sep 2009. The mean fraction of necrotic bole tissue caused by the weevils per sampled plant was 75.4 ± 2.1% and 72.6 ± 2.0% in Ahualulco and Amatitán, respectively. In Ahualulco, there was a positive correlation between the number of weevils caught in the pheromone traps and the number of adult weevils on the plants, whereas the number of weevils caught by the pheromone traps at Amatitán was positively correlated with the total number of weevils on the plants. We found that the sex ratio of weevils recovered from agave plants was approximately 1:1 at both experimental sites, and this trend did not vary seasonally; conversely, the sex ratio of captured weevils in pheromone traps was female-biased year-round.

Key Words: agave tequilero, agave weevil, population fluctuation, aggregation pheromone traps

RESUMEN

El picudo del agave, *Scyphophorus acupunctatus* Gyllenhal, es considerado la principal plaga en *Agave tequilana* Weber var. Azul en México. En esta investigación, se utilizaron dos métodos de muestreo para estudiar la dinámica poblacional de *S. acupunctatus* durante 19 meses en plantaciones comerciales de agave tequilero en Ahualulco y Amatitán, Jalisco. Así mismo, se registró el daño directo causado por larvas de picudo en las plantas muestreadas. Adicionalmente, se determinó la posible relación entre el número de picudos encontrados en plantas y el número de picudos capturados en trampas con feromona de agregación sintética. En Ahualulco la mayor densidad poblacional de picudo (todos los estadios de desarrollo) se detectó en marzo de 2008, mientras que la mayor cantidad de picudos capturados en trampas ocurrió en mayo de 2009. En Amatitán, la mayor densidad poblacional de picudo (todos los estadios de desarrollo) se detectó en abril de 2009, mientras que las mayores cantidades de picudos capturados en trampas se obtuvieron en mayo y septiembre de 2009. El daño promedio causado por picudo por planta de agave muestreada fue de 75.4 ± 2.1% y 72.6 ± 2.0% en Ahualulco y Amatitán, respectivamente. En Ahualulco el número de picudos cap-

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turados en trampas estuvo positivamente correlacionado con el número de picudos adultos en plantas. En Amatitán el número de picudos capturados en trampas con feromona estuvo positivamente correlacionado con el número total de picudos en plantas. En ambos sitios experimentales la proporción sexual de los picudos adultos encontrados en plantas fue de aproximadamente 1:1 y esta tendencia no varió a lo largo del tiempo. Sin embargo, en el caso de los picudos capturados en trampas, la mayoría fueron hembras.

Palabras Clave: agave tequilero, picudo del agave, fluctuación poblacional, trampas con feromona de agregación

Tequila is a spirit distilled from the fermented juice of the blue agave plant, *Agave tequilana* Weber var. 'Azul' (Asparagales: Asparagaceae), in Mexico. Tequila is produced in 5 states in Mexico, but the main production areas are located in the state of Jalisco, including the Tequila-Amatitán region and the Jalisco Highlands (Cedeño & Alvarez-Jacobs 1999). The tequila industry continues to grow in Mexico due to increases in both domestic consumption and foreign export; the tequila industry produced 312.1 million liters in 2008 (Valenzuela 2011). However, several abiotic and biotic factors can limit the production of agave plants, and one of the most important phytosanitary problems of blue agave is the weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae). The estimated damage caused by the feeding larvae of this insect on the agave boles tissue is approximately 24.5% (Solís-Aguilar et al. 2001); in addition to direct damage, the feeding weevils may facilitate the entry of phytopathogens, causing bole rot disease (González et al. 2007).

Insecticides are the main control method for *S. acupunctatus* (Solís-Aguilar et al. 2000; Terán-Vargas et al. 2012); however, the effective use of insecticides for controlling the weevil population is hindered by the cryptic behavior of the insect. Additionally, the misuse of insecticides may cause the development of resistance to these products and cause environmental and health problems. Within this context, the chemical ecology of *S. acupunctatus* has recently been investigated (Ruiz-Montiel et al. 2003, 2008, 2009) in the search for alternative management strategies. As with other weevil species, *S. acupunctatus* males release a pheromone that attracts both sexes. Although the aggregation pheromone of *S. acupunctatus* is composed of 2 ketones and 2 alcohols (Ruiz-Montiel et al. 2008), the major pheromone component, 2-methyl-4-octanone (Ruiz-Montiel et al. 2008), is sufficient to achieve captures equivalent to those for the quaternary blend (Rodríguez-Rebollar et al. 2012). In general, pheromone traps catch more *S. acupunctatus* females than males, though it is not known if this is due to a higher percentage of females than males present in the fields when the experiments were performed. Indeed, the potential to reduce the weevil population is much greater if more females than males are captured, yet weevil captures in pheromone traps are not always representative of field populations with respect to the sex ratio (Mitchell & Hardee 1974). Thus, an understanding of the causes for changes in the distribution, abundance, and sex ratio of *S. acupunctatus* may be essential for developing an integrated management system for this insect pest.

