

Biology and Management of Avocado Lace Bug in California

Year 1 of 3

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Benefit to the Industry

As fast as possible, we hope to be able to suggest sustainable solutions for Avocado Lace Bug (ALB) management that are derived from sound scientific research. One emphasis is chemical control. A second emphasis is biological control based strategies that could assist in controlling ALB field populations, thereby reducing source populations that might spread into uninfested areas. There is currently no active research on ALB in the U. S. other than ours and activities overseas are limited.

Summary of Results to Date

1. Pesticide Trials:

1.1. Residual impact of insecticides on ALB:

The residual effect of six insecticides was screened against ALB. Insecticides tested included carbaryl, soil-applied imidacloprid, spinosad, abamectin, petroleum oil, and fenprothrin. Foliar pesticides were applied using a hand sprayer to apply ca. 1 gallon of spray to 10 avocado seedlings per treatment. The avocado seedlings were left exposed to outside weather conditions, leaves were sampled periodically, and bioassays were conducted using Munger cells. Mortality was evaluated 3, 7, 14, 28, 49, 77, and 112 days post-treatment. Pesticide treatment evaluations were dropped for insecticides which showed <20% of ALB corrected mortality after two consecutive evaluations.

Results: The residual effect of the six insecticides on ALB nymphs 3 to 112 days after treatment is shown in Fig. 1. The insecticides carbaryl, imidacloprid, and fenprothrin were the strongest treatments of those evaluated to control ALB nymphs at 112 days post-treatment. Imidacloprid mortality of ALB increased from 23 to 75 to 97.1 to 100% as the soil-applied material was taken up by the potted tree. This treatment remained effective through 112 days after 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 until 77 days after treatment and lower effectiveness 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).

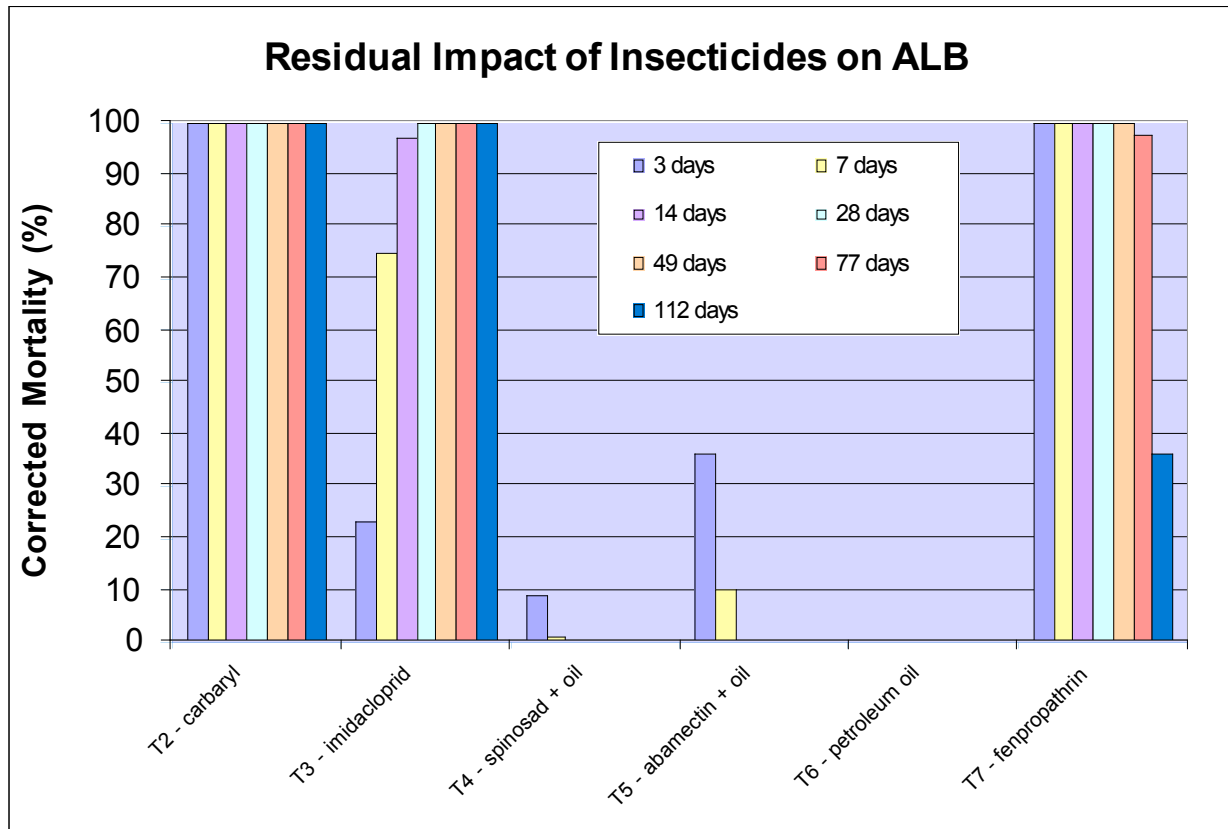


Fig. 1. Residual impact of insecticides on ALB nymphs with mortality evaluations between 3 and 112 days after treatment.

