

Natural History and Ecology of *Scyphophorus acupunctatus* (Coleoptera: Curculionidae) and Its Associated Microbes in Cultivated and Native Agaves

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ABSTRACT A cultivated plant, *Agave americana* var. *expansa* (Jacobi) Gentry, is attacked by the weevil *Scyphophorus acupunctatus* Gyllenhal before flowering, while *Agave palmeri* Engelm., a wild species, is colonized by the weevil only after it blooms. Several explanations for this differential susceptibility are offered, and new host records for *S. acupunctatus* are reported. Microbes associated with the weevil apparently cause agave decline, a pathological condition that accompanies larval *S. acupunctatus* infestation. Agave decline kills afflicted plants.

AN AGAVE WEEVIL, *Scyphophorus acupunctatus* Gyllenhal (Fig. 1B, 2A), is the most important pest of cultivated agaves. Both the *Agave* and *S. acupunctatus* originated in the New World (Sellers 1951, Gentry 1972). However, agave species have been introduced into many arid and tropical regions as a crop for the production of sisal, and *S. acupunctatus* appears to have successfully tracked these introductions. Its range is now coincident with the plant's extended range. The weevil has been a major problem in the tequila and henequen industries of Mexico (Woodruff and Pierce 1973); the sisal industry of Africa and Indonesia (Clinton and Peregrine 1963, Lock 1969, Materu and Hopkinson 1969, Hopkinson and Materu 1970a,b,c); and in the nursery businesses and landscapes of the southwestern United States, where the plant is cultured as an ornamental (Pott 1975). Agave decline, a fatal disease in southwestern ornamental agaves (Fig. 1C) is associated with larval infestations. Agave decline has been a major concern among homeowners in southern Arizona (personal observation; D. Langston, personal communication). Although some weevil control studies have been conducted (Lock 1969, Materu and Hopkinson 1969, Hopkinson and Materu 1970a), and some taxonomic work has been done on *S. acupunctatus* (Dugès 1881, Vaurie 1971), very little is known about the natural history of this insect and its relationship to the host plant (however, see Dugès [1881, 1886]).

More than 300 agave species occur in the southwestern United States and Mexico (Gentry 1972). *A. americana* L. and some other large species are referred to as century plants because of the popularly held belief that they live for many (100

years, before producing a spectacular inflorescence. In reality, individuals grow vegetatively for 10-25 years before producing the tall stalked inflorescence (Fig. 1A). Semelparity or "big bang" reproduction occurs when a plant expends such a large portion of its resources in one sexual reproductive event that it dies shortly thereafter. Agaves are semelparous. During vegetative growth, photosynthate accumulates in the bole or base of the plant. Up to 59% of this stored energy in *A. palmeri* Engelm. is used to produce the inflorescence before sexual reproduction (Howell and Roth 1981).

Several aspects of *S. acupunctatus* biology seem to account for the weevil's role in agave decline. It is a multivoltine species whose adults bore into the bole of the plant, causing mechanical damage as well as consuming the plant's stored resources, and it may be involved in symbiotic relationships with microorganisms that break down plant tissues. Most members of the subfamily (Rhynchophorinae) attack monocotyledonous plants almost exclusively and their larvae are always associated with plant tissue necrosis (Vaurie 1971). A fungus, *Aspergillus niger* van Tiegh, induces rotting in agaves that have been attacked by *S. acupunctatus* (Wallace and Diekmahns 1952, Clinton and Peregrine 1963). Thus, agaves apparently must contend with microbial infection coincident with the insect herbivory.

S. acupunctatus is considered a pest because it creates conditions that cause cultivated and ornamental agaves to die before they bloom or can be harvested. There is a need to understand this complex ecology involving herbivore, microbial symbiotes/decomposers, and host before any effective management protocol can be developed. These relationships in natural stands of indigenous agaves are also of interest from an evolutionary perspective. The relationship between the agave weevil

