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## *Scyphophorus acupunctatus* (Coleoptera: Dryophthoridae): a weevil threatening the production of agave in Mexico

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### Abstract

*Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae), which also is known as agave weevil, is distributed across 5 continents. In Mexico, their presence has been reported in 12 states. Many species of *Agave* (Asparagaceae) are suitable hosts, though not all are equally suitable. Plant infestation can start with either female or male weevils, and their presence can be detected by gummy secretions emanating from feeding sites. The punctures are observed principally in the lower part of the trunk and external roots of the infested plants. Eggs are deposited singly or in clusters of up to 4 eggs at the feeding punctures after tissue decay has begun. The eggs hatch after about 5 d, and young larvae begin to create tunnels in the plant tissue. The larvae display 11 instars, and require about 50 to 90 d for development. The pupae normally require 11 to 14 d. The life cycle lasts between 105 to 137 d depending on the agave species with which the weevil is associated. The active insect can attack during any mo of the yr, although it is more frequent in the rainy season. *Scyphophorus acupunctatus* dispersion is determined by the attraction of volatile agave compounds. This insect apparently introduces different microorganisms that have been associated with the agave maladies. Plant extracts and seed powders have been assessed as potential botanical insecticides on larvae and pupae, with plant extracts causing mortality of 43 to 53% in larvae and 3 to 15% in pupae, and with seed powders causing 90 to 100% larval mortality. Synthetic insecticides have not been successful because the larvae and adults live inside the plant tissues. Alternative effective control tactics are needed to avoid increased damage and destruction of this important crop.

Key Words: Agave; agave weevil; mescal; tequila; pulque

### Resumen

*Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae), que también se conoce como gorgojo del agave, se distribuye en 5 continentes. En México, se ha reportado en 12 estados. Muchas especies de *Agave* (Asparagaceae) son huéspedes adecuados. La infestación de plantas puede comenzar con gorgojos hembra o macho, y su presencia puede detectarse mediante secreciones gomosas que emanan de los sitios de alimentación. Las punciones se observan principalmente en la parte inferior del tronco y las raíces externas de las plantas infestadas. Los huevos se depositan solos o en grupos de hasta 4 huevos durante la pudrición de la planta. Los huevos eclosionan 5 días después de su oviposición, y las larvas jóvenes crean túneles en el tejido de la planta. Las larvas muestran 11 etapas y requieren alrededor de 50 a 90 días para su desarrollo. Las pupas normalmente requieren de 11 a 14 días. El ciclo de vida dura entre 105 y 137 días dependiendo de la especie de agave con la que se asocia el gorgojo. El insecto activo puede atacar durante cualquier mes del año, aunque es más frecuente en la temporada de lluvias. La dispersión de *Scyphophorus acupunctatus* está determinada por la atracción de compuestos volátiles de agave. Aparentemente, este insecto introduce diferentes microorganismos que se han asociado con las enfermedades del agave. Los extractos de plantas y las semillas en polvo se han evaluado como posibles insecticidas botánicos para larvas y pupas. Los extractos de plantas causan una mortalidad de hasta 43 a 53% en larvas y de 3 a 15% en pupas, mientras que algunos polvos de semillas causan mortalidad de larvas de 100 y 90%. Los insecticidas sintéticos no han tenido éxito porque las larvas y los adultos viven dentro de los tejidos vegetales. Se requiere técnicas de control efectivas para evitar un mayor daño y la destrucción de este cultivo importante.

Palabras Claves: Agave; picudo del agave; mezcal; tequila; pulque

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The genus *Agave* (Asparagaceae) consists of 273 species, of which 75% grow in Mexico (García & Galván 1995). These plants have branched roots, a thick cuticle (depending on the species), succulent leaves with sunken stomata, and CAM metabolism (Dominguez et al. 2008). They provide an environmental and an agro-ecological service in the semi-desert areas where they grow (García & Galván 1995).

The *Agave* species that have a commercial use are *Agave tequilana* F.A.C. Weber, *Agave americana* L., *Agave sisalana* Perrine, and *Agave salmiana* Otto ex Salm-Dyck (Terán-Vargas & Azuara-Domínguez 2013). These plants are used commercially for the production of fermented beverages (mescal, tequila, and pulque), in the textile industry for fiber extraction, as biofuel, and for by-product extraction (such as fructose and inulin, among others) (Nikam 1997; Magallán & Hernández 2000; García et al. 2010).

