Pest and Diseases

Survey of Scale Insects Found on Imported Avocado and Dispersal of Scale Insects from Fruit to Host Plants

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Summary
In February 2007, avocados from outside the U.S. were allowed into California for the first time. During the first months of importation, CDFA inspectors at the border between Arizona and California found several species of armored scale insects on avocados imported from Mexico, that were not known to occur in California nor anywhere in the U.S. The detection of these scales on the fruit was not deemed to be important by USDA-APHIS because they had determined that scale insects on fruit in general pose a low risk of population establishment (this is in contrast with scale insects found on imported plants). Partially because of the presence of these scales on avocado and concerns expressed by CDFA and the CAC, this determination was reexamined at a USDA-APHIS science panel in May 8-9, 2007. Following the panel meeting, USDA-APHIS did not change their risk assessment and maintained that armored scale insects pose little risk with this policy affecting all imported fruits and vegetables (all "commodities for consumption"). The low risk associated with living scales on fruit was based in part on the fact that adult female scale insects are sessile and establishment of new populations would need to rely on the movement of the tiny, short-lived, first instars (crawlers) that were thought to mainly move about by walking short distances (< 4 feet) or by being blown around by the wind. We were asked by the CAC initially to determine which scale species were present and how many were present on the imported fruit. We established a sampling program, and developed a quick DNA-based method for the accurate identification of the species present on the fruit, and observed that huge numbers of living scales were imported on Mexican avocados (> 60 million during our initial 8 month sampling period). Many of the species were not known from the U.S. One of the species was even new to science. Next we investigated how crawlers disperse and in contrast to dispersal mainly by wind or walking, we found that crawlers hitchhike along with flying insects and could be transported long distances. Thus crawlers present on fruit do have a method of long-distance dispersal. We have published all of these findings in short order because USDA-APHIS largely considers research data published in refereed journals as possible reasons to revise a pest risk assessment. USDA-APHIS is presently re-examining their previous pest risk assessment and this revision is scheduled to be finished by December 2011.

Subproject 1. Sampling Avocados Entering CA from Mexico. With funding from the CAC and help from the CDFA, we initiated a sampling program in September 2007 to identify the scale species and count the numbers present on the imported fruit. Over an eight month sampling period we discovered: 1) at least 6 described and 2 undescribed species of armored scales were present on avocado shipments entering California from Mexico; 2) we estimated that approximately 20.1 million live eggs and crawlers entered the state on these avocados in addition to 47.6 million live sessile scales (capable of producing additional eggs and crawlers); and, 3) the most common scale was a previously unknown

species. This work was published as a “Forum” (i.e. highly visible) article as listed below in a well-respected core entomology journal.

Morse, J. G., P. F. Rugman-Jones, G. W. Watson, L. J. Robinson, J. L. Bi, and R. Stouthamer. (2009). High Levels of Exotic Armored Scales on Imported Avocados Raise Concerns Regarding USDA-APHIS Phytosanitary Risk Assessment. Journal of Economic Entomology 102: 855-867.

Subproject 2. Rapid Identification of Any Scale Life Stage. One problem with armored scales is determining what species is present. Normally, adult females are mounted on slides (a laborious process) and are identified by one of relatively few experts worldwide knowledgeable regarding armored scale taxonomy based on minute physical characters. Thus, there are delays in how fast specimens can be identified and this requires the presence of undamaged adult females; other life stages are not easily identifiable. To solve this problem, we developed a rapid means of identifying any life stage of armored scales based on simple DNA-based methods that a large number of laboratories are equipped to run. This work was again published in a respected scientific journal as listed below.

Rugman-Jones, P. F., J. G. Morse, and R. Stouthamer. (2009). Rapid Molecular Identification of Armored Scale Insects (Hemiptera: Diaspididae) on Mexican ‘Hass’ Avocados. Journal of Economic Entomology 102: 1948-1953.

Subproject 3. *Abgrallaspis aguacatae* Phylogenetics. The most common armored scale on Mexican Hass avocados identified in the Morse et al. (2009) survey was new to science at the time and was later given the name *Abgrallaspis aguacatae* in 2009 by Evans, Watson and Miller. As a side project to the main purpose of our CAC research, we collaborated with an expert on the molecular genetics of armored scales, Dr. Benjamin Normark of the University of Massachusetts, in a study looking at the phylogenetics of *A. aguacatae* (basically, how it is related in an evolutionary sense to other armored scale insects). A key point of this paper is that the taxonomy of this group of scale insects is not well understood and thus, we do not clearly know how *A. aguacatae* relates to other scale insects. This may make future biological control of this scale (should it establish in CA) more difficult as we would not know which closely related scales might provide parasitoids that would also control *A. aguacatae*. This manuscript helps resolve this situation and points to future research that is needed in this area. Which other parasitoids that are already present in California, can parasitize these scale species is now the subject of another project (CAC 65101). Knowledge of the potential parasitoids for these scales will help in being prepared if this scale species ever establishes on California avocados.

