Development of Pheromone-based Detection & Monitoring Systems for Invasive Scale & Species Infesting Avocado

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This project has two main goals. First, we aim to establish colonies and mass rear exotic scale species that are coming into California on shipments of fresh avocados from Mexico, to provide insects for study. Second, we will use the colonies to identify the sex pheromones of the various species, so that the pheromones can be developed for use as sensitive detection tools for detection of these species if they become established in California. Early detection, while populations are still small and spread over a limited area, will provide the best possible chance of suppressing and eradicating any incursions of these species.

Goal 1: Establish and mass rear cultures of exotic scale species found on commercial shipments of fresh avocados coming in from Mexico.

To address the first goal, for the past 18 months we have been collecting scale from infested fresh avocados coming into the U.S. from Mexico, and trying to establish laboratory colonies of the various species (in quarantine) to provide us the raw material that we need for identification of the pheromones of each species. This is done by several methods. For example, infested avocados are placed in contact with young avocado trees grown in Quarantine, so that crawlers can move over onto the live plants from the degrading fruit and establish on the trees (Figures 1 and 2).

Figure 1. Scale-infested Mexican avocados placed against an avocado sapling to allow infestation

Figure 2. Exotic scale establishing on an avocado sapling in Quarantine (circled in red).

Alternatively, live crawlers can be collected with a fine paintbrush and transferred directly onto avocado saplings or fruit, or other possible host plant materials, to find out which host material they do best on.

To date, our efforts at establishing laboratory colonies of exotic scales collected from commercial shipments of Mexican avocados can be summarized as follows:

1. We have established a strong colony of *Acutaspis albopicta* (Cockerell) and have started trying to identify the pheromone of this sexual species. This is a good exotic scale to start with because it has been reported to infest plants in 14 different genera that are in 13 plant families. Because of its relatively broad host range, it poses quite a risk to CA avocados (it might first establish on other host plants and later move to avocado). As far as we know, this insect is present only in the state of Texas in the U.S.

2. We have a small colony of a sexual strain of *Hemberlesia* nr. *lataniae* (Signoret). This is a similar species to the female-only strain of *H. lataniae* that is present in California. In sexually reproducing armored scales, a sex pheromone is produced by females to attract males, and pheromone traps catch only the males. Thus, a pheromone trap for this species could tell us if the sexual strain from Mexico had established in CA. Pheromone traps would be by far the easiest and fastest way of determining this because the non-sexual CA strain and the sexual MX strain are very similar in appearance. At present, the colony is quite small (building up a colony to levels where we can identify its pheromone takes quite a period of time) but we plan to identify the pheromone of this species next unless we can first get a colony of either of the next three target species going rapidly.

3. We have tried repeatedly to start a colony of *Abgrallaspis aguacatae* Evans et al. but this species is proving difficult because it produces live crawlers instead of eggs, and it only produces 2-3 crawlers at a time (compare with e.g., *A. albopicta* producing 20 or so eggs at a time that are easy to transfer to new host material). In addition, *A. aguacatae* has been present in relatively low numbers in our Mexican scale samples over the last several months. Looking back at our records, November-December seem to be the peak months for this species and we plan to concentrate on it during November-December of 2010. To date (it was described as a new species in 2009), this species is known to be present only in Mexico and it is unknown if it will infest plants other than avocado.

4. A fourth species we are targeting is *Diaspis miranda* (Cockerell). This species is not believed to be present in the U.S. and at present its true host range is not known (so far it has been reported only from avocado and *Achras sapota* L. in Mexico, but these records are probably far from complete).

5. The fifth target of this research is *Pinnaspis strachani* (Cooley). It may be quite difficult to find enough live insects of this species to set up a colony because it was quite rare in our sampling of Mexican avocados. However, it could be a very dangerous species because it is known to have a very broad host range; it has been reported to infest plants in 68 different families and many of these plants are present in California. To date, this species has been reported only from Florida in the U.S.

Goal 2: To identify sex pheromones of exotic scale species from Mexico, so that the pheromones can be developed as trap lures for detection and monitoring of invasive scale species as early as possible.

