Development of pheromone-based detection and monitoring systems for invasive scale species infesting avocado

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Project Overview

As a result of the relaxation of importation restrictions on Mexican avocados, the California avocado industry is at risk from a number of new, potentially invasive armored scale species that are not currently present in the U.S. The overall goal of this project is to identify the sex pheromones of the new species, and develop methods for using the pheromones of each species for detection, monitoring, and eradication of the new species should one or more become established in California. Our first objective was to develop methods of mass rearing one or more of the new species in quarantine to provide the raw material needed for collection of sufficient pheromone to identify. Our follow-on objectives include designing methods of synthesizing the pheromones, and developing protocols for their application for detection and sampling of the various invasive scale species. During the rearing efforts, we will also document the basic biology and life history of the target scale species.

Brief Summary of Research Results

Based on our analysis of armored scale species seen on Mexican Hass avocados entering California (Table 1 below, adapted from Morse et al. 2009), we believe the two species we should prioritize for initial investigation are *Abgrallaspis aguacatae* (because this species is so common on Mexican avocados) and *Acutaspis albopicta* (because of its broad known host range and the fact it is moderately common on Mexican avocados). *Diaspis miranda* would be a third priority (not very common in our samples) and possibly *Pinnaspis strachani* (quite rare but extremely broad known host range).

Developing methods of mass rearing scale and mealybug species can be difficult and time-consuming, because the insect species have to become adapted to the somewhat artificial laboratory rearing conditions, including in some cases, rearing on hosts other than their preferred hosts. Typically, colonies will initially grow very slowly if at all, followed by a sharp rise in reproduction and growth as a particular species becomes adapted to the laboratory rearing conditions.

We have now reached that point with one of our target species, the scale *Acuataspis albopicta*. This species in now growing well in culture (see Fig. 1 below) on butternut squash, an alternate host that we are using because it lasts a lot longer without rotting than avocados. Thus, we can maintain the cultures on individual squash from the crawler stage to the adult, without having to transfer them as the fruit degrades. Furthermore, we can then transfer the squash infested with

adult scales directly into aeration chambers for collection of crude pheromone extracts (see below).

The *Acutaspis albopicta* colony was started from eggs and crawlers collected from Mexican avocados from the Blythe border station. Eggs were collected and transferred to butternut squash adapted from a method used by Hanks (1994). As the colony grows, additional squash are added to the infested fruit allowing crawlers to move to the new fruit on their own. The colony now is well established on both butternut and spaghetti squash. Another colony of *A. albopicta* has been established on an avocado seedling by the hand transfer of crawlers with a metal probe. This is not as easy as it sounds because we discovered that crawlers have suction cup-like appendages that they use to attach themselves to plant materials or items such as the fine tip brushes that are normally used to transfer small insects.

Our second target species, *Abgrallaspis aguacatae* has so far proven difficult to establish in culture for two reasons: (1) recent shipments have contained mostly dead scales and (2) the female gives birth to a small number of live crawlers (eggs hatch internally, and only 2-3 crawlers appear to be associated with each female), and so far, they have not established on any of the host material that we have tried, including 5 different squash varieties (butternut, spaghetti, green acorn, potato squash, and mini gourds), lemons (California red scale does well on this host), seed potatoes, and avocado seedlings. This species also has the same suction cup like appendages as *A. albopicta*, and if anything, the *Abgrallaspis* crawlers seem to grip transfer tools even more tightly than *Acutaspis* or *Hemiberlesia* nr. *latania* and do not let go of the probe to move to the new substrate as willingly.

A strong colony of *Hemiberlesia* nr. *latania* was established on butternut squash, but died when the room it was being reared in failed to hold the proper temperature and humidity. A small colony is currently being restarted on an avocado seedling.

Our immediate plans are to continue to build up our *A. albopicta* culture as rapidly as possible, and to focus on pheromone collection and identification with that species. We will also continue our attempts to get *A. aguacatae* through the “lab rearing bottleneck”, so that we can begin to mass rear it for pheromone work as well.

With the *Acutaspis albopicta* colony doing so well, we have started using some of the infested squash for exploratory collections of pheromone. Thus, the infested squash are transferred into glass aeration chambers, which are swept continuously with clean air. At the outlet of the chamber, the air passes through an activated charcoal trap, which adsorbs all of the odors given off by the scales (and the squash). The traps are changed every few days, and the trapped odor compounds are washed off with methylene chloride solvent. This type of collection system is much more effective than simply killing the insects and soaking them in solvent, because it does not harm the insects, so that we can collect from them for periods of several weeks. With this system, we now have several sequential crude pheromone collections from adult *Acuataspis albopicta*, and we will start coupled gas chromatography-electroantennogram analyses of the extracts to look for the pheromone compounds shortly.

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Table 1. Species of armored scale insects found on avocados from Mexico (adapted from Morse et al. 2009), their known distribution in North America, and their known host range

Armored scale species	Present in the U.S.	Present in California	Known host plant range
Abgrallaspis aguacatae Evans, Watson and Miller	No	No	Unknown other than avocado
Abgrallaspis perseae Davidson	Yes [a]	No	Unknown other than avocado
Acutaspis albopicta (Cockerell)	Yes [b]	No [b]	Broad host range – reported from 14 hosts in 13 plant families
Diaspis miranda (Cockerell)	No	No	Previously known only from the type specimens on sapote
Diaspis sp. near *miranda* [c]	No	No	Unknown other than avocado
Hemiberlesia lataniae (Signoret)	Yes	Yes	Present on avocado and other plants in California
Hemiberlesia sp. near *lataniae* [c]	No	No	Unknown other than avocado
Pinnaspis strachani (Cooley) [d]	Yes	No	Very broad host range – reported from 68 plant families

[a] *Abgrallaspis perseae* is reported from avocado in Georgia (Tippins and Beshear 1970) and Texas (Davidson 1964) in the U.S.

[b] According to Gill (1977), *A. albopicta* was eradicated from nurseries in California and has not been seen since 1960. McDaniel (1968) lists it as being found in Texas.

[c] Genetic data suggest a species different from *Diaspis miranda* and *Hemiberlesia lataniae*, respectively.

[h] Cooley (1899) and Dekle (1954) list *P. strachani* as found on avocado in Florida in the U.S.

Table 2. Known host plants of *A. albopicta* (from ScaleNet - http:/www.sel.barc.usda.gov)

Plant family	Genus
Apocynaceae	Tabernaemontana
Araceae	Aglaonema
Araceae	Philodendron
Bromeliaceae	Tillandsia
Ebenaceae	Brayodendron texanum
Lauraceae	Persea americana
Leguminosae	Inga
Menispermaceae	Hyperbaena denticulate
Oleaceae	Ligustrum vulgare
Palmae	Cocos nucifera
Rubiaceae	Gardenia jasminoides
Rutaceae	Citrus
Tiliaceae	Jacquinia

Fig. 1. Culture of *Acutaspis albopicta* initiated from Mexican Hass avocados.