Pseudacysta perseae
Biology and Management of Avocado Lace Bug in California

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Project Overview

This research project will be ending October 31, 2008 and we are on schedule to accomplish all of the major goals laid out for the project. Although the avocado lace bug (ALB) has not spread into commercial avocado orchards in California, we could not have predicted ahead of time that this would not happen and it is possible a more virulent strain might be introduced into California at some point in the future. In some areas of the world, ALB is a very serious pest on avocados. If a more virulent strain were to be introduced and/or the present strain were to become pestiferous on commercial avocados, we feel well prepared to deal with this insect. We appreciate funding provided for ALB research by the CAC and this funding was leveraged substantially via ALB grants obtained from the UC Exotic/Invasive Pest & Disease Research Program, the UC Thelma Hansen Trust, and grants provided by the California Department of Food & Agriculture. In addition, this work would not have been possible without outstanding cooperation provided by the above listed Cooperators. In particular we thank Bill Roltsch for his assistance on sampling and other portions of the project, the San Diego Ag Commissioner's office for providing research space at their old Insectary building where we could do work inside the ALB Quarantine zone, and Gary Bender for assistance with field trials early in the program.

A manuscript is in press in Florida Entomologist that deals with our evaluation of 3 natural enemies and both persistent pesticides and biorational pesticides for control of avocado lace bug – see the summary listed below. In addition, we have submitted a manuscript on ALB phenology in San Diego County over a 3-year period which includes the results of CDFA/County delimitation surveys – see the summary below.

Summary – Evaluation of Neonicotinoids for ALB Control

A major collaboration with Dr. Frank Byrne has involved evaluating the impact of four systemic neonicotinoids against ALB nymphs. We are have nearly finished with this work and will soon have good dosage – mortality relationships for imidacloprid (Admire Pro), thiamethoxam (Platinum), dinotefuran (Venom), and clothianidin (Clutch) – these materials are quite effective against ALB.

Summary – Influence of Temperature on ALB Development

A second collaboration is with Dr. Lilian Morales Romero who completed her Ph.D. degree in Cuba working on the developmental biology of ALB. We are comparing Dr. Romero's data on the response of the Cuban strain of ALB to temperature with developmental data from the San Diego strain of ALB so that we can better model how far this species might be able to spread in California.

Summary – Foreign Exploration Efforts & DNA Analyses

Dr. Hoddle has made a number of trips to avocado various growing regions to evaluate the severity of ALB populations, search for natural enemies, and collect ALB specimens for genetic analysis. The foreign exploration efforts covered countries in the Caribbean, all of Mexico (especially the bi-coastal regions with a lesser focus on the more arid central highlands), and Guatemala. Colleagues contributed preserved ALB material from South America. Adult ALB from all areas have been processed, DNA extracted, and the COI and 28s-D2 regions have been analyzed. Microsatellite analyses are now underway to further refine the geographic location from which the California ALB population was sourced. Although our search for parasitoids of ALB has so far failed to detect a species which might be imported into California, Dr. Serguei Triapitsyn did an outstanding job examining museum specimens of parasitoids from various areas of the world in the genus *Erythmelus* so that should we find egg parasitoids associated with ALB, we might be able to evaluate their taxonomy in relation to known species. The result of Dr. Triapitsyn's work was the publication of a 64-page monograph (Triapitsyn et al. 2007) which revised/corrected the taxonomy of 12 North American species and provided a key to the 30 known species from Central and South America, the likely areas where natural enemies for ALB would be sourced.

Summary – Evaluation of Natural Enemies and Pesticides for ALB Control

Three natural enemies naturally present in southern California avocado groves were evaluated against different stages of ALB in the laboratory. The natural enemies tested were adult females of a predatory thrips, *Franklinothrips orizabensis*, second instar green lacewing larvae, *Chrysoperla rufilabris*, and a predaceous mite, *Neoseiulus californicus*. The most promising natural enemy from laboratory and subsequent greenhouse evaluations was *C. rufilabris.* In addition to natural enemies, insecticides were also evaluated for *P. perseae* control. The contact impact of less persistent materials on nymphs in the laboratory was assessed. The most effective insecticides based on residual impact studies were carbaryl, imidacloprid, and fenpropathrin, and two materials commonly used on avocados in California, abamectin and spinosad, were ineffective. Among the insecticides evaluated based on contact activity, a pyrethrin mixture was the best treatment followed by petroleum oil and potash soap. The contact insecticides were also evaluated for their impact on second instar *C. rufilabris* larvae. The pyrethrin mixture was less toxic to *C. rufilabris*, and because of its low mammalian toxicity this insecticide may be suitable for use with natural enemy releases for homeowners to manage *P. perseae* populations on backyard avocados.

Details of this study are presented in Humeres, E. C., J. G. Morse, R. Stouthamer, W. Roltsch, and M. S. Hoddle. 2008. Evaluation of Natural Enemies and Insecticides for Control of *Pseudocysta perseae* (Hemiptera: Tingidae) in Southern California. Florida Entomologist (In Press).

Summary – Phenology and Detection Surveys for ALB

The known distribution of *P. perseae* includes the Caribbean, the southeastern USA, Mexico, Guatemala, Venezuela and French Guyana. *P. perseae* was detected for the first time in California (USA) in September 2004 from two residential avocado trees in Chula Vista and San Diego in San Diego County. As part of a management plan for *P. perseae*, surveys were conducted to delineate the range of this pest in California, and at selected sites, long-term phenology studies were initiated to determine population growth trends.

