Amount and Bagging of the Bait Food Affect the Captures of Scyphophorus acupunctatus (Coleoptera: Curculionidae) by Pheromone-Baited Traps

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Amount and bagging of the bait food affect the captures of *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) by pheromone-baited traps

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Abstract

The weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Cuculionidae) is the most important insect pest of wild and cultivated agaves worldwide. This weevil causes direct damage while feeding and egg laying on host plants, and indirect damage due to the wounds on agave tissue that may serve as an entry for plant pathogens. Sampling and control of this weevil is complicated because of its cryptic habits. Thus, the use of synthetic aggregation pheromone-baited traps may be a useful tool for monitoring or control of this pest. The attraction of agave weevil to pheromone traps is synergized by the presence of host plant tissue. In this study, we evaluated the effect of the amount of fresh agave tissue and bagging of the plant tissue on the capture of *S. acupunctatus* with pheromone-baited traps in a blue agave crop. The highest captures of weevils were obtained in traps baited with the synthetic pheromone plus 400 g of agave tissue. Weevil capture rate was increased when the food bait was placed into a plastic bag or plastic container with holes as compared with unbagged food bait.

Key Words: Blue agave, agave tissue quantity, trapping

Resumen

El picudo *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) es uno de los problemas fitosanitarios de mayor importancia económica en agaves cultivados y silvestres en México. La importancia de esta plaga en agaves radica en que provoca daños directos por alimentación y/u oviposición, e indirectos ya que las heridas causadas por esta plaga pueden servir de entrada a microorganismos fitopatógenos del agave. Debido a que todo el ciclo biológico de esta plaga ocurre en el interior de la planta de agave, su muestreo es complicado y por ende resulta difícil monitorear su densidad poblacional en plantaciones de agave para tomar decisiones sobre su manejo; así, el uso de trampas con feromona de agregación sintética es una buena opción para su monitoreo. La respuesta de los picudos a la feromona de agregación sintética es sinergizada por la presencia de la planta hospedera. Por lo tanto es necesario determinar si la cantidad de tejido de agave y si el embolsado del cebo afecta la captura de los insectos. En la presente investigación se evaluaron diferentes cantidades de tejido fresco de agave y dos formas de embolsar el alimento para la captura de *S. acupunctatus* en agave tequilero. Se encontró que las mayores capturas de picudos se obtuvieron en las trampas cebadas con feromona de agregación sintética más 400 g de tejido de agave. El colocar el tejido de agave en bolsa o bote de plástico transparente con orificios incrementó el número de picudos capturados por trampa comparado con el cebo sin embolsar.

Palabras Clave: agave tequilero, cantidad de tejido de agave, trampeo

The weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) is considered the most important pest of agaves worldwide (Solís-Aguilar et al. 2001; González et al. 2007). Both larvae and adults of *S. acupunctatus* cause direct damage while feeding, which is estimated to be about 10 to 25% of the total volume of the agave bole (Solís-Aguilar et al. 2001; González et al. 2007; Aquino Bolaños et al. 2007; Figueroa-Castro et al. 2013). Additionally, the weevil may be a vector of agave pathogens, and wounds caused during weevil feeding may serve as an entry point for these pathogens (Waring & Smith 1986; Aquino Bolaños et al. 2011; González et al. 2007). For instance, there is a positive relationship between blue agave plants with symptoms of agave bole rot disease and level of weevil infestation. At high levels of infestation, approximately 70% of the total volume of the agave bole can be damaged (Figueroa-Castro et al. 2013). The agave weevil has

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several generations per year and its life cycle occurs inside the agave plant, making detection, monitoring, and management difficult.