1.2. Contact impact of insecticides on ALB:

The contact impact of six insecticides was screened against ALB. The insecticides were pyrethrins, pyrethrins + potash soap, pyrethrins + rotenone, extract of neem oil, petroleum oil, and potash soap. 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 pesticides were applied using a small plastic hand sprayer to spray 10 avocado leaves holding ALB nymphs per treatment. Mortality was evaluated 72 hours later.

Results: The contact effect of the six insecticides on ALB nymphs 72 h after treatment is shown in Fig. 2. 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 effect, but was effective as a contact insecticide. Neem oil had little impact on ALB nymphs.

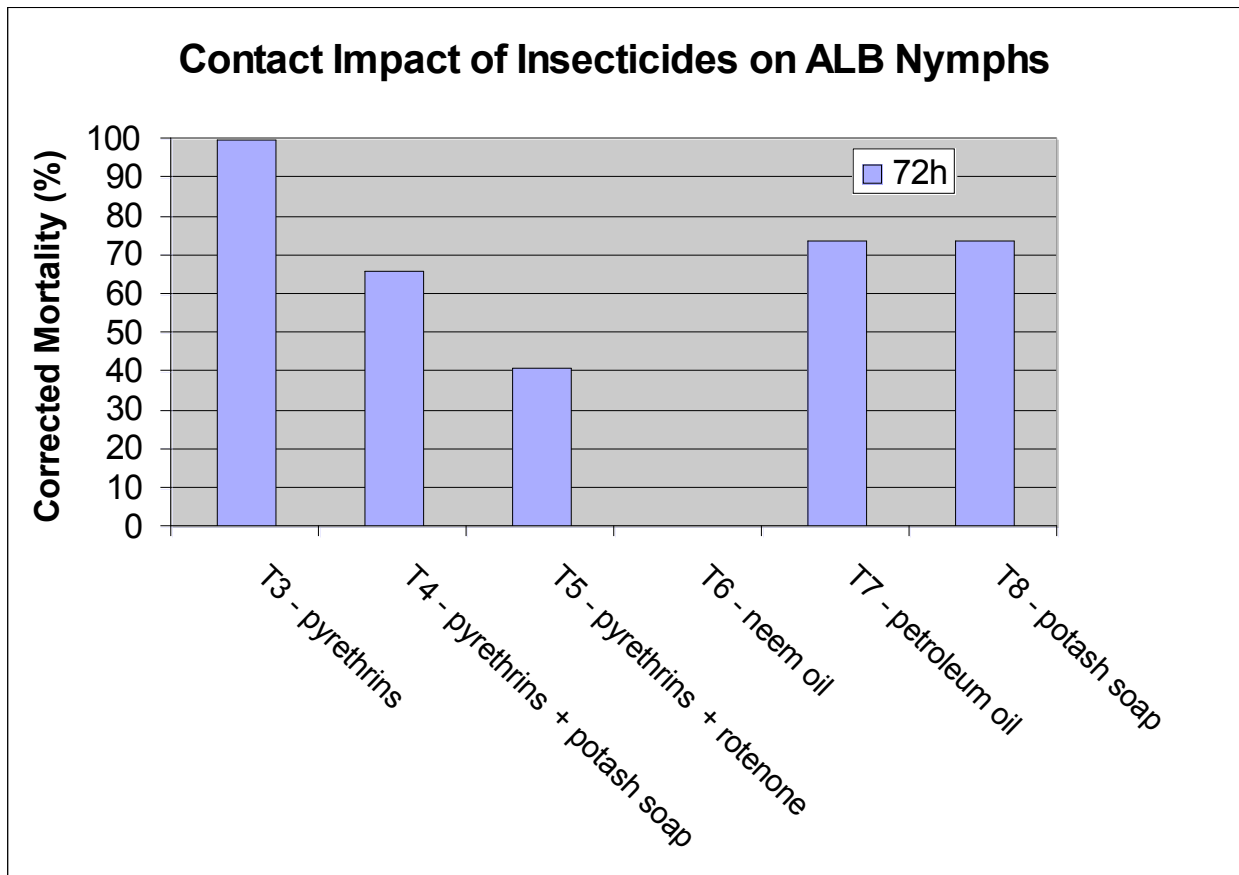


Fig. 2. Contact impact of insecticides on ALB nymphs 72 h after treatment.

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 (see the systemic pesticide report in this issue by Morse, Byrne, and Toscano) showed that Admire is 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.

