CRB-FUNDED RESEARCH PROGRESS REPORT

Photo 1. ACP in cages at the Insectary.

ORGANIC PESTICIDE SCREENING AT THE CHULA VISTA INSECTARY

James Bethke, Marianne Whitehead, Joseph Morse, Frank Byrne, Elizabeth Grafton-Cardwell, Kris Godfrey and Mark Hoddle

BACKGROUND

*In 2012, we began an intensive screening of registered and experimental, conventional and organic insecticides, against the Asian citrus psyllid **(Photo 1)** at the Chula Vista Insectary **(Photo 2)**. Without large populations of ACP in commercial citrus, we needed an effective method of screening products that would give us a scientific basis for making treatment recommendations in California, especially for organic producers.*

To date, we have successfully tested 22 organic products or combinations of products, and we have screened a number of conventional and experimental products. In addition, we have nearly doubled the number of trials conducted in 2013 compared to 2012 due to the increased funding supplied by the Citrus Research Board. In this article, we will concentrate our discussion on the organic product results and potential management tactics using organic insecticides.

It is well known that many of the organic products work mainly on contact and do not persist for an appreciable amount of time (at most 7-10 days, if that long). Some consider this a benefit because of reduced impact on natural enemies, but the lack of persistence also means that a greater number of applications is required to effectively reduce difficult-to-control pests, which means that these pests are likely to remain in citrus groves at low levels. This presents a conundrum when there is an ever-increasing demand for fruit free of conventional pesticides and also the need for high levels of control of a devastating invasive pest such as ACP. The problem is even more serious when pests such as ACP transmit a deadly disease of citrus, namely huanglongbing (HLB). Short-lived insecticides are less effective in preventing disease transmission because they do not kill the insect fast enough, nor do they sufficiently suppress the vector population to prevent feeding and pathogen spread.

The goal of our research is to test as many organic products as we can, in an effort to identify those products that are most effective and persistent against ACP. Some of this research already has been initiated in Florida. We need California-derived data in order to register new insecticides and so that we know how to use them properly under our environmental conditions.

Photo 2. Chula Vista Insectary.

INSECTICIDE TESTING PROTOCOLS

As outlined in our previous article in *Citrograph* [May/June 2012, V3(3):36-40], we have a thriving colony of ACP at the Chula Vista Insectary, and there are strict protocols for entry and movement within the facility to ensure that the release of ACP will not occur. Space at the facility is at a premium, so good coordination and assistance is required to maximize the screening efforts. As such, the plants we use in our trials are grown from seed in greenhouses at University of California Riverside and transported to the Insectary under permit and in an enclosed vehicle. In addition, the plants are sampled and tested for HLB on a monthly basis. One- to two-year-old *Citrus volkameriana* plants in one-gallon pots (#1 nursery cans) were used in all trials.

STUDIES AGAINST ACP NYMPHS

Potted citrus plants were exposed to ACP adults in colony rearing cages for 48 hours and then removed. Following egg hatch (approximately eight days), the number of nymphs on a single terminal shoot (approximately 10 cm long) per plant was recorded. The shoot was marked for further evaluation following the application of the various compounds. The number of single plant replicates per trial varied and was either six or eight replicates. The number of nymphs per plant also varied, so the plants were assigned to treatments to equalize the number of nymphs in each treatment prior to applications. Following the pre-treat-

Photo 3. A citrus plant is infested with ACP, treated and caged. Mortality is assessed by counting the number of live ACP on treated plants.