Two detection surveys for *P. perseae* were performed in San Diego County during spring (March 20 – June 30) and fall (September 20 – October 30) of 2006 to delineate the geographic distribution of this pest. Surveys were conducted using a pest detection trapping grid system established by the California Department of Agriculture (CDFA) in San Diego County. The 2006 fall survey in San Diego County was limited to the area enclosed by two major freeways, Interstate 8 and California State Route 78. The spring 2006 survey for *P. perseae* in San Diego County delimited the distribution of this pest south of the Interstate 8 freeway to the USA-Mexico border (Fig. 1). The survey covered important commercial avocado production regions such as Fallbrook and Escondido where no *P. perseae* were found. *P. perseae* detected in surveys were exclusively restricted to avocado trees growing in residential areas (Fig. 1). During the fall 2006 survey, two new sites near La Jolla were detected north of the Interstate 8 freeway indicating a slight (~16 km) movement north. Survey results indicated that areas infested with *P. perseae* were restricted to the southern coastal region of San Diego County and were within 50 km of the coast. The first year of the *P. perseae* phenology studies conducted in San Diego over 2005-2008 showed that nymphs and adults increased in numbers in early summer (June) and decreased during fall (September) in 2006. During the same time period for 2007 and 2008, *P. perseae* populations remained low with no outbreaks observed across all sampling sites used. Despite the high *P. perseae* infestations at the Naples and Del Sol sites that originally caused heavy leaf damage and defoliation during 2006, these populations didn't display similar population trends in 2007-08 and natural enemy (i.e. egg parasitism and presence of predators) activity did not differ significantly over the course of this study.

The results from distribution monitoring and three years of phenology data are presented in Humeres, E., J. G. Morse, R. Stouthamer, W. Roltsch, and M. S. Hoddle. 2009. Phenology and Detection Survey for *Pseudacysta perseae* on Avocados in California. Florida Entomologist (this Scientific Note was submitted October 7 2008 for peer review).

Background of the Problem

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.

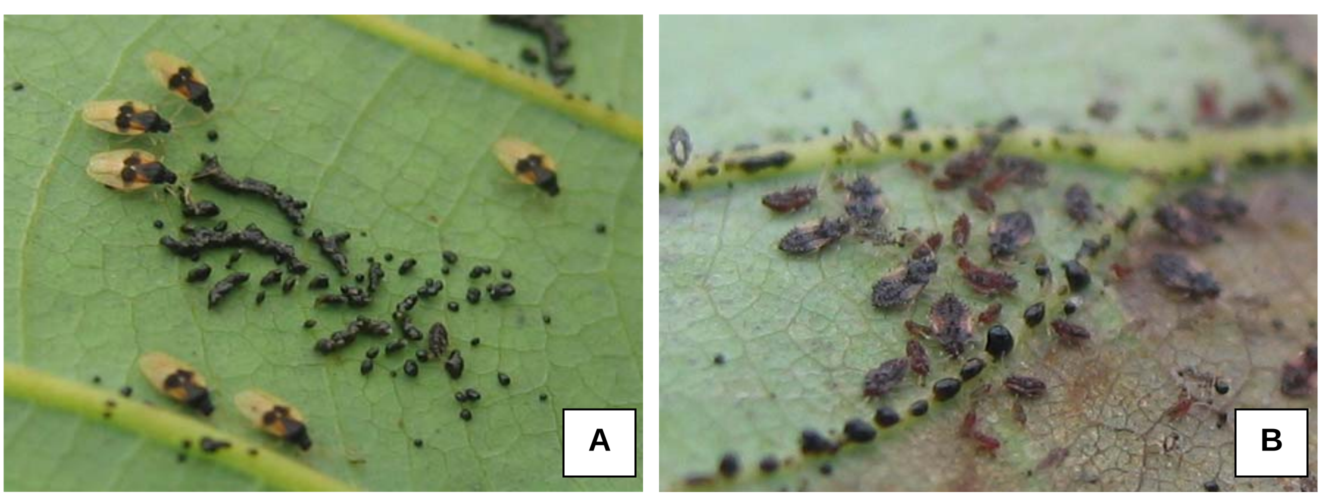


Figure 1. Avocado lace bug (A) adults and egg mass (B) nymphs

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. The results of the ALB surveys in San Diego Co. (2004-05; 2006 spring and 2006 fall) show that 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. 3 & 4).

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.

