

Biology and Management of Avocado Lace Bug (ALB) in California

New Project: Year 1

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Benefit to the Industry

As fast as possible, we hope to be able to suggest sustainable management solutions to the ALB problem that are derived from sound scientific research. Because it may take some time to register new chemicals for ALB control, much of our initial emphasis will be on biological control based strategies which could assist in controlling ALB field populations, thereby reducing the source populations that will spread into uninfested areas. Such an approach may reduce the speed at which ALB spreads in California and subsequent crop losses. There is currently no active research on ALB in the U. S. and activities overseas are limited.

Summary of Results to Date

1. Backyard Pesticide Trial:

Artificial infestation:

Four-foot tall avocado seedlings potted in 5-gallon pots were used in this trial, which took place in the backyard of a cooperator (Charles T. Robinson) in San Diego. Prior to artificial infestation, ALB were collected from different sites in the San Diego area and taken to the lab. The total number of nymphs per leaf was counted and leaves with approximately 50-60 nymphs were placed in a plastic bag to be used to infest each potted seedling. Nymphs ranged from mid to late instars. Each bag was used to infest one tree the following day. The artificial infestation took place on 3 June 2005, clipping the infested leaf to the abaxial surface of the leaf of the pesticide trial tree (Figure 1). Each infested leaf was tagged with a plastic wire to facilitate future counting.

Fig. 1. A) Artificial infestation procedure using paper clips to attach the infested leaf to the leaf of the pesticide trial seedling. **B)** Avocado lace bug adult.



Pre-count and pesticide spray:

The pre-count was taken three days after the artificial infestation (6 June) with an average of 41.2 nymphs recorded per seedling. The following day (7 June) pesticide treatments were applied using a hand sprayer (B&G Equipment Co., Jackson, GA) to apply ca. 0.5 liter of spray to 4 plants (plants were sprayed to runoff). Pesticide concentrations used in the study were the maximum rate for backyard use on fruit trees or avocado. Mortality was evaluated 7 and 14 days later.

Experimental design:

A total of seven treatments and four replicates (one tree per replicate) were used in the pesticide trial.

Results:

Results indicate that imidicloprid, cyfluthrin, carbaryl and malathion were the best treatments of those evaluated to control ALB nymphs 7 and 14 days after treatment. Spinosad and Mineral Oil were less effective and should be discarded from our list of potential control materials. Based on counts in the water control, it appeared a substantial number of nymphs disappeared from the plants prior to the 7 and 14 day counts. In future pesticide trials, we plan to spray avocado leaves in the field, allow the pesticide to weather on the tree in the field, and then bring the leaf back to the San Diego Insectary where ALB nymphs will be bioassayed using a closed system (Munger cell) which does not allow them to escape.

Table 1. Treatments and ALB mortality counts for the seedling spray trial

Treatment	Use rate	Pre-count -1 day 6/6/2005	+7 days	+14 days
		ALB/ Tree	% of Pre-count	% of Pre-count
1. Control	water alone	42.0	53.3	33.9
2. Imidicloprid	1 oz per inch trunk circumference	42.0	0.0	0.0
3. Cyfluthrin	0.5 fl oz per gallon	41.8	0.0	0.0
4. Malathion	0.67 fl oz per gallon	42.8	1.8	3.0
5. Carbaryl	4.5 fl oz per gallon	41.3	0.0	0.0
6. Spinosad	2 fl oz per gallon	40.8	14.7	16.4
7. Mineral Oil	1.5 fl oz per gallon	43.8	15.4	16.3

The formulation of each pesticide used was: 2. Imidacloprid – Bayer Advanced Garden, Tree & Shrub Insect Control Concentrate; 3. Cyfluthrin – Bayer Advanced Garden, Power Force Multi-Insect Killer Concentrate; 4. Malathion – Ortho Malathion Plus; 5. Carbaryl – GardenTech – Sevin Concentrate Bug Killer; 6. Spinosad – Monterey Garden Insect Spray; 7. Mineral Oil – Sunspray Ultra-Fine Year-Round Pesticide Oil.

2. Systemic Pesticide Bioassays:

Experimental design and statistics:

Because avocado lace bug cannot be transported outside the containment area, we collected leaves from the CAC systemic pesticide trial (see the systemic pesticide report in this issue by Byrne, Morse, and Toscano) and bioassayed ALB nymphs on these leaves at the Insectary in San Diego. For each treatment listed below, 10 Munger cells (replicates) per treatment were used to evaluate ALB mortality. In each cell, five small to medium size ALB nymphs were confined (50 nymphs per treatment in total).