3. ALB Population Monitoring:

3.1. ALB sampling sites:

ALB sampling sites were selected based upon high levels of ALB infestation, moderate to large avocado tree size, and so as to achieve some diversity in sample locations. No ALB infested sites were found further inland, therefore the six sites are located along the coastal region of San Diego Co. as shown in Fig. 3. Site #6 had to be replaced because the major sample tree was cut down within the trailer park where it was located. At each site, two sampling methods are used, a field sample and a lab sample. The field sample consists of on-site evaluations of the number of ALB adults present on each of 25-50 leaves around the lower portion of the avocado tree. For the

lab sample, we randomly collect 25-50 avocado leaves around the lower portion of the avocado tree and place them in plastic ziploc bags, which are stored inside a cooler. The number of ALB nymphs, adults, and the presence of ALB leaf damage were assessed on each leaf using a microscope in the lab. Our objective is to compare field vs. lab sampling procedures in order to compare the two methods. Both samples have been taken once per month at each site continuously since August 2005.

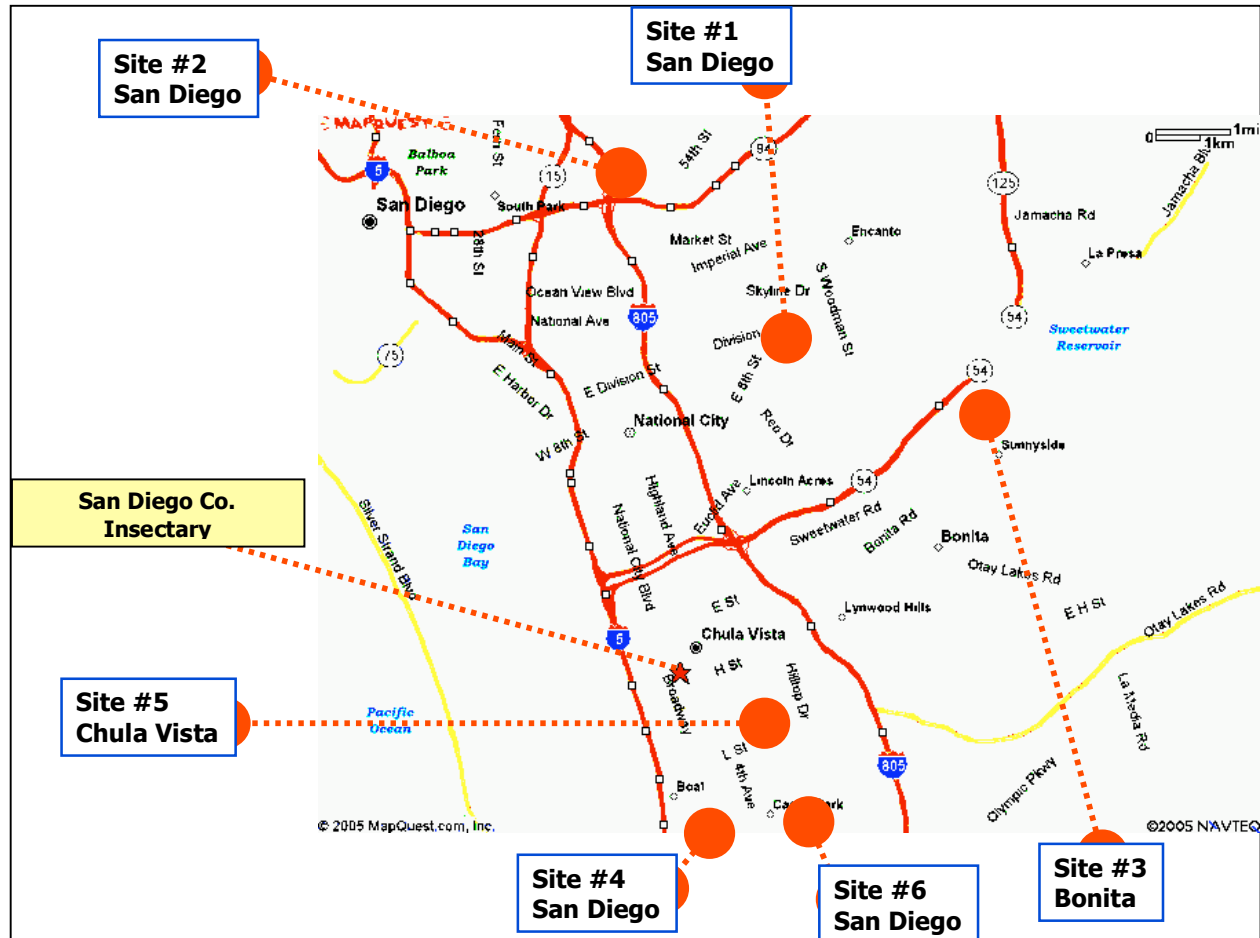


Fig. 3. Map of ALB sampling sites in San Diego Co.

Sampling Results: Determining the seasonal phenology of ALB (nymphs & adults) in different environments in San Diego Co. is important in order to determine ALB activity over the season. Seasonal ALB phenology is shown in Fig. 4. After a year 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.

