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Authors: Pacheco-Sánchez, Cinthia, Villa-Ayala, Patricia, Montes-Belmont, Roberto, Figueroa-Brito, Rodolfo, and Jiménez-Pérez, Alfredo

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REPELLENCY OF HYDROETHANOLIC EXTRACTS OF *RICINUS COMMUNIS* (EUPHORBIACEAE) TO *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA: CURCULIONIDAE) IN THE LABORATORY

CINTHIA PACHECO-SÁNCHEZ, PATRICIA VILLA-AYALA, ROBERTO MONTES-BELMONT, RODOLFO FIGUEROA-BRITO
AND ALFREDO JIMÉNEZ-PÉREZ*

Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional. Carretera Yautepec- Jojutla,
Km. 6 calle CEPROBI No. 8 San Isidro Yautepec, Morelos., C.P. 62731 México

*Corresponding author; E-mail: aljimenez@ipn.mx

ABSTRACT

The agave snout weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) is an ubiquitous insect and the main pest of blue tequila agave, *Agave tequilana* Weber, and other agaves. This study reports the repellent effect of the hydroethanolic extract of the castor oil plant, *Ricinus communis* L. (Euphorbiaceae) wild and 'Mirante' cultivar on the adult's behavior. Females and males visited untreated agave tissue more frequently than agave tissue treated with *R. communis* extracts. Insects visited agaves treated with seed extracts more frequently than those with leave extracts; therefore leaves of *R. communis* deserve a closer look to identify their properties and gauge their potential use as a repellent.

Key words: vector, flavonoids, quercetin, quercitrin, rutin

RESUMEN

El picudo del agave, *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), es un insecto cosmopolita y principal plaga del agave azul *Agave tequilana* Weber y otras agaveáceas. Este estudio informa del efecto repelente de extractos hidroetanólicos de la "Higuerrilla" *Ricinus communis* L. (Euphorbiaceae) variedad "Mirante" y silvestre sobre el adulto. Hembras y machos visitaron más veces pedazos de agave impregnados con extractos hidroetanólicos que aquellos no impregnados. Los insectos visitaron más veces los agaves tratados con extractos hidroetanólicos provenientes de semillas que de hojas, por lo que es necesario estudiar las hojas y evaluar su potencial como repelente de insectos.

Palabras Clave: flavonoides, quercetina, quercitrina, rutina

Resumen elaborado por los autores

The agave weevil (AW) *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) is considered an important pest of major economic relevance; it feeds upon Agavaceae. The insect larvae damage the mature bole of *Agave* spp., the adults pierce the bole and leaves of the plant. Further, AW adults transmit *Erwinia caticida*, *Pantoea agglomerans* and *Pseudomonas* sp. (Jiménez et al. 2004), which can produce a deep red hard necrosis.

AW is controlled through the use of organosynthetic insecticides, culture measures, and plant mobility has been restricted recently. Organosynthetic insecticides do not control this pest due to the difficulty to reach the insects since they lodge deeply inside the plant (Valdés-Rodríguez et al. 2004). Thus the control of AW must focus on the adult when it moves from plant to plant seeking food or shelter; it is at this time when it is exposed and subject to control. Some of the alterna-

tive methods employed to reduce the population of AW include the use of baits (Valdés et al. 2005) and agave volatiles (Altuzar et al. 2007).

Powder and slurries at 1-10% of castor oil plant, *Ricinus communis* L. (Euphorbiaceae) mixed with corn kernel killed *Sitophilus oryzae* L. (Coleoptera: Curculionidae) adults (Niber 1994). In addition, leaf aqueous extract (2.5 g/20 mL) impregnated on sweet pepper foliar discs inhibited consumption of leaf discs on *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) (Palma & Serrano 1997). Biologically active flavonoids as quercetin, quercitrin and rutin has been identified in hydroethanolic extracts of this plant (Pacheco-Sánchez et al. 2012).

The purpose of this study was to determine the repellent effect of the hydroethanolic extracts of the wild and Mirante variety of the castor oil plant on the agave weevil in the laboratory.

