
Pseudacysta perseae **Biology and Management of Avocado Lace Bug in California**

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The avocado lace bug (ALB), *Pseudacysta perseae* (Heidemann) (Fig.1), was discovered in September 2004 feeding on backyard avocado trees in San Diego County. The ALB is known from Florida, the Caribbean and Mexico. ALB adults and nymphs feed in colonies on the undersides of leaves (Fig. 2A). Feeding damage results in necrotic brown spots that can lead to defoliation and reduced fruit yields (no direct damage to fruit) (Fig. 2B). Heavily infested avocado trees in San Diego County have a severely scorched appearance (Fig. 2C). In addition to avocado (*Persea americana*), ALB is known to feed on ornamental camphor (*Cinnamomum camphora*) and red bay (*Persea borbonia*) (all Lauraceae). Thus, in addition to avocado, ALB also poses a pest threat to ornamental plantings in urban areas.

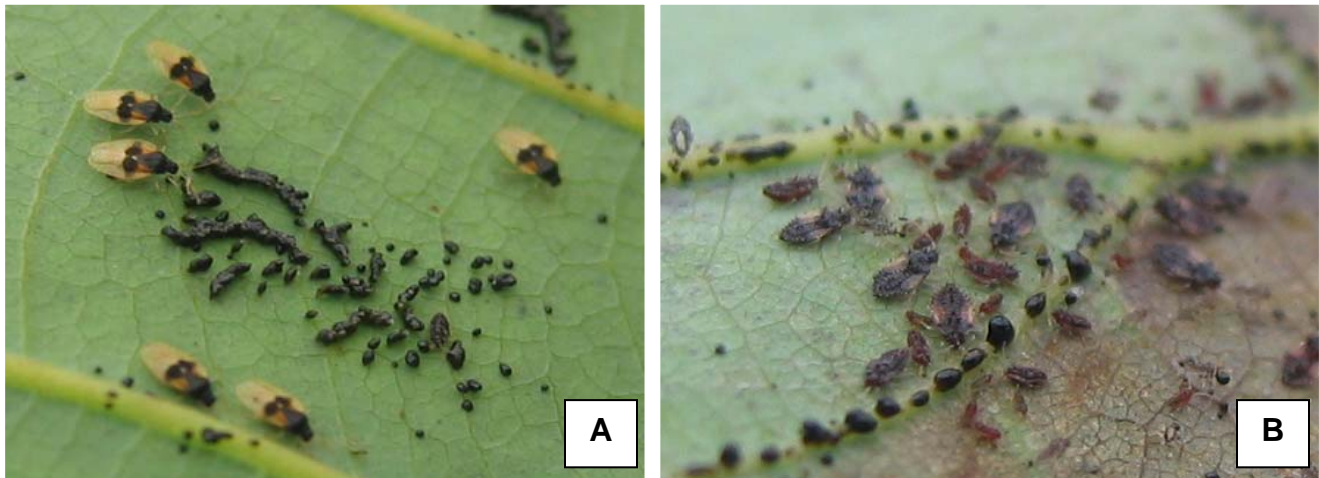


Figure 1. Avocado lace bug (A) adults and egg mass (B) nymphs.

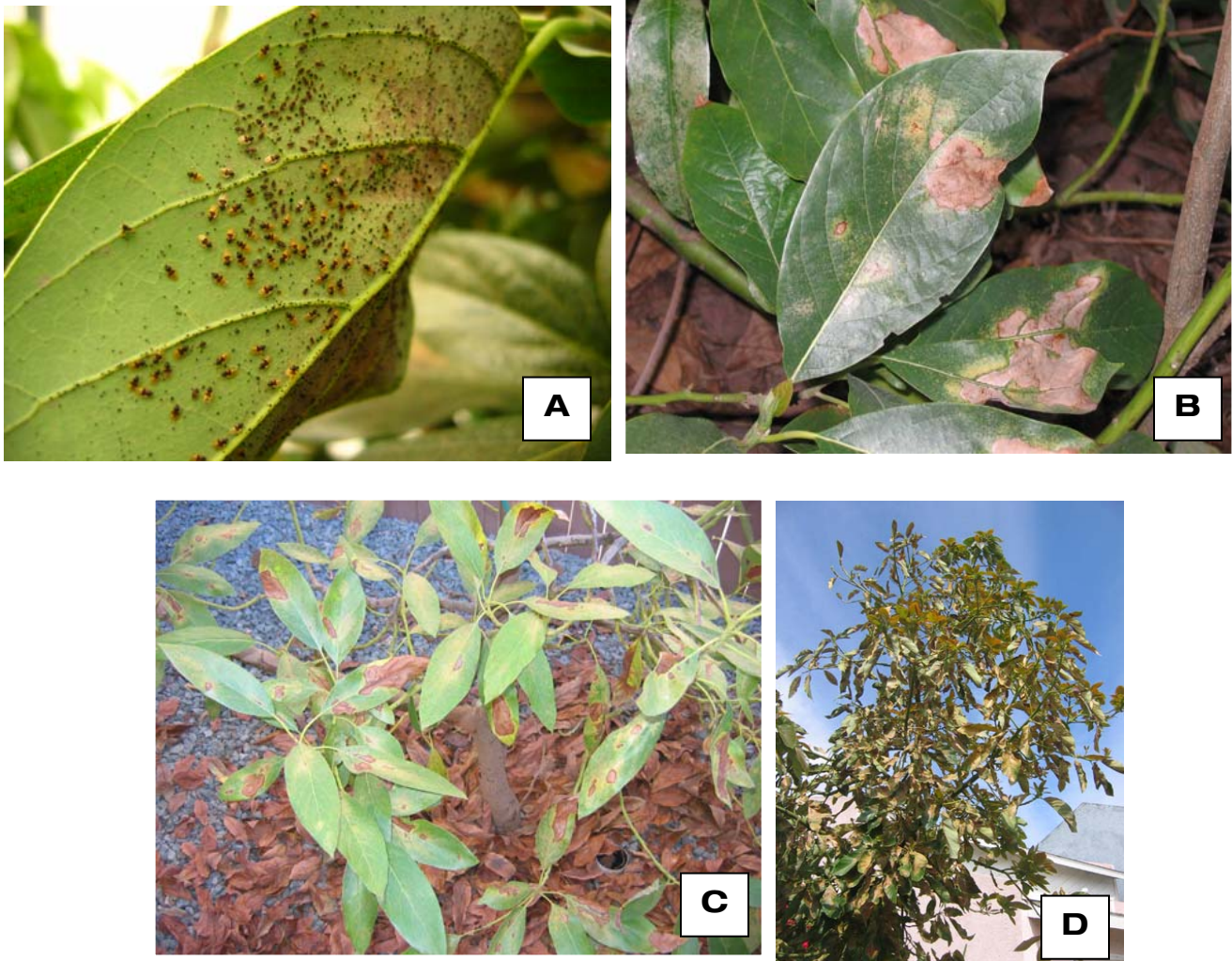


Figure 2. (A) Adults and nymphs feed in colonies on the undersides of leaves, (B) leaf damage, (C) intense leaf damage leads to defoliation, and (D) infested avocado trees have a severely scorched appearance.