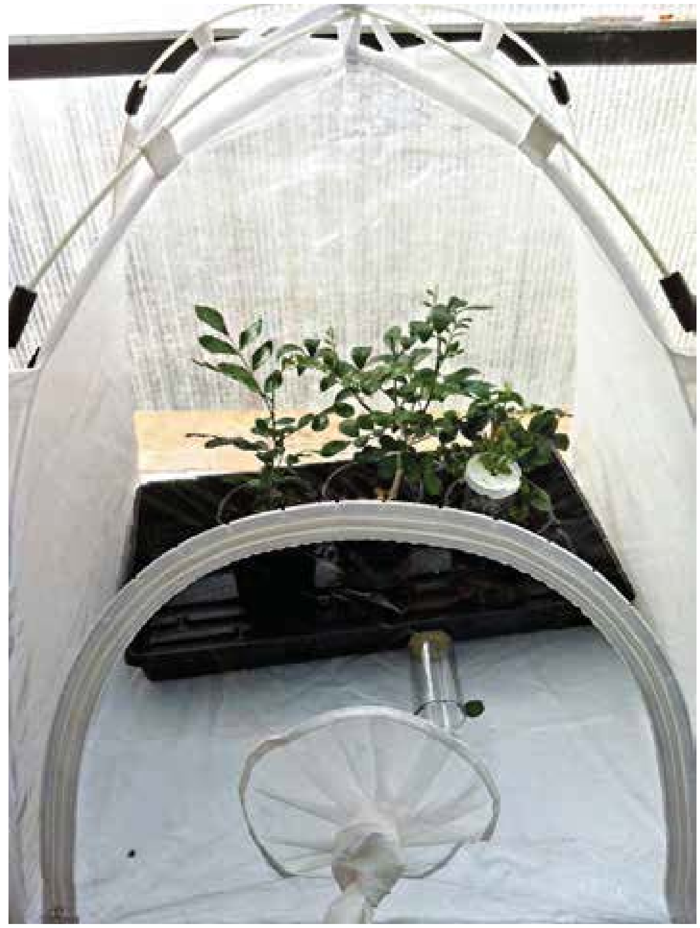

Photo 4. Plastic cage with a cloth sleeve and a zippered front for addition and removal of plants and insects. This cage is called a BugDorm.

ment count, the plants and insects were treated with selected products, allowed to dry and caged ***(Photo 3)***. The cage consisted of a clear plastic 128 oz. jar (110 mm polyethylene round jar). The bottom of the jar was cut off so it could be placed over the plants and the top of the jar was covered with screening material (organza bag) for ventilation.

Mortality was recorded at 48 hours and weekly thereafter until all the adults emerged in the controls. Some organic products required multiple applications and further observations of mortality. It is very difficult to transfer ACP nymphs from one plant to another because they insert their mouthparts into the plant, and transfer causes a high mortality. Therefore, we did not test the survival of nymphs on weathered residues.

STUDIES AGAINST ACP ADULTS

Approximately 10 adult ACP were caged and established on each plant prior to the application of insecticides. Six to eight single plant replicates were used in these trials. Once the ACP adults established (approximately 24 hours), the caged plants were placed into a larger cage (BugDorm, BioQuip) ***(Photo 4)***, the small cage was removed, applications were made to the infested plants and adult psyllids, and the small cages were placed back on the infested plants almost immediately so as not to lose any exposed adults. When the pots were dry and the plants could be moved, they were removed from the large cage, and a pretreatment count of adults present on the plants was made. This pre-treatment count was necessary because during the process of spray applications, some adults were forced from the plants and were unrecoverable.

Mortality of the remaining insects was recorded at 48 hours. Following the 48-hour assessment, all remaining insects were removed. A new set of 10 adult ACP was placed on treated plants at seven-day intervals, and mortality was recorded at 48 hours to determine the persistence of the applications. New adult ACP were placed on treated plants until efficacy declined below 50 percent mortality.

ORGANIC INSECTICIDES EVALUATED

Although it is not a complete list, the organic insecticides presented in our results are listed in ***Table 1***, and they can be broadly categorized by type (biological, botanical or horticultural oil) and by mode of action (insect growth regulator, desiccant, asphyxiant or stomach poison). In addition to the organic products, we are testing many conventional products and some unregistered products, as well. Therefore, some of the results presented may contain those other products, and they are also listed in ***Table 1***.