We currently have 4 separate aeration chambers set up with populations of *Acutaspis albopicta* (Figure 3). One of these has been running since November of last year and is a source of males for the coupled gas chromatography-electroantennogram analyses that are used to screen the extracts for pheromone compounds. The scale-infested fruit in these chambers are used mainly for continuing to build up the colony of this species. We tried treating one chamber full of immature scales with the insect growth regulator (IGR) pyriproxifen, trying to selectively kill the males and leave us a colony of virgin females that would produce pheromone continuously throughout their lives. Female scales stop producing pheromone after they mate, and because each females produces such a tiny amount of pheromone, cohorts of virgin females represent the only chance we have of collecting enough pheromone to be able to identify it. In general, male scales and the related mealybugs are more sensitive to insect growth regulators (and many other insecticides) than females because the males undergo a complete metamorphosis from the immature, sessile feeding form to the fragile winged adult male, whereas females do not. This discriminating IGR method has been used extensively in the identification of pheromones from other scale and mealybug species.

Figure 3. Scale-infested squash in aeration chambers. Odors produced by the scale are collected for analysis on activated charcoal traps.

However, the concentration of IGR used may have been too high because in addition to killing the males, almost all of the females were killed as well. We are currently waiting for more fruit with a sufficient density of scales to repeat the IGR treatment over a lower range of concentrations. In the past couple of weeks we have had a major hatch of eggs and are currently infesting squash with high densities of crawlers. These should be suitable for doing these experiments, and subsequent pheromone collections assuming good survivorship.

Although we knew that the concentrations of pheromone present in the aeration extracts prepared from the 4 mixed-sex cultures described above were likely to be small, we screened these extracts with GC-EAD to see whether we could locate any possible pheromone peaks in the extracts. We first tested the system out with male citrus mealybugs (which are similar in size to the male scales), to ensure that all the instrumentation was functioning properly. We then hooked up antennae from male scales, and screened all ten of the aeration extracts collected to date, both before and after they were concentrated. Although the antennae did show some responses, indicating that the antennal preparation was alive and functioning, we did not see any strong and reproducible responses from the antennae, as would be expected from a possible pheromone component (Figure 4). As described above, it appears that these aerations from mixed sex cultures (in which the females are mated essentially as soon as they start producing pheromone) contain too little pheromone to be detected by our instrumentation. Thus, we have two options. First, we will repeat the IGR treatments with a range of lower doses, to try and find a discriminating dose that both kills the majority of the males while causing

minimal mortality to females. If that proves not to be possible, i.e., if males and females turn out to have similar susceptibilities to IGRs, our second option will be to kill all the immature scale manually with a needle. This is possible because there is sufficient difference in size between immature males and females that they can be reliably discriminated. However, this would clearly be the option of second choice because it is very time consuming and labor intensive.

In summary, we are making progress on a difficult project. It must be emphasized that this is true research, in that we are attempting to rear exotic insects starting from scratch. That is, unlike common pest species which have a voluminous literature describing their life histories and rearing methods, there is essentially no literature background on our study species, so everything is new, and we are learning as we go.

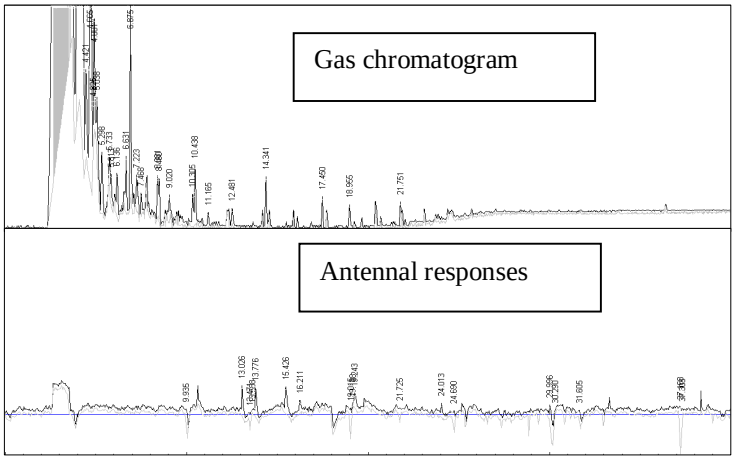

Figure 4. Analysis of a scale aeration extract by coupled GC-EAD. The spikes from the antenna indicate that the antennal preparation is alive and active, but it is not detecting any pheromone in the extract from a mixed-sex culture.