This pest has now infested an area exceeding 250 square miles and only backyard grown avocados are infested. Surveys by the California Department of Food and Agriculture (CDFA) and County of San Diego Department of Agriculture, Weights & Measures (AWM) are monitoring the spread of the ALB in San Diego Co. to determine the distribution of this pest. The results of the ALB surveys in San Diego Co. (2004-05; 2006 spring and 2006 fall) show that the status of the ALB distribution has changed only slightly (Fig. 3). The ALB population remains mostly below the 8 freeway concentrated in the coastal area. However, during the ALB fall 2006 survey, two new sites were detected near the coastal region of La Jolla and San Diego and they are approximately 10 miles above the northernmost previous ALB site (Fig. 4). We have now included these two new sites in the ALB population monitoring in San Diego County as of February 2007.

ALB has not advanced to any commercial avocado groves in San Diego Co. but as observed previously for a number of exotic pests, invading populations can remain static for some time and then expand rapidly after the founding population adapts to the local environment.

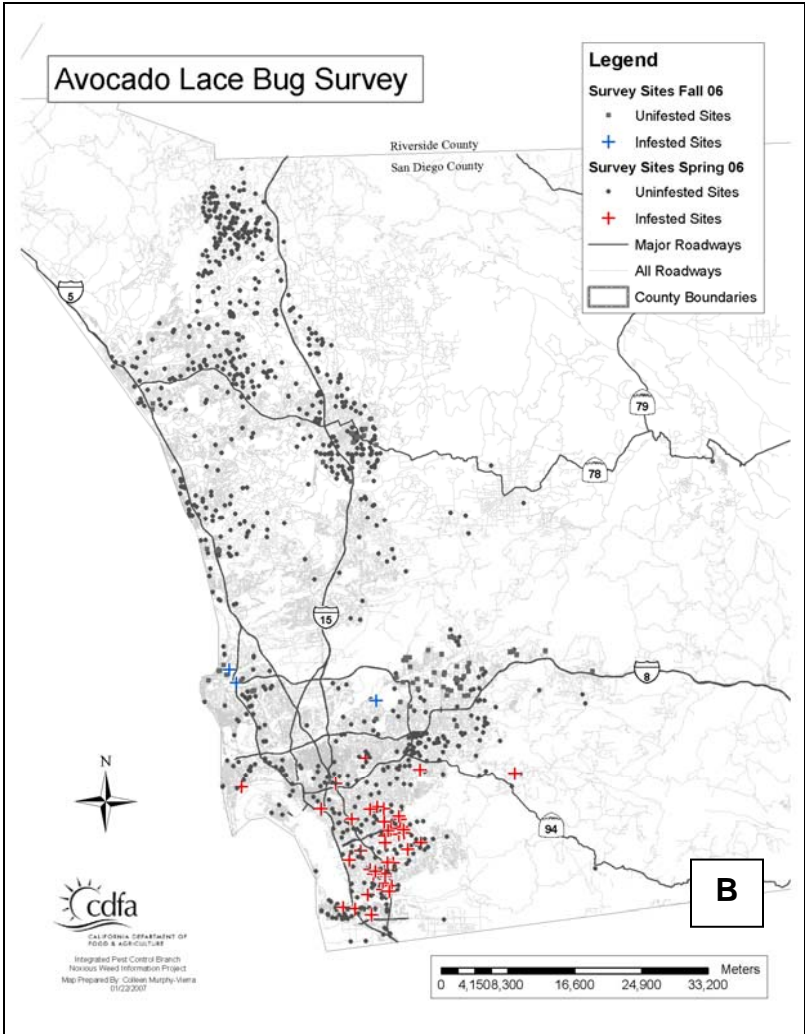
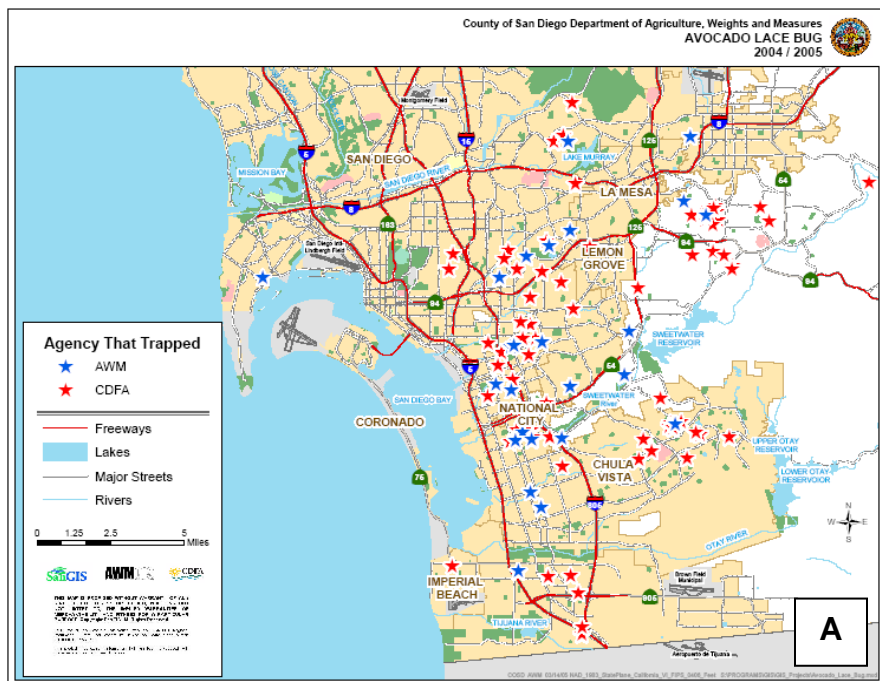


Figure 3. Avocado lace bug survey conducted by the County of San Diego Department of Agriculture, Weights & Measures (AWM) and the California Department of Food and Agriculture (CDFA) during (A) 2004-05 and (B) 2006 (spring and fall) to determine the distribution of the pest in the county.