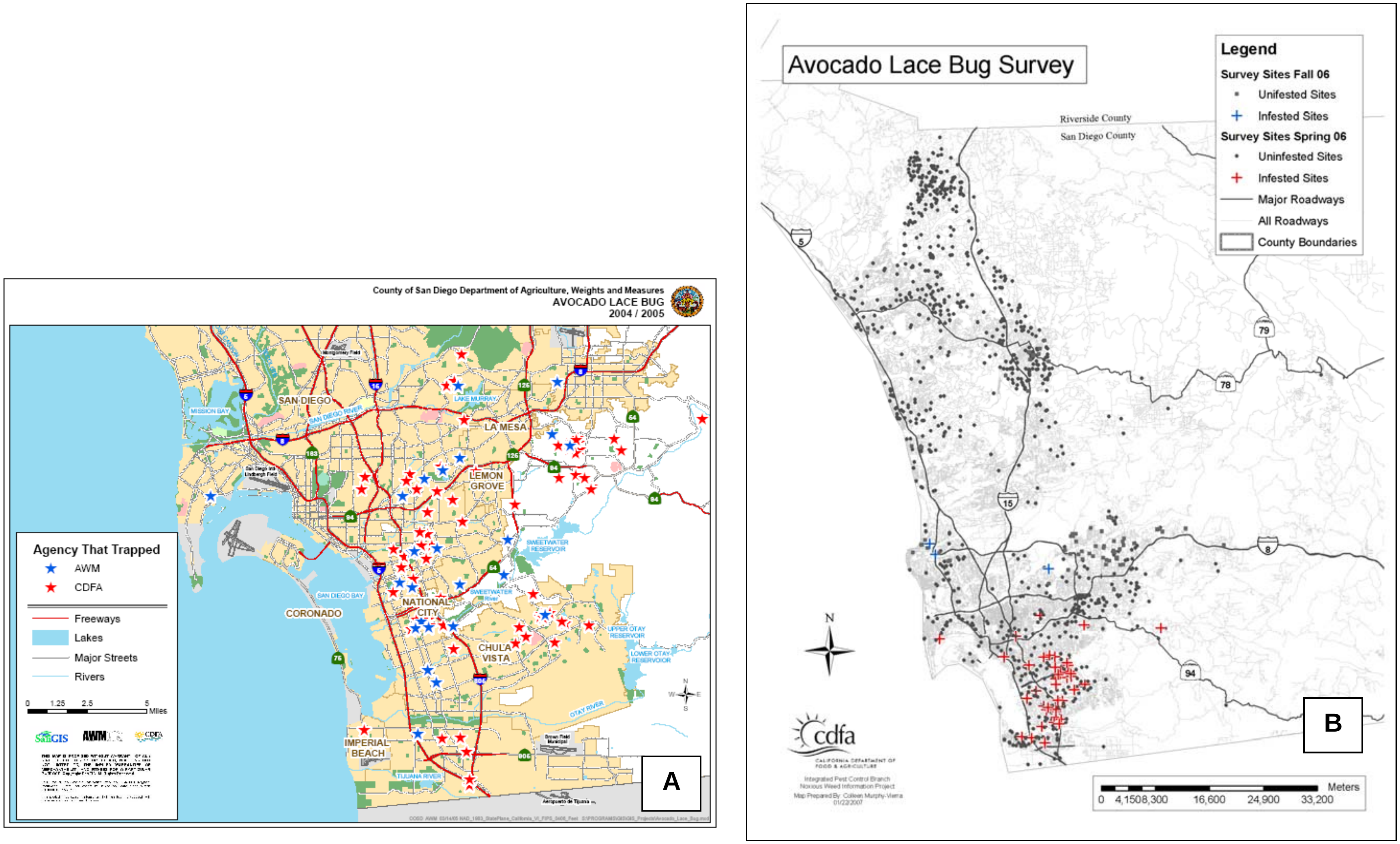
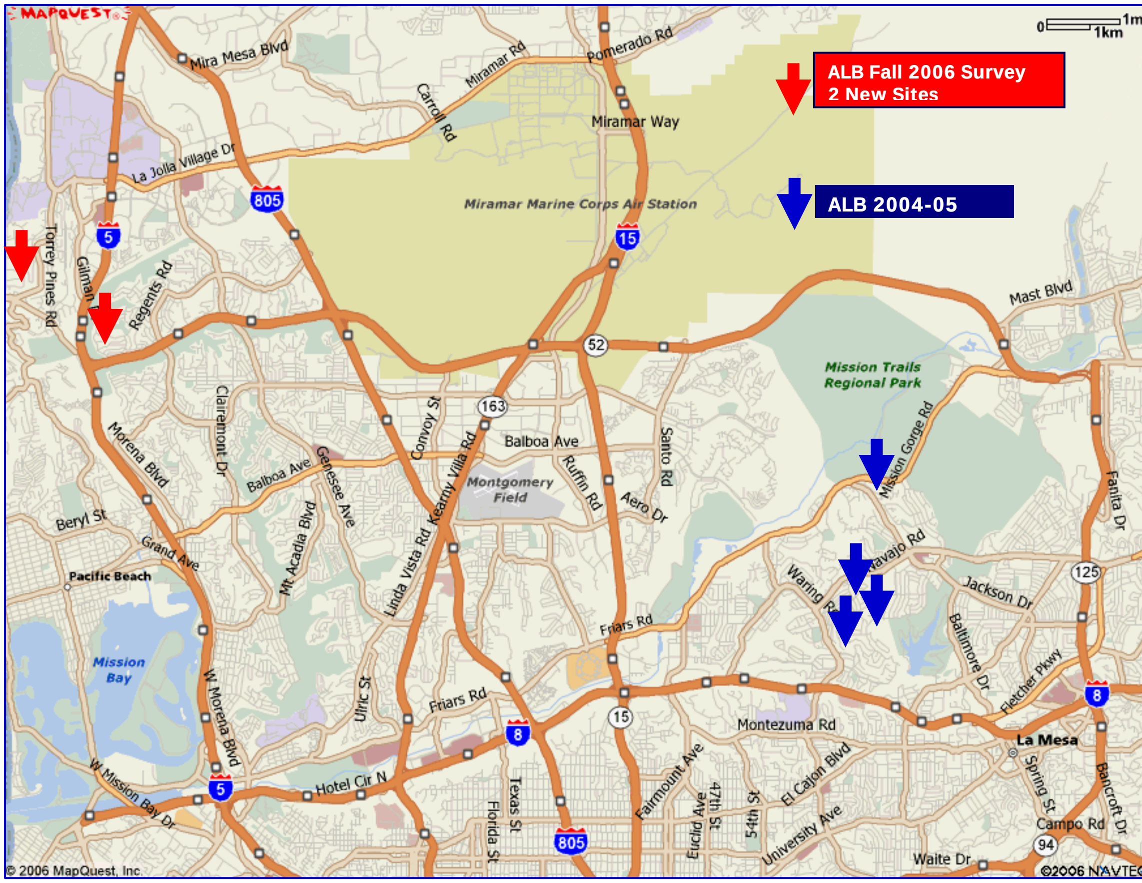


Figure 3. Avocado lace bug survey conducted by the County of San Diego Department of Agriculture, Weights & Measures (AWM) and the California Department of Food and Agriculture (CDFA) during (A) 2004-05 and (B) 2006 (spring and fall) to determine the distribution of the pest in the county.