Using two sampling methods, we investigated the population fluctuations of *S. acupunctatus* for 19 months in two blue agave fields. We recorded the direct damage caused by weevils on sampled agave plants, and we further determined the possible relationship between the number of *S. acupunctatus* caught by pheromone traps and the density of weevils located on the plants.

Materials and Methods

Experimental Sites

The experiments were conducted in 2 commercial agave plantations in Jalisco, Mexico, each containing 7-yr-old blue agave plants. The first plantation, "El Molino", is located in the municipality of Ahualulco de Mercado (N 20° 37' W 103° 52', 1,300 m asl). This municipality has an average annual rainfall of 871.4 mm, with a rainy season normally occurring from Jun to Sep; the average annual temperature is 20.6 °C, with Jun as the hottest month and Jan as the coldest (Ruiz et al. 2004). The second plantation, "Loma Norte", is located in the municipality of Amatitán (N 20° 42' W 103° 37', 1,260 m asl). This region has an average annual temperature of 23.2 °C, with Jun as the hottest month and Jan as the coldest; the average annual rainfall is 951.7 mm, and the rainy season generally occurs from Jun to Sep (García 1987).

Direct Sampling on Whole Infested Plants

Each month, 10 agave plants per experimental site were selected for sampling ("jimadas"). The plants selected exhibited symptoms of bole rot disease with damage levels of 4 and 5 according to the conventional scale (Table 1) proposed by Consejo Regulador del Tequila (CRT 1999). A relationship between weevil infestation and bole root disease has been previously observed:

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TABLE 1. SCALE USED FOR DETERMINING THE DAMAGE LEVEL OF BOLE ROT DISEASE IN BLUE AGAVE, PROPOSED BY CONSEJO REGULADOR DEL TEQUILA (1999).

Damage level	Symptoms description
1	Apparently healthy plant
2	From 1 to 5 necrotic injuries points from 1 to 30 cm length, starting in the apical or lateral spines.
3	One or more necrotic injuries over 30 cm length, or more than 6 injuries from 5 to 30 cm.
4	Necrotic injuries in the head (crown), but healthy bole or "piña", the disease has not yet reached the "piña".
5	Head completely damaged and "piña" affected, the rot disease had invaded the "piña". Plant obviously dead.

plants more severely affected by the disease (4-5 on a scale of 1-5) regularly showed the highest number of weevils (Solís-Aguilar et al. 2001). We first removed the leaves of the plants, and the bole was carefully cut to search for developmental stages (except eggs) of *S. acupunctatus*. For each plant, the number of larvae, pupae, and adults was recorded, and the adults were placed in plastic containers and transported to the laboratory to be sexed according to Ramírez-Choza (1993). Additionally, we evaluated the damage caused by feeding larvae in the bole of the sampled plants on a visual basis by cutting the agave bole and observing the proportion of damaged bole tissue with respect to the total bole volume. We distinguished the direct damage resulting from the feeding larvae versus the disease because feeding larvae produce galleries in the tissue, which may be surrounded by necrotic tissue due to a chemical reaction by the plant. Data for the temperature, relative humidity, and precipitation were obtained from meteorological stations near the experimental sites.