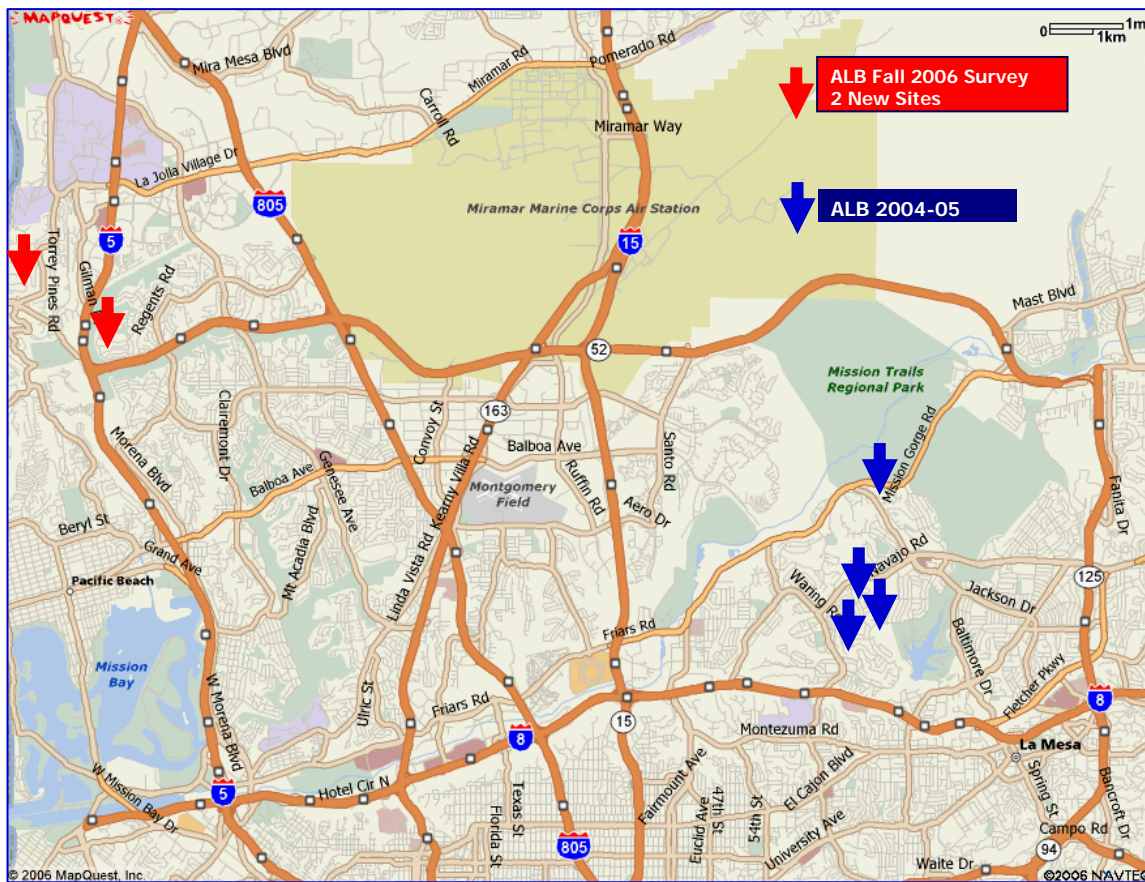


Figure 4. Two new ALB sites detected during the ALB fall 2006 survey, conducted by the County of San Diego Department of Agriculture, Weights & Measures (AWM) and the California Department of Food and Agriculture (CDFA). ALB was detected in the coastal region of La Jolla and San Diego approximately 10 miles above the northernmost populations found in the previous ALB surveys of 2004-05 in San Diego Co.

There are few published data on avocado lace bug biology, ecology, natural enemies, or chemical control. There is a paper from Florida that has been of some use but its application to California conditions is tenuous (Peña et al., 1998). More recently, a Ph.D. dissertation by Morales (2005) from Cuba studied bio-ecological aspects of ALB and control using an entomopathogenic fungus. ALB is a serious pest of 'Hass' avocados in The Dominican Republic and Puerto Rico. In 2001, Hoddle witnessed first-hand, massive outbreaks of ALB in commercial Hass orchards in the Dominican Republic while searching for avocado thrips natural enemies. Similar outbreaks, should they occur in California, would be devastating and would cost the avocado industry millions of dollars in pesticide applications and reduced crop yields. Additional chemical use for avocado lace bug control would also increase the likelihood of secondary pest flare ups (especially problematic are induced outbreaks of several mite and lepidopteran species).

The objectives of the ALB research program are to screen commercially available natural enemies and insecticides for control of this pest in residential areas and commercial avocado orchards; acquire basic information on the phenology of ALB in California; conduct foreign exploration for ALB natural enemies in the home range of the pest; and genotype the ALB population in California and populations collected throughout the home range of ALB to determine exactly where the invading population in California came from and confirm species identity of the California population.

1. Pesticide Trials:

In the pesticide screening research we observed the **residual impact** (foliar pesticides applied to avocado seedlings and left exposed to outside weather conditions and sampling leaves periodically post-treatment to evaluate ALB mortality) and/or **contact effect** (pesticides applied directly on leaves holding ALB nymphs) on ALB. Our goal was to obtain a list of effective pesticides for potential use by homeowners, avocado growers, as well as for the County of San Diego Department of Agriculture, Weights & Measures (AWM) and the California Department of Food and Agriculture (CDFA) if necessary for eradication of small highly localized populations of ALB detected outside of San Diego Co.

1.1. *Residual impact of insecticides on ALB:*

The residual effect of six insecticides on ALB nymphs 3 to 112 days after treatment is shown in Fig. 5. Insecticides tested included carbaryl (GardenTech - Sevin Concentrate Bug Killer), soil-applied imidacloprid (BAYER Advance Garden - Tree & Shrubs Insect Control Concentrate), spinosad (Success 2SC), abamectin (Agri-Mek 0.15 EC), petroleum oil (415 spray oil), and fenpropathrin (Danitol 2.4 EC Spray).

The insecticides carbaryl, imidacloprid, and fenpropathrin were the strongest treatments of those evaluated to control ALB nymphs. Imidacloprid mortality of ALB increased from 23 to 75 to 97.1 to 100% as the soil-applied material was taken up by potted trees. This treatment remained effective through 112 days post-treatment. The best foliar treatment was the insecticide carbaryl with 100% ALB mortality in all bioassays (3 to 112 days after treatment). The second best foliar treatment was fenpropathrin with 100% ALB mortality up to 77 days post-treatment and lower effectiveness was observed at 112 days (36.6%). Spinosad and abamectin plus oil were eliminated as candidate treatments because ALB mortality dropped below 20% in the first two bioassays (3 and 7 days after treatment). It is important to mention that two pesticides widely used in commercial avocado groves in California (abamectin and spinosad) have low efficacy against ALB.

1.2. *Contact impact of insecticides on ALB:*

The contact effect of the six insecticides on ALB nymphs 72 h after treatment is shown in Fig. 6. The insecticides tested were pyrethrins (PyGanic® Crop Protection EC), pyrethrins + potash soap (SAFER BRAND Yard & Garden Insect Killer II Concentrate), pyrethrins + rotenone (Pyrellin E.C.), extract of neem oil (Green Light Neem Concentrate), petroleum oil (415 spray oil), and potash soap (M-Pede Insecticide Fungicide).

Petroleum oil had no effect as a residual pesticide as shown in our previous residual effect bioassay so we decided to evaluate its contact impact. The pyrethrins were the best contact treatment of those evaluated to control ALB. The two other pyrethrin treatments (i.e. pyrethrins mixed with potash soap or rotenone) were not as effective as using the pyrethrins alone. Petroleum oil and potash soap tied as the second most effective treatments. Petroleum oil had no residual impact, but was effective as a contact insecticide. Neem oil had little impact on ALB nymphs.

