Determination of the Likelihood that Scale Insects, Originating from Imported Fruit, Establish on Avocado Trees in California

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Project Overview

Between 1914 and 2007, a quarantine protected California avocado groves from pests that might be introduced into the state along with fresh, imported avocados. Soon after Mexican avocados were first allowed entry on 1 February 2007, live specimens of several species of armored scales (Hemiptera: Diaspididae) not believed to be present in California were detected on 'Hass' avocados entering the state from Mexico. Initially, the California Department of Food and Agriculture (CDFA) prevented avocados infested with these scales from entering the state or required that they be fumigated with an approved treatment such as methyl bromide. Following a Science Advisory Panel meeting in May 2007, USDA-APHIS reaffirmed its position that armored scales on shipments of fruits for consumption (including avocados) pose a "low risk" for pest establishment. In compliance with APHIS protocols, as of 18 July 2007, CDFA altered its policy to allow shipments of scale-infested avocados into the state without treatment.

The objective of this project is to evaluate the likelihood that armored scale insects from imported avocado fruit will start a new infestation in California. The following approaches have been taken. First we needed to determine the "propagule pressure" and for that we needed to know how much fruit was imported, the fraction of the imported fruit that was infested, and the number and species identity of the living scales on infested fruit that are capable of producing live crawlers. We sampled avocados as they entered California, counting and removing live armored scales for DNA and morphology-based identification.

To determine the chance that crawlers from fruit will end up on trees, we had planned to study the following four mechanisms whereby they might reach avocado trees: 1) wind, 2) humans, 3) rodents, and 4) birds. In the process of investigating wind, we found a significant new method of crawler dispersal, phoresy (movement via "hitch-hiking" on flying insects), which has kept us quite busy recently.

The May 2007 Science Panel report left the door open for APHIS to re-examine their policy should new information be presented showing that armored scales on imported avocados pose a significant risk. Generally, such information is not given strong consideration unless it is presented as a scientific, peer-reviewed publication in a reputable journal. The focus of our work over the past several years has been to investigate this situation and publish our results in such venues. To date, one publication has appeared in print, two are in press, and a fourth is being prepared for submission. We summarize the key findings of each of these publications below. Additional research is in progress and will lead to further publications.

Brief Summary of Research Results

<u>Subproject 1</u>. Propagule pressure from armored scales entering California on Mexican Hass avocados - numbers and species identities

Publication #1 -- Morse, J. G., P. F. Rugman-Jones, G. W. Watson, L. J. Robinson, J. L. Bi, and R. Stouthamer. 2009. High Levels of Armored Scales on Imported Avocados Raise Concerns Regarding USDA-APHIS' Phytosanitary Risk Assessment. Journal of Economic Entomology 102(3): 855-867.

CDFA records indicated that among their inspection stations, the Blythe station received the highest transit volume of Mexican 'Hass' avocados. With the assistance of CDFA inspectors at Blythe, we sampled levels of armored scales on commercial shipments of avocados entering California from Mexico 11 times over the period September 2007 through April 2008. We destructively sampled one carton of fruit from each truck, for a total 140 trucks. The inspectors indicated that peak numbers of trucks were seen on Thursdays and Sundays, allowing shipments to arrive at markets on Fridays and Mondays. To maximize data capture, each 4.5-day sampling period started at Wednesday midnight and ended the following Monday between 10:30 a.m. and noon.

Each piece of cut fruit was inspected and the species determined (based on digital pictures and feedback from molecular identifications). The cover of each scale was lifted using a small probe; viability was based on the condition of the scale body; dead scales being shriveled and dry whereas live scales were turgid and actively exuded body fluids when pierced During each sampling period, a sub-sample of live, adult scales of each taxon was placed in 95% ethanol individually and stored at 4°C for subsequent molecular characterization. In addition, any parasitoid emergence holes found were recorded, and any larval, pupal, or adult parasitoids preserved individually for subsequent molecular identification. Additional specimens were collected and placed into 70% ethanol for taxonomic identification by G. Watson.

A single carton of Mexican 'Hass' avocados was sampled from each of 140 trucks entering California in our survey. This resulted in a total of 7,343 avocados being inspected. These 140 trucks contained a total of 189,325 boxes of avocados with 10.4 million fruit in total.