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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.

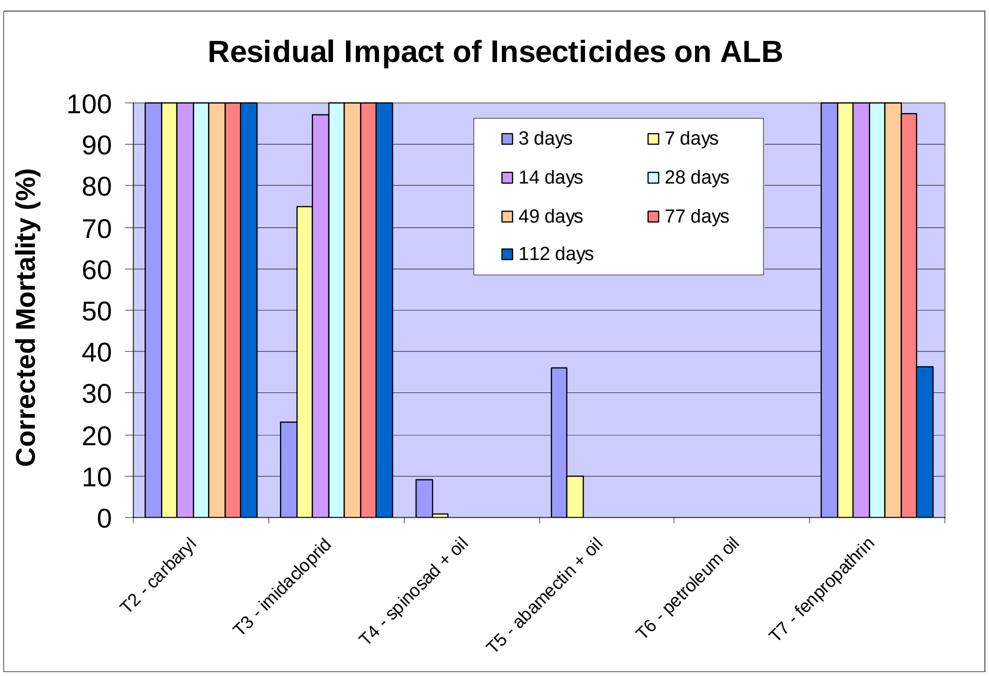

Figure 5. Residual impact of insecticides on ALB nymphs with mortality evaluations between 3 and 112 days after treatment (pesticide treatment evaluations were dropped for insecticides which showed <20% ALB mortality after two consecutive evaluations).

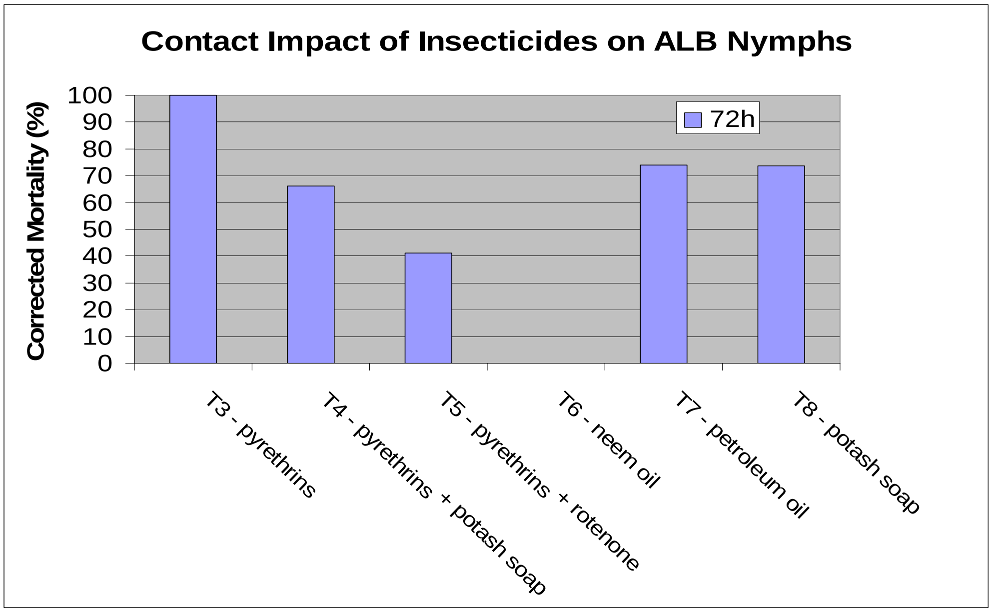

Figure 6. Contact impact of insecticides on ALB nymphs 72 h after treatment.

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.