GEOGRAPHICAL DISTRIBUTION OF *SCYPHOPHORUS ACUPUNCTATUS*

The principal pest that attacks *Agave* crops is *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Dryophthoridae), which also is known as agave weevil. It is a polyphagous species in the family Dryophthoridae (or Curculionidae) (Solís-Aguilar et al. 2001). Currently, it is distributed across 5 continents (Table 1). In Mexico, the presence of *S. acupunctatus* has been reported in 12 states (Fig. 1): Guanajuato; Baja California Sur; Puebla; Queretaro; Tlaxcala; Yucatan (Halffter 1956; Molina 2013); Morelos (municipality of Emiliano Zapata; Valdés-Estrada et al. 2010); Jalisco (municipalities of Aluahuco de Mercado, Ameca, Amatitan, Zapotlanejo, Tepatlitan, and Tequila; Figueroa-Castro et al. 2013); Guerrero (municipalities of Quetzalapa, Huitzuco de los Figueroa; Figueroa-Castro et al. 2016); Tamaulipas (Terán-Vargas & Azuara-Domínguez 2013); Oaxaca (municipalities of Tlacolula, Totolapan, Santa del Valle, Yauteppec, and Matatlan; Espinosa-Paz et al. 2005; Aquino-Bolaños et al. 2006, 2007, 2010; Bravo 2003); and Hidalgo (municipalities of Apan, Emiliano Zapata, Voladores, and Almoloya; Terán-Vargas & Azuara-Domínguez 2013).

ECONOMIC IMPORTANCE OF AGAVE

Tequila is an alcoholic beverage obtained from the blue agave (*Agave tequilana* F.A.C. Weber). Blue agave is grown in the Mexican states of Jalisco, Guanajuato, Nayarit, Michoacan, and Tamaulipas, with a planted area of 111,420 ha in 2016. The production of tequila represents 1.25% of agricultural Gross Domestic Product (GDP), and exports were valued at 1,203 million dollars (SAGARPA 2017). The Tequila Regulatory Council (CRT) reported that 17,500 farmers and more than 50,000 jobs were dependent on blue agave (CRT 2009). The Secre-

taria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (2010) records an inventory of 310 million plants, 150 tequila factories, 60,000 direct jobs, and 900,000 day farmers.

In addition, agave serves as a host for insect larvae (*Comadia redtenbacheri* Hammerschmidt [Lepidoptera: Cossidae], *Aegiale hesperiaris* Walker [Lepidoptera: Hesperidae]) that are highly valued in both national and international cuisine (Erlwein et al. 2009). The maguey red worm (*Comadia redtenbacheri*) called “chinicuil” or “tecol,” contains 7 essential amino acids and has an energy value of 607.9 kcal per 100 g; this worm is found in roots of the maguey (Llanderal-Cázares et al. 2010). The maguey white worm (*Aegiale hesperiaris*) is found in the maguey leaves and roots, and has a high nutritional value. Also, *Liometopum apiculatum* Mayr (Hymenoptera: Formicidae) brood are a delicacy known as “escamoles”; these ants commonly nest under the agave plants (Rafael-Valdez et al. 2017).

Pulque is an ancestral Mexican drink that is still being produced in a traditional way in the Mexican central region. The raw material for pulque is principally *Agave salmiana* (maguey pulquero). During the 19th and early 20th centuries, when pulque was very popular, pulque production was one of the principal economic activities of the region. Currently, the pulque industry no longer exists on a large scale; however, some producers realize a weekly income of approximately US \$200 (Erlwein et al. 2009).

Nowadays, there are many industries engaged in obtaining other derivatives of the agave, such as inulin, mixiotes (cuticle), fiber for the textile industry, mescal, etc. However, the agave weevil can drastically affect agave fields and, therefore, the economic situation of the producers.

Bud rot is a disease characterized by the presence of necrotic lesions on the agave leaf tips at the beginning of the disease (Rubio-Cortes 2007). The lesions advance to the center until they reach the center of the pineapple causing soft rot (Martínez-Ramírez 2011). The progress of the disease depends on the level of moisture that accumulates in the heart (DGSV-CNRF 2017). Bud rot in mezcalero agave is associated with high populations of *S. acupunctatus* (Espinosa-Paz et al. 2005). Microorganisms have been detected on the external surface of the insect, which enter the plant through the perforations caused by the insect when feeding (Rubio-Cortes 2017).

In Jalisco, one of the most important states for production of *A. tequilana*, bud rot associated with agave weevil has an incidence between 25 and 93% in some localities (Castro-Valera 2003). In Hidalgo, Tlaxcala, and Mexico states, the principal producers of *A. salmiana*, bud rot has been reported to cause damage to 30% of the cultivated plants (Ruvalcaba 1983). In Yucatán State, the principal producer of *A. fourcroydes* Lem., the weevil has caused damage of up to 40% (Valdés-Rodríguez et al. 2004; Solís-Aguilar et al. 2001; SIAP 2017).