As part of the integrated pest management strategies for this weevil, there is an increasing interest in developing a semiochemical-based system for monitoring or mass trapping of this pest. A number of studies have investigated the chemical ecology of this weevil, resulting in the identification of an aggregation pheromone (Ruiz-Montiel et al. 2003; Rojas et al. 2006; Ruiz-Montiel et al. 2008; Rodríguez-Rebollar et al. 2012). These studies also reported that the response of agave weevil to the pheromone is synergized by the presence of the host plant volatiles. Currently, a trapping system using traps baited with aggregation pheromone plus fresh agave tissue is used for monitoring *S. acupunctatus* at blue agave (*Agave tequilana* Weber var. Azul; Asparagaceae) plantations (SENASICA 2014). However, little is known about the factors that affect the efficiency of the food bait used for monitoring the agave weevil. In this study, we investigated the effect of amount of fresh agave tissue and bagging of the plant tissue on capturing *S. acupunctatus* in blue agave.

Materials and Methods

EXPERIMENTAL SITE

All experiments were conducted in the plantation "El Molino" (20.7166°N, 103.9166°W, 1,314 masl), located at Ahualulco de Mercado, Jalisco, Mexico. This farm had 60 ha sown with 2-yr-old blue agave at the time of the experiments.

TRAPS

The trap used consisted of a 4-L white plastic bucket with 4 circular holes (4.0 cm diam) made equidistant at 1 cm above the trap base. On each of these holes a transparent plastic cone (3.5 cm long; 4.0 and 3.0 cm entry and exit diam, respectively) was attached to the inner wall of the bucket to allow the weevils to enter but obstructing their exit from the bucket. Traps were placed at ground level, close to an agave plant; the between-trap distance was 100 m.

SYNTHETIC AGGREGATION PHEROMONE

The synthetic aggregation pheromone (Tequilur®) was obtained from a commercial source (FeroComps, Mexico City, Mexico). According to the supplier, each pheromone dispenser contained 350 mg of 2-methyl-4-octanone. The pheromone dispenser was hung by a piece of wire tied to the center and inside the trap lid. New pheromone dispensers were supplied at the beginning of each experiment.

FOOD BAIT

Pieces of fresh (recently cut) agave tissue were used as food bait. The agave food bait was sprinkled with 50 mL of water plus insecticide (Malathion 1000®, Agricultura Nacional de Jalisco, S. A. de C. V., Tlaquepaque, Jalisco, Mexico) (10 mL Malathion per L of water). In studies where containers were used to hold the bait, the insects were able to access the bait through the holes in the containers, and thereby poisoned. The agave tissue was replaced at 21 d intervals.

EFFECT OF AMOUNT OF AGAVE TISSUE ON WEEVIL CAPTURE

Two experiments were performed to determine the effect of different agave tissue quantities used as food baits for trapping *S. acupunctatus*. In the 1st experiment (18 Jan–8 Feb 2013), the following 5 treatments were evaluated: (1) pheromone alone, (2) 200 g of agave tissue alone, (3) pheromone + 200 g of agave tissue, (4) pheromone + 400 g of agave tissue and (5) pheromone + 600 g of agave tissue. We used fresh (recently cut) agave tissue in all treatments. This experiment was performed in a completely randomized experimental design with 4 replications. Only 1 collection of captured weevils in traps was made at 21 d after the experiment was established.

In the 2nd experiment (11 Apr–23 May 2013), the following 7 treatments were evaluated: (1) pheromone alone, (2) 200 g agave tissue alone, (3) pheromone + 100 g of agave tissue, (4) pheromone + 200 g of agave tissue, (5) pheromone + 300 g of agave tissue, (6) pheromone + 400 g of agave tissue, (7) pheromone + 500 g of agave tissue. This experiment was performed in a completely randomized experimental design with 4 replications. Two collections of captured weevils in traps were made at 21 d intervals after the experiment was established.

EFFECT OF BAGGING THE FOOD BAIT ON WEEVIL CAPTURE

In previous trapping experiments with this weevil on blue agave completed in 2008, we observed that the agave tissue used as food bait in traps became dehydrated within 15 d or less. One of us (R. R. C.) observed that when agave tissue is enclosed in a plastic bag with holes, the tissue dehydrates more slowly and thus remains attractive longer; consequently, capture of weevils is increased. Based on this preliminary observation, we evaluated whether bagging of the food bait affected the capture of weevils in pheromone-baited traps. This experiment was performed from 24 May to 14 Jun 2013.