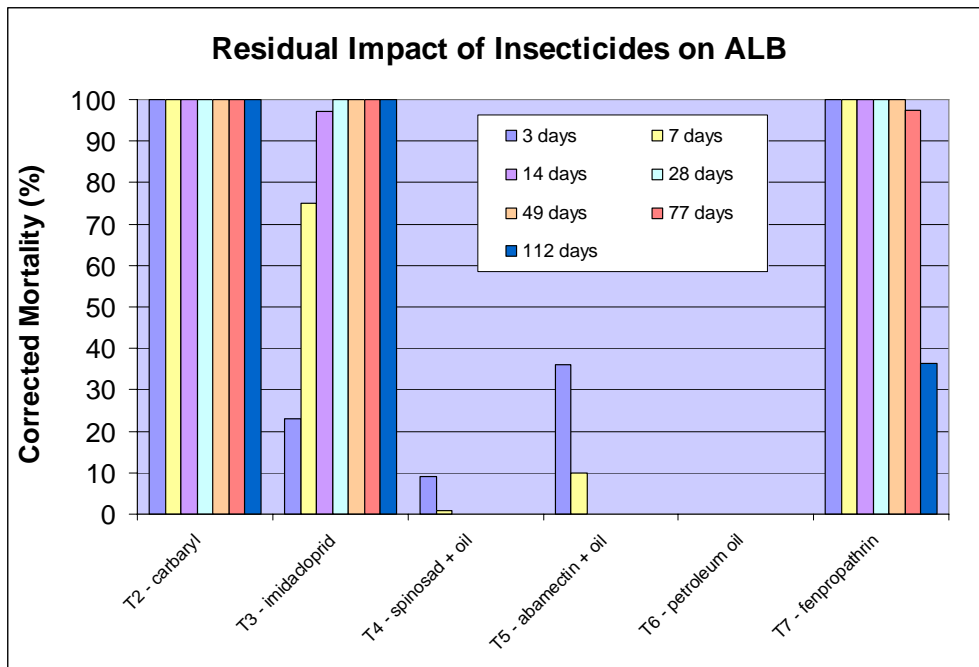


Figure 5. Residual impact of insecticides on ALB nymphs with mortality evaluations between 3 and 112 days after treatment (pesticide treatment evaluations were dropped for insecticides which showed <20% ALB mortality after two consecutive evaluations).

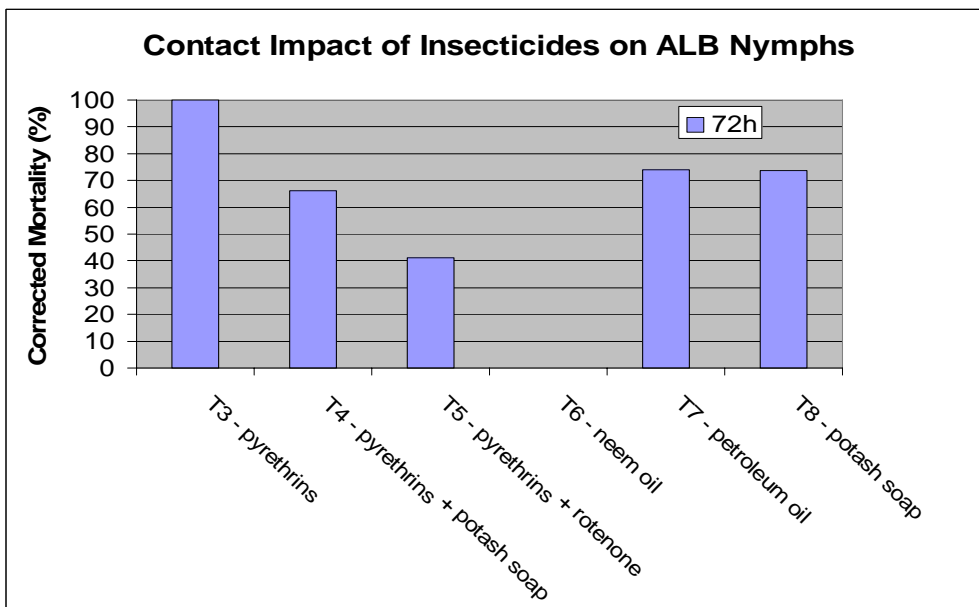


Figure 6. Contact impact of insecticides on ALB nymphs 72 h after treatment.

2. Systemic Pesticide Bioassays:

2.1. Systemic insecticide imidacloprid effect on ALB:

Quantification of systemic levels within xylem fluid and leaves was conducted by Dr. Frank Byrne. These data showed that imidacloprid was effective against the ALB in bioassays conducted with both potted and commercial trees. We have shown that it is possible to use systemically-applied imidacloprid to achieve adequate levels of toxicant within the tree and that this will give excellent control of ALB. These data

supported the 24c registration of Admire Pro® in 2006. Evaluations of imidacloprid uptake and efficacy against ALB are ongoing.

3. ALB Population Monitoring:

3.1. ALB sampling sites:

Determining the seasonal phenology of ALB (nymphs & adults) in different environments in San Diego Co. is important in order to determine ALB activity in areas with differing climatic conditions over the season. The six ALB sampling sites are located along the coastal region of San Diego Co. To date, no ALB infested sites have been found further inland, as shown in Fig. 7.

