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Attraction range and inter-trap distance of pheromone-baited traps for monitoring *Scyphophorus acupunctatus* (Coleoptera: Dryophthoridae) on blue agave

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Abstract

Scyphophorus acupunctatus Gyllenhal (Coleoptera: Dryophthoridae) is one of the most important insect pests of wild and cultivated agaves in Mexico. For managing this weevil, it is important to have a method for detecting and sampling its population density. The weevil's life cycle takes place inside the agave plant, which makes sampling of the weevil difficult. The use of traps baited with synthetic pheromone plus agave tissue is a useful tool for sampling the population density of this pest. Using the capture-mark-release-recapture method, we investigated the attraction range of traps baited with synthetic pheromone plus agave tissue to capture *S. acupunctatus*. We also evaluated several inter-trap distances to determine the best density of traps for monitoring this insect. Our results showed that these traps attracted *S. acupunctatus* up to a range of 120 m. In addition, we found that the cardinal point at which weevils were released affected their recapture. The results also showed that, in general, experiments with the longest inter-trap distances captured the most weevils. Thus, traps placed at 100 m in the 1st experiment, 200 m in the 2nd experiment, and 250 m in the 3rd experiment captured more weevils than traps placed at shorter distances. Based on our results of attraction range and inter-trap distances, we suggest that these pheromone-baited traps could be used for monitoring *S. acupunctatus* at densities of 1 trap per 6 ha of blue agave crop.

Key Words: aggregation pheromone; trap density; sampling

Resumen

Scyphophorus acupunctatus Gyllenhal (Coleoptera: Dryophthoridae) es la plaga insectil de mayor importancia en agaves silvestres y cultivados en México. Para el manejo de este picudo es importante tener un método de muestreo para monitorear su densidad poblacional, pero debido a que todo su ciclo biológico ocurre en el interior de la planta, su muestreo es difícil; así, el uso de trampas con feromona sintética es una opción para su monitoreo. En este estudio, se investigó el radio de atracción de trampas cebadas con feromona sintética y tejido de agave para la captura de *S. acupunctatus*, mediante la técnica de captura-marcaje-liberación y recaptura. También se evaluaron diferentes distancias entre trampas para el monitoreo de este insecto. Se encontró que las trampas cebadas con feromona sintética y tejido de agave atrajeron a *S. acupunctatus* hasta en un rango de 120 m. Adicionalmente, encontramos que el punto cardinal en donde se liberaron los insectos afectan la recaptura de los picudos. En cuanto a distancia entre trampas, se encontró que las trampas colocadas a las mayores distancias entre sí capturaron más picudos. Así, las trampas colocadas a las mayores distancias evaluadas (100, 200 o 250 m) capturaron más picudos que las trampas colocadas a menores distancias. En base a los resultados de este estudio, para el monitoreo de *S. acupunctatus* se sugiere usar estas trampas cebadas con feromona sintética y tejido de agave a una densidad de una trampa por cada 6 hectáreas de agave tequilero.

Palabras Clave: feromona de agregación; densidad de trampas; monitoreo

Scyphophorus acupunctatus Gyllenhal (Coleoptera: Dryophthoridae) damages a number of wild and cultivated agaves (Asparagales: Asparagaceae) in many countries (Vaurie 1971; Waring & Smith 1986). In Mexico, this insect is considered the most important pest for several cultivated agaves including the blue agave (*Agave tequilana* Weber 'Azul') (Solís-Aguilar et al. 2001), agave "espadín" (*Agave angustifolia* Haw.), agave "papalote" (*Agave cupreata* Trel. & Berger) (Barrios et al. 2006; Aquino Bolaños et al. 2007), henequen (*Agave fourcroydes* Lem.) (Ramírez-Choza 1993), and tuberose (*Polianthes tuberosa* L.) (Camino

et al. 2002). This insect pest causes direct damage on agave plants when larvae feed on the "bole" or head of agaves, drilling and making galleries; the adults also feed on agave, but the damage they cause is considered of minor importance. The damage by feeding larvae in blue agave has been estimated at about 24.5% of the total volume of the blue agave boles (Solís-Aguilar et al. 2001), and about 10.3% in agave "espadín" (Aquino Bolaños et al. 2007). Figueroa-Castro et al. (2013) found that on blue agave plants with symptoms of bole rot disease, the direct damage by the weevils was about 70% of the total tissue of