Table 1. Distribution of *Scyphophorus acupunctatus* in the world.

| Continent | Country/Region                                                                                                                                                                                                                                                                                                                                        | References                                                                                                                                                                                     |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Asian     | Indonesia, Java, Kalimantan, Sumatra, Israel, Saudi Arabia                                                                                                                                                                                                                                                                                            | Vaurie 1971; Kalshoven 1981; Servin et al. 2006; Molina 2013; EPPO 2014                                                                                                                        |
| African   | Kenya, South Africa, Tanzania                                                                                                                                                                                                                                                                                                                         | Servin et al. 2006; Smith et al. 2012; Molina 2013; EPPO 2014                                                                                                                                  |
| American  | Mexico, USA (Arizona, Arkansas, California, Colorado, Florida, Georgia, Hawaii, Kansas, Nevada, New Mexico, Texas), Belize, Cayman Islands, Costa Rica, Cuba, Curaçao, Dominican Republic, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Netherlands, Antilles, Nicaragua, Puerto Rico, US Virgin Islands, Argentina, Brazil, Colombia, Venezuela | Halffter 1956; NHM 1967; Vaurie 1971; Ramirez 1978a, b; Van Rossem et al. 1981; O’Brien & Wibmer 1982; Servin et al. 2006; Setliff & Anderson 2011; Molina 2013; Aquino et al. 2014; EPPO 2014 |
| European  | Cyprus, France, Corsica, Italy (mainland), Sicily, Netherlands, Portugal, Spain (Alicante, Barcelona, Murcia, and Valencia), United Kingdom                                                                                                                                                                                                           | Van Rossem et al. 1981; EPPO 2014                                                                                                                                                              |
| Oceania   | Australia, Queensland, South Australia, Fiji, New Zealand                                                                                                                                                                                                                                                                                             | Vaurie 1971; Van Rossem et al. 1981; Servin et al. 2006; Riba I Linch & Alonso-Zarazaga 2007; Molina 2013; EPPO 2014                                                                           |



Fig. 1. Distribution of *Scyphophorus acupunctatus* in Mexico.

#### HOST PLANTS OF *SCYPHOPHORUS ACUPUNCTATUS*

*Scyphophorus acupunctatus* can attack many plants from the family Asparagaceae, including *Dracaena draco* L., *Furcraea cubensis* (Jacq.) Vent., *F. tuberosa* (Mill.), *F. foetida* (L.) Haw., *Polianthes tuberosa* L., *Beaucarnea* spp., *Dasyllirion longissimum* Lem., *Yucca aloifolia* L., *Y. elephantipes* L., *Y. glauca* Nutt., *Y. whipplei*, (Torr.) Baker, (Riba I Linch & Alonso-Zarazaga 2007; Terán-Vargas & Azuara-Domínguez 2013; Vassilis & Pavlos 2015), *Y. valida* Brandeguee (Servin et al. 2006), *Sansevieria trifasciata* Prain, *Beaucarnea recurvata* Lem., and *Dasyllirion longissimum* Lem. (Vassilis & Pavlos 2015). However, agave weevil prefers plants of the genus Agave: *A. americana* L., *A. fourcroydes* Lemaire, *A. lechuguilla* Torr., *A. salmiana* Otto ex Salm-Dyck, *A. sisalana* Perrine, *A. tequilana* F.A.C. Weber (Riba I Linch & Alonso-Zarazaga 2007), *A. angustifolia* Haw., *A. mexicana* Lam., *A. shawii* Engelm., *A. cubensis* Jacq., *A. attenuata* Salm-Dyck, *A. ferdinandi-regis* A. Berger, *A. cocui* Trel., *A. pismulae* Trel., *A. atrovirens* Karw. Ex Salm-Dyck, *A. longispala* Tod., *A. palmeri* Engelm., *A. mapisaga* Trel., *A. schottii* Engelm., *A. parryi* var. 'Huachucensis' Engelm., *A. karchovei* Lem., *A. pseudo-tequilana* Trel., *A. pacifica* Trel., *A. peacockii* Croucher, and *A. subtilis* Trel. (Velázquez et al. 2006; Terán-Vargas & Azuara-Domínguez 2013).

#### MECHANISMS OF HOST INVASION BY *SCYPHOPHORUS ACUPUNCTATUS*

*Scyphophorus acupunctatus* act in a similar manner among the different species of agave they attack (Terán-Vargas & Azuara-Domínguez

2013). According to Figueroa-Castro et al. (2015), *S. acupunctatus* colonization of tequila, espagnet, and papalote agaves can be initiated by both males and females. The initial damage observed is the presence of gummy secretions: a dark brown, viscous, and sticky exudate near the perforations, with a very characteristic odor (Riba I Linch & Alonso-Zarazaga 2007; SAGARPA 2017). The adults and larvae typically bore into the agave and produce physiological damage by decreasing water and nutrient flow, and mechanical damage by fracturing stems and leaves, consequently affecting production negatively. The losses caused by the mechanical damage are additionally important because they delay the crop harvest (Lock 1957; Terán-Vargas & Azuara-Domínguez 2013).