Table 1. Organic pesticides recently screened at the Chula Vista Insectary against ACP.

Trade Name	Active Ingredient	Manufacturer	Type	Mode of Action
Organic Products				
AzaGuard	Azadirachtin	Biosafe Systems LLC.	Botanical	IGR
Azera	Azadirachtin + Pyrethrins	MGK Corp.	Botanical + Botanical	IGR + Paralytic
DE	Diatomaceous earth	Brandt	Biopesticide	Desiccant
Entrust 80%	Spinosad	Dow	Biological	Gut Poison
Grandevo	*Chromobacterium subtsugae*	Marrone	Biological	Multiple/Complex
Met52	*Metarhizium anisopliae* Strain F53	Novozymes Biologicals	Biological	Insect Pathogen
Gavicide Green (NR 415)	Mineral oil	Loveland	Horticultural Oil	Membrane Disruptor
Preferal (PFR-97)	*Isaria fumosoroseus*	Certis	Biological	Insect Pathogen
Pyganic	Pyrethrins	MGK Corp.	Botanical	Paralytic
Tritek (Saf-T-Side, NR 438.5)	Spray oil	Brandt	Horticultural Oil	Membrane Disruptor
Widespread Organic	Polymethylsiloxane	Loveland	Horticultural Oil	Membrane Disruptor
Nonorganic Products				
Danitol	Fenpropathrin	Valent	Synthetic Pyrethroid	Paralytic
BotaniGard ES*	*Beauveria bassiana*	BioWorks	Biological	Insect Pathogen
Orocit CA**	Citrus oil	Oro-Agri	Horticultural Oil	Membrane Disruptor
Silwet L-77***	Organosilicone surfactant	Helena Chemical Co.	Organosilicone Surfactant	Membrane Disruptor
Unregistered Products				
MBI-206 (4 Formulations)	*Burkholderia* spp.	Marrone	Biological	Multiple/Complex
Sivanto	Flupyradifurone	Bayer	Synthetic Butenolide	Feeding inhibitor

* Mycotrol O is a similar product formulated for use in organic production.

** Oroboost is a similar product formulated for use in organic production.

*** Silwet Eco is a similar product formulated for use in organic production.

Several of the organic products we tested were either biological in nature or derived from a biological source. Those that were bacteria-based were Grandevo and MBI-206 from Marrone Bio Innovations and Entrust from Dow AgroSciences. Grandevo and MBI-206 contain a number of compounds that contribute to the creation of complex modes of action. Entrust contains spinosad, a compound found in the bacterial species *Saccharopolyspora spinosad*. Spinosad acts as a stomach poison.

BotaniGard, Met-52 and Preferal (PFR97) contain entomopathogenic fungi. These products usually contain spores of the pathogenic fungus that attach to the body of the insects. Under the right conditions, the spores germinate through the insect's outer skin and kill the insect.

The botanicals include Pyganic and the neem-based products. Pyganic is a chrysanthemum-derived product that includes several pyrethrins. Pyrethrins cause paralysis in insects. The neem-based products contain azadirachtin, which acts as an insect growth regulator and a feeding deterrent or a repellant. Lastly, there are the horticultural oils, which have long been used as insecticides and have been effective against ACP. We examined two narrow range horticultural oils, a 415 oil called Gavicide Green and a 438.5 oil called TriTek. We studied whether they would kill psyllids on their own and/or whether they would enhance the performance of some of existing organic insecticides. Additionally, we studied the effect these oils would have on ACP if we applied them at several different labeled rates.

In addition to the insecticides, we included a water treated control (check) in all experiments. We also included the pyrethroid Danitol (fenpropathrin), which ensured that our methods would demonstrate mortality (i.e. served as a positive control). If an adjuvant was used in combination with one or more of the products, we included it as a stand-alone treatment, so that we could observe any additive effects due to the presence of the adjuvant. We included Gavicide Green, Orocit (Oroboost is a similar product, and it is the organic version of Orocit) and Widespread Organic as adjuvants in some of our trials. Silwet L77 was used as an adjuvant for one treatment application (Grandevo; Silwet Eco is an Orangic Materials Review Institute listed version of Silwet).