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the agave bole. In addition to direct damage, the galleries and wounds produced by larvae and egg-laying females may be used as an entry for pathogenic organisms (Waring & Smith 1986; González Hernández et al. 2007). There is also a possibility that this weevil may be a vector of some plant pathogens (Waring & Smith 1986; Aquino Bolaños et al. 2011).

The chemical ecology of this species has been studied recently in order to develop a strategy for monitoring or controlling this weevil (Ruiz-Montiel et al. 2003, 2008, 2009; Rojas et al. 2006). *Scyphophorus acupunctatus* males produce an aggregation pheromone, composed of 2 ketones and 2 alcohols (Ruiz-Montiel et al. 2008) of which 2-methyl-4-octanone is the major compound. Field experiments showed that this compound is sufficient for capturing *S. acupunctatus* adults (Rodríguez-Rebollar et al. 2012). Recently, a study demonstrated that there was a correlation between the weevil population density and the weevils captured on pheromone-baited traps on agave plants (Figueroa-Castro et al. 2013), suggesting that pheromone traps could be used for monitoring this weevil. Nevertheless, several factors associated with the trap and attractants need to be optimized before a pheromone-mediated monitoring system for this weevil can be developed (Rojas et al. 2006).

This study was conducted in order to investigate the attraction range of traps baited with a synthetic aggregation pheromone plus agave tissue, using the capture-mark-release-recapture method, and to evaluate several inter-trap distances to determine the best trap density for monitoring the population density of *S. acupunctatus* on blue agave plantations.

Materials and Methods

TRAP

The trap used was a 4 L white plastic bucket with 4 circular holes (each hole 4.0 cm in diameter) made at 1 cm above the trap base. On each of these holes, a transparent plastic cone (3.5 cm long, with 4.0 cm entry and 3.0 cm exit diameter) was attached to the inner wall of the bucket to allow the weevils to enter and obstruct their exit from the bucket. The materials for making the traps were obtained locally, and traps were handmade.

LURE

Lures were obtained from commercial sources (Tequilur, FerroComps, Mexico City, Mexico), and each lure contained 350 mg of 2-methyl-4-octanone. The pheromone lure dispenser was hung on a wire tied to the center and inside the trap lid. In addition, pieces of recently cut agave tissue (200 g) were placed in each trap. The agave tissue was sprayed with 50 mL of malathion insecticide (Malathion 1000®, Agricultura Nacional de Jalisco, S. A. de C. V., Tlaquepaque, Jalisco, Mexico) (10 mL/L of water) and then placed inside a polyethylene bag (18.5 × 24.5 cm) on which 40 holes (5 mm in diameter) were made to allow the insecticide to contact the incoming weevils. The bag with the agave tissue was placed inside the trap. The trap was placed at ground level close to agave plants at the agave plantation, or in the center of the uncultivated plots. The pheromone dispenser was changed monthly, and the agave tissue was replaced every 15 d.

PHEROMONE ATTRACTION RANGE

Three experiments were performed to investigate the pheromone attraction range. The 1st experiment was conducted from Aug to Oct 2008 in a 7-yr-old blue agave plantation "Loma Norte" (20.700000°N,

103.616667°W, 1,260 m asl) located in the municipality of Amatitán, Jalisco. The 2nd experiment was conducted from Jan to Feb 2012 in a 2.5 ha uncultivated plot "El Amatito" (18.333333°N, 99.166667°W, 911 m asl). The 3rd experiment was conducted from Feb to Mar 2012 in a 3.5 ha uncultivated plot "El Terrero" (18.350000°N, 99.166667°W, 953 m asl). Plots used in experiments 2 and 3 were weed free at the time of the trials. The uncultivated plots were semi flat in topography and were located in Quetzalapa at the municipality of Huitzuco de los Figueroa, Guerrero.