Pheromone Traps for Monitoring Adults

Five traps baited with a synthetic aggregation pheromone were placed at each experimental site. One trap was placed at the center, and 4 other traps were placed at each cardinal direction; the traps were spaced 100 m apart from each other. The trap design used in this study consisted in a 4-L white plastic bucket with five 3 × 9-cm entry slits cut into the side to allow the entrance of the weevils (Fig. 1). A commercially available pheromone lure (each dispenser was loaded with 350 mg of 2-methyl-4-octanone) (FeroComps, Mexico City), the dispenser was hung from a wire tied to at the center and inside the lid of the container. The approximate release rates of the pheromone lures at Ahualulco de Mercado and Amatitán were 8.8 and 8.6 mg/day, respectively. The release rates were determined by the weight loss of the release devises maintained in the field in Ahualulco de Mercado and Amatitán at mean (±SD) temperatures of 20.3 ± 3.4 and 22.4 ± 2.3 °C, respectively. Pieces of agave leaves (200 g) sprinkled with 20 mL of malathion insecticide (Agricultura Nacional de Jalisco, S. A. de C. V., Tlaquepaque, Jalisco, Mexico) (5 mL/L of water) were placed inside a polyethylene bag (18.5 × 24.5 cm) in which 40 holes (5 mm diam) were made to allow the insecticide to contact the incoming weevils. Previous experiments have shown that malathion does not affect the response of weevils to its pheromone (Rodríguez 2011). The polyethylene bag with the agave tissue was placed inside the plastic bucket, and the trap was placed at ground level close to agave plants. Every 15 days, the weevils caught by each trap were recorded, and the agave tissue was replaced. The pheromone lures were changed monthly. The captured weevils were placed in plastic containers and transported to the laboratory where they were sexed.

Statistical Analysis

Correlation analyses were performed where appropriate to determine correlations among the number of adults, total number of weevils (including all developmental stages except eggs) on the plants, number of weevils caught by pheromone traps, pluvial precipitation, temperature, and relative humidity. We applied a Chi-squared test to determine whether there were significant differences between the number of females and males caught in the traps. All of the analyses were performed using SAS statistical software version 9.0 (SAS Institute, Cary, NC).

RESULTS

Direct Sampling on Whole Infested Plants

In Ahualulco, the highest agave weevil population density (all developmental stages except eggs) was detected in Mar 2008, with 70.2 weevils/plant (Fig. 2A). The highest number of larvae occurred in Mar 2008 and Sep 2009, whereas the number of weevil pupae peaked in Apr 2008. The highest population of adults was observed in Apr 2008 and May 2009 (Fig. 2A), and the proportion of females and males collected in plants averaged 51.2:48.8. The mean damage to the agave boles caused by the agave weevils per sampled agave plant was 75.42 ± 2.1 (sample size = 190 agave

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Fig. 1. Trap used to capture *Scyphophorus acupunctatus* in commercial plantations of *Agave tequilana* Weber var. 'Azul' in Jalisco, Mexico. The trap was designed by Rangel (2007).

plants) (Table 2). Negative correlations were found between the number of adults on plants and relative humidity (r = 0.76, P = 0.002) and the total number of weevils (including all developmental stages except eggs) on plants and relative humidity (r = 0.67, P = 0.009). The number of adults and the total number of weevils (including all developmental stages except eggs) on the plants were not correlated with temperature or precipitation.

In Amatitán, the highest agave weevil population density (all developmental stages except eggs) was found in Apr 2009 with 120.3 weevils/plant (Fig. 2B). The number of larvae peaked in Apr 2009, whereas the highest number of pupae occurred in Feb-Mar 2009. The adult population density at this site showed 3 peaks: May 2008 and Feb and Apr 2009 (Fig. 2B). On average, the proportion of females and males collected on the plants was 50.7:49.3. The mean damage to the agave boles (fraction of necrotic tissue in the boles) caused by the agave weevils per sampled agave plant was 72.57 ± 2.0 (sample size = 170 agave plants) (Table 2). Negative correlations were found between the number of adult weevils on the plants and relative humidity (r = 0.72, P = 0.002), the total number of weevils (including all developmental stages except eggs) on the plants and relative humidity (r = 0.59, P = 0.02), and the number of adult weevils on the plants and precipitation (r = 0.53, P = 0.03). The number of adults and total number of weevils were not correlated with temperature, and the total number of weevils on the plants was not associated with precipitation at this site.

