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Does cycad aulacaspis scale (*Aulacaspis yasumatsui*, Hemiptera: Diaspididae) play a direct role in causing soil phytotoxicity?

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Cycad aulacaspis scale (CAS, *Aulacaspis yasumatsui*, Hemiptera: Diaspididae) was accidentally introduced to Guam in 2003, and has caused acute mortality of the dominant, endemic forest tree *Cycas micronesica*. A phytotoxic legacy in the soils beneath cycad trees killed by CAS over a period of about three years has been demonstrated. The origin of the toxicity may be large quantities of CAS-encrusted cycad leaf litter. We explore the possibility that a major contribution to this toxic legacy may come from the scale insects, not just from the plant material.

Keywords: cycads, *Cycas micronesica*, Guam, invasive species, scale cover

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Introduction

Since the accidental introduction of cycad aulacaspis scale (CAS, *Aulacaspis yasumatsui*, Hemiptera: Diaspididae) in 2003, infestation of cycads (Cycadaceae) in Guam and Rota has resulted in thick encrustations of living and dead scale insects on the leaves (**Fig. 1**). The development of very high density populations of armored scale insects is the consequence of an introduced species with limited natural dispersal abilities thriving in an environment without natural enemies to suppress its numbers, and is not unique to CAS. A similar situation resulted from the accidental introduction of *Aspidiotus rigidus* (Diaspididae) to Sangi Island in Indonesia, where total encrustation of *Cocos nucifera* leaves was documented.[1] Similar encrustations occur on coconut palms when *As. destructor* invades islands in the absence of its natural enemies.[2] However, on Guam and Rota, such massive levels of cycad infestation are unique to CAS, and can be used in the field to distinguish it from other, similar species on cycads.

Chronic infestations of CAS on *Cycas micronesica* trees in Guam and Rota have killed many of the host trees.[3] In some habitats, the gaps created by *C. micronesica* mortality remain barren for years, suggesting that a phytotoxic legacy may persist in the soil. Plants grown in soil from these barren sites revealed improved growth following the addition of activated charcoal,[4] indicating the presence of a putative organic molecule that inhibits plant growth. *C. micronesica* plants in high density do not deposit this substance if there is no CAS-induced leaf or plant death.

Cycads are known to produce a range of compounds that are toxic to other organisms.[5] Armored scales ingest the contents of individual phloem sieve tubes and possibly other plant cells.[6] The toxins that are inadvertently ingested may accumulate in the scale insect's body or cover, then pass into the soil following chronic litterfall and decay of scale-infested leaves. Under the scale insect outbreak conditions in Guam, phytotoxic molecules may be being accumulated by, or even generated by the scale insects themselves.

Armored scale cover formation

A unique feature of armored scale insects (Hemiptera: Diaspididae) is the construction of a non-living scale cover over the insect's soft body to conserve humidity around it, conceal it from predators and parasitoids, and protect it from physical and environmental threats.[7] The scale cover is initiated by the first-instar larva as a tangle of filaments secreted by specialized integumentary glands. At each molt,

Figure 1. An infestation of *Aulacaspis yasumatsui* on *Cycas revoluta* in Guam (top). The development of thick encrustations of living and dead scale insects on the cycad leaves (bottom) is common in *A. yasumatsui* but is not seen in other armored scale insect species on the island.

the cast exuviae are incorporated into the cover. Unlike most insects, armored scales grow allometrically between molts, so the cover is enlarged between molts by addition of material to its margins.[8] Loose material is glued together with liquid from the anal opening, and felted and shaped by movements of the insect beneath.[9]

The largest components in an adult female diaspidid's cover are two exuviae of chitin and sclerotin (the cast cuticles of the immature instars), which confer structural strength. The felted part of the cover consists of secreted filaments (and sometimes dirt and plant fragments) glued together to form a more-or-less flexible cover. The filaments are secreted by specialized glands whose ducts open on the pygidium (several abdominal segments fused together at the posterior end of the body). The glands obtain the raw materials for their secretions from the insect's hemolymph. Movements of the insect's body assist in extrusion and positioning of the filaments.[9] Each filament is shaped by the cuticular structure of the spinneret into a white ribbon consisting of two or three hollow strands stuck together.[10] The cement component of the scale cover is a proteinaceous liquid released from the anal opening during scale formation, which is mixed with the filaments by movements of the insect's pygidium. The liquid hardens soon after contact with the air.[7]