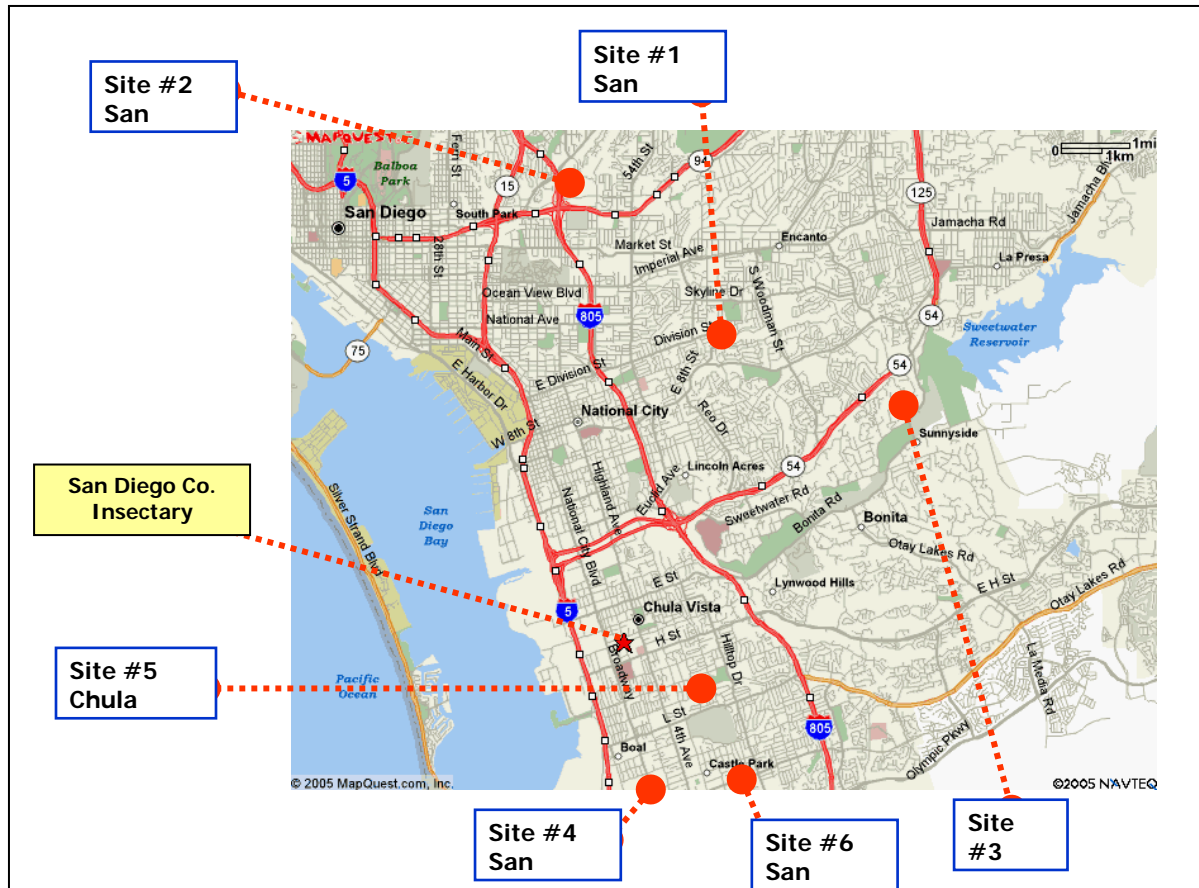


Figure 7. Map of ALB sampling sites in San Diego Co.

Seasonal ALB phenology is shown in Fig. 8. After 19 months of ALB sampling, the highest densities of adult ALB in San Diego Co. were reached between the months of August – December with the peak occurring in October. The peak was much higher during 2007 compared with 2006. Despite the freezing temperatures observed during the month of February 2007, which severely affected the avocado industry, ALB remained present in almost all sites after the freeze. This may suggest that the colonization phase of ALB at monitored sites is over, and the exponential phase of the invasion is about to begin. Continued monitoring will be needed to verify this.

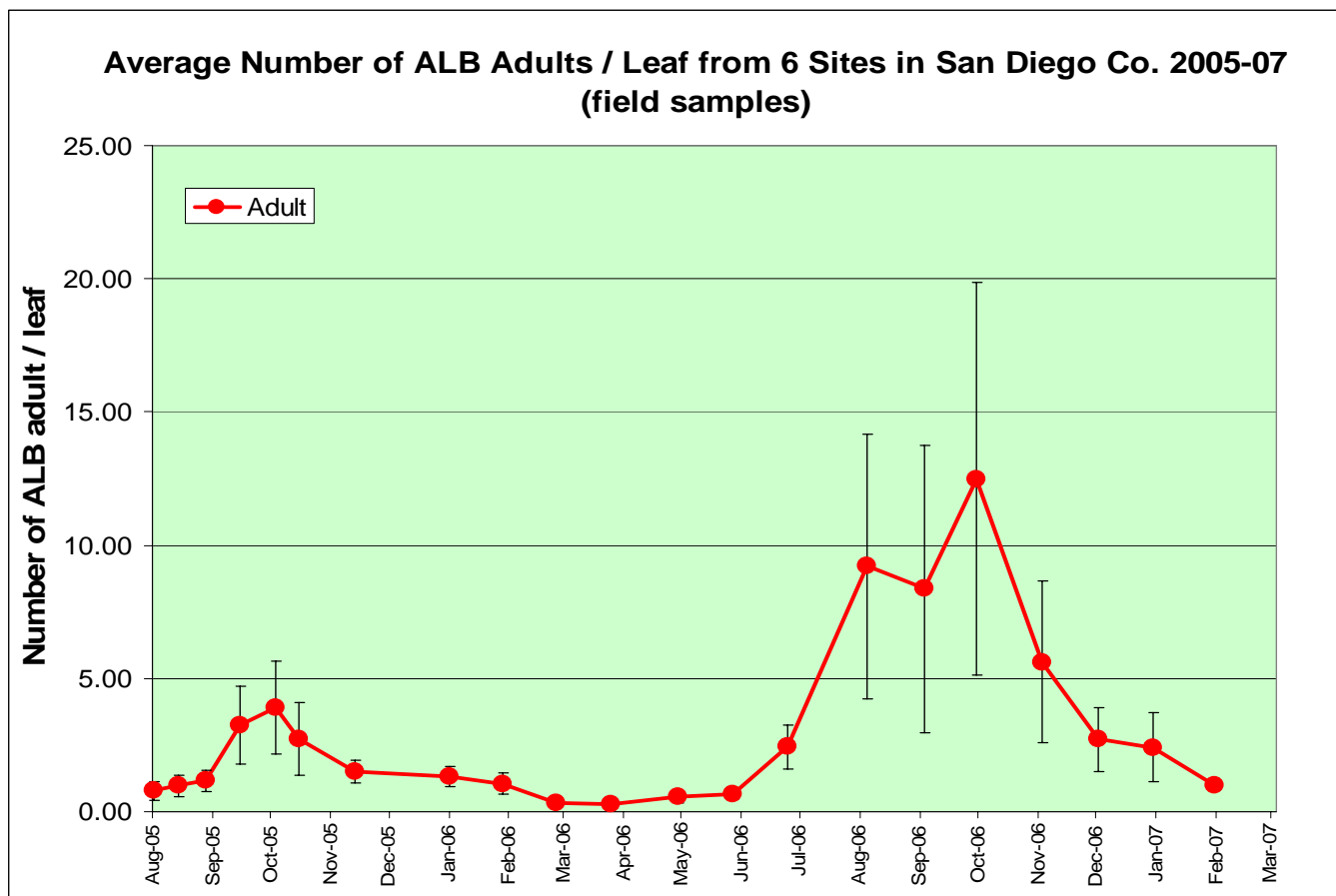


Figure 8. Seasonal ALB phenology in San Diego Co.

3.2. Determination of the scion variety of the avocado trees ALB sampling sites:

The majority of the trees at the ALB sampling sites are unknown avocado varieties that were grown by germinating the seeds of a commercial avocado variety. Tree variety identification can help with interpreting differences in ALB seasonal phenology across sites that may result because of different avocado varieties. Young leaves from spring flush were collected from each ALB sampling site and taken to the Dr. Richard Stouthamer's lab (Department of Entomology, UCR) for DNA extraction. Extracted and purified DNA was sent to Dr. Michael T. Clegg at UC Irvine (Biological Sciences, Ecology & Evolutionary Biology, School of Biological Sciences) where further genetics tests were performed to determine the variety of the avocado trees. The results from the manual and computer analyses were similar and concluded that two sites had Bacon and Fuerte varieties, while the remaining sites appear to be a complex hybrid of several varieties such as Hass, Bacon, and Zutano.