Data were analyzed using ANOVA and were transformed as necessary to satisfy the assumptions of analysis of variance. Means were separated by Fisher's Least Significant Difference test ($P = 0.05$). The data are presented in bar graphs as means (columnar bars) and standard errors (error bars) per treatment per sample day.

RESULTS

In this article, we chose to highlight nine of the 21 trials we conducted that answer some of the most pressing questions. *Remember that these are greenhouse screening trials, and that they represent the best-case scenario of coverage of the plant with insecticide*. In other words, we are confident of contacting the insects with our applications, and we are confident of the resulting mortality. The products may not kill psyllids as well in field trials because of incomplete coverage, degradation by light and heat, or if the product is diluted to a greater extent (to standardize, rates were based on 100-gpa). *It will be important to validate our results in field trials*.

EFFICACY AGAINST NYMPHS

Figure 1 shows the mortality caused by one of the more popular botanical, organic insecticides, Pyganic. In this trial, Pyganic was compared to two conventional products, Danitol and Sivanto (unregistered experimental product) and to Gavicide Green, a narrow range 415 oil. The mortality imparted by Pyganic to the nymph stage of ACP was comparable to the conventional products, Danitol and Sivanto; and the mortality caused by Gavicide was significantly less.

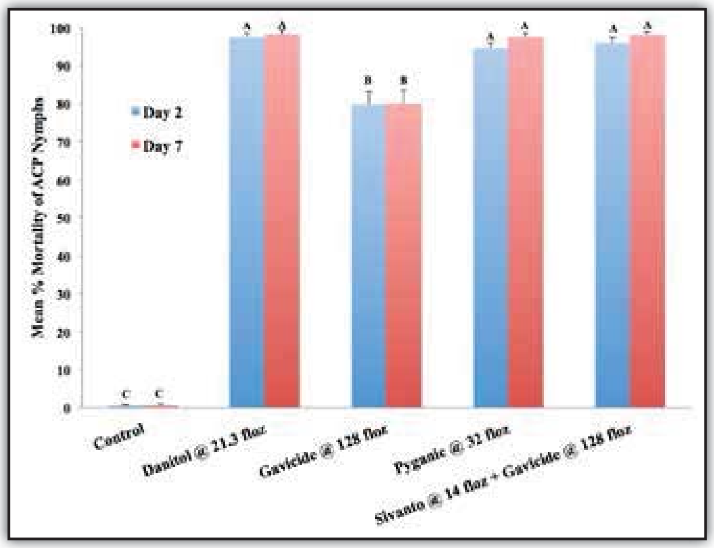

*Figure 1. Mean percent mortality of ACP nymphs on citrus treated with Pyganic and compared to two conventional products, Danitol and Sivanto. Gavicide Green was used as an adjuvant at 1 gallon/100 gallons, both as a stand-alone application and at the same rate in combination with selected treatment applications.**

The efficacy of various entomopathogenic fungi, a bacterial by-product and diatomaceous earth against ACP nymphs can be found in ***Figure 2***. With good contact, these products were highly effective causing >90 percent mortality of ACP. Note that the Orocit alone was as efficacious as the Orocit+microbials.