The adult insect burrows into the agave stalk to feed on the succulent stem in the plants, leaving punctures of about 1 cm in diam (Harris 1936). The punctures are observed principally in the lower part of the trunk and portions of the external roots of the infested plants. Adult insects cluster in galleries that can contain between 25 and 39 insects (Fig. 2a). Females use feeding sites with decaying tissue (Harris 1936) for the placement of eggs deep in the agave, and also place eggs on the surface of water-storing tissues (leaves and stems). The larvae hatch and form tunnels, leaving frass in the tunnels inside the leaves and stems (Terán-Vargas & Azuara-Domínguez 2013). Before pupation, the larvae build a cocoon with fibrous tissue from the plant (Lock 1957; Terán-Vargas & Azuara-Domínguez 2013). Cocoons are found at the base of leaves of certain agave species (Waring & Smith 1986).

In the adult stage, insect activity is crepuscular (SAGARPA 2017), with most activity observed between 9:00 AM to 5:00 PM. They can



**Fig. 2.** Infestation of *Agave salmiana* by *Scyphophorus acupunctatus*: (a) damage by adults in the beam, (b, c) channels built by larvae, and (d) agave heart rot.

attack during any mo of the yr, although attack is more frequent in the rainy season (Terán-Vargas & Azuara-Domínguez 2013). However, activity varies among different species and geographic location. For example, the highest activity occurs on *A. tequilana* during the mo of Feb to Jul (Solís-Aguilar et al. 2001). On *Agave angustifolia* Haw., in the Oaxaca central valleys, maximum activity has been observed in the mo of Jun to Oct, which represents the wettest and warmest period of the year (Aquino-Bolaños et al. 2007). Figueroa-Castro (2009) found the largest populations of agave weevil on *A. angustifolia* during Apr, May, and Jun in Ahualulco and Amatitán (Oaxaca State).

When the insect infests mature plants (4–7 yr old), the trunk withers and rots, and the plant tilts toward the ground and dies (Terán-Vargas & Azuara-Domínguez 2013). The aggressive attack on plants 4 yr or older is due to the fact that in this stage they produce large amounts of sugars (Solís-Aguilar et al. 2001). *Agave tequilana* plants attaining 6 to 8 yr of age have high sugar levels of up to 80% of non-structural carbohydrates (Cedeño 1995). Carbohydrates, especially fructose and sucrose, represent an important source of energy for many insects in the larval stage (Valentine 1983).

It is unknown how far insects can move in their adult stage and whether climate contributes to their dispersion (Huxman et al. 1997). In observations made in Caldes d' Estrac park (Barcelona, Spain), new generations of adult agave weevils attack nearby agaves, generally within less than 20 m (Riba I Flinch & Alonso-Zarazaga 2007). On the other hand, using capture-mark-release and recapture techniques, agave weevils were found up to 100 m away (Beltrán 2005).

*Scyphophorus acupunctatus* dispersion is determined by their attraction to volatile compounds produced by the agave, or by the synergized response of these plant volatiles, and of aggregation pheromones (Ruiz-Montiel et al. 2008). Valdés-Rodríguez et al. (2004) found 5 compounds in henequen extracts (*A. fourcroydes*) that are attractive to the weevil, which they identified as 4-ethylcumene, *p*-methoxy-ethyl-benzene, 1-methyl-4 (1-methyl-ethyl) -cyclohexanol, *p*-mentha-1-5-dien-8-ol, and butyrophenone. Agave weevils also are attracted by the volatiles generated during fermentation (Rojas et al. 2006).

Punctures made by adult insects (Fig. 2b) and the tunnels created by the larvae (Fig. 2c) allow the entrance of secondary pests and different opportunistic microorganisms that cause rot and accelerate plant destruction (Fig. 2d; Gold & Messiaen 2000). The lesion often becomes noticeable only after 1 to 2 yr, when a large percentage of discolored fibers draws attention to the damage (Schwencke 1934). When weevils attack young plants or shoots, decay does not occur (González et al. 2007).

