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

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End of the Year Summary

Before February 2007, avocados from outside the U.S. were not allowed entry into California. However, after these import restrictions were lifted, the CDFA detected several species of armored scale insects on avocados imported from Mexico, that were not known to occur in California nor in some cases, anywhere in the U.S. The detection of these scales on the fruit was not deemed to be important by USDA-APHIS because they 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).

USDA-APHIS considers research data published in refereed journals as the major criteria for considering new information that might inform a revised pest risk assessment. Thus, one of our major efforts over the past several years has been to clearly document what we are finding in respected scientific journals.

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 in armored scale taxonomy. Thus, there are delays in how fast specimens can be identified and this requires the presence of undamaged adult females. To solve this problem, we developed a rapid means of identifying any life stage of armored scales based on simple molecular 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' avocado. 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 (one that did not take much extra time but we think was valuable), 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 (and might support funding from other agencies to resolve armored scale taxonomy).

Rugman-Jones, P.F., J. Andersen, J. G. Morse, B. Normark, and R. Stouthamer. (2010). Molecular Phylogenetic Placement of *Abgrallaspis aguacatae* Evans, Watson & Miller 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 if they do not settle close to their mother on the same host plant, and they instead allow themselves to be taken up by the wind in hope of 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 fast wind speeds, we made photographs of their legs using a scanning electron microscope. Our research was published as listed below.

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.

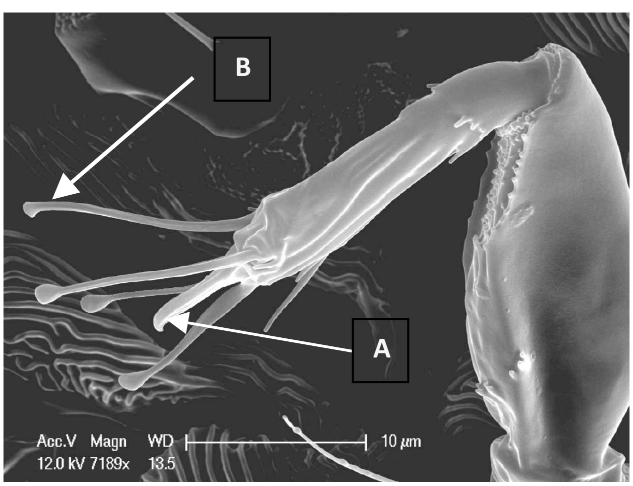

Figure 1. Close up of the leg of a crawler of the scale insect *Hemiberlesia lataniae*. A. Tarsal claw, B hairs with suction cup-like structures at their tip.

These pictures showed some surprising features; at the end of each of their legs, the crawlers had a hook-like structure (see A in Fig. 1), and four hairs, each with a suction cup-like structure at its end (B in Fig. 1). 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 are now trying to determine if indeed 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.

In summary, we believe this project has been extremely productive over the last several years and we look forward to completing the work in our last year, 2010-11.

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 process Mexican avocados to continue to document levels of scales of each species and in particular, during this last year of the project, focus 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 (as is being done 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 pheromone. The amount of pheromone produced by each female is so small that a large colony is needed (see the progress report for that project).

Over the last year (Dec. 2009-April 2010), we have processed samples from 24 additional shipments of Mexican avocados, looking for live scales and parasitoids. 23 of 24 samples contained armored scales of one species or another and overall, 83.0% of the scales were dead. *Abgrallaspis aguacatae* (found to be the most common species on Mexican fruit per Morse et al. 2009) was found in 15 of the 24 samples and 85.7% of the scales of this species were dead.

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. In the 24 shipments sampled this year, 18 contained live eggs or crawlers of one or more species of armored scale and 9 contained live *A. aguacatae* crawlers. Eight of the 24 samples contained live parasitoids, which were collected for genetic identification (genetic work is in progress); 62% of the parasitoids were collected from *Acutaspis albopicta*, 22% from *Abgrallaspis aguacatae*, and 17% from what we assume is *Hemiberlesia* nr *lataniae* (but genetic examination will be done to confirm this).

Over this last year of the project (11/10-10/11) we plan to revise how we examine Mexican scale shipments to focus on processing larger number of avocados containing live scales and parasitoids and de-emphasize data collection to document the relative frequency of different species of scales. The emphasis will be on trying to establish colonies of exotic scales in Quarantine other than *A. albopicata* (for the pheromone work of project #65105) and to collect larger numbers of parasitoids (for this project).

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. Throughout the summer we have been conducting 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 for trapping 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. However (and in hindsight), we do not know how quickly crawlers dismount (or are lost) from their "transport" after it reaches the wall of the Malaise trap. Thus, we do not know if the numbers of crawlers detected in the collecting vessel is a true reflection of the numbers actually transported to the wall of the Malaise trap. We are currently designing a set of laboratory trials to test this over the fall/winter and are also investigating alternative means of "trapping" that avoid any such problem.