Little work has been done on the chemistry of armored scale insect covers or the molecules of which they are composed. Chemical analysis of the covers of six diaspidid species showed a range of wax / lipid content between 13% and 50%.[6] The cover of *Aonidiella aurantii* is composed of approximately 45% waxes, 47% proteins, and 8% chitin by weight; its wax component melts at 86–88 °C and consists of chains of 27–30 carbon atoms.[11] In *Comstockaspis perniciosa* the lipid fraction also includes 9.8% hydrocarbons and substantial amounts of fatty acids other than wax esters.[12] Each secreted filament consists of an inner glycoprotein core and a thin external wax coat.[10]

The non-waxy component in the cover of *Chrysomphalus aonidum* does not contain any DNA, RNA or carbohydrate, but contains 3.7% nitrogen and 15% of the total weight consists of amino acids. Most of the fraction consists of material with a molecular weight of at least 200,000, which may indicate polyphenol or melanin-like compounds. Hardening of the scale cover occurs soon after exposure of the anal fluid to air[7] and probably involves enzymatic polymerization of a tyrosine derivative.[13]

Feeding and excretion

Diaspidid scale insects have an unusual gut configuration compared with most insects; there is no continuity between the "stomach" and the hind intestine, so all ingested materials must be absorbed and pass into the hemocoel. Nothing can pass directly through the gut and be egested.[6] Honeydew is not produced, and very little material passes out of the anal opening.[6,7] The manner in which diaspidids dispose of unwanted metabolites or excess nutrients is not known, but these materials may be returned to the plant system via the salivary glands (possibly damaging plant tissues at the feeding site) or be stored in the stomach or in its epithelial cell lining.[14,15] An example is the armored scale *Aspidiotus nerii*, which sequesters phytotoxins from its host plant, *Nerium oleander*.[16]

Discussion

What is the fate of cycad-derived toxins after inadvertent ingestion by an armored scale insect? If the toxins are returned to the plant in injected saliva,[14,15] this would further raise the toxin concentration in the cycad leaf. Any molecules too large for absorption by the epithelial cells lining the "stomach" may accumulate in the "stomach" lumen.[14] In that case, dissections or histological sections of armored scales may reveal a distention of the "stomach" in CAS compared with other species of *Aulacaspis* feeding on other host-plant species. The same technique might indicate whether heavily infested cycads respond to infestation by increasing phytotoxin production; CAS from heavily infested cycads would exhibit larger "stomachs" than CAS from lightly infested plants.

Any phytotoxins absorbed by the "stomach" lining may be stored in the epithelial cells[15] (which might be evident in histological sections) or released into the hemocoel. If the phytotoxins accumulated in the hemolymph, the entire body of the insect would become toxic unless a subsequent process detoxified or removed them.

All scale insects (Hemiptera: Sternorrhyncha: Coccoidea) contain endosymbionts housed in specialized cells (mycetocytes) in the hemocoel. The function of the endosymbionts is not understood but the evolution of specialized cells to house them suggests that their presence increases the fitness of the insects.[17] In mealybugs (Pseudococcidae) the mycetocytes have been shown to produce abundant enzymes in spite of their relatively small mass.[18] *Stigmacoccus asper* (Stigmacoccidae) can produce novel metabolites,[19] a process possibly involving its endosymbionts. The endosymbionts in CAS may absorb and detoxify the cycad toxins, possibly metabolizing them into other compound(s) that are toxic to plants but not to the scale insect. Alternatively, the cycad phytotoxins may be taken up by the Malpighian tubules and excreted into the rectum for subsequent elimination in the anal fluid.

If the armored scale anal fluid contained concentrated toxins, the use of anal fluid in scale cover construction would result in relatively low toxin levels in the insect's body, but high toxin levels in the scale cover. This hypothesis might be tested by bioassaying CAS bodies and scale covers separately for phytotoxic activity. Such an experiment would also demonstrate whether the scale insects' body contents were phytotoxic.

Application

In situations where massive armored scale insect infestations occur due to the absence of natural enemies, the influence of scale-encrusted litterfall on all aspects of biogeochemistry may differ substantially from that exhibited in the native habitat of the same armored scale species, where predation and parasitism suppress population size. If CAS bodies and/or scale covers accumulate toxins synthesized by the plant, these toxins will be present in greater amounts in CAS-killed leaves than in senescent, uninfested leaves. Additionally, if the CAS metabolome includes any de novo-synthesized components that are phytotoxic, these would be present in greater amounts in heavily CAS-infested leaf litter than in lightly infested or uninfested leaves.

Regardless of their putative origin, phytotoxins are undoubtedly released into the soil by leaching or decomposition of CAS-encrusted cycad leaves after they fall to the soil surface. The phytotoxic legacy that persists in soils following CAS-induced plant death[4] may result from a dose effect due to the massive numbers of dead scale bodies and covers on the leaf litter. In addition to toxins affecting plant growth, others may be released that could impact members of the soil biota.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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