The weevils used in the 1st experiment were collected in the blue agave plantation where the experiment was conducted. The weevils used in the 2nd and 3rd experiments were collected in plantations of *A. angustifolia* near to the places where these experiments were established. Collected weevils were separated by sex (Ramírez-Choza 1993) and kept in plastic containers of 2 L capacity with agave tissue for 1 d before they were marked and released. In the 1st experiment, weevils were marked in the intersegmental area of the thorax and elytra with a Neon Red fluorescent pigment (DayGlo Color Corp. Cleveland, Ohio) that is used as marker for other insects (Quintero-Fong et al. 2009). In the other experiments, weevils were marked on the elytra and pronotum with nail polish (Amour®) of various colors to differentiate the weevils released in each evaluated distance. In a preliminary trial, we observed that these paints did not affect the survival or behavior of weevils. Marked weevils always were released in the afternoon between 3:00 and 6:00 p.m.

In the 1st experiment, 240 weevils were used, and 10 weevils (5 males and 5 females) were released at 6 distances from the trap (1, 5, 10, 20, 50, and 100 m) in each of 4 cardinal points. In the 2nd experiment, 280 weevils were used, and 14 weevils (7 males and 7 females) were released at 20, 40, 60, 80, and 100 m from the trap in each of 4 cardinal points. In the 3rd experiment, 160 weevils were used, and 20 weevils (10 males and 10 females) were released at 60 and 120 m from the trap in each of 4 cardinal points. In these experiments, we evaluated if the responses of weevils to pheromone-baited traps change when traps are placed at short (e.g., 1, 5, 10 m) or long (e.g., 20, 50, 60 m) distances. The number of individuals released in the experiments depended on the number of weevils available when the experiments were performed. In the 1st experiment, weevils were recaptured from the traps 5 d after release; in the 2nd and 3rd experiments, weevils were recaptured each 3rd day, but sometimes, because of logistic problems, the recollections were performed each 4th or 5th day. The number and sex of weevils recaptured in each release distance were recorded.

EFFECT OF THE POINT OF RELEASE ON WEEVIL CAPTURES

The effect of cardinal points where weevils were released on weevil captures was evaluated in 1 experiment. The experiment was conducted from Jan to Feb 2012 in the 2.5 ha uncultivated plot "El Amatito." In this experiment, 120 weevils were used, and 30 weevils (15 males and 15 females) were released at 50 m distance from the trap in each of 4 cardinal points. Insects used in the experiment were collected in plantations of *A. angustifolia* near to the places where this experiment was performed, and they were marked on the elytra and pronotum with nail polish (Amour®) of various colors to differentiate the weevils released in each cardinal point. Weevils were recaptured at 1, 2, 4, 8, 11, 15, and 20 d after release.

EFFECT OF INTER-TRAP DISTANCE ON WEEVIL CAPTURES

Three experiments were conducted to determine the best inter-trap distance for monitoring *S. acupunctatus* on blue agave. The 1st ex-

periment was performed from Nov to Dec 2007 in a 5-yr-old blue agave plantation “Bajío Norte” (20.700000°N, 103.616667°W, 1,450 m asl) located in the municipality of El Arenal, Jalisco. The 2nd and 3rd experiments were carried out from Sep 2012 to Jan 2013 in a 2-yr-old blue agave 60 ha plantation “El Molino” (20.716667°N, 103.916667°W, 1,314 m asl) located in the municipality of Ahualulco de Mercado, Jalisco.