Three treatments were evaluated in this experiment: (1) 200 g of fresh agave tissue placed without a plastic bag (control), (2) 200 g of fresh agave tissue placed in a plastic bag (15.8 × 24.5 cm) with 40 circular holes (5 mm diam), (3) 200 g of fresh agave tissue placed in a transparent 0.5 L plastic container with 8 circular holes (1 cm diam) made on the lateral walls of the container. In addition to plant material, a pheromone lure was placed into the trap as described above. This experiment was performed in a completely randomized experimental design with 9 replications. The collection of captured weevils was made 21 d after the experiment was established.

Weevils captured in all experiments were placed in plastic containers and taken to the Entomology Laboratory at Colegio de Postgraduados, Campus Montecillo, Texcoco, Mexico, where weevils were counted and sexed (Ramírez-Choza 1993). The number and sex of weevils captured in each treatment and in each trap (replication) were recorded.

STATISTICAL ANALYSIS

All statistical analyses were made using SAS statistical software version 9.0 (SAS Institute 2004). The data were analyzed by an analysis of variance (ANOVA) using the procedure PROC GLM. Data from the first experiment of agave amount and data from the bagging experiment were $\sqrt{y + 0.5}$ transformed to meet the assumptions of ANOVA. Treatment means were separated using Tukey's test ($\alpha = 0.05$). A Chi-squared test was applied to determine whether there was a difference in the numbers of male and female weevils captured.

Results

EFFECT OF FOOD BAIT AMOUNT ON WEEVIL CAPTURE

In the first experiment, the number of agave weevils captured was affected by the treatment used ($F = 7.40$; df = 4,15; $P = 0.0017$). Traps baited with pheromone plus 400 g of agave captured more weevils in comparison to traps baited with pheromone alone or 200 g of agave

alone. The number of weevils caught by traps baited with 200 g of agave plus pheromone and 600 g of agave plus pheromone were intermediate and not significantly different from capture by traps baited with pheromone alone, 200 g of agave alone, or 400 g of agave plus pheromone (Fig. 1A). The traps captured more females than males (χ^2 = 93.61; *P* = < 0.0001), although apparently not in the case of agave tissue used alone as bait (Table 1).

In the second experiment, the capture of weevils was affected by treatment (*F* = 10.69; df = 6,21; *P* = < 0.0001). Traps baited with pheromone plus 400 g of agave captured more weevils compared to traps baited with pheromone alone, 200 g of agave alone, and 200 g of agave plus pheromone. Captures of traps baited with 100 g of agave plus pheromone, 300 g of agave plus pheromone, and 500 g of agave plus pheromone were intermediate and not significantly different from the captures in traps baited with 400 g of agave plus pheromone (Fig. 1B). In the same manner as the previous experiment, the traps captured more females than males (χ^2 = 43.76; *P* = < 0.0001) (Table 1) except that traps baited with agave alone captured similar numbers of females and males.

EFFECT OF BAGGING THE FOOD BAIT ON WEEVIL CAPTURE

Bagging of the agave tissue affected the number of captured weevils (*F* = 17.37; df = 2, 24; *P* <0.0001). Traps in which the food bait was placed into a plastic bag with holes or in a plastic container with holes captured significantly more weevils than traps in which the food bait was placed directly in the trap without a bag or plastic container (Fig. 2). Traps captured a higher number of females (89%) than males (χ^2 = 388.65; *P* < 0.0001).