Rugman-Jones, P.F., J. Andersen, J. G. Morse, B. Normark, and R. Stouthamer. (2010). Molecular Phylogenetic Placement of the Recently Described Armored Scale *Abgrallaspis aguacatae* and Several Congeners (Hemiptera: Diaspididae). Annals of the Entomological Society of America 103: 30-38.

Subproject 4. Laboratory Crawler Dispersal Studies. We also studied how crawlers disperse over relatively long distances. The commonly accepted idea is that long-distance dispersal is accomplished by their allowing themselves to be taken up by the wind and potentially landing on another suitable host plant. After a number of experiments, we came to the conclusion that the assumption that wind is the key to crawler long distance dispersal is likely wrong. To determine how it was possible that these crawlers could hang onto surfaces such as glass despite very high wind speeds, we made photographs of their legs using a scanning electron microscope. Our research was published as listed below.

These pictures showed some surprising features -- at the end of each of their legs, the crawlers have a hook-like structure, and four hairs, each with a suction cup-like structure at its end (Fig 1A). Similar structures are known from mites that use other insects to transport themselves to favorable habitats; this phenomenon is called phoresy. To determine if crawlers could also use other insects to transport themselves to new habitats, we did several experiments in the laboratory and determined that indeed the crawlers hitchhiked along with all of the insect species we tried, including ants, beetles, and flies (Magsig-Castillo et al. 2010). It appeared even possible for crawlers to remain attached to flying insects over some distance in the laboratory (at least 6 ft). After transport, some of the crawlers would dismount from the transporting insect and settle on the substrate we supplied. Because this was all done in the lab, we later determined that this also happens in the field. Our initial experiments show that when we create a compost pile with crawler producing squashes, obtained through Joe Barcinas (F.A.R. Insectary, Corona), we indeed find evidence for the transport of crawlers by insects visiting these compost piles.

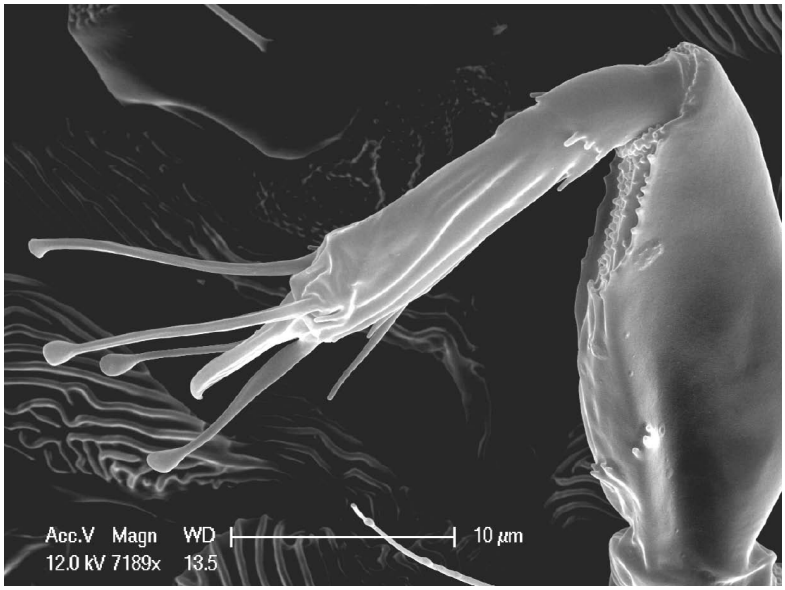
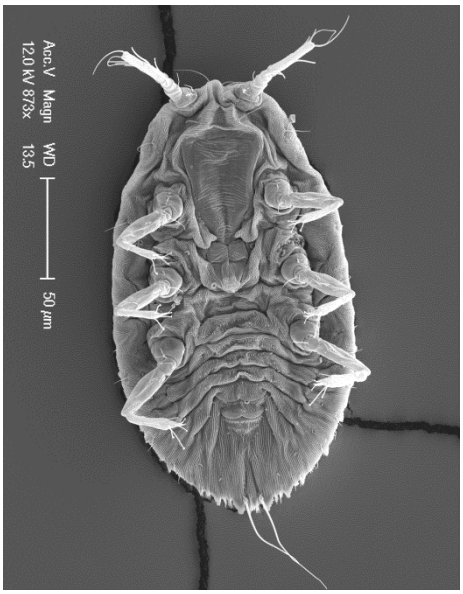


Figure 1A (right): Close up of the leg of a crawler; note the four hairs, each with a suction cup-like structure at their tip; 1B (below): Close up of the crawler of a scale insect showing the hairs with suction cup-like structures at the tip of each leg.



Magsig-Castillo, J., J. G. Morse, G. P. Walker, J. L. Bi, P. F. Rugman-Jones, and R. Stouthamer (2010). Phoretic Dispersal of Armored Scale Crawlers. Journal of Economic Entomology 103(4): 1172-1179.

Subproject 5. Additional Scale and Parasitoid Sampling. Following publication of the Morse et al. (2009) manuscript documenting which armored scale species are coming into California from Mexico and their levels, we have continued to sample Mexican shipments under both this and the related project #65105 (Millar & Morse). These 2 projects have very different objectives but there is a cost savings to the CAC by conducting the two types of work simultaneously on Mexican fruit.