Pheromone Traps for Monitoring Adults

In Ahualulco, few weevils were captured during the first 3 months of the experiment (Apr-Jun 2008), whereas the number of weevils caught increased thereafter until reaching a peak in May 2009 after which the captures decreased (Fig. 3A). The pheromone traps captured significantly (P < 0.001) more females than males (an average of 89.97% of females were captured), and this trend did not vary throughout the study. A negative correlation between the weevils caught by

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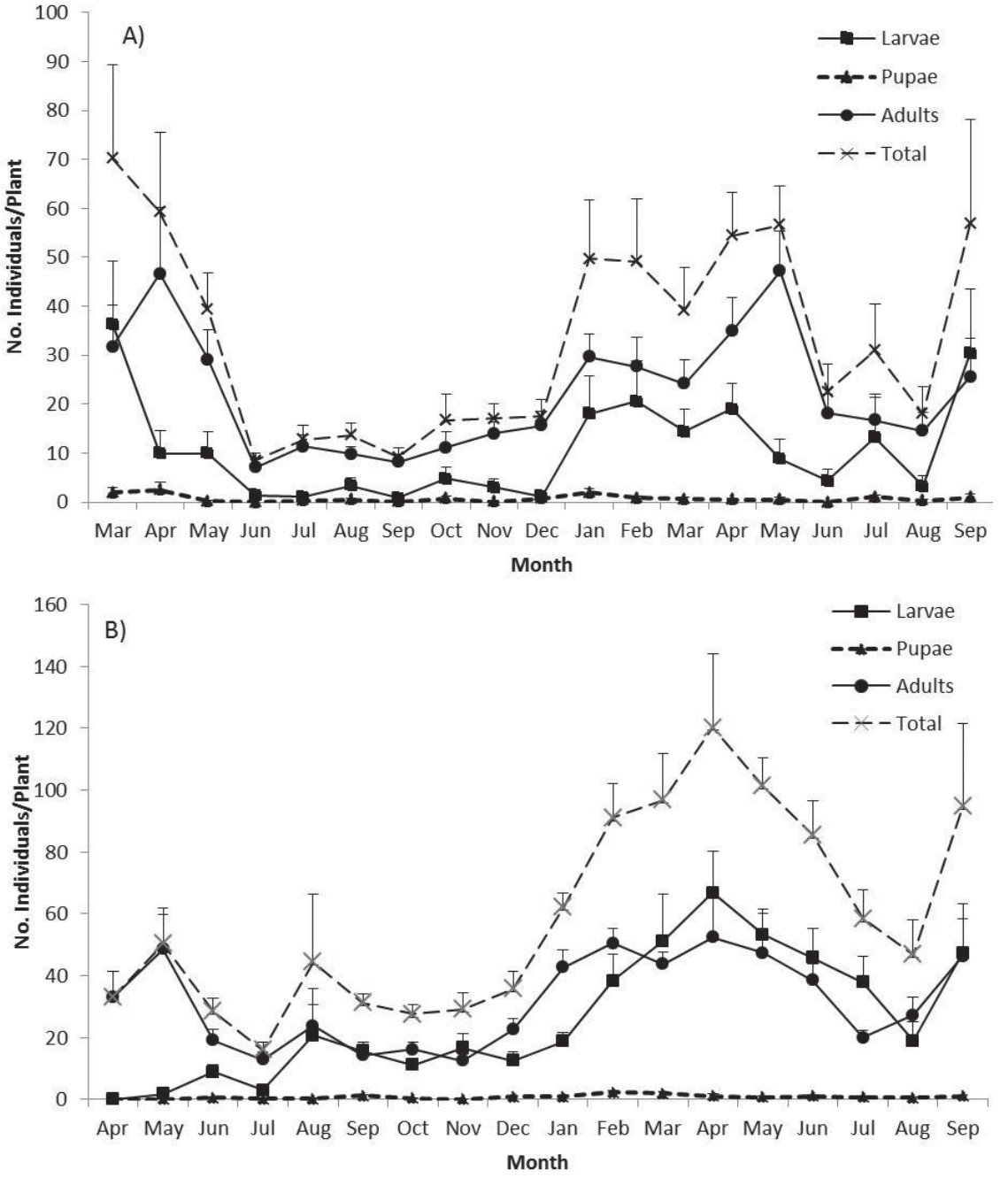


Fig. 2. Mean (+SEM) number of larvae, pupae, adults and total number of *Scyphophorus acupunctatus* in blue agave plants in Ahualulco (A) and Amatitán (B), Jalisco, Mexico. Each month 10 plants were sampled in each plantation during Mar 2008 to Sep 2009 (Ahualulco) and Apr 2008 to Sep 2009 (Amatitán).

pheromone traps and relative humidity ($r = 0.60$, $P = 0.02$) was found, but the number of weevils caught was not correlated with temperature or precipitation.