Mortality criterion:

Mortality was assessed at 48 and 72 hours after nymphs were placed on leaves. Nymphs were considered dead if they did not move twice their body length after being prodded with a brush.

Results:

Quantification of systemic imidacloprid levels within xylem fluid and leaves was conducted by Dr. Frank Byrne. These data (see the systemic pesticide report in this issue by Byrne, Morse, and Toscano) showed that Admire uptake was more effective in small trees than in large trees. In the smaller trees, levels of pesticide in mature leaves were generally higher than in younger leaves, and this correlated well with the bioassay results. Although ALB will show a preference for more mature leaves, when confined to either young or mature leaves, ALB mortality was

governed by the relative levels of pesticide in the leaf. Control mortality on both young and mature leaves was minimal indicating no significant ‘natural’ mortality. For ALB bioassays reported below, we selected 10 leaves from each of two small trees – one treated at the 14 fl oz/acre rate and a second treated at the 28 fl oz/acre rate. The trees were chosen based upon imidacloprid uptake levels determined from xylem fluid tests.

Bioassay 34 days after systemic pesticide treatment:

Treatment	Percent ALB corrected mortality 13 July (+34 d)
<u>Large Trees:</u>	
Untreated (control), large trees, mature leaves	2.0
Admire Pro 28 oz, large trees, mature leaves	0.0
Platinum 11 oz, large trees, mature leaves	79.6
<u>Small Trees:</u>	
Untreated (control), small trees, mature leaves	2.0
Admire Pro 14 oz, small trees, mature leaves	91.8
AdmirePro 28 oz, small trees, mature leaves	100.0

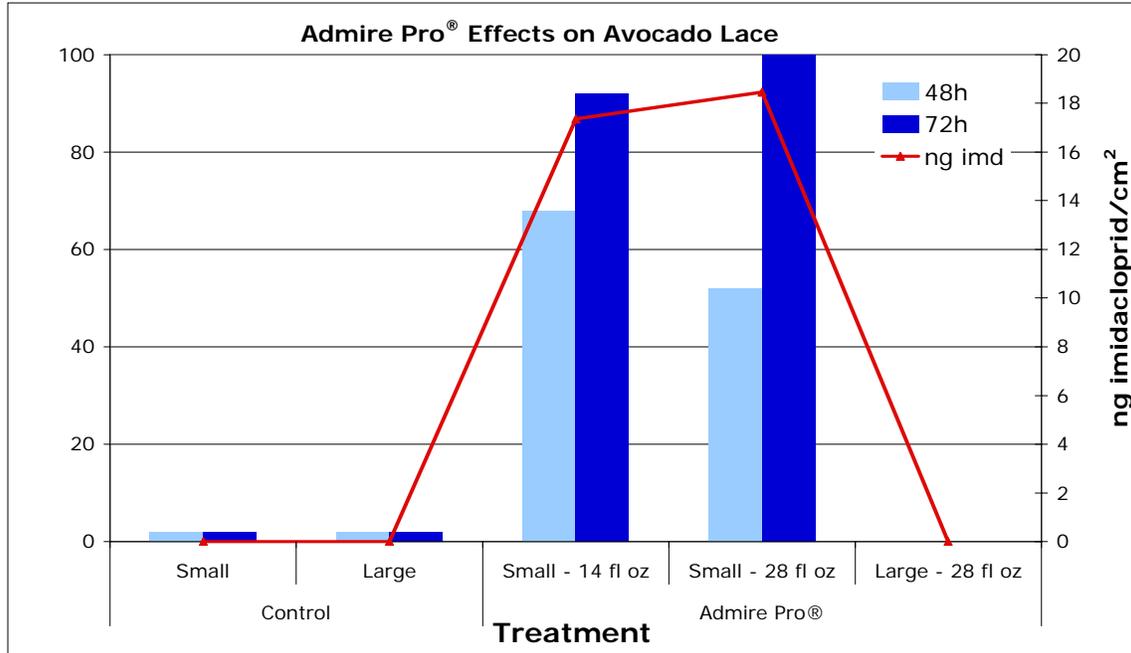


Fig. 2. Correlation of ALB mortality with imidacloprid residues in avocado leaves. Leaves containing appreciable levels of imidacloprid resulted in excellent mortality of ALB in bioassays. Optimal mortality was achieved in bioassays after 72 hours exposure. At the time of the bioassay, residues within leaves taken from mature trees were insufficient to result in ALB mortality.