Under this project (#65102), we processed Mexican avocados to continue to document levels of scales of each species and in particular, during this last year of the project, focused on identifying parasitoids and other natural enemies present in the shipments. We are building a database of which parasitoids appear to be present in Mexico versus those that are present in California (under project #65101).

Under the Millar & Morse project (#65105), we process Mexican avocados to find live crawler-producing or egg-producing females (the majority of scales are dead or non-reproductive) so that we can establish colonies to be used for the collection, identification, and synthesis of pheromones. The amount of pheromone produced by each female is so small that a large colony is needed (see the progress report for that project).

Only the eggs or crawlers can establish a new population, although adult females present on shipments can produce additional eggs or crawlers after they arrive in California. Some of the species of armored scales (such as *Acutaspis albopicta*, the species we are rearing inside Quarantine) lay eggs that later hatch into crawlers. Other species (such as *Abgrallaspis aguacatae*) birth living crawlers, i.e. the eggs hatch inside the female.

We have continued to document what species of parasitoids are associated with Mexican armored scales and the data suggest that overall, parasitism on scales present on fruit imported into California is relatively low. It is very interesting that so far, only a single species of parasitoid present in California is found in Mexican samples (the two countries might benefit greatly from an exchange of parasitoid species). In addition we have found several species of *Signophora* in scales on the imported fruit. *Signiphora* species can either be primary parasitoid or can have a hyperparasitic lifestyle. Hyperparasitoids parasitize primary parasitoids thus reducing the effectiveness of biological control exerted by the primary parasitoids. The presence of hyperparasitoids on avocado scales in Mexico was recently reported in the literature, thus providing another good reason to reduce the presence of scale insects on imported fruit. Hyperparasitoids are generally not restricted to hosts found on only one crop, consequently the establishment of new species of hyperparasitoids in California may lead to a reduction of the biocontrol efficiency in other tree crops as well.

Subproject 6. Field Crawler Dispersal Studies. We feel it is critical to follow-up our laboratory studies on crawler dispersal (Magsig-Castillo et al. 2010) with field studies that document that scale phoresy occurs under natural conditions. We have conducted experiments to investigate crawler dispersal of two armored scale species. In one set of experiments, we created a pile of banana squash infested with crawler-producing oleander scale (supplied by F.A.R.) (mimicking culled fruit at a packing house) and set up Malaise traps at varying distances from the pile. A Malaise trap is a tent-like structure used to trap flying insects. Insects fly into the tent wall (netting) and walk up the wall, eventually being "funneled" into a collecting vessel attached to the highest point. We already know from laboratory tests that crawlers cannot make their way into the traps under their own locomotion, and because of the trap design, are unlikely to be blown (by the wind) into the traps. Thus, we can assume that the only way crawlers can make it into the trap is if they are "carried" by other insects, which first visit the pile of squash and then fly or walk to the trap. The squash pile was refreshed and the contents of the traps examined weekly. In a second (more natural) set of experiments, we set up Malaise traps in two citrus groves infested with high levels of California red scale, one near Redlands and one near Bakersfield

(we are grateful to Joe Barcinas [F.A.R.] and John Gless Jr. [Gless Ranch], respectively, for access to these groves). While we have detected crawlers in the Malaise traps, the numbers of crawlers recovered in both sets of experiments has been much lower than we expected. We therefore did additional experiments to determine why the number of crawlers in the trap was so low, and we found that after landing in on the vertical wall of the trap the crawlers start to dismount from their host. Within a minute, 65% of the crawlers had dismounted, this increased to 66% after 5 min, and to 78% after 20 minutes. Malaise trap experiments showed that only 4% of the original crawler number per host when the host landed on the trap reached the ethanol. This experiment explains the low number of crawlers found in our field traps. In conclusion, scale crawlers hitchhike along with insect hosts, their numbers may be substantial but are difficult to ascertain in the field because upon landing on the Malaise trap many crawlers dismount and will never reach the alcohol trap.

Future Research Plans. Although this research project (CAC 65102) was concluded 10-31-11, we will continue research with armored scales, their parasitoids, and pheromone detection of exotic species under CAC projects 65101 (Survey for armored scales and their natural enemies on California avocados, Morse & Stouthamer) and 65105 (Development of pheromone-based detection and monitoring systems for invasive scale species infesting avocado, Millar & Morse). Both 65101 and 65105 are scheduled to continue until 10-31-12. Unfortunately, based on the levels of live armored scales entering California on Mexican Hass avocados, we believe it is only a matter of time before an exotic species, i.e. one not yet present in California or the U.S., establishes in California. If so, hopefully it will be one that project 65105 has produced a pheromone trap for, it can be caught early, and it can be eradicated before it has spread widely. Project 65101 is being conducted largely to determine what species of parasitoids are present in California. If an exotic scale appears on California avocado and cannot be eradicated, the ideal long-term solution would be biological control. To the degree possible, Project 65101 is trying to prepare us for that eventuality, i.e. establishment of an exotic scale insect species and its potential effective biological control on California avocados.