4. Natural Enemy Studies:

4.1. Testing natural enemies as potential augmentative control agents for ALB:

We have tested three natural enemies in a closed arena environment (Munger cells) as potential augmentative control agents for ALB. Selected natural enemies for evaluation are those frequently found or used in southern California avocado groves:

Franklinothrips orizabensis (a predatory thrips), *Chrysoperla rufilabris* (predatory green lacewing larvae), and *Neoseiulus californicus* (a predaceous mite).

Adult female *Franklinothrips* preyed mostly on small nymphs (60% mortality) compared with only 6% and 0% mortality on medium instar and adult ALB, respectively (Fig. 9.). Given that a substantial fraction of the ALB population would escape predation (i.e. anything larger than a small nymph), we don't consider the results with *Franklinothrips* to be promising.

Second instar larvae of *Chrysoperla rufilabris* efficiently preyed on all ALB stages tested (Fig. 10). The highest mortality was medium sized ALB nymphs (96%), followed by adults (71.4%), and small nymphs (60%). The promising results led us to test the efficacy of second instar larvae of *C. rufilabris* to control ALB nymphs on potted cloned avocado seedlings under greenhouse conditions. The results indicated that second instar lacewing larvae preyed on ALB nymphs under greenhouse conditions causing 13.4% mortality in contrast with 3.7% natural mortality in the control treatment (Fig. 11). In a closed arena environment (Munger cells), the efficacy of lacewing larvae preying on ALB nymphs was significantly higher than in an open environment (greenhouse). Closed access to the prey was a significant difference between both bioassays and the area to be searched by predators most likely affected recorded mortality rates. Different lacewing densities per avocado seedling should be tested in order to see if predator efficacy increases.

Adult female *N. californicus* did not effectively feed on small ALB stages (Fig. 12). A behavioral study was performed to investigate *N. californicus* predation on ALB eggs. The behavioral categories recorded were: **exploring egg mass** (making physical contact with the egg mass); **feeding on eggs**; **resting**; **grooming** (cleaning body parts); **exploring the arena** (individual is motile presumably searching for prey). The predatory behavior of *N. californicus* towards ALB egg stage is shown in Fig 13. The results indicate that 46.9% of the time, the predatory female mite explores the arena, followed by resting (39.4%) and grooming (11.3%). The contact of the predatory mite with the egg was minimal, only 2.5% of observations indicated that females examined ALB eggs, and 0% feeding events were observed on ALB eggs.

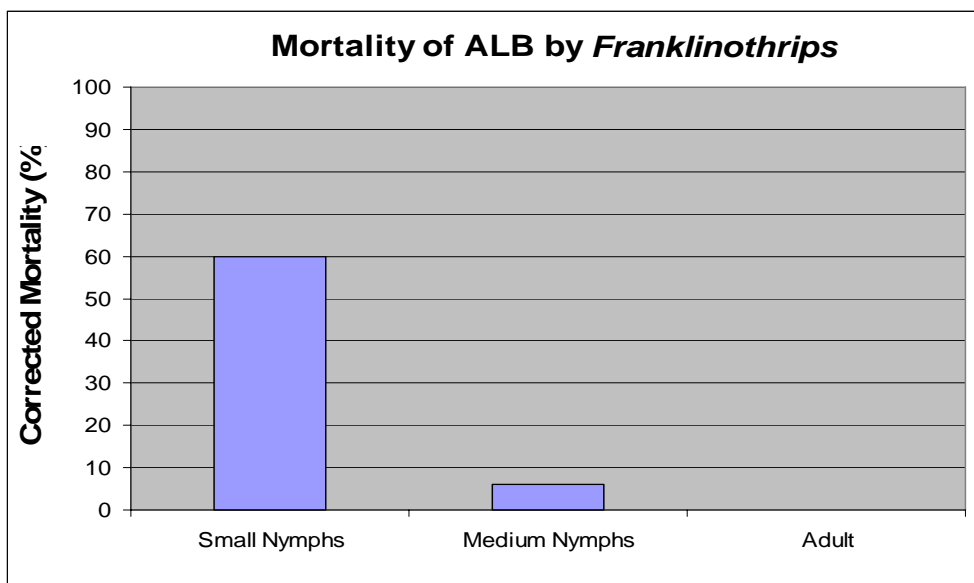


Figure 9. Munger cell bioassay evaluating adult *Franklinothrips* predation on three stages of ALB.

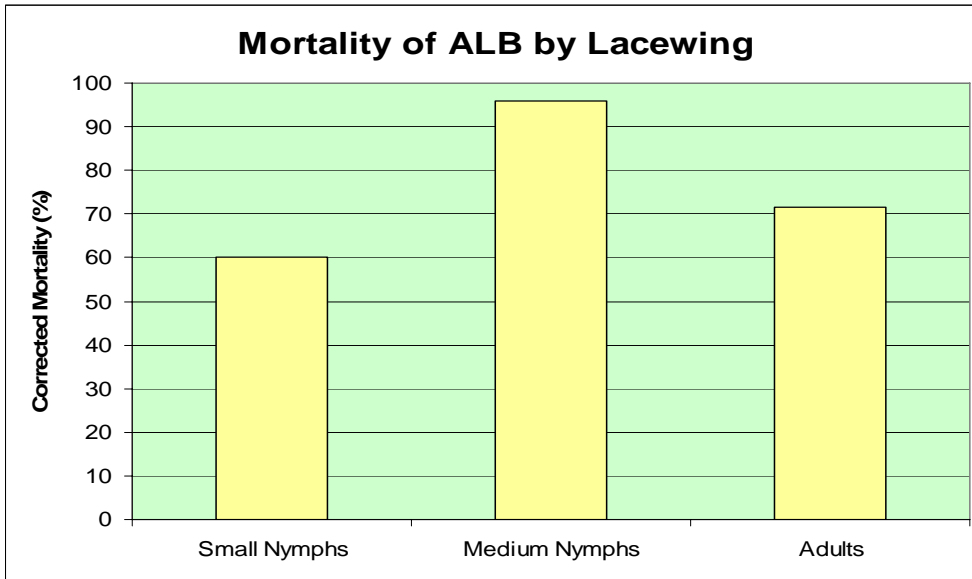


Figure 10. Munger cell bioassay with second instar larvae of *C. rufilabris* preying on three stages of ALB.