#### LIFE CYCLE OF *SCYPHOPHORUS ACUPUNCTATUS*

As with all holometabolous insects, *Scyphophorus acupunctatus* has 4 stages: egg, larva, pupa, and adult (Terán-Vargas & Azuara-Domínguez 2013). Adults are active most of the yr, with varying longevity and duration of their life cycle based on the species of agave with which they are associated. The adult females oviposit near the base of the plant; eggs can be deposited singly or in small groups of 2 to 4 eggs. During its life span, an adult female can oviposit from 30 to 50 eggs (Solís-Aguilar et al. 2001).

Ramírez (1993) reported that adult longevity of the agave weevil under laboratory conditions was more than 1 yr. The cycle duration from eggs to adult was 133 to 137 d, when cultured at an average temperature of 27 °C and RH of 62 to 93%, and a photoperiod of 12:12 h (LD). When fed *A. salmiana*, the life cycle from egg to adult under laboratory conditions was 81 d (Fig. 3). The average time of egg incubation was 8 d, the larval stage went through 3 instars during the autumn in 58 d, and the pupa required 13 d (range 12 to 14 d). In culture medium with juice of *A. tequilana*, the cycle averaged 111 d (5 d in the egg stage, 85 d in the larval stage, 4 in the pre-pupal stage, 11 d in the pupal stage, and 6 d for the adults to escape the cocoon). However, when the weevils were fed healthy tissue of *A. tequilana*, the life cycle lasted an average of 105 d (5 d in the egg stage, 78 d in the larval stage, 6 in the pre-pupal stage, 11 d in the pupal stage, and 5 d as an adult inside the cocoon). In both cases, the time from copulation to oviposition is 21 d (Beltrán 2005).

### EGG

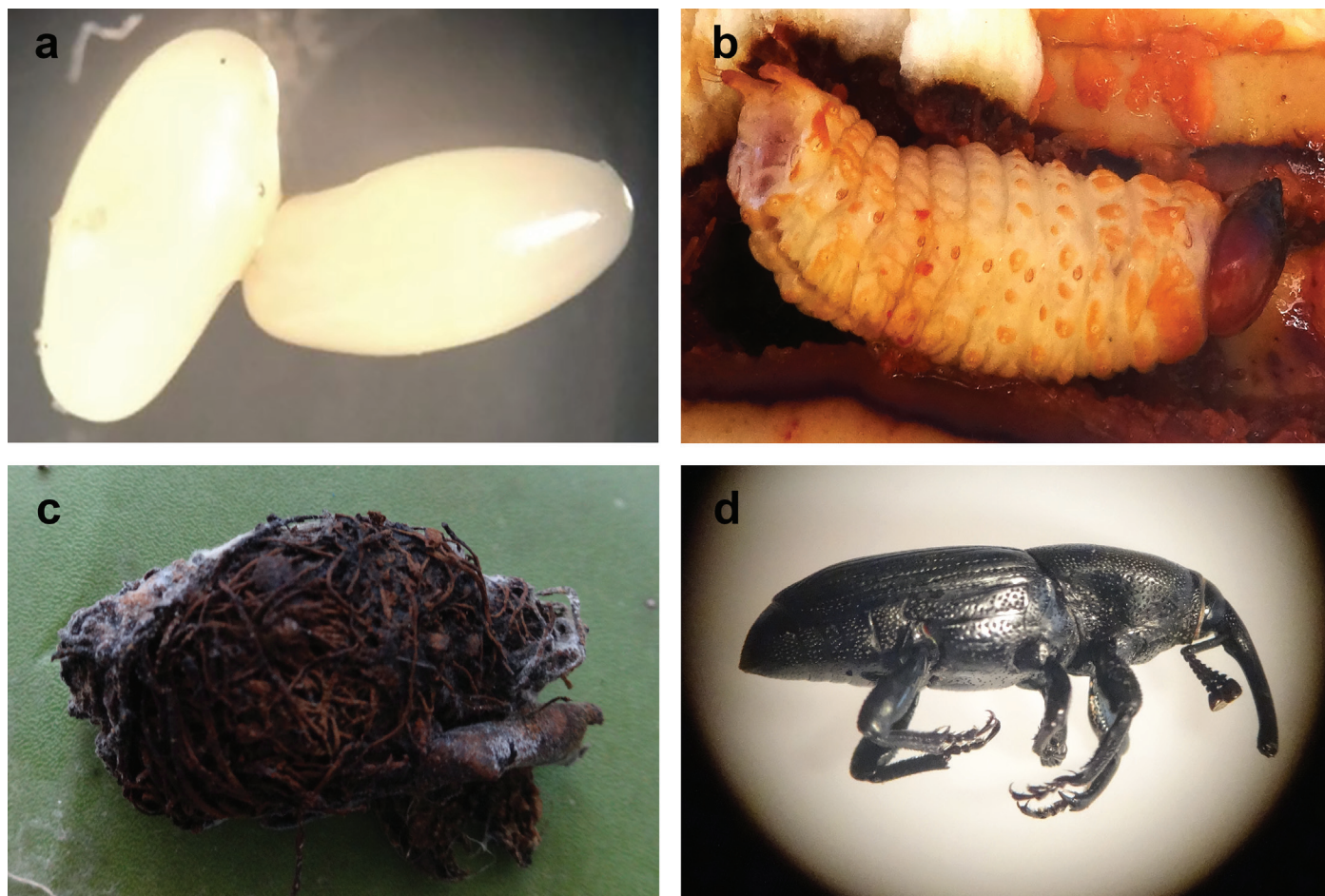
The egg is elongate-ovoid, with a length of 1.3 to 1.7 mm and width of 0.5 to 0.7 mm. When newly deposited, the egg is creamy white, with a soft, thin, membranous chorion. As it approaches hatching, it becomes slightly yellow (Lock 1957; Ramírez 1993; Terán-Vargas & Azuara-Domínguez 2013). Under the microscope, the cross-linking of the chorion can be distinguished, and in mature eggs the head capsule of the developing larva can be observed (Siller-Jasso 1985).