We further tested Grandevo against ACP nymphs in search of a surfactant that might increase efficacy ***(Figure 3)***. Although the efficacy of each product combination is above 90 percent mortality, there is a statistically significant improvement in the efficacy of Grandevo+Orocit compared to Grandevo applied

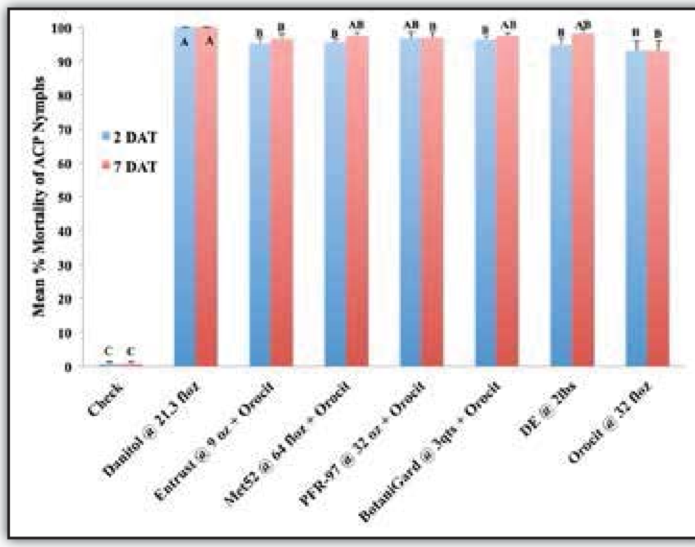

*Figure 2. Mean percent mortality of ACP nymphs on citrus treated with various biological insecticides and diatomaceous earth. Orocit was used as an adjuvant at 32 fluid oz./100 gallons, both as a stand-alone application and at the same rate in combination with selected treatment applications.**

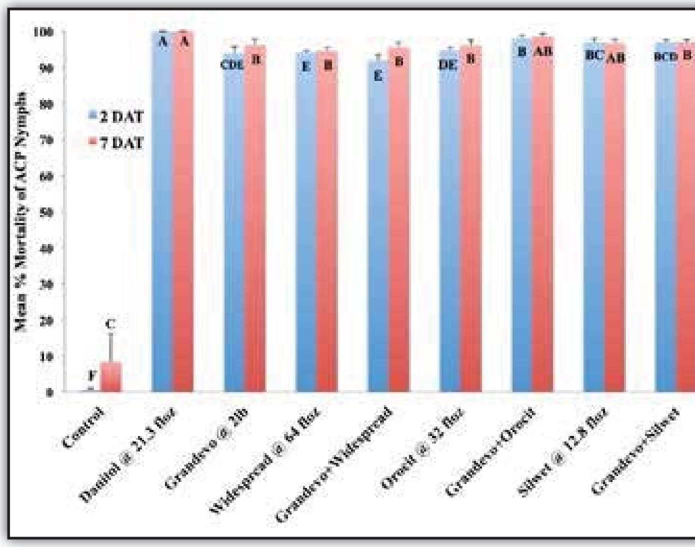

*Figure 3. Mean percent mortality of ACP nymphs on citrus treated with Grandevo and Grandevo plus three different adjuvants. Adjuvants were used as a stand-alone application and at the same rate in combination with selected treatment applications.**

by itself in the first 48 hours after application. A week later, the efficacy of Grandevo+Orocit was not statistically different than Grandevo alone.

Lastly, we tested two new formulations (E1 and E3) of a new product from Marrone, MBI-206, against ACP nymphs ***(Figure 4)***. Gavicide Green was added to each treatment in this trial, and three rates of MBI-206 were tested for each formulation. Both formulations were equally effective at the high rate of 1 gallon/100 gallons, and they were equally as effective as Gavicide Green alone and Grandevo. There was a clear rate response for the E1 formulation, whereas the E3 formulation only showed a reduction in efficacy at the lowest rate (0.25 gallon/100 gallons). In this trial, we also observed Gavicide by itself to be quite effective (>90 percent mortality).