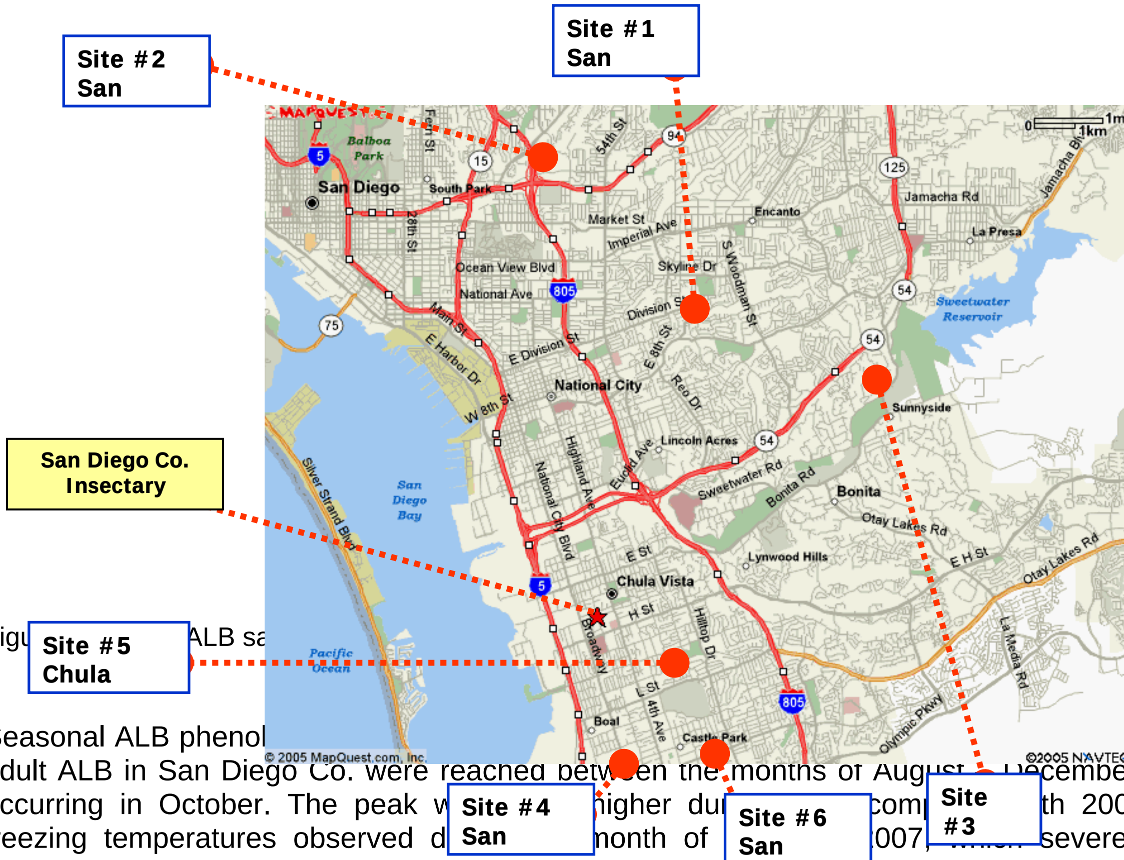

Figure ... ALB sa...

Seasonal ALB phenol... est densities of adult ALB in San Diego Co. were reached between the months of August ... December with the peak occurring in October. The peak w... higher du... comp... th 2006. Despite the freezing temperatures observed d... month of ...007, 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.

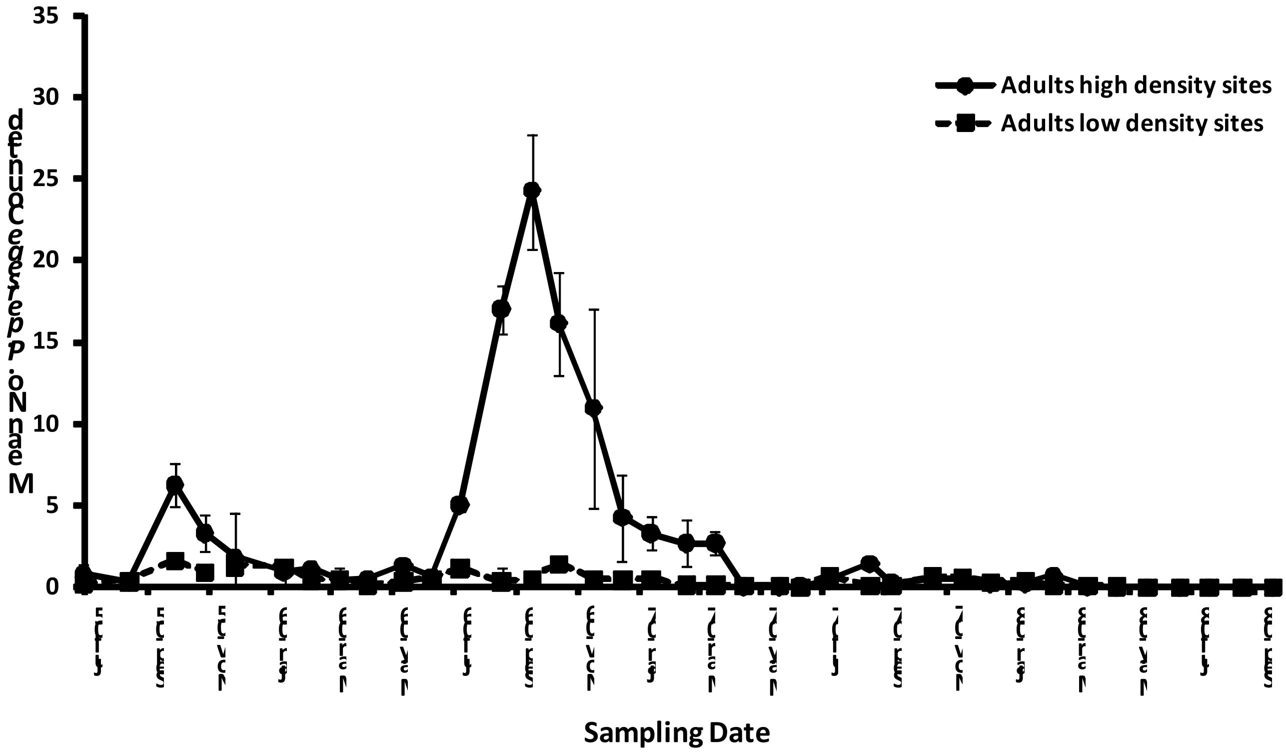

Figure 8. Mean numbers of adult avocado lace bugs / leaf in San Diego Co. 2005-08. from low ALB density sites (Division, 47th Street, Margaret) and high ALB density sites (Naples and Del Sol).