A similar trend in trap captures was noted in Amatitán, though 2 peaks were observed: one in May 2009 and another in Sep 2009 (Fig. 3B). Additionally, the traps caught significantly ($P < 0.001$) more females than males (in average, 93.86% of females were captured), and this trend was consistent throughout the study. The weevil captures were positively correlated with temperature ($r =$

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TABLE 2. MEAN (± SEM) DAMAGE BY AGAVE WEEVIL IN SAMPLED BLUE AGAVE PLANTS IN AHUALULCO AND AMATITÁN, JALISCO. TEN AGAVE PLANTS WERE SAMPLED EACH MONTH AT EACH SITE.

Month	Ahualulco	Amatitán
Mar 08	78.50 ± 9.4	
Apr 08	85.00 ± 6.4	
May 08	87.00 ± 2.4	96.00 ± 3.0
Jun 08	97.00 ± 1.1	95.25 ± 1.6
Jul 08	95.50 ± 1.4	88.50 ± 4.7
Aug 08	90.50 ± 1.6	95.00 ± 1.3
Sep 08	89.00 ± 1.8	78.00 ± 1.1
Oct 08	91.00 ± 1.9	91.50 ± 1.5
Nov 08	90.50 ± 1.9	91.50 ± 1.6
Dec 08	88.50 ± 2.2	90.00 ± 6.5
Jan 09	73.00 ± 11.0	89.00 ± 8.0
Feb 09	83.50 ± 5.2	73.50 ± 8.2
Mar 09	87.50 ± 7.6	58.00 ± 6.2
Apr 09	64.50 ± 8.3	45.00 ± 5.1
May 09	84.00 ± 4.8	60.00 ± 3.5
Jun 09	42.50 ± 8.2	55.50 ± 5.1
Jul 09	41.00 ± 8.0	40.00 ± 3.5
Aug 09	39.50 ± 10.2	37.90 ± 6.0
Sep 09	25.00 ± 6.1	49.00 ± 11.0
Mean	75.42 ± 2.1	72.57 ± 2.0

0.56, $P = 0.03$) but not with relative humidity or precipitation.

At Ahualulco, the number of weevils captured by pheromone traps was positively correlated with the number of adult weevils on the plants ($r = 0.46$, $P = 0.05$) but was not correlated with the total number of weevils (i.e., number of larvae, pupae and adults) on the plants ($r = 0.46$, $P = 0.0547$). The number of weevils captured in pheromone traps at Amatitán was positively correlated with the total number of weevils on the plants ($r = 0.58$, $P = 0.02$), though no significant correlation between the number of adult weevils on the plants was observed ($r = 0.40$, $P = 0.11$).

DISCUSSION

In this study, we investigated the population fluctuation and seasonality of *S. acupunctatus* on blue agave in Amatitán and Ahualulco, Jalisco. We found that this weevil species is present in the agave plantations year-round, though there are 1 or 2 population density peaks during the yr. A similar situation was reported on blue agave for other regions of Jalisco (Solís-Aguilar et al. 2001). In Tucson, Arizona, weevils were found to colonize cultivated *Agave americana* L. from May to Oct, whereas populations of *S. acupunctatus* were observed on wild *Agave palmeri* Englemann from Apr to Sep, a time when some of the plants bloomed (Waring & Smith 1986).

The degree of damage (fraction of the tissue of the bole that has galleries and is necrotic) caused by the weevil to the agave boles per sampled plant in the present study was higher than in previous studies. For example, Solís-Aguilar et al. (2001) reported an average of 24.5% of damage by the weevil of boles of *Agave tequilana* Weber in Jalisco. In Oaxaca, 10.3 and 13.4% weevil damage was reported for the boles of *Agave angustifolia* Haw and *A. tequilana*, respectively (Aquino et al. 2007). These differences were mainly caused by the sampling methodology, as our sampling was directed to plants with symptoms of bole rot disease in the plantations. In contrast, Solís-Aguilar et al. (2001) directly sampled harvested boles at a Tequila factory, and Aquino et al. (2007) sampled harvested boles at a Mezcal factory. Regardless, the present study confirmed the previously reported (Solís-Aguilar et al. 2001) relationship between bole rot disease and agave weevil attack, in that the presence of weevils was detected on all of the sampled agave plants, which displayed bole rot disease symptoms. However, it remains to be investigated whether the adult agave weevil is a vector or transmitter of this rot disease. Waring & Smith (1986) have reported that microbes associated with *S. acupunctatus* apparently cause agave decline, a disease that accompanies larval infestation.