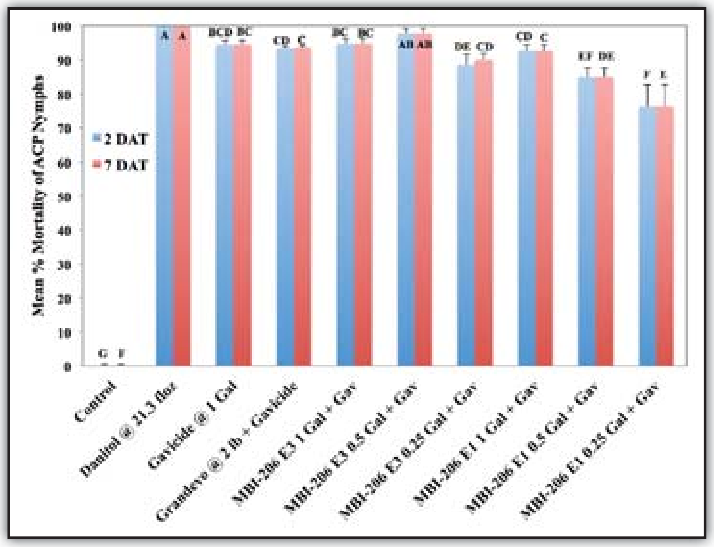

*Figure 4. Mean percent mortality of ACP nymphs on citrus treated with three different rates of two experimental formulations of MBI-206. Gavicide Green was used as an adjuvant at 1 gallon/100 gallons, both as a stand-alone application and at the same rate in combination with all treatment applications except Danitol.**

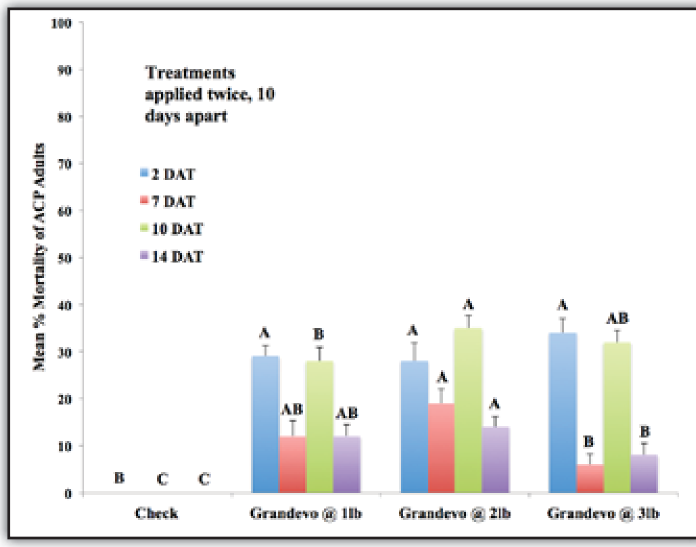

*Figure 6. Mean percent mortality of ACP adults placed on citrus treated with varying rates of Grandevo. Two applications were made ten days apart. New insects were placed on treated plants once the applications were dry. No adjuvants were used in this trial.**

EFFICACY AGAINST ADULTS

Figure 5 shows the mortality caused by the most commonly-used organic, botanical insecticides, Pyganic (pyrethrum from chrysanthemums) and two neem-based products, Azera and Azaguard. All three botanicals provided good initial control (80-95 percent) two days after treatment (2 DAT), but efficacy fell rapidly over time. The products were only effective for approximately seven days before falling to <60 percent mortality at day 14. Orocit, the surfactant used in the trial, provided about 60 percent mortality to adult ACP 2 DAT, and efficacy fell rapidly over time.

In the trial depicted in ***Figure 6***, Grandevo was applied twice ten days apart against adult ACP. Grandevo exhibited low efficacy (< 40 percent mortality) against adults at any of the three rates tested, and efficacy did not improve with time.

The efficacy of two narrow range oils, 415 and 439, tested at increasing rates between 0.25 to 4 gallons/100 gallons against ACP adults, can be found in ***Figures 7 and 8*** respectively. First, there is a clear rate response, which means the more you use, the more effective it is (4 gallons/100 gallons of Gavicide Green or TriTek caused >90 percent mortality). Efficacy, however, dropped off rather quickly with time and lasted less than 14 days. It appeared that TriTek, which is a heavier oil (438.5), was somewhat more effective than the Gavicide Green (415).