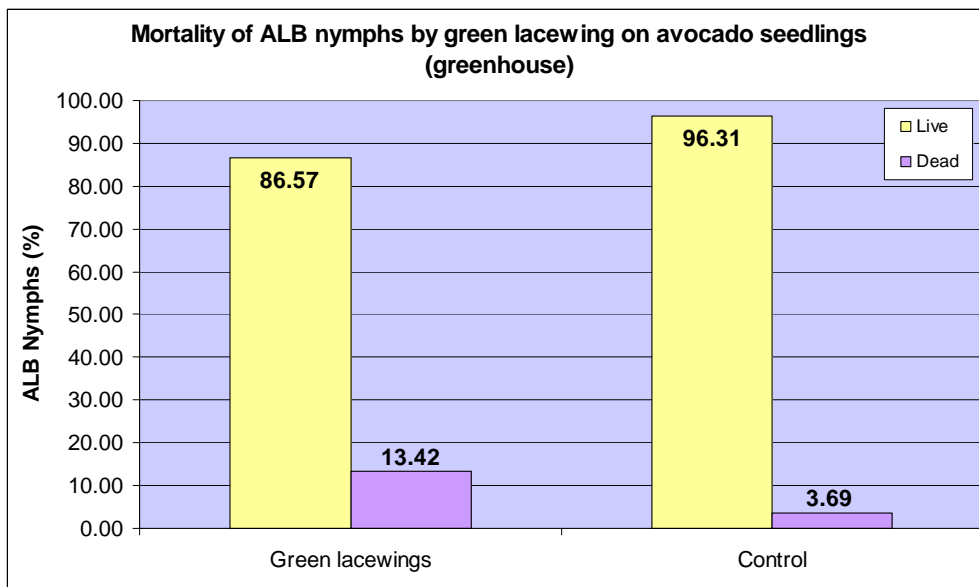


Figure 11. Mortality of ALB nymphs by second instar larvae of *C. rufilabris* on potted avocado seedlings under greenhouse conditions.

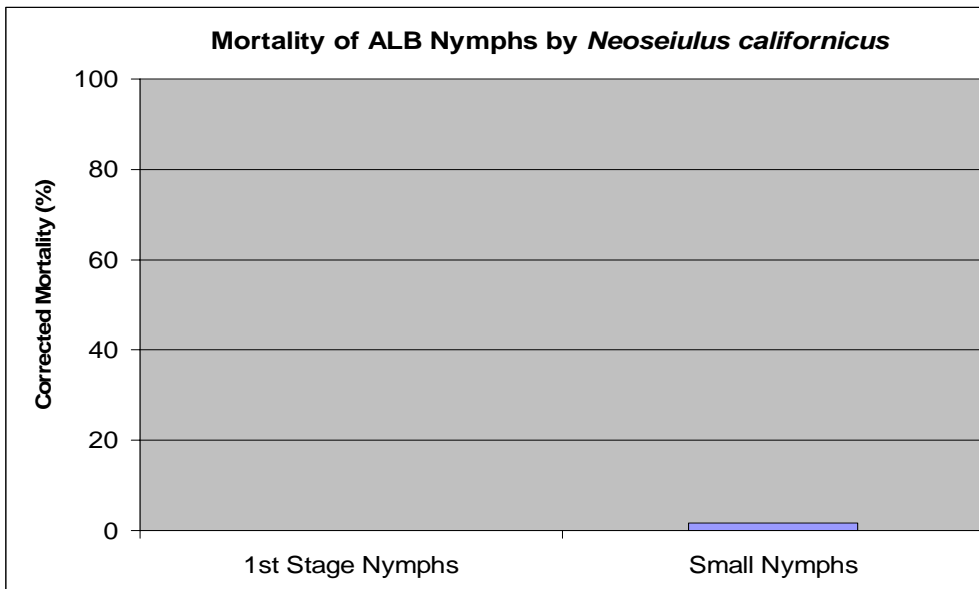


Figure 12. Munger cell bioassay for adult female *N. californicus* on two ALB life stages.

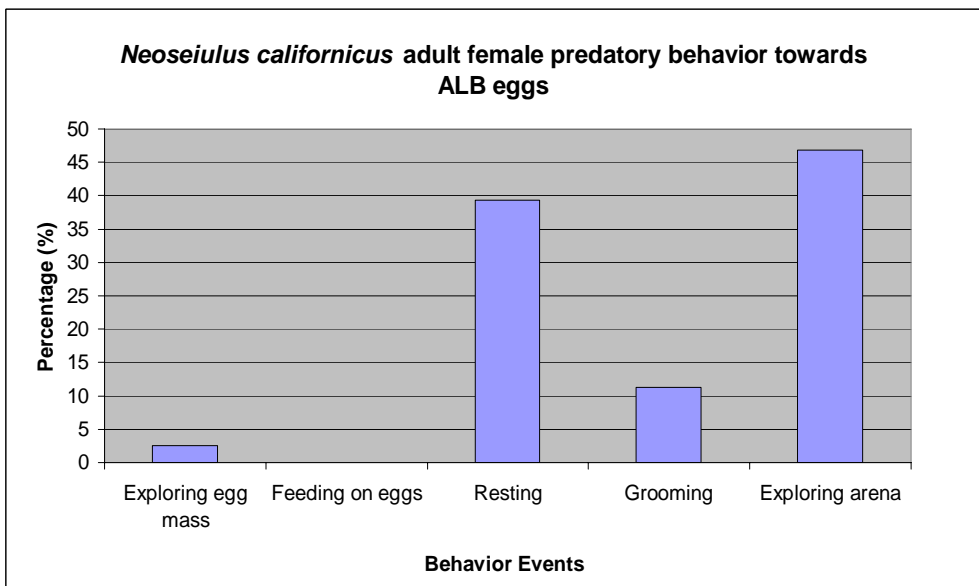


Figure 13. Adult female *N. californicus* behaviors recorded in Munger cells with ALB eggs.

4.2. Susceptibility of *Chrysoperla rufilabris* larvae to imidacloprid:

We were interested in seeing if treatment of avocado trees with imidacloprid (*Admire Pro*®) would affect mortality rates of the lacewing predator, *C. rufilabris*. Avocado leaves were collected from an experimental site in Temecula where *Admire* had been injected into the irrigation water. The results of the bioassay are shown in Fig. 14. What is important to observe is that there was a significant difference between treatments 2 and 4, where second lacewings larvae preyed on ALB nymphs feeding on the imidacloprid treated leaf compared with the control treatment where ALB nymphs were not feeding on leaves treated with imidacloprid. These results suggest that avocado trees treated with imidacloprid can adversely affect lacewing mortality because of food chain impacts, i.e., due to lacewing larvae acquiring a lethal dose of imidicloprid by feeding on intoxicated ALB nymphs.