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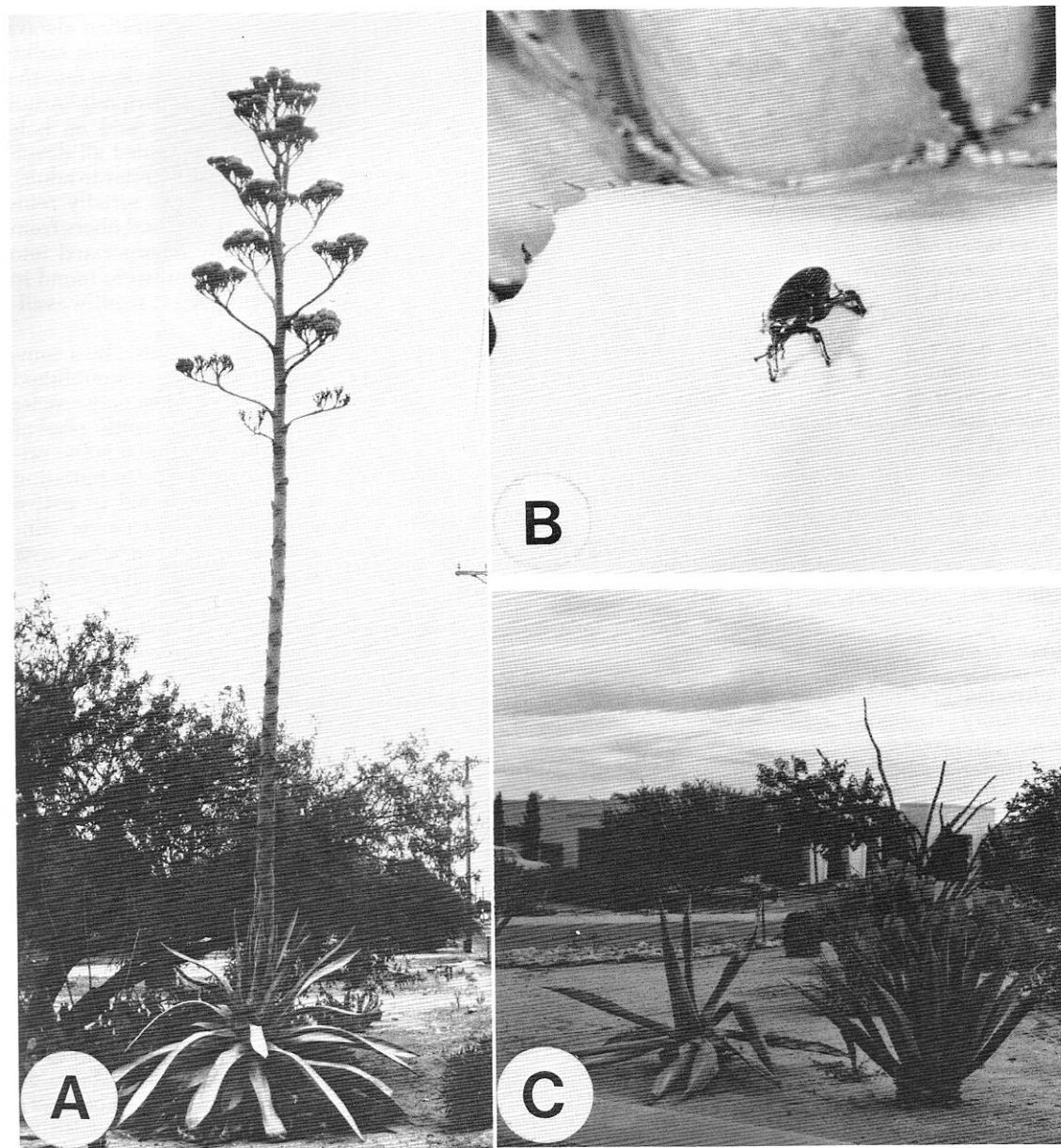


Fig. 1. (A) *Agave americana* in bloom, (B) adult female *S. acupunctatus* on agave leaf, (C) healthy ornamental *A. americana* (right) adjacent to individual (left) in agave decline.

and wild native agaves has not previously been investigated.

A. palmeri is common in, and endemic to, the foothills of mountain ranges in southern Arizona, and *A. americana* var. *expansa* (Jacobi) Gentry (endemic to northeastern Mexico [Gentry 1972]) is widely cultivated as an ornamental in the urban areas of southern Arizona. The agave weevil is common in both ecosystems. This study was undertaken to elucidate the natural history of *S. acupunctatus* and its associated microbes and to compare the ecological relationships of these organisms

in ornamental and native agaves in southern Arizona.