METHODS AND MATERIALS

Insects

Agave weevil adults of different ages were manually collected, from Apr to May 2009 in tequila agave, *Agave tequilana* Weber (Asparagales: Agavaceae) commercial plantations in the municipality of Yautepec, state of Morelos, México.

Six and 7 yr old plants damaged by the insect were dissected to collect the adults. These adults were separated by sex based on the size and shape of the last abdominal segment. In females, this segment is narrow and acuminate while in males it is wide and truncated (Ramírez-Choza 1993). The males and females were held separately in an incubator (Precision Scientific® model 818), at 26 ± 1 °C and $50 \pm 10\%$ RH and 12:12 h L:D in clear 1 L-plastic containers with agave leaves as food. The insects were fed on agave boles from the same plots where they were had been collected and the food was changed every 14 d.

A sample of 10 ♀ and 10 ♂ was identified by Dr. Héctor González-Hernández from the Entomology Department at Institute of Plant Protection, Colegio de Postgraduados, Campus Montecillos, Texcoco state, México.

Agave tequilana Plant Material

Leaves and seeds from wild and the “Mirante L. *vialacahui*” variety of castor oil plant were used. “Mirante” seeds were supplied by Ceres Internacional de Semilla S. A. de C. V., Morelos, México. Mirante seeds were planted and the resulting plants developed healthy mature leaves and fruits from October 2008 to January 2009. Wild leaves and seeds were collected during the same period beside the Yautepec–Cuautla road, at Yautepec, Morelos. These plants were identified at the Autonomous University of Morelos State Herbarium (HUMO), where voucher specimens were deposited (registration number 27002).

Fruits and leaves were dried in the shade. The fruits were opened manually to extract the seeds. The leaves were ground in a hand mill (Estrella® model 41B) and in an electrical one (Moulinex®), and then sieved through a 30 mesh screen to obtain a fine powder. The seeds were ground in an industrial mill (Siemens Pulvex Plastic®) and a greasy paste was obtained.

Leaf powder and the greasy paste from the seeds were deposited in 1-L amber bottles and in polyethylene bags, respectively, and stored in a cool, dry place away from light (Ramos-López et al. 2010).

Hydroethanolic Extracts

One kilogram each; powder and paste were immersed for 24 hr in a 70:30 ethanol and distilled

water solution in 5 L and 20 L Erlenmeyer flasks, respectively. The product was filtered through cotton cheese cloth in a plastic funnel and into a 3 L Erlenmeyer flask and concentrated by rotary evaporator (Büchi R-114) at 55 °C and 30 rpm (Silva et al. 2009).

The crude extracts were then freeze dried (Heto Drywinner® DW-3), put into 0.5 L amber glass jars and kept in a refrigerator at -4 °C. A sample of these extracts was used to test for flavonoids according to Pacheco-Sánchez et al. (2012).

Treatments

The hydroethanolic extracts of wild castor oil plant (WS) and ‘Mirante’ (MS) seeds, and of wild plant (WL) and ‘Mirante’ (ML) leaves were tested at 1% (10,000 ppm) obtained by mixing 0.5 g of each extract in 5 mL of acetone (10% solution) and then diluting this solution to 1% following the methodology of Lagunes-Tejeda & Vázquez-Navarro (1994). Agave pieces dipped in 95% acetone (95 acetone/5 distilled water, v/v), as explained below, were used as the agave control.

Bioassay

Two-hundred adult insects of different ages were used (100 ♂ and 100 ♀). Ten arenas were used per sex with 10 specimens per arena. Each arena was circular (28 ø cm, 12 cm high) made of transparent plastic and covered with a plastic lid (Fackelmann®). Five equidistant circles 5 cm in diam, each 9.5 cm from the center of the arena were drawn to delimit response areas. At the center of each of these areas a piece (3 × 3 × 3 cm) of agave was placed. These pieces had been submerged for 10 s in the appropriate agave extract and allowed to dry at room temperature for 30 s before testing. Each experimental arena contained 5 pieces of agave (1 per extract plus a control) randomly distributed and affixed to the center of the response area by means of a pin according to the modified methodology of Capps et al. (2010). The insects were kept inside a plastic cup for 60 s in the center of the arena, before being released. The arena was held at 30 ± 1 °C and 60% RH.