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

The majority of the trees at the ALB sampling sites were 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.

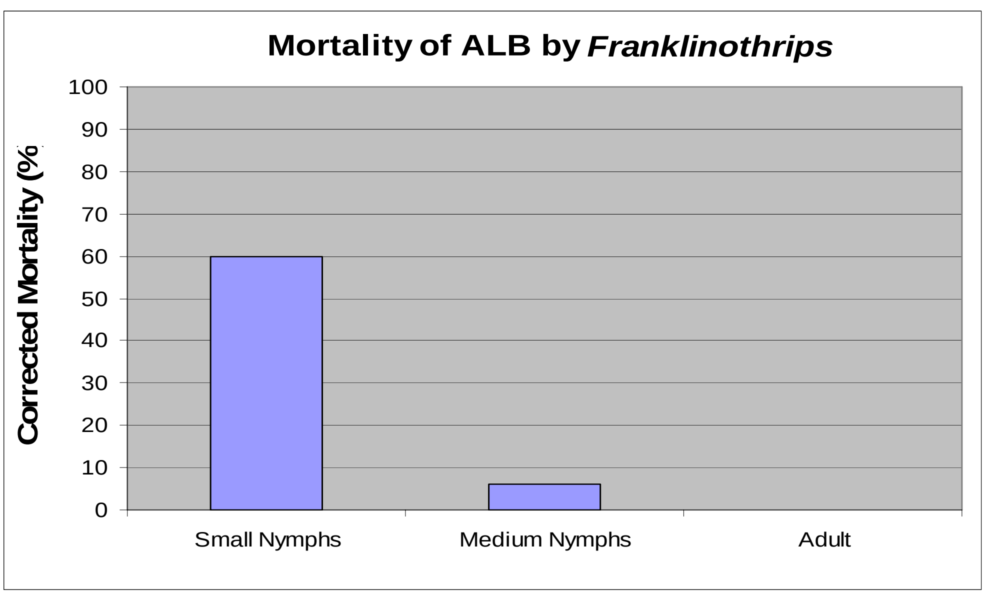

Figure 9. Munger cell bioassay evaluating adult *Franklinothrips* predation on three stages of ALB.

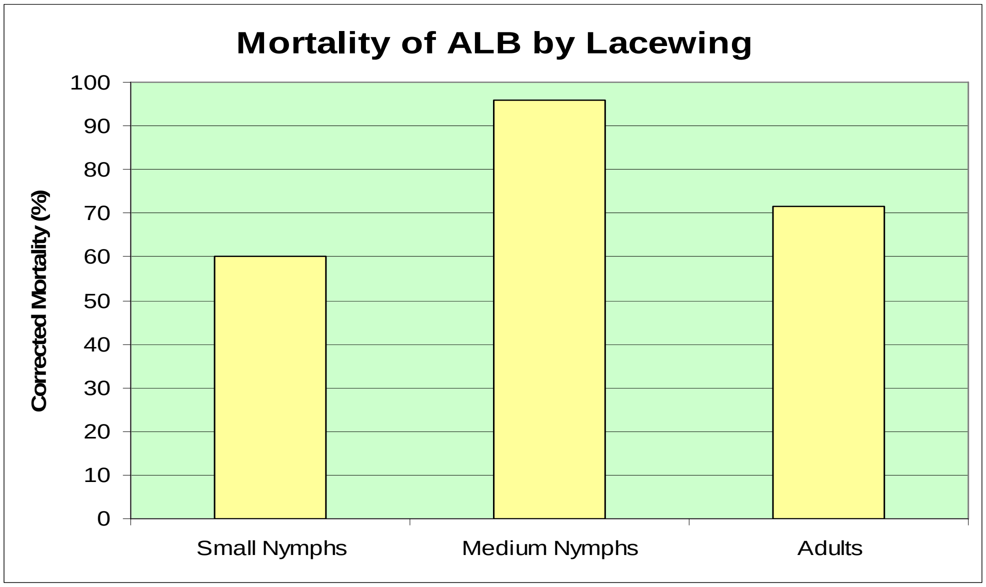

Figure 10. Munger cell bioassay with second instar larvae of *C. rufilabris* preying on three stages of ALB.