Materials and Methods

Six roadside populations of wild *A. palmeri*, all within 160 km of Tucson, Ariz., were randomly selected for study. The following data were recorded for each plant: 1) plant size (in cm, estimated by length of two leaves arising from the middle of the rosette); 2) presence of *S. acupunctatus* (if present, densities, distribution on or in the

plant, and developmental stages represented); and 3) condition of the plant, including presence of inflorescence (stalk), rotting tissue, tissue pH, general condition (i.e., healthy, wilting, chlorotic). Additional data on weevil activity were taken by examining the remains of 18 *A. palmeri* that had bloomed and died in the previous year.

Cultivated *A. americana* were studied within a 1.5-km² residential area in Tucson. The same data were taken as for wild plants. In addition, a survey of homeowners provided information on the age of their cultivated plants, and on cultural practices (i.e., watering, fertilization, and use of pesticides). Other agave species and weevil/host associations in and around Tucson were noted.

The natural history of *S. acupunctatus* in cultivated and native agaves was observed from March through November 1980. Frequencies of weevils on blooming and nonblooming plants of both species were compared by 2 × 2 contingency tables (Nie and Hull 1981) to discern if adult beetles discriminate among plants' reproductive condition. The Pearson's correlation test (Nie and Hull 1981) was applied to these data to determine if the distribution of weevils was influenced by the ratio of blooming to nonblooming plants.

Necrotic and healthy plant tissues from both cultivated and native agaves were collected for laboratory studies. Plant tissues (necrotic and sound) and weevils were sampled in the laboratory for bacteria and fungi, using nonacidified (YM) and acidified yeast maltose agar (AYM) (Starmer et al. 1976). Isolated bacteria and yeast samples were cultured on pectate agar to test for pectolytic activity. Yeasts cultured from rotting agaves were identified by W. T. Starmer (Syracuse University). Two water-stressed *A. americana* were inoculated with *A. niger* to validate earlier reports that *A. niger* causes pathology in agave.

Results

Natural History and Colonization. Populations of *S. acupunctatus* were observed on *A. palmeri* from the time that the plants bloomed in April through September. During this time the weevils occurred on the leaf surfaces of blooming and nonblooming plants. Only one weevil was observed flying between nonblooming plants, but this and the presence of distinctive chewing marks (2- to 3-mm oval indentations) on the upper leaves of many plants that did not become colonized (infested with larvae) suggested that the weevils regularly test plants and find some to be unsuitable as reproductive hosts. A clear viscous acidic (pH 3-4) exudate was often secreted from feeding marks in healthy plant tissue. Weevils stopped feeding on healthy agaves when this material filled feeding excavations.

On suitable hosts, from 1 to 15 weevils were observed boring into the base of the flower stalk. Feeding marks were frequently found on the up-

per leaves of these plants, but colonization always occurred through the base of the flowering stalks. Colonizing adults were found boring deep into the bole of the plants, presumably to oviposit. Adult weevils fed on the bases of leaves and on bole tissues. Many infested agaves contained all developmental stages of the weevil (early instar to adult), suggesting that the plants had been serially reinfested by residents or immigrants. Sisal fibers from the agave leaves were typically incorporated into the cocoons (Fig. 2B). Most cocoons were found in the bases of the leaves where sisal is readily available to larvae.

Although advanced rots are inhabited by a community of many arthropod species (unpublished observations), *S. acupunctatus* was the only species observed in five new rots and consistently present in all rots. This suggests that the weevil is the primary colonizer species and has a role in initiating a rot. *S. acupunctatus* was also found in native *Agave schottii* Engelm., and *Agave parryi* (Engelm.). *A. palmeri*, *A. parryi* var. *huachucensis* (Baker), and *A. schottii* are all new host records for the weevil.

In Tucson, *S. acupunctatus* colonized cultivated *A. americana* from May to October. Adult weevil feeding marks were seen on the upper leaves of these plants, but actual colonization occurred through the roots and between lower leaves. No entry holes were found in stalks of blooming *A. americana*. Pupae were found in the soil beneath the plants and their cocoons usually consisted of more soil than sisal (Fig. 2C). Thus, *S. acupunctatus* patterns of host entry and cocoon construction varied with host species. *A. americana* var. *variegata* Trelease, *A. lophantha* Schiede, and *A. vilmoriniana* Berger were found to host weevils; these are new ornamental host records.