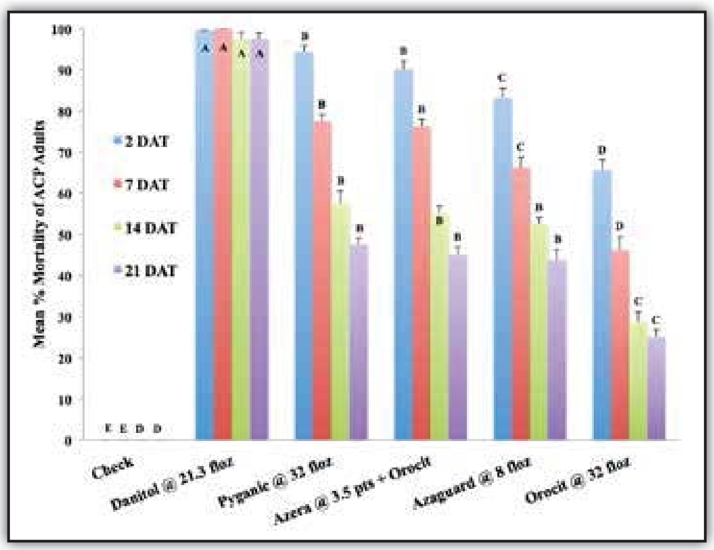

*Figure 5. Mean percent mortality of ACP adults placed on pyrethrum and neem-treated citrus over time. Orocit was used as an adjuvant at 32 fluid oz./100 gallons, both as a stand-alone application and at the same rate in combination with selected treatment applications.**

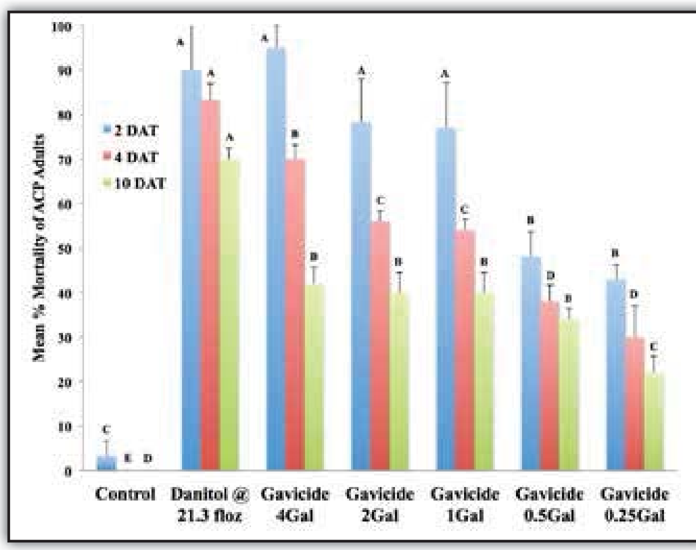

*Figure 7. Mean percent mortality of ACP adults placed on plants treated with Gavicide, a narrow range 415 oil. Gavicide was not added to the Danitol treatment application. New insects were placed on treated plants on a weekly basis.**