Bioassay 55 days after systemic pesticide treatment:

In this bioassay we also picked out specific small trees treated with either the 14 or 28 oz rate of Admire and compared ALB mortality on young versus old leaves.

<u>Treatment</u>	<u>Percent ALB corrected mortality 3 Aug. (+55 d)</u>
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Small Trees:

Untreated (control), small trees, mature leaves	2.0
Untreated (control), small trees, young leaves	2.0
Admire Pro 14 oz, small tree #3, mature leaves	93.9
Admire Pro 14 oz, small tree #3, young leaves	95.9
Admire Pro 28 oz, small tree #8, mature leaves	95.9
Admire Pro 28 oz, small tree #8, young leaves	79.6

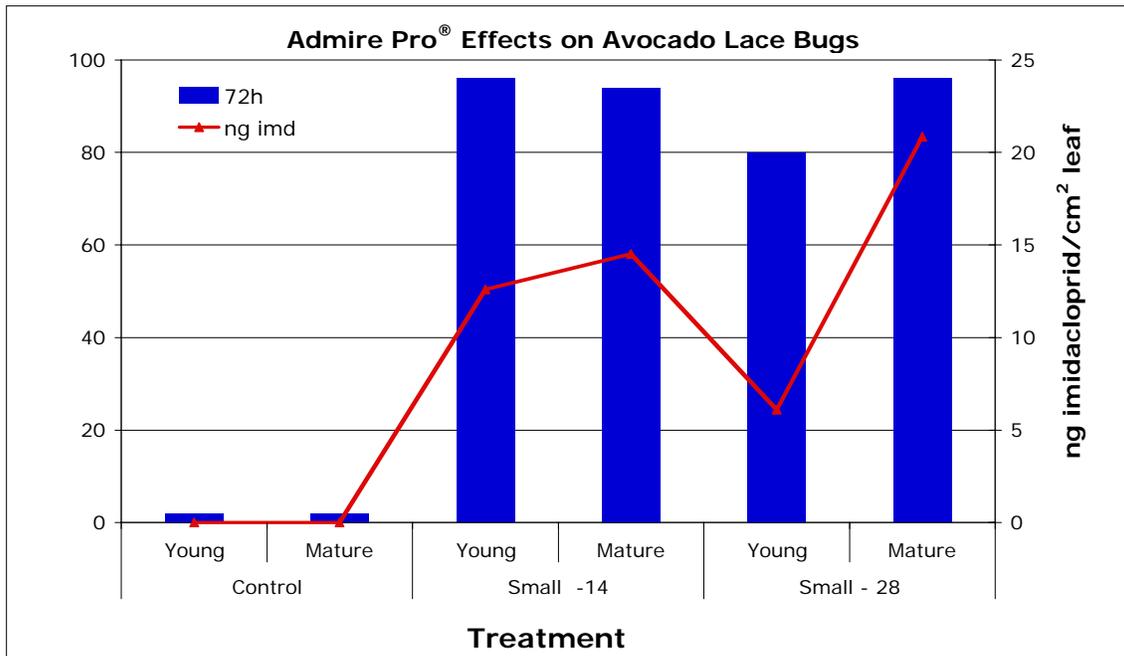


Fig. 3. Correlation of ALB mortality with imidacloprid residues in avocado leaves. Similar levels of imidacloprid in trees treated at either 14 or 28 fl oz/acre resulted in equitable mortality in bioassays. The lower residue measurement for the young leaves in the tree treated at the 32 fl oz rate resulted in a concomitant decrease in mortality.

Imidacloprid has been shown to be effective against the ALB in bioassays conducted with both potted and commercial trees. Effective uptake of imidacloprid will be governed by several factors, some of which we are addressing in this and other projects. We have shown that it is possible to use systemically-applied imidacloprid to achieve adequate levels of toxicant within the tree that will give excellent control of ALB.