### LARVA

The larva is robust, with a maximum width of 9 mm, 2.5× longer than wide, with spiracles in the abdominal segments (Chamorro et al. 2016). It is creamy white in color, striated, with soft texture, and without legs. The last abdominal segment is curved upwards and has 2 small fleshy extensions known as urogomphi (Lock 1957; Ramírez 1993; Terán-Vargas & Azuara-Domínguez 2013). The head is dark brown or brownish with convergent stripes. The dorsal surface is non-pigmented, slightly longer than wide. Head width is 4.0 to 4.5 mm, and bears jaws that are dark brown or black (Cotton 1924; Harris 1936; Anderson 1948). When it is fully developed, larvae measure 1 to 2.3 cm long (Terán-Vargas & Azuara-Domínguez 2013). Spiracles are bicuspid, with an ill-defined rim surrounded by a pigmented semi-elliptical marking. In the last abdominal segment, there are 2 slightly sclerotized extensions, each bearing 3 long setae (Siller-Jasso 1985; Solís-Aguilar 2001).

### PUPA

The pupa is pale yellow, and later darkens as black pigment accumulates and the pupa turns black (Harris 1936). The pupal stage lasts 9 to 10 d. Developing wings are apparent on the dorsal side of the pupa, with the head, snout, eyes, and antennae on the ventral side (Valdés-Estrada et al. 2010). The pupa measures 15 to 19 mm in length (Harris 1936).



**Fig. 3.** Stages of the *Scyphophorus acupunctatus* metamorphosis: (a) ventral view of eggs, (b) ventral view of larvae, (c) ventral view of pupa, and (d) lateral view of adult.

## ADULT

The adult stage characteristics are not highly variable between female and male (SAGARPA 2017). The face is elongated, but shorter in males. It has a well-developed, bent beak. It does not have functional wings, so the adult moves by walking. The body has a matte black coloration. The size of the adult insect is between 15 and 18 mm long (Siller-Jasso 1985). The morphology of the tip of the abdomen can be used to differentiate sexes; the last abdominal segment is narrow and pointed in females, whereas in males it is wider and blunt (Siller-Jasso 1985; Solís-Aguilar et al. 2001).

MICROORGANISMS ASSOCIATED WITH *SCYPHOPHORUS ACUPUNCTATUS*

The agave weevil makes it possible for pathogens to enter into the agave (Rodríguez 1999); bacteria, algae, and fungi have been detected in the reddened tissues near the galleries caused by the agave weevil larvae (Waring & Smith 1986). The microorganisms isolated and identified with the BioMérieux's API biochemical test system indicate the presence of Gram negative bacteria such as *Pseudomonas paucimobilis* Holmes (Sphingomonadaceae) and *Erwinia* (Enterobacteriaceae) spp., and Gram positive bacteria such as *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem (Leuconostocaceae). In addition, some yeasts have been reported: *Candida moris*, *C. famata* (Harrison) Novák & Zsolt (Saccharomycetaceae), *C. glabrata* (Anderson) Mey. & Yarrow (unassigned), and *Rhodotorula mucilaginosa* (Jörg.) Harrison (unassigned). In agave tissues with a higher level of disease, species such as *Candida*, *Rhodotorula*, and *Leuconostoc* have been reported, whereas *Pseudomonas* and *Erwinia* have been found in less damaged tissues (Beltrán 2005). *Pseudomonas fluorescens* Migula (Pseudomonadaceae) biotype I has been isolated from the body surface of the weevil and rotting agave plants (Fucikovsky 2001).