In the 1st experiment, 5 inter-trap distances (5, 10, 20, 50, and 100 m) with 4 replications were evaluated. In the 2nd experiment, 3 inter-trap distances (100, 150, and 200 m) with 8 replications were evaluated. In the 3rd experiment, 2 inter-trap distances (200 and 250 m) with 4 replications were evaluated. All experiments were carried out in a completely randomized experimental design. Weevils were collected from traps every 15 d. The number and sex of weevils captured in each trap were recorded.

STATISTICAL ANALYSES

All statistical analyses were made using SAS statistical software version 9.0 (SAS Institute 2004). The recapture percentage at each evaluated distance in the pheromone attraction range experiments was calculated using the following formula: recapture percentage = [(number of recaptured weevils) / (100)] / number of marked released weevils. A correlation analysis between the release distance of weevils and the recapture percentage was conducted for the first 3 experiments. When a correlation was found, the data were fitted to a linear regression model [$Y = \beta_0 + \beta_1 X$, where: Y = recapture percentage, X = release distance, β_0 = intercept, β_1 = model parameter (unknown)]. In the 3rd experiment of attraction range of pheromone-baited traps, the data were analyzed with a chi-squared test as a contingency table 2×2 (recapture \times distance). Data from the experiment on the effect of cardinal points on weevil captures were also analyzed with chi-squared tests as a contingency table 2×4 (recapture \times cardinal point). The data of the 1st experiment of attraction range of pheromone-baited traps, and the data of the 3 inter-trap distance experiments were analyzed by an analysis of variance using the procedure Proc GLM; previously, data were analyzed for normality (Shapiro–Wilk test) and variance homogeneity (Bartlett test) and transformed if necessary. Treatment means were separated with Tukey’s test ($\alpha = 0.05$). A chi-squared test was applied to determine if significant differences between the number of females and males caught in the traps existed.

Results

PHEROMONE ATTRACTION RANGE

In the 1st experiment (blue agave plantation), there were differences between treatments ($F = 14.23$; $df = 5, 18$; $P < 0.0001$), but there was no correlation between the release distance of weevils and the recapture percentage ($r = -0.78$; $P = 0.119$). The highest recapture percentage was obtained when weevils were released 1 m from the trap (Fig. 1). More females (81%) than males were recaptured ($\chi^2 = 19.69$; $P < 0.001$).

In the 2nd experiment (uncultivated plot), we observed that most of the recaptured weevils were recovered in the trap during the first 4 d after release (Fig. 2), but some weevils were recaptured up to 22 d after release. There was a negative correlation between the release distance of weevils and the recapture percentage ($r = -0.97$; $P = 0.0046$), and the estimated regression model was $Y = 63.389 - 0.56245$ (distance), $r^2 = 0.9513$. In this experiment, the same proportion of males and females ($\chi^2 = 3.48$; $P = 0.06$) was recaptured.

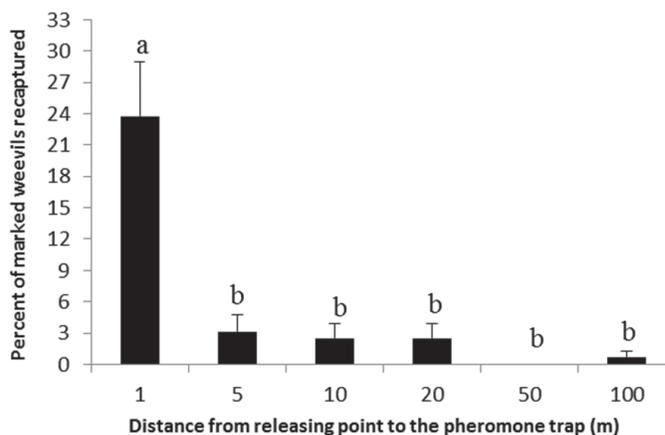


Fig. 1. Percentage of marked weevils recaptured in an experiment performed in a blue agave plantation to evaluate the attraction range of pheromone-baited traps. Weevils were released at 6 distances from pheromone-baited traps and recovered 5 d after release. Bars with similar letters are not significantly different (Tukey’s test, $\alpha = 0.05$).