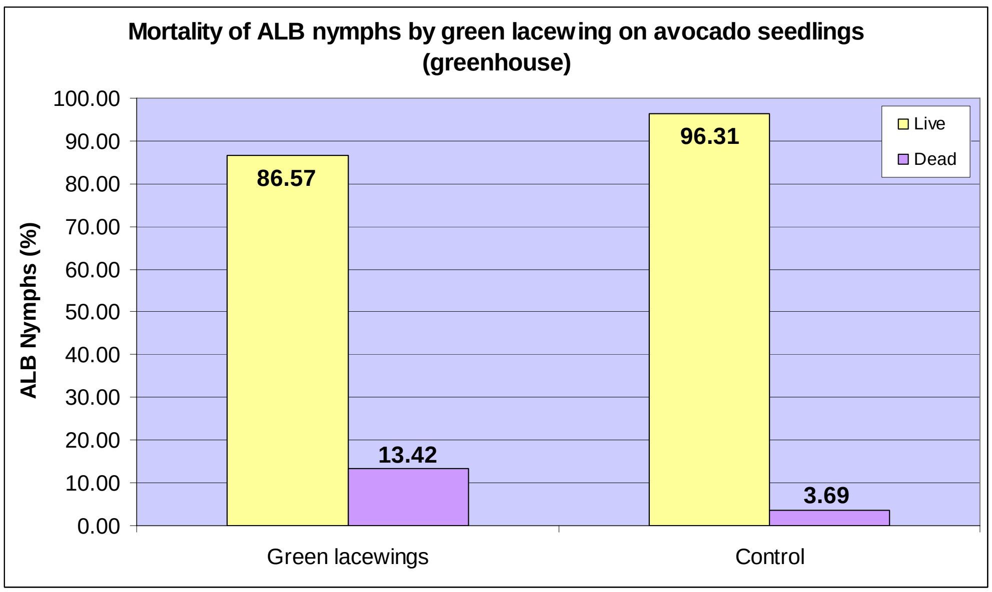

Figure 11. Mortality of ALB nymphs by second instar larvae of *C. rufilabris* on potted avocado seedlings under greenhouse conditions.

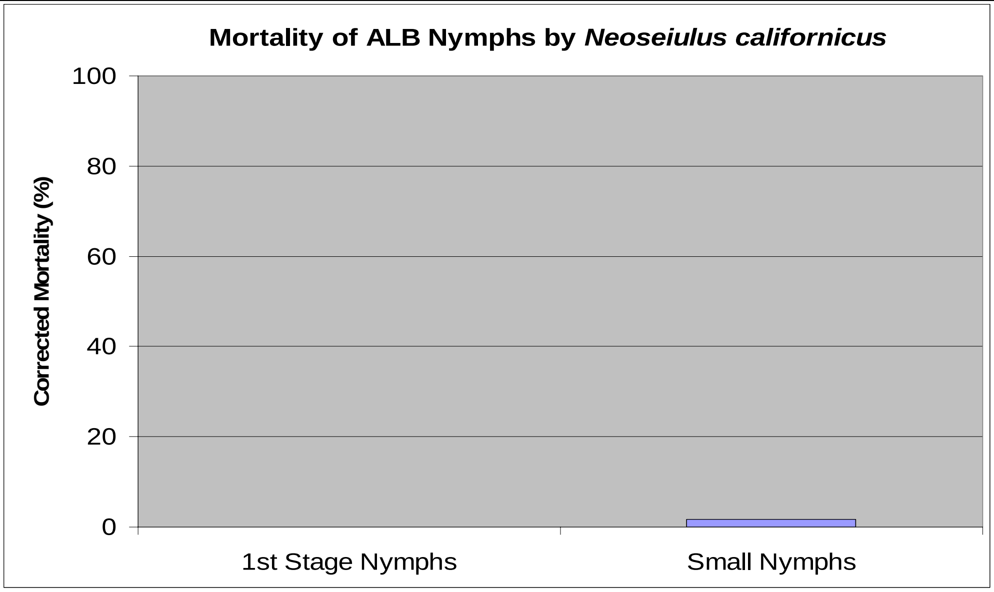

Figure 12. Munger cell bioassay for adult female *N. californicus* on two ALB life stages.

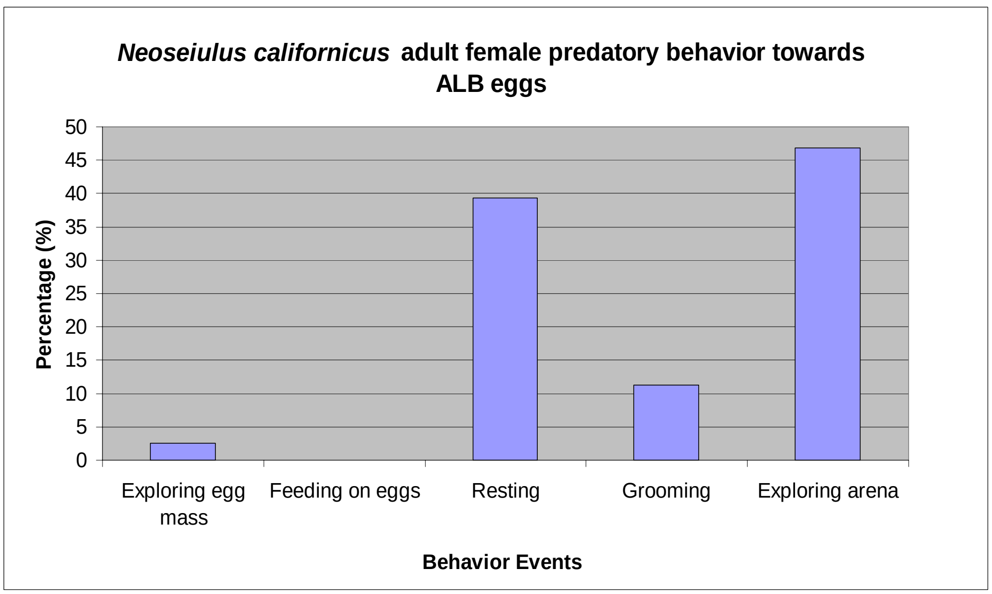

Figure 13. Adult female *N. californicus* behaviors recorded in Munger cells with ALB eggs.