*Rhodotorula*, *Leuconostoc*, *Pseudomonas*, and *Erwinia* bacteria were isolated from agave weevil larvae and grown in artificial media (Beltrán 2005). From the body surface of *S. acupunctatus* and decaying agave plants, other microorganisms have been isolated, such as *Pectobacterium carotovorum* (Jones) Waldee, emend. Hauben, emend. Gardan (Enterobacteriaceae) (*Erwinia carotovora* [van Hall] Dye) (Enterobacteriaceae), *Erwinia cacticida* Alcorn (Enterobacteriaceae), *Pantoea agglomerans* (Ewing & Fife) Gavini (Enterobacteriaceae), *Pseudomonas* (Pseudomonaceae) spp., the alga *Prototheca* (Chlorellaceae) sp., the fungi *Aspergillus niger* Kirk (Trichocomaceae), *Kluyveromyces marxianus* (Hansen) Van der Walt (Saccharomycetaceae), *Pichia amethionina* Starmer (not assigned) var. 'amethionina,' and several *Candida* spp. (Velázquez et al. 2006; González et al. 2007; Ruiz-Montiel et al. 2008).

## MICROORGANISMS ASSOCIATED WITH AGAVE BUD ROT

Several species of bacteria associated with agave bud rot have been reported. Vélez et al. (1996) isolated *Erwinia* sp. of the Carotovora group from *A. tequilana*. Later, Martínez-Ramírez (2011) confirmed that *E. carotovora* is the causal agent of soft rot in *A. tequilana*. Additionally, other microorganisms have been associated with the rot in *A. tequilana*, such as *E. cacticida*, *P. agglomerans*, *Pseudomonas* sp., *Bacillus pumilis* (Bacillaceae), *Arthrobacter* (Micrococcaceae) sp., *Streptomyces* (Streptomycetaceae) sp., and the fungus *Fusarium oxysporum* Schltdl. (Nectriaceae) (Rodríguez 1999; Espinosa-Paz et al. 2005; Jiménez-Hidalgo et al. 2004; Rincón-Enríquez et al. 2014).

BIOLOGICAL CONTROL OF *SCYPHOPHORUS ACUPUNCTATUS*

## INSECTS

Natural biological suppression of *S. acupunctatus* results from the activities of several parasitic and predatory insects, as well as insect pathogens. For example, Martínez et al. (2003) and Velázquez et al. (2006) noted the importance of 2 parasitoids, *Cyclaulacidea* sp. (Hymenoptera: Braconidae) and *Alienoclypeus insolitus* Shenefelt (Hymenoptera: Braconidae), and Velázquez et al. (2006) reported 4 predators, *Hololepta quadridentata* F. (Coleoptera: Histeridae), *Phileurus valgus* Olivier (Coleoptera: Dynastidae), *Ectatomma ruidum* Roger (Hymenoptera: Formicidae), and *Odontomachus bauri* Emery (Hymenoptera: Formicidae). According to Velázquez et al. (2006), *Cyclaulacidea* sp. was only detected in 3 *S. acupunctatus* pupae out of 76 collected (3.9% parasitism) and in 2 pupae out of 86 (2.3% parasitism). The genus *Cyclaulacidea* occurs naturally from central Mexico to southern South America (Velázquez et al. 2006), but evidently does not provide high levels of parasitism. The ants *E. ruidum* and *O. bauri* attack *S. acupunctatus* larvae (Velázquez et al. 2006). *Ectatomma ruidum* is omnivorous and feeds on arthropods and honeydew secretions (Fernández 1991).

## PATHOGENS

The fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae) applied in laboratory assays at a concentration of  $2.12 \times 10^7$  spores per mL caused higher mortality of adult weevils ( $86.6 \pm 12\%$ ) compared to the fungi *Metarhizium anisopliae* (Metschn.) Sorokin (Clavicipitaceae) and *Isaria fumosorosea* Wize (Cordycipitaceae). *Beauveria bassiana*, *M. anisopliae*, and *I. fumosorosea*-treated insects that perished following treatment developed external fungal growth and sporulated on both adults and larvae regardless of the spore concentration (Gkonti et al. 2015). *Beauveria bassiana* infection results in a white, powdery, or cottony growth that sometimes completely envelops the insect (Velázquez et al. 2006). This widespread entomopathogenic fungus affects numerous insects, principally Coleoptera, but its effectiveness is affected by environmental conditions (Alves 1986).