In the 3rd experiment (uncultivated plot), we found that the agave weevils were attracted to the pheromone-baited traps up to 120 m (Fig. 3). More weevils were recaptured when the marked weevils were released at 60 m (recapture = 21.25%) than at 120 m (recapture = 6.25%) ($\chi^2 = 8.18$; $P = 0.0042$). Most of the recaptured weevils were recovered during the first 8 d after release, but some weevils were recaptured at 20 d after release. More females (77.27%) than males were recaptured ($\chi^2 = 6.55$; $P = 0.011$).

EFFECT OF THE POINT OF RELEASE ON WEEVIL CAPTURES

The results showed that the cardinal point at which weevils were released affected the recapture percentage (Fig. 4) ($\chi^2 = 25.91$; $P < 0.001$). From the north, 53% of marked weevils released at this point were recaptured, from the south 50%, from the east 43%, and from the west 27%. Most of the weevils were recaptured during the first 8 d after release, but some weevils were recaptured up to 15 d after release. There was no significant difference in the sex ratio of recaptured weevils ($\chi^2 = 1.23$; $P = 0.267$).

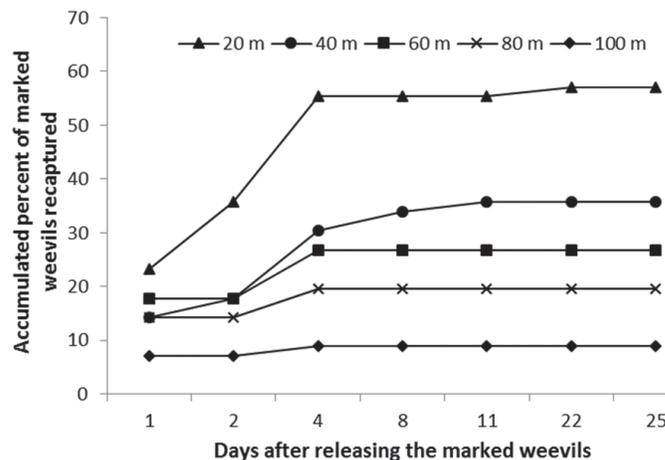


Fig. 2. Cumulative percentage of marked weevils recaptured in an experiment performed in an uncultivated plot to evaluate the attraction range of pheromone-baited traps. Weevils were released at 5 distances from pheromone-baited traps and recovered at different days after release.

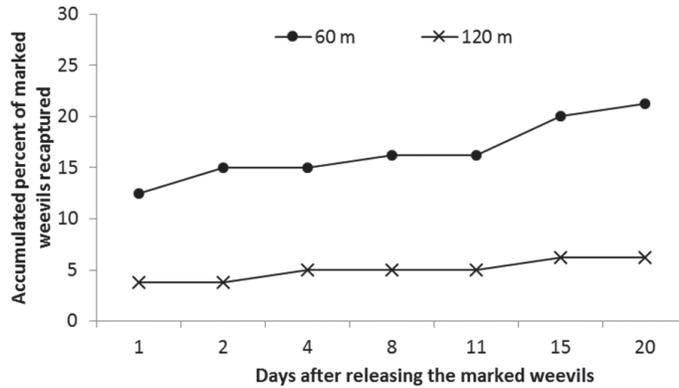


Fig. 3. Cumulative percentage of marked weevils recaptured in an experiment performed in an uncultivated plot to evaluate the attraction range of pheromone-baited traps. Weevils were released at 2 distances from pheromone-baited traps and recovered at different days after release.

EFFECT OF INTER-TRAP DISTANCE ON WEEVIL CAPTURES

In the 1st experiment, we found that the number of weevils caught was affected by the inter-trap distance ($F = 4.75$; $df = 4,15$; $P = 0.011$). Pheromone-baited traps placed at 50 and 100 m captured more weevils than traps placed at 5, 10, and 20 m (Fig. 5A). There was no difference between the number of weevils captured by traps at 50 and 100 m. Traps captured more females (89%) than males ($\chi^2 = 295.48$; $P < 0.001$).