Host Plant Selection. Of the 155 *A. palmeri* studied, 56 (36%) were blooming and 41 (73%) of those were infested with weevils. None of the 99 nonblooming plants had weevils, larvae, or boring adults. Adults were found on the leaf surfaces of 11 nonblooming individuals, however. The preference of weevils for blooming *A. palmeri* is significant ($\chi^2 = 55.13$, $P < 0.005$). Furthermore, 17 of the 18 dead postbloom plants examined were riddled with insect mines and most contained weevils, adult weevil remains, or pupal cases. It is, therefore, clear that most blooming *A. palmeri* become infested with *S. acupunctatus*.

Because weevils prefer blooming plants, we assumed that they would occur on nonblooming plants only if few blooming plants were available. However, no relationship between ratio of blooming and nonblooming plants and of weevil occurrence on nonblooming plants was revealed ($R^2 = 0.06$).

Of the 142 *A. americana* examined in Tucson, 10% ($n = 14$) were infested with weevil larvae and were dying. None of these was blooming. Of four blooming plants outside the sample, all were in-

ceptibility of individual plants. Two of the largest plants that died of agave decline were estimated to be 18 and 25 years old, and both were probably near flowering. In both cases, the agaves were adjacent to conspecifics of similar size that were not infested by weevils.

Supplemental watering, a cultural practice by homeowners at 20 residences, had no effect on *A. americana* susceptibility to weevil infestation ($R^2 = 0.353$). No homeowners applied fertilizer or insecticides to their ornamental agaves. In the urban sample area, 11 of 20 residences landscaped with agaves were losing or had lost them within the preceding years. It is, therefore, clear that non-blooming urban agaves are regularly infested.

Microbial Activity. All weevil-infested agaves had rotten boles. In field observation and laboratory experiments ($n = 78$ plants), the onset of agave decline began with weevil colonization. Weevils (adults and larvae) that were observed boring into plants were always surrounded by dark red, foul-smelling tissue; healthy white tissue had a fresh pungent odor. Where measured ($n = 10$ rots), we found that rotting tissues consistently preceded larval excavations by 1 cm at most. This was true of two plants that had been colonized by weevils in the previous year. In both cases, roughly one-half of the bole was colonized by weevils and only that half contained decomposing tissue, while the other half was sound.

The ability of *S. acupunctatus* and its associated microbes to induce rotting was also demonstrated in the laboratory. All agave tissue samples ($n = 5$) rotted when weevil larvae were allowed to feed on them; control tissue, not exposed to larvae, did not rot. An adult weevil that was starved for 30 days induced rotting when allowed to bore into a healthy plant.

Cultures from field-collected rot samples ($n = 15$ plants) yielded a variety of bacteria (not identified), an alga (*Prototheca* sp.), and fungi, including *Kluyveromyces marxianus* (Hansen) Van der Walt (pectolytic), *Pichia amethionina* var. *amethionina* Starmer et al., and a species of *Pichia* being described by Hansen. Cryptococcaceae species included *Candida lusitanae* van Uden & do Carmo-Sousa, *C. valida* (Leberle) van Uden & Buckley, and an undetermined species of *Candida*. Most rotten plants contained at least three yeast species. Yeasts seemed to predominate in early rots, which generally had a pH of from 3 to 4. Bacteria and mycelial fungi were more common in later stages when the pH was higher (>5). The AYM agar used to culture yeasts had a pH ranging from 4 to 5, close to that of newly attacked agaves. This pH precluded bacterial growth on the plates and may likewise have inhibited bacterial development in the plants. Stressed plants ($n = 2$) inoculated with the fungus *A. niger* did not become infected.

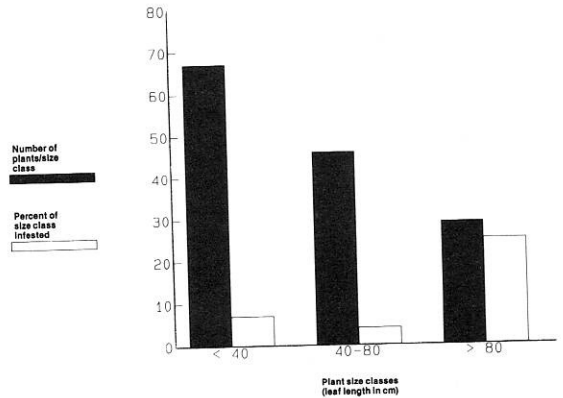


Fig. 3. Size distribution of weevil-infested *A. americana* from urban Tucson, Ariz., sample.