In one study in the field and in the laboratory, 3 nematodes were evaluated to determine their pathogenicity to *S. acupunctatus* (as *S. interstitialis*): *Steinernema carpocapsae* Weissner (Rhabditida: Steinernematidae), *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae), and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae). Under laboratory conditions, the nematodes killed 100% of larvae in all treatments, 24 d after inoculation. In adults, the nematodes reduced between 70 to 100% of the population of insects at 60 d. Under field conditions, the percent reduction in the population of adult weevils was 52 to 89% (Aquino-Bolaños et al. 2006).

## PLANTS

Plants contain many bioactive chemicals that have potential to be used as botanical insecticides. Extracts of *Argemone mexicana* L. (Papaveraceae), *Allium sativum* L. (Amaryllidaceae), and *Tagetes erecta* L. (Asteraceae) at controlled conditions caused 43 to 53% mortality in *S. acupunctatus* larvae, whereas extracts of *Prosopis laevigata* (Willd.) M. C. Johnst. (Fabaceae), *Bursera grandifolia* (Schltdl.) Engl. (Burseraceae), *Chenopodium ambrosioides* L. (Amaranthaceae), and *Tagetes erecta* L. (Asteraceae) caused less than 26% larval mortality. The 3 extracts that produced the greatest weight reduction in larvae at 12 d were from *A. sativum*, *A. mexicana*, and *P. laevigata*. The lowest mean larval weights at 12 and 24 d were associated with *A. sativum* extract. Significant effects of plant extracts on weight of both pupae and adults were observed for nearly all extracts. Extracts from *T. erecta*, *P. lae-*

*vigata*, *A. sativum*, *B. grandifolia*, *C. ambrosioides*, *T. erecta*, and *A. mexicana* also caused mortality of pupae (3–15%; Valdés-Estrada et al. 2016).

The seed powders of *Trichilia havanensis* Jacq. (Meliaceae), *Annona cherimola* Mill. (Annonaceae), and *Carica papaya* L. (Caricaceae) were evaluated for their ability to produce mortality and growth inhibition in *S. acupunctatus*. Seeds of *T. havanensis* and *C. papaya* at 15% were very toxic to *S. acupunctatus*, causing 100 and 90% larval mortality, respectively, whereas seed powder of *A. cherimola* killed 63% of the weevils. All powders inhibited the growth of the insect in vitro. *Annona cherimola* was most effective, decreasing the weight of larvae, pupae, and adults by 98.5, 40.6, and 45.0%, respectively (Valdés-Estrada et al. 2014). The results obtained in the laboratory with plant extracts and seed powder show the possibility of applying them in field conditions without seriously affecting the environment because they are derived from natural products.

Pacheco-Sánchez et al. (2012), tried *Ricinus communis* extracts to control *Scyphophorus acupunctatus* in bioassays conducted in the laboratory. They concluded that these extracts are not very toxic to adult weevils, but they do have an effect on weevil weight. According to Pacheco-Sánchez et al. (2012) the extract possibly was not able to penetrate into the insect and, therefore, did not have a toxic effect, even though acetone was used as a solvent, and organic solvents normally facilitate penetration of insecticides into the insect cuticle. Or perhaps the molecules evaluated penetrated, but did not have a toxic effect on the weevils.

#### CHEMICAL CONTROL OF *SCYPHOPHORUS ACUPUNCTATUS*

Synthetic insecticides are the principal tactic for the elimination of *S. acupunctatus* currently. The insecticides malathion, endosulfan, methomyl, and fipronil have shown to provide 98 to 100% suppression. In contrast, other materials such as the insecticides cypermethrin, lambda-cyhalothrin, deltamethrin, and beta-cyfluthrin are less effective (12% to 47%). The lack of control of *S. acupunctatus* can be attributed to the use of insecticides in the pyrethroids toxicological group. However, it is recommended that these be used in rotation with different insecticides classes, with different mechanisms of activity, or in conjunction with other alternatives, such as food attractants, entomopathogenic fungi, and aggregation pheromones, to obtain better control of *S. acupunctatus* under field conditions (Terán-Vargas et al. 2012). Also, the control of *S. acupunctatus* with insecticides applied directly to the plants can be difficult because the larvae are located in tunnels inside the plant (Valdez et al. 2004).

Overall, *S. acupunctatus* (agave weevil) is a very important factor in the survival of plants in the genus *Agave*. The incidence of weevils in agaves may exceed 90% in some communities in Mexico. Unfortunately, the presence of weevils often is observed only after insect infestation has occurred. The principal damage is caused by the feeding of the larvae, and the microorganisms associated with it. In addition to mechanical damage by feeding, this insect facilitates infection with phytopathogens, and causes putrescence. Presently, neither biological nor chemical control methods have shown significant efficacy for control of this pest. However, it is necessary to develop new methods to prevent the occurrence of the insect in plantations and to protect this economically important industry.

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