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.

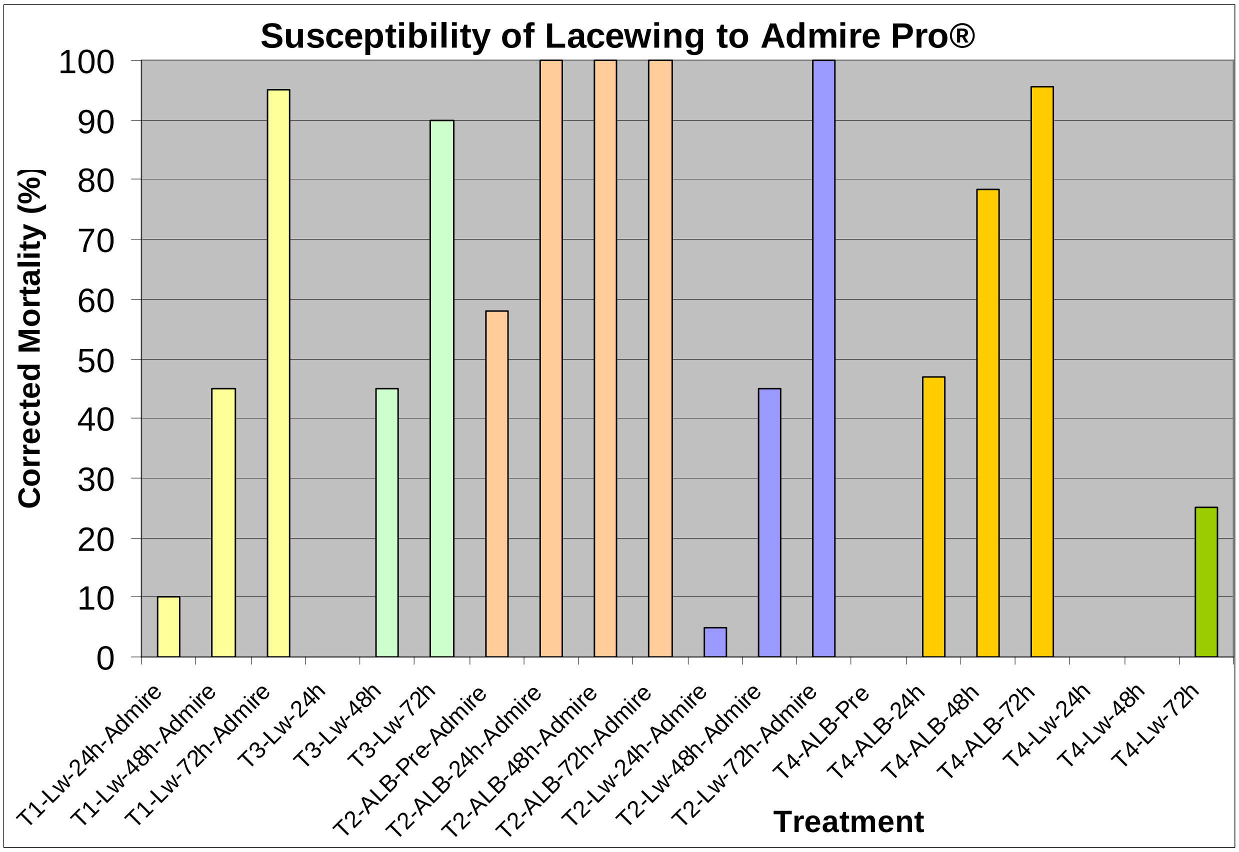

Figure 14. Susceptibility of *Chrysoperla rufilabris* to imidacloprid. (LW – lacewing ; ALB – avocado lace bug)
Treatment 1: second instar lacewing larvae placed on a leaf treated with Admire with no ALB.
Treatment 2: second instar lacewing larvae placed on a leaf treated with Admire with ALB nymphs 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 addition to exploration in eastern areas of Mexico, the central highlands and west coast regions of Mexico were surveyed for ALB. ALB was very common on avocados along the west coast of Mexico, and to the best of our knowledge, these records may be the first “official” collections of ALB along the west coast of Mexico. ALB populations were very rare in high altitude extremely xeric locations, but they did exist, suggesting that this pest may have a broader tolerance for cold dry conditions than previously suspected. ALB was discovered in Guatemala for the first time during work on *Stenoma*. 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.

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; Oxkutcab, 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. Emerging results from recent analyses of ALB collected from the west coast of Mexico are beginning to suggest that the possible source of the invading population into California may have originated from this area.

Recent publications resulting from this research

Hoddle, M. S., G. S. Bender, J. G. Morse, D. Kellum, R. Dowell, and G. W. Witney. 2005. Avocado Lace Bug. AvoResearch. Spring 2005. 2 pp.

Hoddle, M. S., J. G. Morse, and R. Stouthamer. 2005. Biology and Management of Avocado Lace Bug (ALB) in California. Pp. 1-13, *In*: *Proceedings*, California Avocado Commission Research Symposium, October 29, 2005, California Avocado Commission, Santa Ana, CA. 133 pp.

Morse, J. G., F. Byrne, and N. C. Toscano. 2005. Evaluation of Systemic Chemicals for Avocado Thrips and Avocado Lace Bug Management. Pp. 24-33, *In*: *Proceedings*, California Avocado Commission Research Symposium, October 29, 2005, California Avocado Commission, Santa Ana, CA. 133 pp.

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