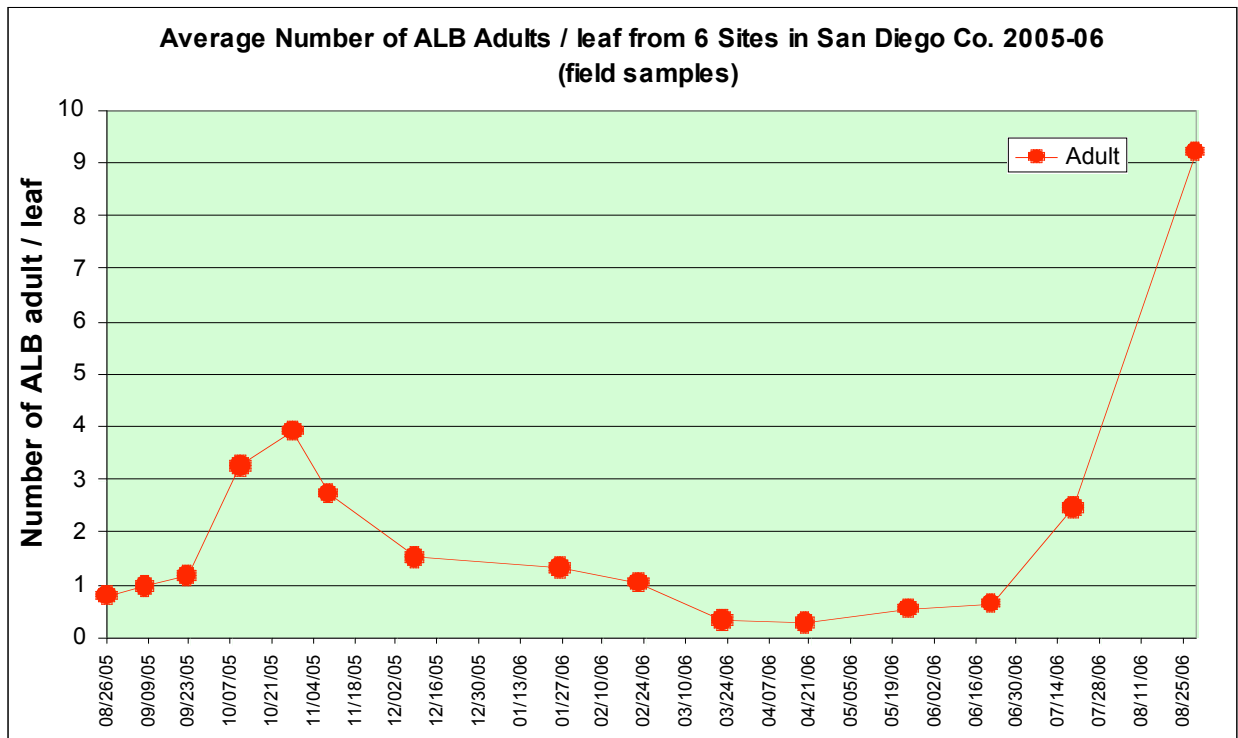


Fig. 4. Seasonal ALB phenology in San Diego Co.

4. Natural Enemy Studies:

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

4.1.1. *Franklinothrips orizabensis* - thrips predator:

Pupal *F. orizabensis* (Buena Biosystems, Ventura, CA) were reared on irradiated *Ephestia kuehniella* (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 an avocado leaf held within a Munger cell. 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 individually transferred into new Munger cells 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. A total of six treatments and 10 cells (replicates) per treatment were used for this study. A ratio of one adult female *Franklinothrips* per five ALB per cell was the predator/prey ratio used in each cell (1 adult *Franklinothrips* with 5 nymphal or adult ALB).

Results: 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. 5). 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 to be promising.