At least six described armored scale species and two other probably undescribed species were present in the samples collected during our survey (see Table 1 below). We did not slide mount or sequence all 18,890 sessile scales found, so there could have been additional species present. The most common species found was the recently described *Abgrallaspis aguacatae.*

Live eggs or crawlers were found in 84 of the 140 sampled cartons (60.0%). Fifty-eight cartons contained live *A. aguacatae* crawlers, 44 had live eggs or crawlers of other species of armored scale, and 18 cartons contained live eggs or crawlers of both *A. aguacatae* and at least one other armored scale species. Live, sessile armored scales were found in 129 cartons (92.1%); 93 had live *A. aguacatae*, 86 had live scales of at least one other species, and in 50 cartons there were live specimens of both *A. aguacatae* and other species of armored scale. Including dead scales, only two (1.4%) of the 140 cartons sampled failed to yield at least one fruit infested with an armored scale. This is remarkable as sometimes there were relatively few fruit in the carton (this varied from 24 to 92 fruit per carton with a mean [± SD] of 52.5 ± 13.9). On average, each inspected fruit was home to 0.71 live, sessile scales and 0.34 eggs and/or crawlers (all species combined).

Data collected from the single carton sampled from each truck were extrapolated to the total number of fruit present on that truck based on data from the Bills of Lading. The number of cartons on each truck varied from 80 to 1,680 and the estimated number of scales on each truck was calculated separately before calculating the totals. Based on this extrapolation, 52 of the 140 trucks contained more than 5,000 live *A. aguacatae* crawlers, 29 had more than 5,000 live eggs and crawlers of other scale species, and 69 trucks contained more than 5,000 live eggs and crawlers of some species of armored

scale (i.e. 12 trucks had more than 5,000 live eggs and/or crawlers of both categories [*A. aguacatae* versus others] of scales). Ten trucks each contained more than an estimated 50,000 live *A. aguacatae* crawlers, with one truck containing an estimated 243,097 live crawlers (Fig. 2a). Twenty trucks each contained more than an estimated 50,000 live second and third instar female *A. aguacatae* scales and one contained an estimated 1.35 million such scales.

Sample data were also extrapolated to estimate the number of scales present on the total volume of Mexican 'Hass' avocados entering California over the 8-month sample period. There were ca. 10.4 million fruit on the 140 sampled trucks and an estimated 67.0 million fruit entering California over this period. Based on the samples from 140 trucks carrying an estimated 15.6% of the total fruit entering California over this period, a total estimated 47.6 million live sessile scales, and an additional 20.1 million live eggs and crawlers, entered California on Mexican 'Hass' avocados over the 8-month sample period.

In contrast to the USDA-APHIS opinion, we believe the volume of shipments and levels of live scales they contain present a significant risk to California's $300-million avocado industry and to other crops that might become infested by one or more of these exotic species.

Table 1. Species of armored scale insects found on avocados from Mexico (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.

<u>Subproject 2</u>. Additional sampling beyond what was published in the *Journal of Economic Entomology* article noted above

Data presented in the paper noted above were sound but they represented a snapshot over 8 months. So that we have a strong data set should our results be questioned, we believe it is important to gather additional samples. In addition, in recent sampling, we are focusing on any parasitoids present on the fruit and are also using live scales to attempt to start colonies inside UC Riverside's Quarantine Facility.

We have continued sampling and now have data from one carton of fruit from each of 14 additional 3.5-day sample periods (thus 25 in total) and 134 additional trucks (thus, 274 trucks in total). Additional sampling is planned.

<u>Subproject 3</u>. Rapid molecular methods developed to differentiate armored scales seen on Mexican avocados

Publication #2 -- Rugman-Jones, P. F., J. G. Morse, and R. Stouthamer 2010. Rapid Molecular Identification of Armored Scale Insects (Hemiptera: Diaspididae) on Mexican Hass Avocados. Journal of Economic Entomology (In Press).

Hass avocado fruit imported into California from Mexico are infested with high levels of armored scale insects constituting several species. Morphological characters traditionally used to identify armored scale species are often very difficult to interpret, and require careful preparation of slide-mounted specimens and expert knowledge of the group. In this publication, we presented a simple, quick and accurate means to identify armored scales on Mexican avocados, based on amplification of a section of ribosomal DNA, using the polymerase chain reaction (PCR). This region appears to show a high level of intra-specific conformity among scale specimens originating from different localities. A suite of species-specific reverse PCR primers are combined in a single reaction, with a universal forward PCR primer, and produce a single PCR product of a unique size, which following standard gel electrophoresis, allows the direct diagnosis of six diaspidid species: *Abgrallaspis aguacatae*, *Hemiberlesia lataniae*, *H.* sp. near *lataniae*, *H. rapax*, *Acutaspis albopicta*, and *Pinnaspis strachani*. Two further species, *Diaspis miranda* and *D.* sp. near *miranda*, are also separated from the others using this method, and are subsequently diagnosed by secondary digestion of the PCR product with the restriction endonuclease *sma*I.