A total of 16 observations were made in the course of 4 h, 1 every 15 min, and the number of insects in each response area was tallied, and how many of them were in contact with the piece of agave was recorded. After each observation the arena was rotated.

The pooled numbers of insects that visited each extract were subjected to a Friedman test for repeated measurements and then to a Tukey test. These procedures were carried out using Sigma Stat 3.5 (Systat, Software Inc. 2006).

RESULTS AND DISCUSSION

The numbers of females that visited the agave pieces treated with ML, WL and WS were significantly smaller than the numbers that visited MS treated agave pieces and the agave control dipped in 95:5% acetone ($\chi^2 = 36.40$; $gl = 4$; $P < 0.0001$) (Fig. 1). Thus relative to the number of females that visited the agave control, such visitations of females were reduced by 66, 54 and 37% by treatment with ML, WL and WS respectively. However extracts of 'Mirante' seeds were not significantly more repellent to females (Fig. 1) or to males (Fig. 2) than the agave control, and extracts of wild agave seeds were not significantly more repellent than those of "Mirante" seeds or the untreated control to either males or females (Figs. 1 and 2).

Relative to the number of males that visited the agave control or MS treated agave pieces, such visitations by males were reduced by 53 and 48% by WL and ML, respectively ($\chi^2 = 41.27$; $gl = 4$; $p < 0.0001$, Fig. 2). In both bioassays, the responses of the weevils to the control (95% acetone applied to agave pieces) was not significantly different from the seed extract treatments. Shapiro et al. (2000) and Bittner et al. (2008) also used acetone as a control in experiments with other weevil species.

Weevils detect and discriminate toxic and food factors before choosing a host, and their response can be positive (attraction) or negative (repellency) for food, shelter, or oviposition (Reeves & Lorch 2009; Blake et al. 2011; Germinara et al. 2012).

In this study (Figs. 1 and 2), both sexes of AW visited the pieces of agave treated with leaf extracts less frequently than pieces not treated

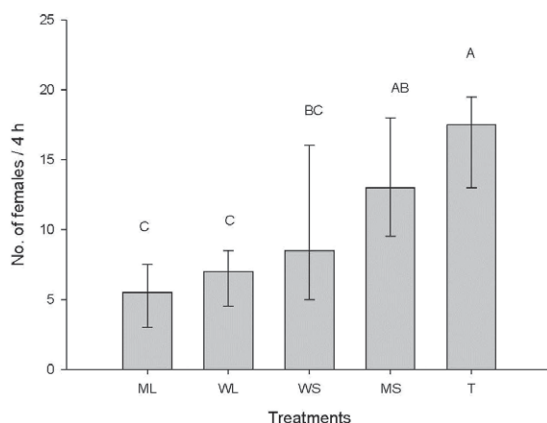


Fig. 1. Number of females of *Scyphophorus acupunctatus* (median, Q_1 and Q_3) that visited a piece of agave treated with hydroethanolic extract from *Ricinus communis* during 4 h. Bars topped with the same letter are not significantly different. Tukey ($P > 0.05$). WL = Wild Leaf, ML = 'Mirante' Leaf, WS = Wild Seed, MS = 'Mirante' Seed and T = Control.

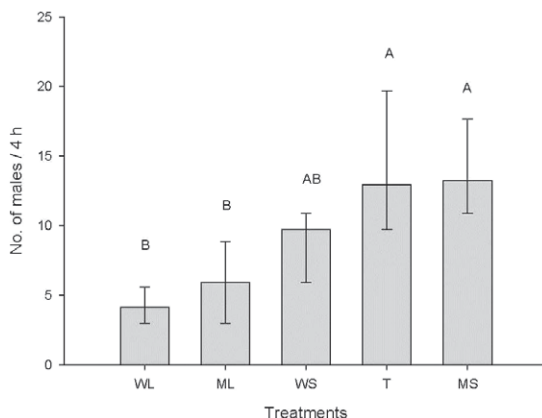


Fig. 2. Number of males of *Scyphophorus acupunctatus* (median, Q_1 and Q_3) that visited a piece treated with *Ricinus communis* extract during 4 h. Bars topped with the same letter are not significantly different. Tukey ($P > 0.05$). WL = Wild Leaf, ML = 'Mirante' Leaf, WS = Wild Seed, MS = 'Mirante' Seed and T = Control.