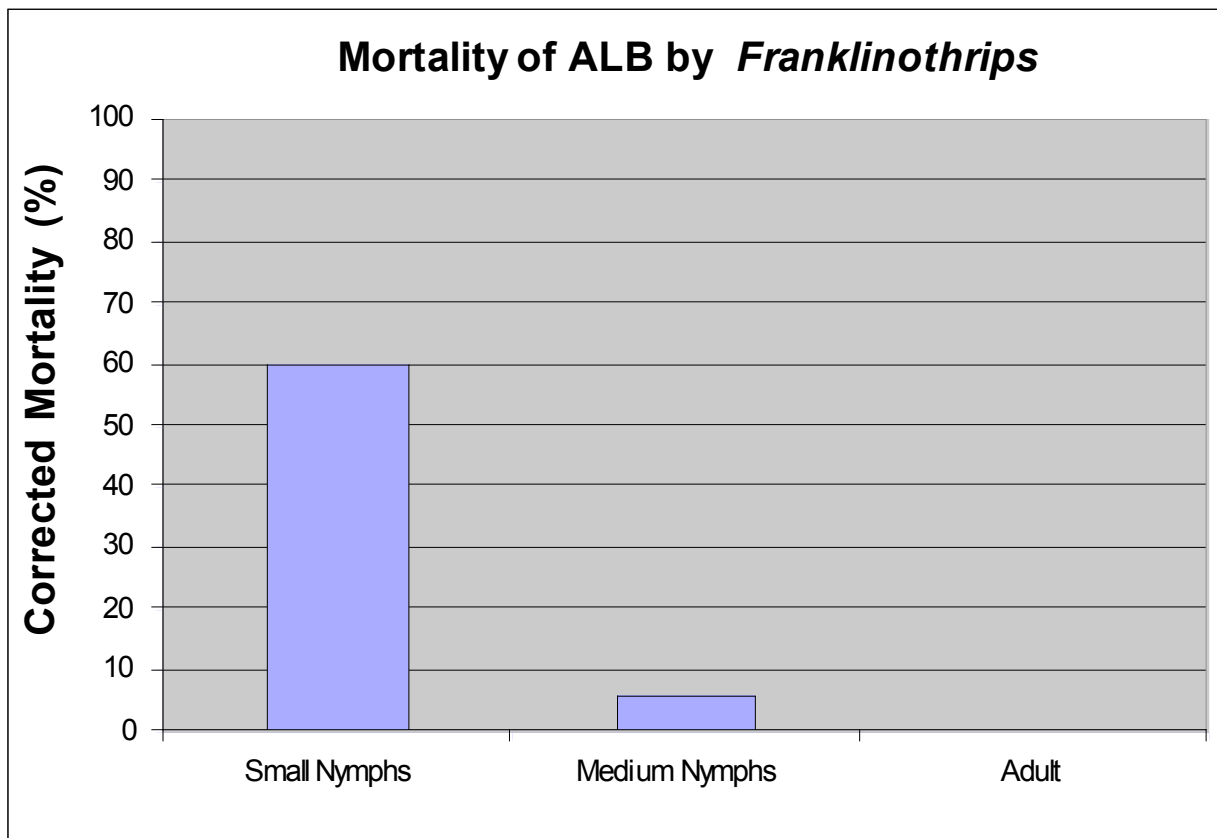


Fig 5. Munger cell bioassay evaluating adult *Franklinothrips* predation on three stages of ALB.

4.1.2. Chrysoperla rufilabris - lacewing:

Second instar larvae of *C. rufilabris* purchased from a commercial insectary (Buena Biosystems, Ventura, CA) were used in these studies. The same ALB nymph age criteria (small and medium size nymphs and adults) and experimental set up as used in the *Franklinothrips orizabensis* bioassays were used. A ratio of one second instar *C. rufilabris* larva per five ALB per experimental cell was evaluated. Lacewing larvae were starved prior to the bioassay for at least 24 hours prior to exposure to the ALB nymphs and adults. ALB mortality was assessed 24 hours after prey were exposed to lacewings.

Results: Second instar larvae of *Chrysoperla rufilabris* efficiently preyed on all ALB stages tested (Fig. 6). The highest mortality was on the ALB medium size nymphs (96%) followed by adults (71.4%), and small size nymphs (60%).