Discussion

We confirmed that host volatiles synergize the attraction of the agave weevil to its synthetic aggregation pheromone. Traps baited with pheromone alone or agave tissue alone captured fewer weevils than traps baited with pheromone plus agave. Earlier, Rojas et al. (2006) pointed out the possibility of synergism between the synthetic pheromone and the agave tissue in trapping this weevil. García-Ramírez et

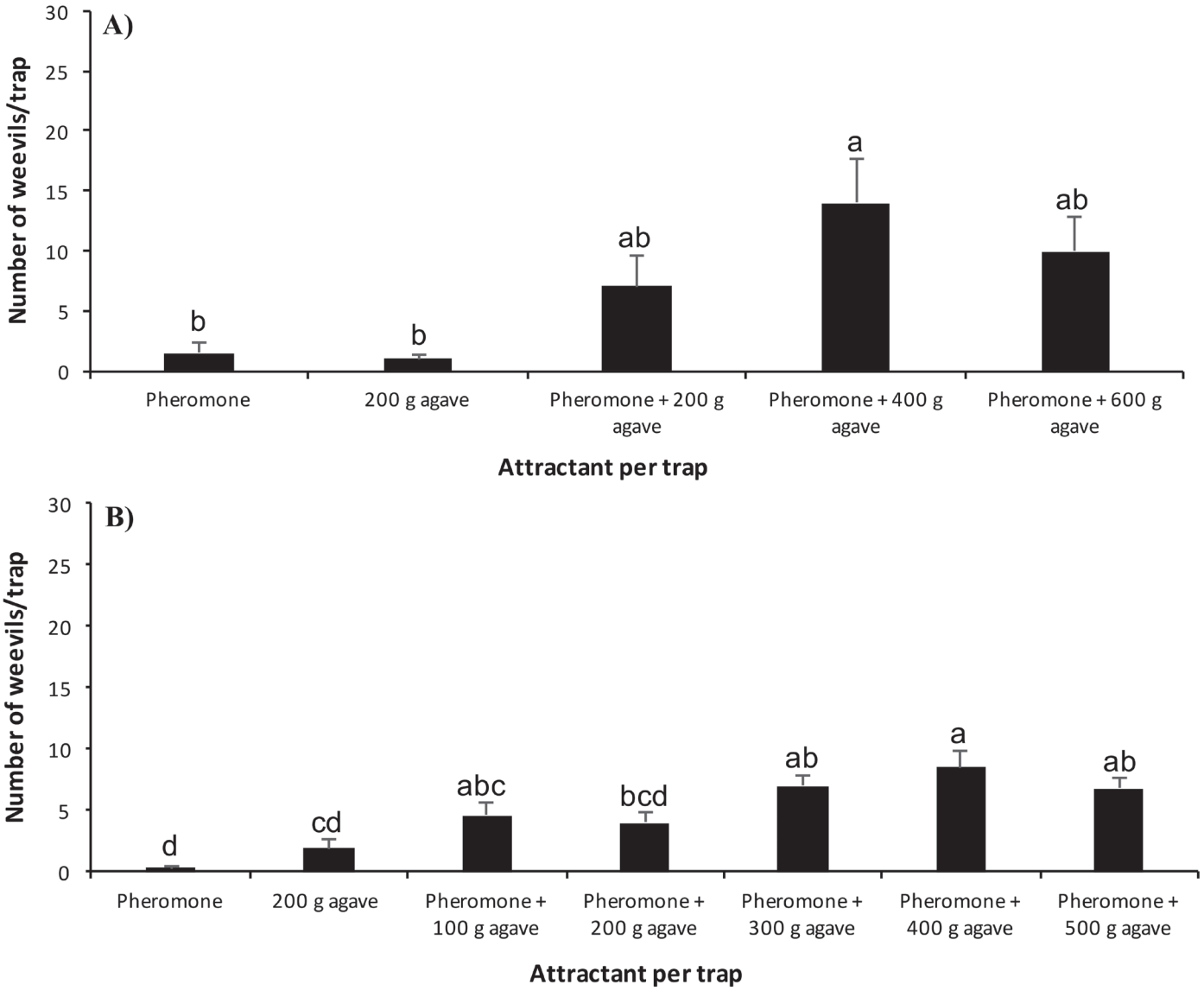

Fig. 1. Mean (+ SE) number of *Scyphophorus acupunctatus* captured per trap baited with different amounts of fresh agave tissue in 2 trials. The 1st trial was performed from 18 Jan to 8 Feb 2013 (A), while the 2nd trial was carried out from 11 Apr to 23 May 2013 (B). Bars with the same letter are not significantly different (Tukey's, α = 0.05).