or treated with seed extract. In the leaf extracts evaluated, the following flavonoids were identified: quercetin, quercitrin and rutin (Pacheco-Sánchez et al. 2012). Many flavonoids seem to have low toxicity and moderate physiologic activity on many insects (Morimoto et al. 2003; Onyilagha et al. 2004; Diaz-Napal et al. 2010). Quercetin has been reported to deter insect feeding (Frazier & Chyb 1995). Rutin (10 to 150 $\mu\text{mol/g}$) stimulated feeding of the desert locust *Schistocerca gregaria* Forsk (Orthoptera: Acrididae), while 2% rutin stimulated feeding of the American grasshopper, *Schistocerca americana* Drury (Orthoptera: Acrididae), and rutin was a feeding stimulant of the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), while quercitrin was a feeding deterrent of the cotton boll weevil (Bird & Hedin 1986; Louveaux et al. 1998; Bernays & Chapman 2000). Quercitrin and rutin either increase the feeding rates of insects, they are toxic to them depending on concentration (Schoonhoven 1972).

The response of an organism to a substance depends on its concentration; so a low concentration may attract only one sex, produce no attraction, or repel the insect (Rodríguez 2004). Altuzar et al. (2007) reported that the freshly cut leaves of healthy *A. tequilana* var. 'blue plants' in Y-tube olfactometer bioassays showed that males and females of *S. acupunctatus* were attracted to α -pinene, 3-carene, γ -terpinene and/or linalool at doses of 1 to 10 μg , while weevils were repelled by linalool at a dose of 100 μg .

The extracts obtained from the host plant, pheromones, feces or insect parts or the opposite sex may prove attractive to insects. In contrast, extracts made from non-host plants may be non attractive to them. For example, essential oil of

Vernonia amygdalin (750 mg) repelled the storage-grain pest, *Sitophilus zeamais* (Motschulsky) (Asawalam & Hassanali 2006), however it was attractive to other weevil, *Eurhinus magnificus* Gyllenhal (Ulmer et al. 2007).

Castor oil plant has been used as a barrier within fields of crops or around houses to repel insects. In this respect, a test showed that in a free feeding choice, the mix of castor oil plant issue with 50 g of undamaged pinto bean *Phaseolus vulgaris* var. 'UI 114' at a concentration of 10% repelled the bean weevil, *Zabrotes subfasciatus* Boheman (Coleoptera: Bruchidae) (Cortez-Rocha et al. 1993). Castor oil displayed a repellent effect on the adult distribution of *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) for 4 d in bean seeds (Ratnasekera & Rajapakse 2009). Flavonoids such as quercetin, isolated from the aqueous extract of *R. communis* leaves, showed potential effects as an insecticide, oviposition deterrent and as ovicide on *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) (Upasani et al. 2003). Babarinde et al. (2011) established that the ethanolic and hydroethanolic extracts from castor oil plant seeds impregnated on filter paper repelled adults and larvae of the flour weevil *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae).

In this study, hydroethanolic extracts from leaves and seeds of 'Mirante' and wild castor oil plant applied at 10,000 ppm onto pieces of *A. tequilana* showed repellent activity on *S. acupunctatus* adults; however the leaf extracts showed a much larger effect than the seed extracts. Evaluation of the leaf extracts in a single- or double-choice bioassay would provide valuable information to more precisely gauge the potential of castor oil plant as a source of repellents to protect crops against weevils. Nevertheless, given the simplicity of making hydroethanolic extracts of a *Ricinus*, a common and abundant plant, they should be evaluated replicated agave field trials.

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