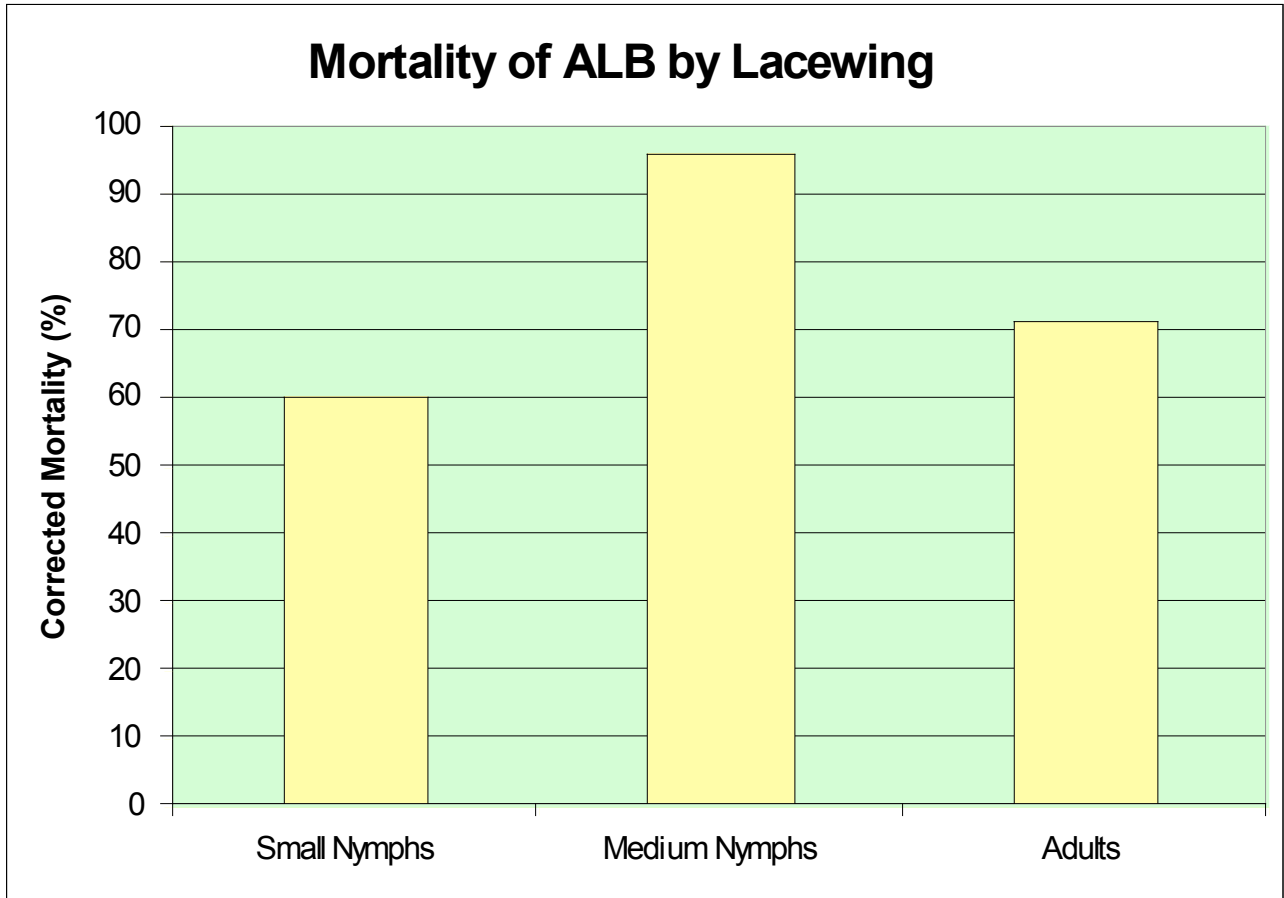


Fig 6. Munger cell bioassay for second instar larvae of *C. rufilabris* preying on three stages of ALB.

4.1.3. Neoseiulus californicus - predaceous mite:

Adult female *N. californicus* were used in these studies (Sterling Insectary, Delano, CA). The same ALB nymph age criteria (first instar and small size nymphs) and experimental design as used in the *Franklinothrips orizabensis* bioassays were used here. A ratio of one female adult *N. californicus* per five ALB per experimental cell was evaluated. *N. californicus* were starved prior to the bioassay for at least 24 hours just before exposure to the ALB nymphs. ALB mortality was assessed 24 hours after prey were exposed to *N. californicus*.

Results: The results show that female adult *N. californicus* did not effectively feed on small ALB stages (Fig. 7). In further studies, we will test whether *N. californicus* might prey on ALB eggs.