Table 1. Percent of *Scyphophorus acupunctatus* females and males captured in baited traps.

Experiment	Treatment	Females (%)	Males (%)	X^2	*P*
1	Pheromone alone	83.33	16.67		
	200 g agave tissue	50.00	50.00		
	Pheromone + 200 g agave tissue	92.86	7.14		
	Pheromone + 400 g agave tissue	91.07	8.93		
	Pheromone + 600 g agave tissue	97.50	2.50		
	Means of experiment 1	91.79	8.21	93.61	< 0.0001
2	Pheromone	100.00	0.00		
	200 g agave tissue	53.33	46.67		
	Pheromone + 100 g agave tissue	91.89	8.11		
	Pheromone + 200 g agave tissue	81.25	18.75		
	Pheromone + 300 g agave tissue	76.79	23.21		
	Pheromone + 400 g agave tissue	79.41	20.59		
	Pheromone + 500 g agave tissue	75.93	24.07		
	Means of experiment 2	78.79	21.21	43.76	< 0.0001

al. (2014) performed field experiments to investigate the influence of the food bait (200 g of pineapple) on the capture of *S. acupunctatus* in Mexican tuberose (*Polianthes tuberosa* L.; Asparagaceae) crop. In 1 trial they found that traps baited with synthetic pheromone plus food bait captured a higher number of weevils than traps baited with pheromone alone, but in a 2nd trial no effect of the food bait was found. The fact that plant volatiles synergize the responses of weevils to its pheromone has been reported for several species of the Rhynchophorinae subfamily (Gries et al. 1994; Oehlschlager et al. 1993; Giblin-Davis et al. 1996; Hallett et al. 1999; Rochat et al. 2000; Wibe et al. 2014). For example, Gries et al. (1994) found that traps baited with palm tissue or pheromone captured few *Rhynchophorus phoenicis* F. (Coleoptera: Curculionidae), in comparison to traps baited with palm pieces plus pheromone. Hallet et al. (1999) reported that the addition of palm volatiles synergized the attraction of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) to its aggregation pheromone. The addition of the major flower volatile from wild strawberry flowers, *Fragaria vesca* L. (Rosaceae), to the aggregation pheromone of *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) increased the trap captures by over 2–fold, as compared to the pheromone alone (Wibe et al. 2014). In contrast, Tafoya et al. (2007) found that the addition of prickly pear did not synergize the attraction of *Metamasius spinolae* (Gyllenhal) (Coleoptera: Curculionidae) to its aggregation pheromone.

Our results indicate that the amount of agave tissue influenced the capture of *S. acupunctatus*. In the 2 trials, we found that traps baited with 400 g of agave tissue captured more weevils than traps baited with other amounts of agave. Previously, Giblin-Davis et al. (1996) found that traps baited with 1.5 kg of chopped Sabal palmetto stem plus aggregation pheromone, cruentol, captured almost 3 times more *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae) than traps baited with 0.5 kg of palm tissue plus cruentol. Tinzaara et al. (2007) reported that traps baited with pheromone plus 100 or 500 g of tissue of banana stem (fermented) captured a similar number of banana weevils as traps baited with pheromone alone; however, traps baited with pheromone plus 1,000 g of stem tissue of banana had higher weevil numbers. Al-Saoud & Ajlan (2013) found that pheromone-traps baited with 550 g of date fruit captured fewer red palm weevils than traps baited with pheromone plus 350 or 450 g of date fruit. In our case, it is likely that lower amounts of agave tissue emit less of the volatile compounds, which would explain the lower captures of *S. acupunctatus*. Interestingly, a larger amount of agave likely would produce more volatiles, and this also seemed to affect the attraction of the weevils, though the reason for this is not apparent. Thus, our data suggest that there is an optimal amount of plant tissue for trapping *S. acupunctatus*.