The results of the 2nd experiment showed that trap captures were affected by the inter-trap distances ($F = 12.64$; $df = 2,21$; $P < 0.0001$). Traps placed at 150 m captured fewer weevils compared with traps placed at 100 and 200 m (Fig. 5B). There was no difference in the number of weevils captured when traps were placed at 100 and 200 m. Traps captured more females (79%) than males ($\chi^2 = 742.59$; $P < 0.001$).

Results of the 3rd experiment showed that there was no difference in the number of weevils captured in traps placed at 200 and 250 m ($F = 0.84$; $df = 1,6$; $P = 0.374$) (Fig. 5C). Traps captured more females (73%) than males ($\chi^2 = 32.52$; $P < 0.0001$).

Discussion

In this study, we found that most of the marked and released *S. acupunctatus* were recovered during the 1st week after the release of

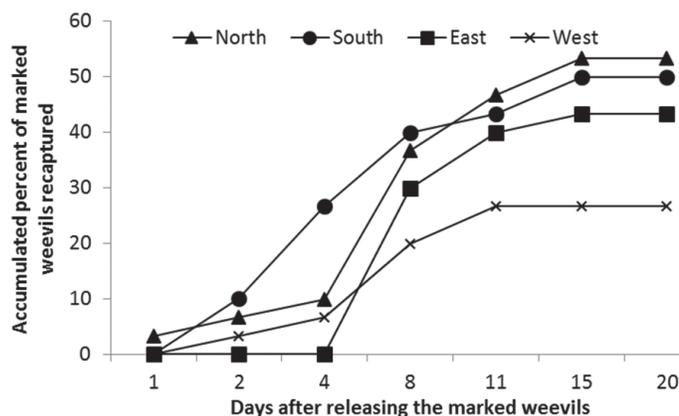


Fig. 4. Cumulative percentage of marked weevils recaptured in the experiment performed in an uncultivated plot to evaluate the effect of point of release of weevils on their responses to pheromone-baited traps. Weevils were released at 50 m from pheromone-baited traps in each cardinal point.

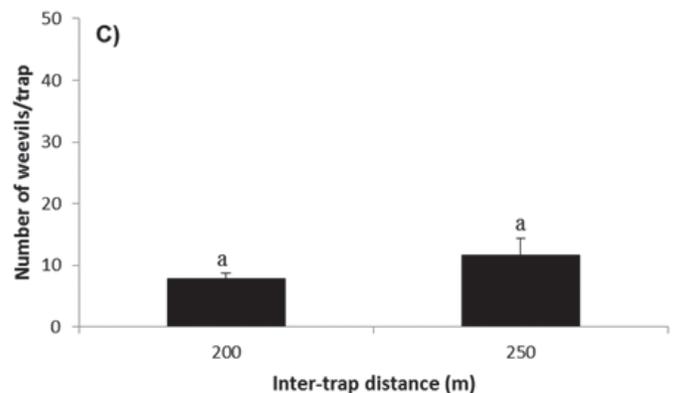
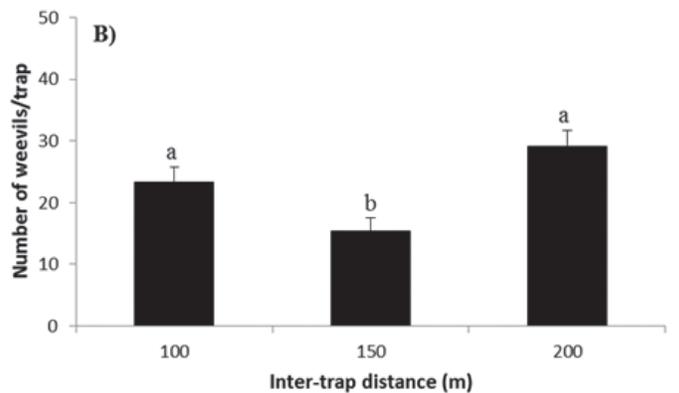
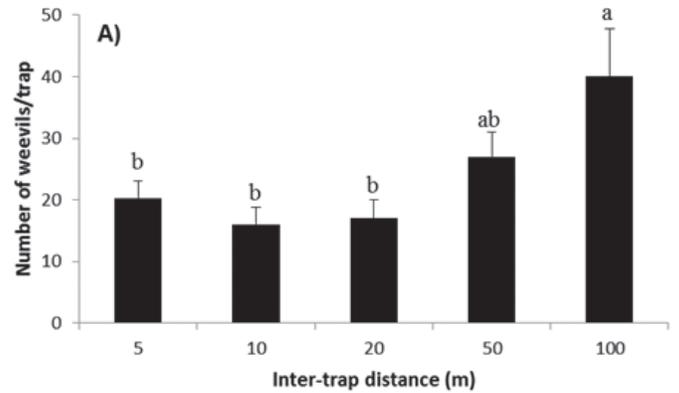