We found relatively few pupae compared to larvae and adults during our direct sampling. Solís-Aguilar et al. (2001) made a similar finding when sampling blue agave plants in 3 other regions of Jalisco, Mexico. The fact that few pupae were recovered in these studies may be ex-

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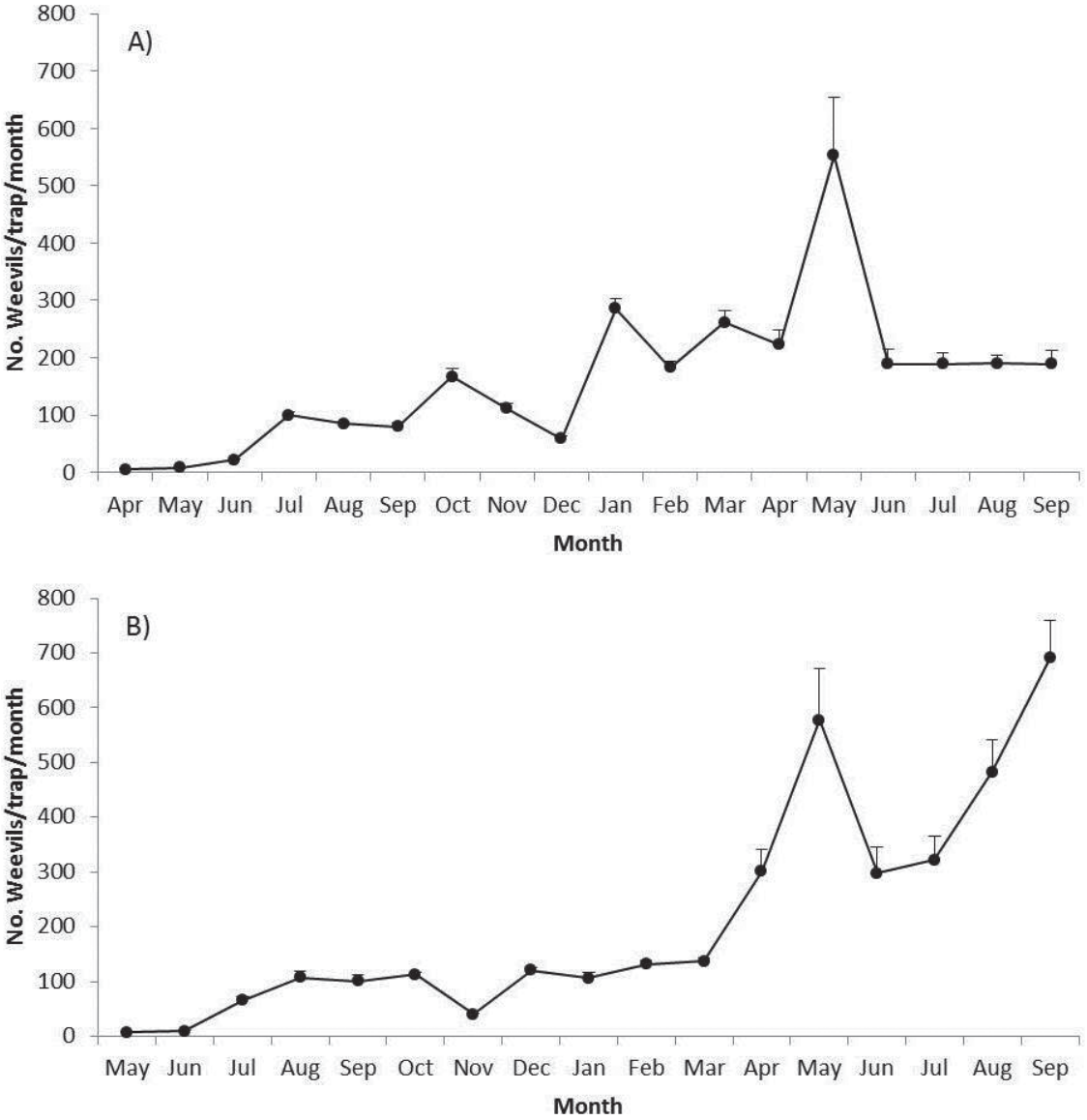