In contrast to traditional morphology-based identification keys for armored scale insects, this work has provided us with a quick and simple method to identify the species of armored scale that we now know are present on Hass avocados imported from Mexico. Our entire protocol from DNA extraction through to species identification, can be performed in a single day, and can be employed by workers with only basic laboratory skills. Removing the need to sequence the DNA of each specimen represents a significant saving both in time and money. A further important advantage of this method (over morphological IDs) is its ability to identify male and immature specimens. Morphological identification is based largely on characters of adult females and as a result, males and immature stages can only be identified by their proximity to an adult female specimen. We estimate that using our method, a single laboratory technician could readily diagnose 100 live or recently deceased specimens (regardless of sex or life stage) in a normal working day. As such, our multiplex PCR represents a valuable tool for monitoring the armored scale fauna entering California on Mexican Hass avocados. Furthermore, with concern rising over the possible accidental introduction of a potentially damaging exotic armored scale, the method provides an efficient means of monitoring resident/native armored scale populations in California and other avocado producing regions around the world.

<u>Subproject 4</u>. Collaboration with the Normark laboratory to study the systematics of the most common armored scale on Mexican Hass avocados – the newly described *A. aguacatae*.

Publication #3 -- Rugman-Jones, P. F., J. C. Andersen, J. G. Morse, B. B. Normark, and R. Stouthamer. 2010. Molecular Phylogenetic Placement of the Recently Described Armored Scale Insect *Abgrallaspis aguacatae* and Several Congeners (Hemiptera: Diaspididae). Annals of the Entomological Society of America (In Press).

Hass avocado fruit being imported into California from Mexico are infested with high levels of a previously unknown species of armored scale insect. This species was recently described and given the name *Abgrallaspis aguacatae*. However, the validity of morphological characters used to diagnose genera within the Tribe Aspidiotini (Diaspididae) is the subject of continued debate. Here, we seek to circumnavigate the inherent problems associated with interpretation of somewhat plastic phenotypic characters. We use two different nuclear gene regions (28S and EF1a) and three different analysis methods (maximum parsimony, maximum likelihood, and Bayesian analyses) to infer phylogeny from DNA sequence data for 35 aspidiotine species. Our analyses suggest that the new species is misplaced in the genus *Abgrallaspis* Balachowsky and that this genus and several closely allied genera are paraphyletic or polyphyletic. The findings of our analyses are discussed specifically in relation to the current placement of *A. aguacatae*, and more broadly in relation to the long-recognized problem of defining generic boundaries between *Abgrallaspis*, *Diaspidiotus* Berlese, and *Hemiberlesia* Cockerell.

We were fortunate to have the assistance of Dr. Ben Normark and one of his graduate students, J. C. Anderson in this study looking at how molecular methods can help better determine how various armored scale species are related to one another. This is important for several reasons but the one of perhaps greatest interest to California growers is in helping us to determine how we best proceed with biological control efforts. One would expect that parasitoids that are fairly specific to closely related scale species would be the most effective biological control agents to concentrate on. In addition, Quarantine research on what scales various parasitoids attack would likely be needed before foreign species could be released into California after they are imported and studied in Quarantine. Thus, progress in understanding how various scale species are correctly related to each other is important. This was an interesting small side project resulting from our work that did not detract from our major efforts (subprojects 1, 2, and 5).

<u>Subproject 5</u>. Studying the Dispersal of Armored Scale Crawlers – Insect Phoresy May be Key to Scale Dispersal and Appears to have been Missed to a Large Degree by Previous Researchers

Publication #4 -- Castillo, J., J. G. Morse, G. P. Walker, J. L. Bi, and R. Stouthamer. 2010. Phoretic Dispersal of Armored Scale Crawlers (Hemiptera: Diaspididae) To be submitted to the Journal of Economic Entomology.

One of the main reasons that USDA-APHIS decided that scale insects on fruit are not a significant threat to agriculture in the U.S. is they believe that the mobile stage of scales, the crawler, can only cover long distances via wind dispersal. In our studies we found that the crawlers were very difficult to dislodge from a perch by wind. Upon closer inspection of the morphology of the legs, we found that attached to the tip of each leg are in addition to a tarsal claw, four hairs that each end in a structure that functions like a “suction cup”. Similar structures have been found in mites that move around by phoresy (i.e. by attaching themselves to mobile organisms). This led us to determine if diaspid crawlers are also transported by phoresy. Results of our lab experiments show that crawlers will attach themselves to other insects (house flies, *Drosophila*, ants, and ladybird beetles), they can be transported quite some distance by phoresy, and they subsequently leave their “host” to settle on a substrate. We expect that this will also take place in the field and consequently, the argument that scale insects found on imported fruit pose only a low risk to U.S. agriculture should be re-examined.