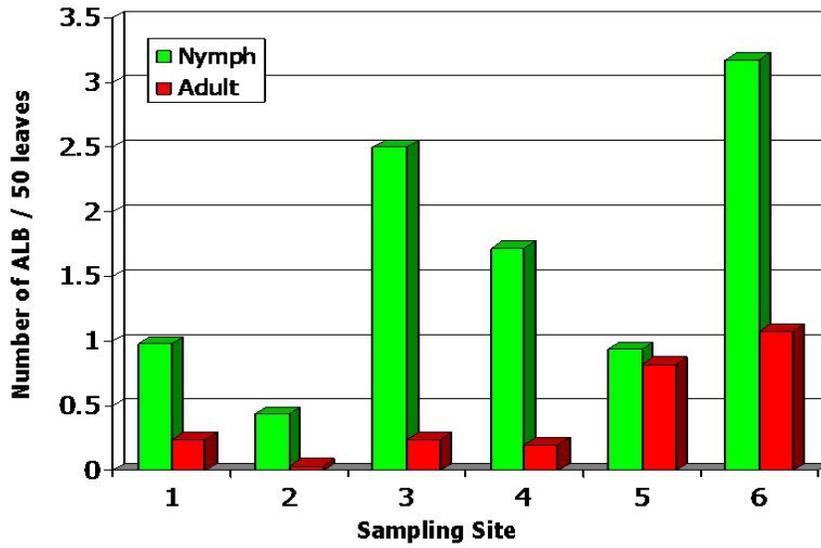
3. ALB Sampling Survey:

3.1. ALB leaf sampling

ALB sampling sites:

ALB sampling sites were selected based upon high levels of ALB infestation, avocado moderate to large tree size, and diversity in location. Different site locations ranging from the coast to inland San Diego Co. provided alternative environments for ALB sampling. Six sites were chosen for the ALB sampling survey as shown in Fig. 4.

Avocado Lace Bug in Home Avocado Trees, San Diego, CA
(sampling date: 12 July 2005)



Avocado Lace Bug in Home Avocado Trees in San Diego, CA
(sampling date: 26 August 2005)

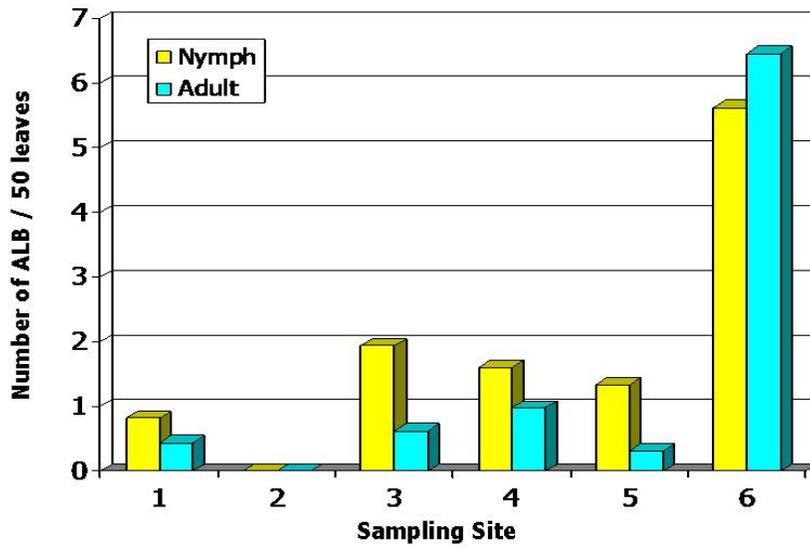


Fig. 5. Mean number of avocado lace bugs in backyard avocado trees in San Diego, 2005.

Table 2. Number of avocado lace bugs in backyard avocado trees, San Diego, CA.

Date	Sampling site	Mean (n = 50 leaves)	
		Nymph	Adult
July 12	1	0.98	0.24
July 12	2	0.44	0.04
July 12	3	2.50	0.24
July 12	4	1.72	0.20
July 12	5	0.94	0.82
July 12	6	3.18	1.06
August 26	1	0.82	0.44
August 26	2	0.00	0.00
August 26	3	1.94	0.62
August 26	4	1.60	0.98
August 26	5	1.34	0.32
August 26	6	5.62	6.46

A second ALB sampling procedure has been initiated in parallel with that described above. It consists of counting adult ALB at the site without leaf removal to the lab. This has the advantage of not removing leaves from the tree, maintaining tree populations if they are low, and improves sampling speed. The results indicate that this sampling procedure is promising but it requires further evaluation (Figure 6 and Table 3).