Fig. 3. Mean (± SEM) number of *Scyphophorus acupunctatus* weevils captured with pheromone-baited traps at 2 blue agave commercial plantations in Ahualulco (A) and Amatitán (B), Jalisco, Mexico. Traps (5 replicate) were deployed in the plantations during Apr 2008 to Sep 2009 at Ahualulco and May 2008 to Sep 2009 at Amatitán.

plained by different factors that are not mutually exclusive. 1) Pupal stadia were not accurately detected because the sampling was performed monthly and the pupal stadium only lasts 9-13 days (Ramírez-Choza 1993; Valdés et al. 2010). 2) After emerging, adult weevils may remain inside the brood host plants, or weevils from neighbor plants may migrate to the infested plants. It has been reported that *S. acupunctatus* adults may live more than 1 yr (Ramírez-Choza 1993; Valdés et al. 2010), and Waring & Smith (1986) suggested that infested agave plants could be re-infested by resident or immigrant weevils. 3) Pupation may occur outside the agave plants; for example, Waring & Smith (1986) found *S. acupunctatus* cocoons in the soil beneath plants, though pupae were also found inside the base of leaves.

The relationship between the environmental factors recorded in this study and the weevil populations on blue agave plants or the number of weevils caught by pheromone traps was not consistent at both experimental sites. This may be explained by our use of data from meteorological stations close to the experimental sites rather

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than directly collecting the data from the sites. The local site microclimate conditions may be more important in affecting insect captures by pheromone traps than regional climatic conditions. For example, Jones et al. (1992) found that variations in microclimate conditions around individual pheromone traps are responsible for considerable inter-trap variation in the capture of *Anthonomus grandis* Boheman. Using an automated weather instrument at an experimental site, Rhino et al. (2010) found that temperature, wind speed, and relative humidity had a significant effect on the capture of *Cosmopolites sordidus* Germar. Further studies are necessary to evaluate the effects of local microclimate conditions on the capture of *S. acupunctatus* by pheromone traps.

We found that the pheromone-baited traps caught more females than males, in agreement with Ruiz-Montiel et al. (2008) and Rodríguez-Rebollar et al. (2012), who mentioned the possibility that more females than males were present in their experimental sites when the experiments were performed. However, the results of the present study clearly show that the proportion of females to males on agave plants was approximately 1:1, and this did not vary seasonally. Mitchell & Hardee (1974) found that boll weevil traps are not representative of field populations with regard to sex ratio, reporting that pheromone traps captured more *A. grandis* females than males as the squaring by cotton plants declined, whereas direct sampling during this time showed more males than females on the plants. Sallam et al. (2007) found that traps baited with sugarcane caught more *Rhabdoscelus obscurus* (Boisduval) males than females from Feb to Apr, though the ratio was more balanced later. However, these authors also found that the sex ratio of captured weevils in pheromone traps was female biased all yr round, except during May.

The fact that more females than males are attracted to baited aggregation pheromone traps may indicate that the primary function of this male-produced aggregation pheromone is to attract females as potential mates, though other males may use it for locating congregated females and plant resources, as suggested for a number of beetle species (Landolt 1997; Hodges et al. 2002; Edde et al. 2005). However, field experiments have shown that traps baited with live males as a lure caught an equal number of males and females (Ruiz-Montiel et al. 2003, 2008; Rodríguez-Rebollar et al. 2012). Alternatively, the higher amount of pheromone released by lures may affect the male's response to the pheromone. It has been estimated that a male releases 1.4 to 11.4 ng/hr of pheromone (Ruiz-Montiel et al. 2009), whereas a synthetic lure emits approximately ten thousand times more pheromone than males. We can speculate that males can become sensorially fatigued when they contact a high pheromone concentration and they therefore stop responding to the pheromone, yet it is not clear why females do not become sensorially fatigued. In wind tunnel experiments, Sanders (1997) found that the flight of a male moth is affected when approaching a high pheromone concentration. Whether the pheromone of *S. acupunctatus* males functions as a sex signal and whether males become sensorially fatigued when contacting a high concentration of pheromone should be investigated in future studies.

In summary, this study showed that *S. acupunctatus* is present year-round at both of the sites examined, with certain months showing the highest population. At one site (Ahualulco), we found a positive correlation between the number of adult weevils on the plants and the number of agave weevils caught in pheromone traps. At the other site (Amatitán), we found positive correlation between the total number of weevils on the plants and the number of weevils caught by the pheromone traps. These results show the possibility of using pheromone traps for monitoring the populations of this pest and suggest their use in control strategies.

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