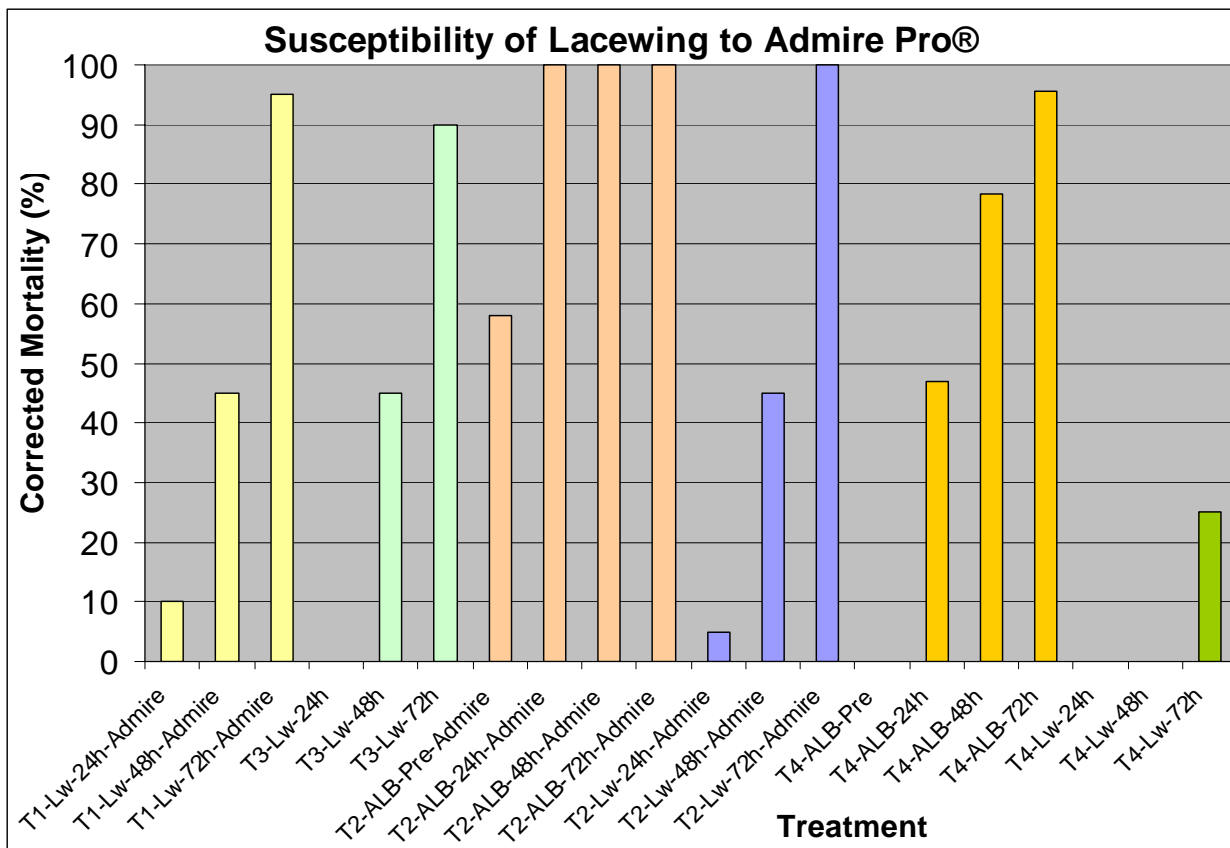


Figure 14. Susceptibility of *Chrysoperla rufilabris* to imidacloprid. (LW – lacewing; ALB – avocado lace bug)

Treatment 1: second instar lacewing larvae placed on a leaf treated with Admire with no ALB.

Treatment 2: second instar lacewing larvae placed on a leaf treated with Admire with ALB nymphs feeding on the Admire leaf.

Treatment 3: second instar lacewing larvae placed on a leaf not treated with Admire with no ALB.

Treatment 4: second instar lacewing larvae placed on a leaf not treated with Admire with ALB nymphs feeding on the leaf.

5. Foreign Exploration:

Foreign exploration for ALB was initiated in March 2006 and a 4 week expedition located ALB in Jamaica, Puerto Rico, St. Thomas, St. John, Veracruz, and Yucatan. ALB was not found in Barbados, Trinidad, or Tobago. Specimens have been received from Texas and material has been received for analyses from St. Kitts and French Guyana. Material from Cuba has been requested. In countries where ALB was found, thousands of ALB eggs on avocado leaves were shipped to the UC Riverside Insectary and Quarantine facility. No parasitoids emerged from shipped material. This may have occurred because prospecting was conducted at the wrong time of year. Because ALB nymphs successfully hatched from eggs in quarantine, shipping was not considered a problem. Adult ALB collected during foreign exploration was used for the genotyping project. No foreign exploration has been conducted in 2007 because of budget cuts due to the freeze.

6. Genotyping ALB:

DNA extracted from the San Diego ALB population 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 have been developed to amplify these microsatellites, which will be tested against the DNA of a number of different individual ALB. 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 parasitoids with the correct pest species, 2) If we try to use the microsatellites on two different species, our analysis will be confounded.

So far we have looked at 14 ALB populations: Chula Vista, San Diego Co., California, USA; Weslaco, Hidalgo Co., Texas, USA; Dade Co., Florida, USA; Oxnacab, Yucatan, Mexico; Hunucma, Yucatan, Mexico; Merida, Yucatan, Mexico; Veracruz, Mexico; Santo Domingo, Dominican Republic; Jamaica; Puerto Rico; St. Thomas & St. John; St. Lucia; St. Kitts, and French Guyana

In terms of the variation in CO1 sequences, there are three different genotypes detected in the CO1 region. Except for the population from Veracruz, Mexico, all other populations are monotypic at the mitochondrial region. The California population shares the same CO1 sequences with that from Weslaco, Texas and shares part of the sequence from the Veracruz, Mexico collection. The sequence of the Florida population is the same as that of ALB from the Caribbean islands. The data strongly suggest that the populations that have settled in the U.S. (i.e. FL vs. TX and CA) have different origins.

Five out of 24 microsatellite markers showed variation between the California, Weslaco, and Veracruz populations. There was also population variation within the California individuals. On the other hand, there was no variation detected within Weslaco and Veracruz populations.

7. Summary of results:

To date, we have accomplished the following.

- Established an ALB colony for our research project at the Chula Vista facility.
- Screened pesticides for control of ALB, indicating that the home-garden insecticides carbaryl and imidacloprid are potentially useful insecticides for homeowners. On the other hand, pyrethrins, petroleum oil, potash soap, and fenpropathrin were the most effective agriculture pesticides among the pesticides tested. Further evaluations under more realistic field conditions are needed before final recommendations can be made with confidence.
- Our trial data for ALB supported the 24c registration of Admire Pro® in 2006. Further studies are needed to develop other chemically-oriented control options.
- We are continuing to monitor the seasonal phenology of ALB in San Diego Co. and we expect to gather data for at least two full seasons. So far the highest ALB densities were observed between August and December.
- Among the natural enemies tested as potential augmentative control agents for ALB, *Franklinothrips orizabensis* (a predatory thrips), *Neoseiulus californicus* (a predaceous mite) and *Chrysoperla rufilabris* (predatory green

lacewing larvae), only the lacewing larvae showed promise. This natural enemy efficiently preyed on all ALB stages tested in the lab and greenhouse.

- Treatment of avocado trees with imidacloprid (Admire Pro®) can adversely affect lacewing mortality because of food chain impacts that result from lacewing larvae acquiring a lethal dose of imidacloprid after on intoxicated ALB nymphs.

8. Future Research Plans:

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

- Further test selected pesticides for ALB control.
- Test green lacewing susceptibility to other promising pesticides for control of ALB.
- Continue ALB biology studies on Hass and Bacon leaves under different temperatures
- Continue endemic egg parasitoid surveys at selected survey sites.
- Analyze ALB sampling data to determine an optimal field sample size.
- Select a reliable method to preserve avocado leaves in the rearing room for the natural enemy survey.
- Continue ALB biology studies testing different temperatures, humidities, and avocado cultivars and develop a day-degree model for use in CLIMEX.
- Continue foreign exploration for ALB for genotyping and importation of associated parasitoids from Florida and Mexico.

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