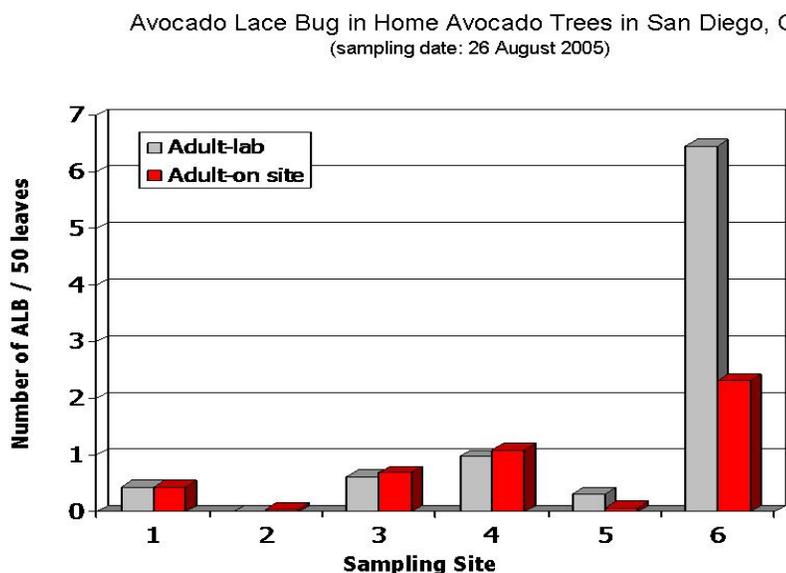


Fig. 6. Mean numbers of adult of avocado lace bugs in backyard avocado trees in San Diego, CA 2005.

Table 3. Number of adult avocado lace bugs in backyard avocado trees counted at the site versus in samples brought back to the lab, San Diego, CA.

Date	Sampling site	Mean (n = 50 leaves)	
		Adult-lab	Adult-on site
August 26	1	0.44	0.44
August 26	2	0.00	0.06
August 26	3	0.62	0.70
August 26	4	0.98	1.10
August 26	5	0.32	0.08
August 26	6	6.46	2.32

4. Natural Enemy Study:

4.1. Testing *Franklinothrips* sp. as a potential augmentative control agent for ALB

Protocol:

Franklinothrips orizabensis colony:

Pupal *F. orizabensis* (Buena Biosystems, Ventura, CA) were reared on irradiated *Ephestia kuehniella* (Lepidoptera: Pyralidae) (Beneficial Insectary, Redding, CA) eggs through several generations in the lab before being used in evaluations. Two to three second instar female larvae were transferred to a Munger cell with an avocado leaf. Small, circular filter papers (0.5 mm) were placed along the avocado leaf surface so the larvae could pupate underneath them. *Ephestia* eggs were supplied as food for the larvae. Once they pupated, the filter papers were transferred into new Munger cells individually until 2-3 adults emerged. Only adult females were used in the bioassay and they were starved prior to the bioassay for at least 24 hours. Adult *Franklinothrips* used in the study were 1-2 days old.

ALB nymphs:

Since we haven't begun the ALB biology studies and therefore haven't determined the number of ALB nymphal stages, we classified the nymphs used in the present study as small or medium size. In addition, predation on adult ALB was evaluated.

Experimental design:

A total of six treatments and 10 cells (replicates) per treatment were used in the present study. A ratio of one adult female *Franklinothrips* per five ALB per cell was the prey/pest ratio used in each cell (1 adult *Franklinothrips* with 5 nymphal or adult ALB).

Mortality criterion:

ALB mortality was assessed 24 hours after the prey was exposed to the thrips and the ALB nymphs were considered dead if they were sucked dry by the predator.

Results:

Adult female *Franklinothrips* prey mostly on small nymphs (60% mortality) compared with only 6% and 0% mortality on medium instar and adult ALB, respectively. Given that a substantial amount of the ALB population would escape predation (i.e. anything larger than a small nymph), we don't consider the results to be promising.

Table 4. Results of Munger cell bioassay for adult *Franklinothrips* predation on three stages of ALB.