Fig. 5. Mean (\pm SE) number of captured *Scyphophorus acupunctatus* weevils per trap at various inter-trap distances, in the 1st (A), 2nd (B), and 3rd (C) inter-trap distance experiments. It was not possible to compare the captures between experiments because they were made at different agave plantations and times of the year. Bars with similar letters are not significantly different (Tukey's test, $\alpha = 0.05$).

weevils. These results are similar to those obtained by Rieske & Raffa (1990), who performed mark-recapture experiments with the weevil *Hylobius pales* Herbst (Coleoptera: Curculionidae) in Christmas tree plantations and found that most of the recaptured weevils were recovered in traps during the first 7 d after release.

The distance between the pheromone-baited trap and the point of release affected the recapture percentage of *S. acupunctatus* on the blue agave plantation. The greatest recapture numbers were obtained when the weevils were released at 1 m away from the trap. With the results of the 2nd experiment, performed in the uncultivated plot, we confirmed that the recapture percentage of *S. acupunctatus* was

negatively correlated with the distance at which the marked weevils were released. The results of the 3rd experiment indicated that traps baited with the synthetic aggregation pheromone and agave tissue are able to attract *S. acupunctatus* up to a range of 120 m. Mason et al. (1990), studying the sweet potato weevil, *Cylas formicarius elegantulus* (Summers) (Coleoptera: Curculionidae), found that this weevil was recaptured in pheromone-baited traps up to a distance of 280 m. Dodds & Ross (2002) found that most individuals of the bark beetle *Dendroctonus pseudotsugae* Hopkins (Curculionidae: Scolytinae) were recaptured in the pheromone-baited trap placed up to 200 m away from the release point.

We further observed that the accumulated percentage of marked weevils recaptured was lower when the experiment was performed in a blue agave plantation than when experiments were performed in uncultivated plots without the agave host. This may be because in a blue agave plantation, some plants may have been infested with *S. acupunctatus* and released weevils were attracted to conspecific males. In addition, weevils are attracted to agave plants (Altuzar et al. 2007). On the other hand, when we conducted the experiments in the uncultivated plots without agave plants, the pheromone + agave tissue-baited trap was the only attraction source for marked weevils.

In this study, we found that most of the recaptured *S. acupunctatus* weevils arrived from the north and from the south. This may be due to the wind direction, because in the uncultivated plot where this experiment was conducted, the prevailing wind direction was from south to north in the morning and from north to south in the afternoon. Turner et al. (1978) studied the effect of wind speed and direction on the attraction of *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) to a synthetic pheromone, and found a weak evidence that wind speed influences attraction of this insect to the pheromone trap, but found a strong evidence for a tendency that the attracted insects fly upwind to the pheromone source.