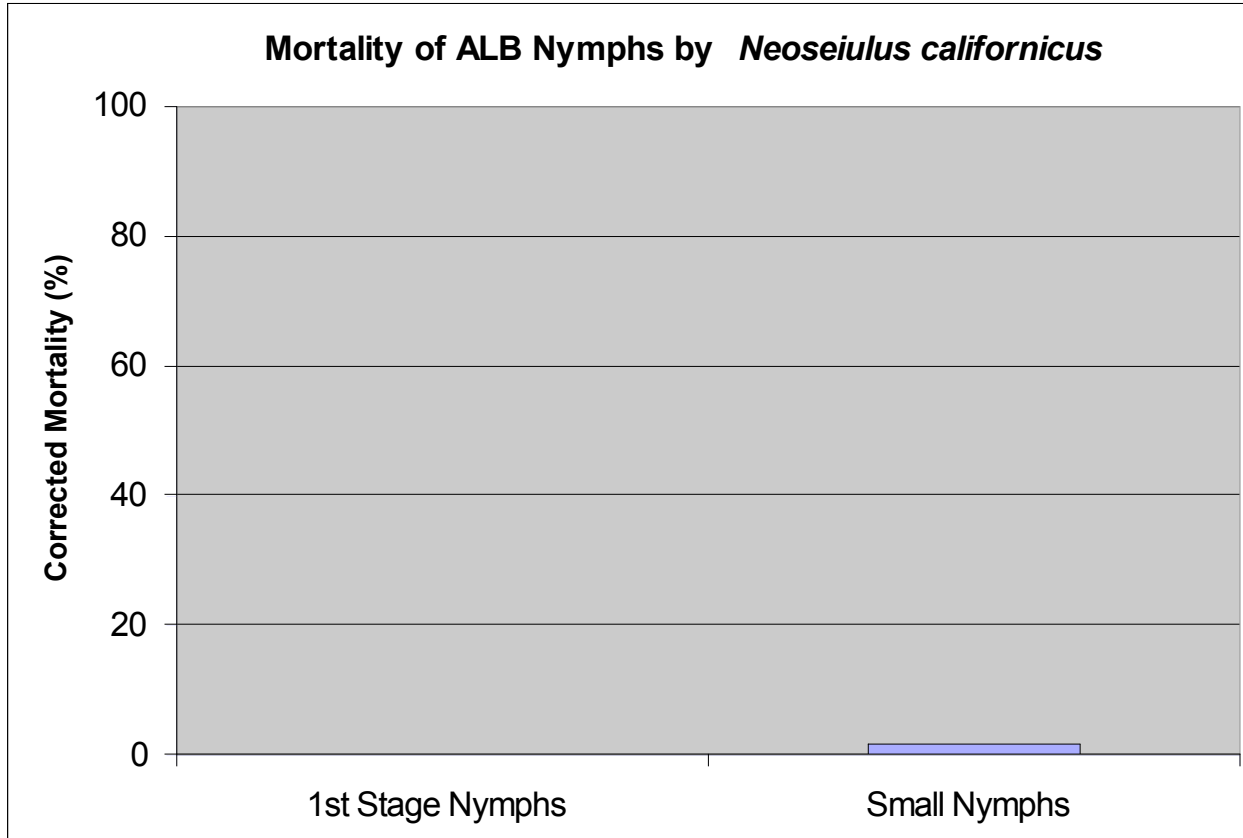


Fig 7. Munger cell bioassay for adult female *N. californicus* on two ALB life stages.

4.2. Susceptibility of *Chrysoperla rufilabris* to imidacloprid:

We were interested in seeing if avocado trees treated with imidacloprid (*Admire Pro*®) would affect mortality of the lacewing predator *C. rufilabris*. Therefore, avocado leaves were collected from an experimental site in Temecula where *Admire* had been injected into irrigation water. Tree #13 from this site was used because of the high levels of imidacloprid present at the time of the bioassay. The bioassay used 2nd instar lacewing larvae (starved for 24 h prior to the bioassay) and ALB nymphs that had fed on the imidacloprid-treated leaf for 24 h. ALB mortality was assessed 24, 48, and 72 hours after the prey were exposed to lacewings. A ratio of one second instar *C. rufilabris* per 10-15 ALB per experimental cell was evaluated.

The following treatments were tested:

Treatment 1: second instar lacewing larvae starved for 24 h and placed on a leaf treated with *Admire* (T1-Lw-24h-*Admire*, T1-Lw-48h-*Admire* and T1-Lw-72h-*Admire*).

Treatment 2: second instar lacewing larvae starved for 24 h and placed on a leaf treated with *Admire* with ALB nymphs feeding on the *Admire* leaf for 24 h (T2-ALB-Pre-*Admire*, T2-ALB-24h-*Admire*, T2-ALB-48h-*Admire*, T2-ALB-72h-*Admire*, T2-Lw-24h-*Admire*, T2-Lw-48h-*Admire* and T2-Lw-72h-*Admire*).

Treatment 3: second instar lacewing larvae starved for 24 h and placed on a leaf not treated with *Admire* (T3-Lw-24h, T3-Lw-48h and T3-Lw-72h).

Treatment 4: second instar lacewing larvae starved for 24 h and placed on a leaf not treated with Admire with ALB nymphs feeding on the leaf for 24 h (T4-ALB-Pre, T4-ALB-24h, T4-ALB-48h, T4-ALB-72h, T4-Lw-24h, T4-Lw-48h and T4-Lw-72h).

Results: The results of the bioassay are shown in Fig. 7. No significant difference was found between treatments #1 and #3, testing second instar lacewing larvae starved for 24 h and placed on a leaf treated with Admire vs. the leaf not treated with Admire. On the other hand, significant differences were found between treatments #2 and #4, where second instar lacewing larvae were starved for 24 h and were then placed on a leaf treated with Admire with ALB nymphs feeding on the Admire leaf for 24 h vs. the leaf not being treated with Admire. The data indicate that avocado trees treated with imidacloprid can affect lacewing mortality because of food chain impacts (i.e. by lacewings being exposed to the imidacloprid via the ALB).