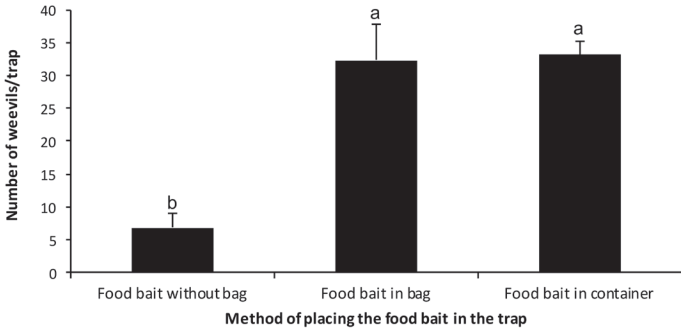

Fig. 2. Mean (+ SE) number of *Scyphophorus acupunctatus* captured per trap baited with pheromone plus bagged or unbagged fresh agave tissue. The experiment was performed from 24 May to 14 Jun 2013. Bars with the same letter are not significantly different (Tukey's, $\alpha = 0.05$).

Bagging the food bait affected the capture of *S. acupunctatus*. One explanation for this result is that the plastic bag and plastic container help the agave tissue to maintain freshness longer, thus allowing the agave tissue to release volatiles longer in comparison to unbagged food bait. Unbagged agave tissues dry sooner in comparison to bagged plant material. However, we do not know if weevils captured by traps baited with unbagged agave tissue occurred during the 1st days, when the agave was not dry, which would support this explanation. Another possibility is that the bagged agave could produce fermentation volatiles that synergize the attraction of *S. acupuncatus* to its aggregation pheromone. However, Rodríguez-Rebollar (2011) did not find any differences in the captures of *S. acupuncatus* by pheromone-baited traps plus agave tissue bagged for 1, 5, 10, and 15 d. Although, agave weevils can be attracted to plant volatiles (Altuzar et al. 2007), they also respond to general fermentation volatiles (Valdés-Rodríguez et al. 2005). Future studies could clarify whether specific volatiles from agave plants or general fermentation volatiles are the pheromone synergist in agave weevil.

In all experiments, more females than males were captured, although the sex ratio for *S. acupunctatus* on agave plants is 1:1 (Figueroa-Castro et al. 2013). Previous studies have reported similar results when trapping this weevil species in agave and Mexican tuberose crops (Ruiz-Montiel et al. 2008, López-Martínez et al. 2011, Rodríguez-Rebollar et al. 2012, Figueroa-Castro et al. 2013, Figueroa-Castro et al. 2016a, b, c). Our results indicate that the synthetic aggregation pheromone could be responsible for attracting more females than males to the

traps because traps without pheromone (i.e., agave tissue alone) captured equivalent proportions of females and males. Traps baited with pheromone, even traps in which pheromone alone was used, caught more females than males. Our hypothesis is that synthetic aggregation pheromone could work as sexual signal sometimes, but this needs to be studied in future studies. With respect to this, Figueroa-Castro et al. (2015) noted that colonization of new agave plants could be started by females and males. In boll weevil, the male pheromone attracts both sexes (aggregation pheromone), or only females (sex pheromone), depending on time of year (Hardee & Harris 2003). In agave weevil, however, pheromone-baited traps capture more females than males during the entire year (Figueroa-Castro et al. 2013).

In summary, this study showed that the amount of food bait, and bagging the food bait, in the pheromone-baited traps affected the number of captured agave weevils. We also confirmed the synergism between the food bait and the synthetic aggregation pheromone. We suggest that traps baited with the synthetic aggregation pheromone plus 400 g fresh agave tissue bagged in plastic bags with holes be used for monitoring or mass trapping the agave weevil.

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