Regarding the sex ratio of recaptured weevils in experiment 1, in which the marked weevils were released in a blue agave plantation, the pheromone traps recaptured more females than males. These results show that—although in an agave plantation the sex ratio of *S. acupunctatus* is 1:1 (Figueroa-Castro et al. 2013)—the traps captured a greater number of females than males. Tinzaara et al. (2011) recaptured more females than males using pheromone-baited traps for trapping the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in banana fields. There was a similar trend in our experiments examining inter-trap distances in blue agave plantations, where we found that traps captured significantly more females than males. These results agree with those of Ruiz-Montiel et al. (2008), who studied trapping of *S. acupunctatus* in plantations of blue agave and found that pheromone-baited traps captured more females than males. López-Martínez et al. (2011) studied the daily activity of *S. acupunctatus* by using pheromone-baited traps in a commercial field of Mexican tuberose plant *P. tuberosa* and captured more females than males. Figueroa-Castro et al. (2013), working in blue agave plantations in Jalisco, Mexico, found that the sex ratio of *S. acupunctatus* populations sampled in agave plants was 1:1, but pheromone-baited traps placed in the same plantations captured more females than males. The fact that the sex ratio of captures in pheromone-baited traps is not representative of the sex ratio of insect populations in the field was also reported by Mitchell & Hardee (1974). They studied the cotton weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) and found that pheromone-baited traps captured more females than males as the squaring (bud formation) by cotton plants declined, whereas with direct sampling during the same period they found more males than females on the cotton plants.

In contrast, in our experiments of pheromone attraction range conducted in an uncultivated plot, most traps recaptured a similar sex ratio of males and females. Why pheromone-baited traps captured the same sex ratio of males and females when the host (agave plant) was absent but captured more females than males when the host was present in the experimental site is not clear. This result suggests that the presence of the host plant interferes with the response of males to pheromone-baited traps. It is possible that males colonize the host plants and then release the pheromone to attract females. This would explain the low attraction of males to pheromone-baited traps when the host plants are present. However, further experiments are needed to understand the function of the pheromone in the behavior of *S. acupunctatus*.

The results of the experiments of inter-trap distances showed that when pheromone-baited traps were placed further apart (250 m), they captured more weevils than traps placed at shorter distances. This result may indicate that the pheromone-baited traps placed at short distances can cause an interference effect. Bacca et al. (2006) experimented with the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) and found an interference effect in traps placed at distances shorter than 10 m. Based on the results of the present experiments (1, 2, and 3), we can report that traps placed at 100 (experiment 1), 200 (experiment 2), and 250 m (experiment 3) captured a greater number of weevils than traps placed at shorter distances. As traps attracted the agave weevil up to a range of 120 m and traps placed at 200 and 250 m captured a greater number of weevils than traps at shorter distances, we suggest that traps for monitoring *S. acupunctatus* could be placed 250 m apart. Thus, they could be used at densities of 1 trap per 6 ha of blue agave crop. Bacca et al. (2006) indicated that for monitoring *L. coffeella*, the use of 1 pheromone trap for 3.5 to 4.0 ha would be adequate. Bacca et al. (2008) proposed a sampling plan for *L. coffeella* in which they suggested using 1 trap per 4 ha. For the American palm weevil in coconut, Cortazar & Carrillo (1999) suggested the use of 1 trap per 2 ha.

In summary, this study showed that traps baited with synthetic aggregation pheromone plus agave tissue attract *S. acupunctatus* up to a range of 120 m, most of the recaptured marked weevils were recovered during the first 8 d after their release, and traps placed at distances of 200 and 250 m captured more weevils than traps placed at shorter distances. In this context, we recommend that in experiments of mark-release-recapture with *S. acupunctatus*, the traps should be checked within the first 8 d after placement in the field to record weevil captures. We also recommend the use of these traps for monitoring the agave weevil in blue agave crop, placed at inter-trap distances of 200 (1 trap per 4 ha) or 250 m (1 trap per 6 ha).

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