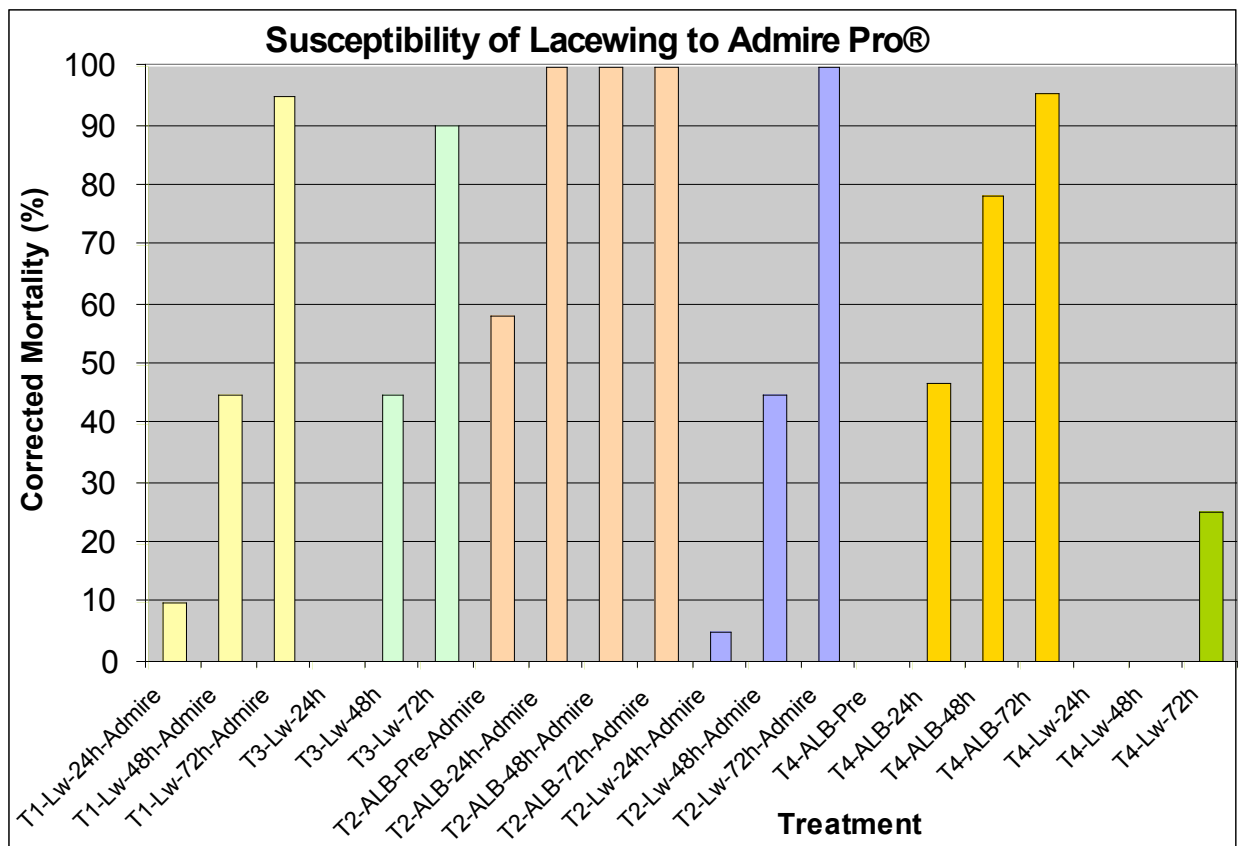


Fig 8. Susceptibility of *Chrysoperla rufilabris* to imidacloprid.

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 could potentially be sent from St. Kitts, St. Croix, and French Guyana. 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 are to be used for the genotyping 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. 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.

So far we looked at 14 ALB populations:

Chula Vista, San Diego Co., California, USA

Weslaco, Hidalgo Co., Texas, USA

Dade Co., Florida, USA

Oxkucab, 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

French Guyana

Data from the 14 ALB collections breaks down into three different ALB genotypes based on CO1 sequences. Except for the population from Veracruz, Mexico, all other populations are monotypic (i.e. DNA that was sequenced from each individual from these populations was similar). The California population has the same CO1 sequence as the Weslaco, Texas population and our California CO1 sequence is identical to one of the haplotypes found in Veracruz, Mexico. The sequence of the Florida population is the same as that of ALB from the Caribbean islands, French Guyana, and the Yucatan peninsula. Consequently, it is highly unlikely that Florida was the source of the California invasion. Five out of 24 microsatellite markers showed variation in the California, Weslaco, and Veracruz populations. Within the California population different alleles of the microsatellite loci were found. On the other hand, there was no variation detected within Weslaco and Veracruz populations. The California population differed from the Weslaco and Vera Cruz populations. Tentatively, we can conclude that neither the Weslaco nor the Vera Cruz populations were the direct source of the California invasion and consequently some other population must have been the source of our ALB invasion.

7. ALB Research lab at the San Diego County Insectary:

Substantial work has been done on the San Diego County Insectary in Chula Vista to optimize it 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, and 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.

At this point, the two greenhouses are working properly and two ALB colonies are being reared as source material for our ALB experiments. Two new insect growth chambers were purchased, have been set up and calibrated, and will be used in future ALB biology studies.

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.
- 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.
- Initiate ALB biology studies testing different temperatures, humidities, and avocado cultivars.
- Continue foreign exploration for ALB for genotyping and importation of associated parasitoids from Florida and Mexico.

Acknowledgments:

Progress made to date 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 use his backyard avocado trees in several aspects of our research. Bill Roltsch (CDFA) 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 and personnel from CDFA and the San Diego Ag. Commissioner's Office helped locate field research sites. 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).