Treatment	Corrected mortality (%)
Control - ALB small nymphs	0
Control - ALB medium nymphs	0
Control - ALB adults	0
<i>Frankliniella</i> + ALB small nymphs	60
<i>Frankliniella</i> + ALB medium nymphs	6
<i>Frankliniella</i> + ALB adults	0

5. Preparing the San Diego Greenhouses & Insectary for Work on ALB:

Substantial work has been done on the San Diego County Insectary in Chula Vista for the ALB project. This has included the installation of new swamp coolers and associated electrical apparatus to operate the swamp coolers; new lights; temperature and humidity monitoring devices; replacement of rusted metal framing that was compromising the integrity of rearing greenhouses; purchasing of potted avocado plants for the ALB colony; transformation of an old storage room into an insect rearing room with a new swamp cooler and thermostat to maintain uniform temperatures inside the room for bioassays and biological studies. Most of this work and associated materials was paid for by the California Department of Food and Agriculture and CDFA BioControl Scientist, Dr. Bill Roltsch assisted with all aspects of this portion of the project.

6. Genotyping ALB:

DNA has been extracted from the San Diego ALB population and was sent to GIS (Genetic ID Services) where it has been exposed to various treatments to construct a microsatellite library of ALB DNA. Subsequently, this library has been enriched for microsatellite DNA sequences and these sequences have been determined. Following this, stage sets of primers will be developed to amplify these microsatellites, which will be tested against the DNA of a number of different individual ALB that we have sent them. The successful primers will be sent to us and we will use these to study the population found here in California and compare it to potential source populations. Currently, GIS is testing various primers against the DNA extracted from individual

ALB sent them. We expect to receive the primer sets within 4 weeks. Once these primer sets are available, we will optimize their reaction conditions to determine the optimal amplification. After optimization the California population will be compared to different potential source populations.

To ensure that we are dealing with only a single species of ALB we have determined the DNA sequence of a number of genes that are used to ascertain the species status of different populations. It is important for us to know if we are dealing with a single species of ALB for a number of reasons: 1) It will allow us to match the parasitoids with the correct pest species, 2) If we try to use the microsatellites on two different species our analysis will be confounded.

We have sequenced the mitochondrial gene COI from one population of ALB from Florida (20 individuals) and California (7 individuals). These sequences differ consistently by a single base pair. This tentatively indicates that the CA population is not derived from the tested Florida population; however, the sample size is much too small to state this with high confidence. In addition we sequenced the D2 region of the ribosomal complex and found that the California and Florida populations were identical. The D2 region is generally a good indicator for species status because it is a highly conserved piece of DNA. If two populations differ in this sequence they often belong to different species, and if they are the same, the chance that the two populations belong to the same species is substantial. Therefore, we may tentatively state that the ALB in the Florida population we sampled and our California population are likely the same species. Obviously, additional research on this subject is needed and will be done.

7. Future Research Plans:

In the near future we intend to initiate and/or complete the following:

- Further test selected pesticides for ALB control (new protocol described above).
- Test green lacewings and predaceous mites as ALB augmentative control agents.
- Initiate endemic egg parasitoid survey at selected sites.
- Analyze ALB sampling data to determine an optimal field sample size.
- Investigate lab ALB rearing procedures for further biological studies.
- Select a reliable method to preserve avocado leaves in the rearing room for the natural enemy survey.
- Rear ALB in the greenhouse.
- Commence foreign exploration for ALB for genotyping and importation of associated parasitoids in Florida and the Caribbean.
- Start the mymarid parasitoid identification work at the Smithsonian Museum.

Acknowledgments

Obviously, progress made to date in ALB research would not have been possible without the substantial assistance of a large number of individuals and organizations. We thank Charles T. Robinson for allowing us to run the potted seedling pesticide trial in his backyard. Bill Roltsch has assisted with field ALB sampling, made a trip to Florida to evaluate the ALB situation there, and has been of great assistance in renovating the San Diego Insectary facility (partial funding by

CDFA; use of the facility was volunteered by the San Diego Ag. Commissioner's Office; special thanks to David Kellum who assisted in upgrades to the facility). Guy Witney and the California Avocado Commission helped provide potted avocado plants used in several aspects of the study. Gary Bender has been of great assistance setting up avocado seedlings and an automatic watering system to maintain plant viability. Gary Bender and personnel from CDFA and the San Diego Ag. Commissioner's Office helped locate field research sites. Linda Feeley, Dinna Estrella, and Robert Roma helped collect ALB nymphs for the seedling pesticide trial. Funding was provided in part by the California Avocado Commission, California Dept. of Food & Agriculture, UC Thelma Hansen Trust Fund, and UC Exotic Pest & Disease Research Program (through a grant from USDA CSREES).