Discussion

Wild *A. palmeri* plants are defended against *S. acupunctatus* during the vegetative phase of their development. *A. palmeri* are, however, readily attacked once blooming is well under way. This relationship seems to be evolutionarily stable. The plant apparently invests in defensive substances to protect its vegetative parts and photosynthate store until the onset of flowering. This defense is relaxed when herbivory and microbial infection are no longer biologically relevant (after blooming is well under way) for the plant. *S. acupunctatus* is apparently a force that maintains the plant's defenses. By constantly testing nonblooming as well as blooming plants, the weevil selects well-defended preblooming individuals.

Agaves produce high concentrations of saponins (Gentry 1982), which are toxic to many herbivorous insects. Appelbaum and Birk (1979) reported that saponin contents of several plant species including *Saponaria officinales* L. and *Polemonium caeruleum* L. diminish in underground storage organs during plant reproduction and increase in the reproductive structures. Saponin concentrations are known to change in developing agaves (Dawidar and Fayez 1961). Thus, shunting of saponins among plant structures during development may occur in agaves and explain the plant boll's increased susceptibility to *S. acupunctatus* during reproduction.

Low pH appears to play an important role in plant defense against herbivores. Roever (1964) observed mortality of mining *Agathymus* spp. larvae (Lepidoptera: Megathymidae) in response to sapping out or the occurrence of acidic exudate in response to wounding. Our results indicate that this wounding response deters colonization by *S. acupunctatus* and its associated microbes.

Nonblooming *A. americana*, particularly larger, older individuals, were far more susceptible to

herbivory by *S. acupunctatus* (and agave decline) than *A. palmeri* (10% versus 0% infestation rate, respectively); this disparity may involve both natural differences and cultivation practices specifically, relaxed selection due to vegetative propagation.

Homeowner practices on ornamental agaves were not correlated with frequency or levels of infestation. However, we observed that *A. americana* was sometimes colonized through its roots. Perhaps this occurs because soil around landscape plants is less well compacted than in the natural environment. Weevils also often entered boles of cultivated plants between the leaves rather than through the roots.

Perhaps relaxed selection due to domestication best explains the observed differential susceptibility and higher mortality in *A. americana*. Intense artificial selection for rapid growth, showiness, and other desired characteristics can severely compromise a plant line's natural defenses (Pimentel 1976). Ornamental *A. americana* is propagated vegetatively by nursery men and homeowners alike. The pups (vegetative propagules that arise from the parent plant) frequently repopulate urban landscapes after parent plants have died of agave decline.

Weevil larvae are consistently present in rotting agaves, and their associated microbes induce rotting of agave tissues. Although the nature of the weevil/microbe relationship is not fully understood, our data suggest at least a facultative symbiosis. Symbiotic relationships between microbes and rhynchophorid weevils have been reported (Musgrave and Miller 1956, Crawford et al. 1960, Graham 1967). Agaves are patchy in distribution (both temporally and spatially), so rot-inducing microbes probably depend on *S. acupunctatus* for dispersal as well as physical entry into the host plant. *S. acupunctatus* may depend on the products of microbial activity to break down tissue (e.g., pectin) and synthesize various nutrients; this dependence occurs in other herbivores (Hagen 1966, Graham 1967, Barras 1972, Wright et al. 1980, Johnson and O'Keefe 1981). Our data suggest that microbial succession may track changes in pH. Yeasts were more tolerant of acidic conditions that existed at colonization, while later rots contained a higher proportion of bacteria and mycelial fungi. Suberkropp and Klug (1976) found a similar succession in decomposing leaf litter of woodland streams.

The disparate susceptibilities of these two agave species to *S. acupunctatus* and its complex of associated microbial symbionts is in need of further investigation. Future studies designed to focus on the precise nature of weevil/microbe interactions as well as the mechanisms that enable *A. palmeri* to defend itself against this complex may contribute significantly to a general understanding of the evolution of plant-herbivore interactions.

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