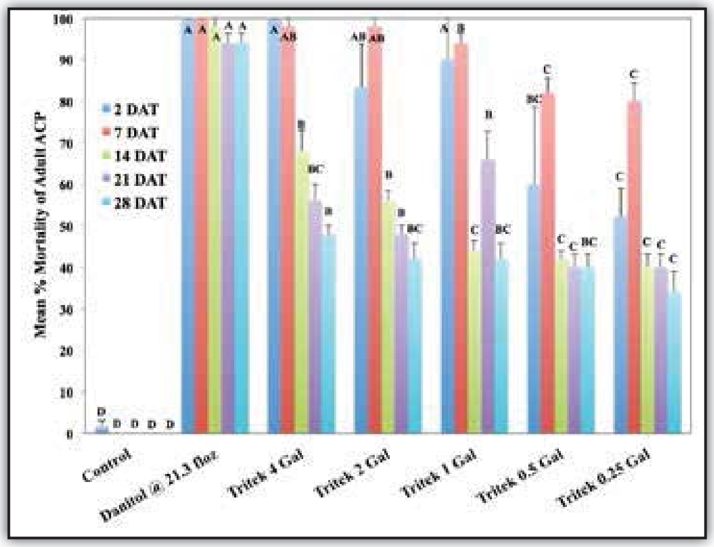

*Figure 8. Mean percent mortality of ACP adults placed on plants treated with narrow range 438.5 oil. TriTek was not added to the Danitol treatment application. New insects were placed on treated plants on a weekly basis.**

Figure 9 shows additional tests of various entomopathogenic fungi, a bacterial byproduct and diatomaceous earth (DE) against ACP adults. With good contact, these products caused 80-90 percent mortality initially (2 DAT); but as with the other products described above, efficacy against adults dropped off very rapidly. These products do not appear to be persistent against adult ACP.

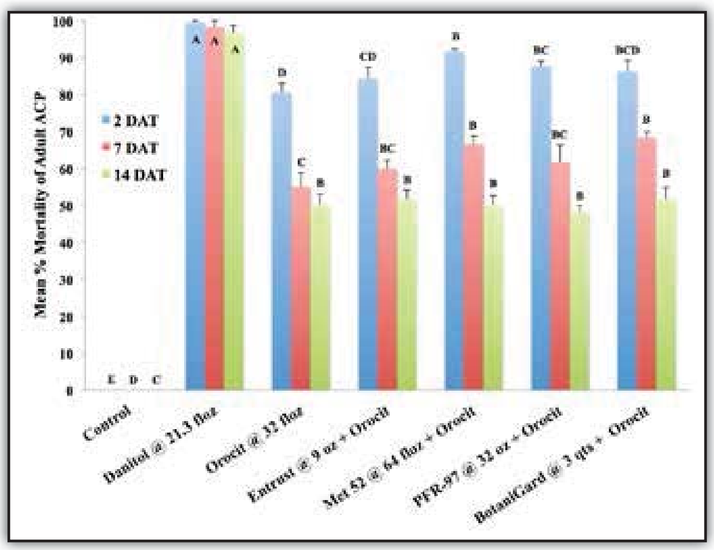

*Figure 9. Mean percent mortality of ACP adults placed on plants treated with various biological insecticides. New insects were placed on treated plants on a weekly basis. Orocit was used as an adjuvant at 32 fluid oz./100 gallons, both as a stand-alone application and at the same rate in combination with selected treatment applications.**

**Data were analyzed using ANOVA and means were separated by Fisher's Least Significant Difference test (P = 0.05). The same colored bars with the same letters are not significantly different.*

CONCLUSIONS

Two important general conclusions can be made from our work so far. First, if direct contact is made, nymphs are fairly easy to kill, but adult ACP are difficult to kill with the registered organic insecticides tested. Second, the organic pesticides tested are clearly short lived on plant surfaces, even under our best-case scenario in greenhouse trials.

This means that in order to control immigrating populations, repeated applications will be necessary to maintain low levels of psyllids in commercial groves. Fortunately, we show that there are products that can achieve 80-95 percent short-term kill. Some of the organic products may be subject to high levels of photo-degradation, and we note again that it is important this work be validated under field conditions. The manufacturers of the biologicals recommend the use of a surfactant with their products, and this is likely to be more important for outdoor field trees than on greenhouse plants.

It appears that the oils or surfactants used in these trials can be as effective as some of the insecticides we tested, especially against ACP nymphs. It also appears that Pyganic, neem-based products and narrow-range oils are effective organic pesticide options against ACP adults. However, in our trials in the greenhouse, these products did not show much persistence. We expect that they would be even less persistent in the field. For example, Pyganic is known to break down quickly in sunlight. Our work also shows that as the rate and distillation point of oil increases, greater mortality of ACP nymphs is observed. Oils need to be used with care, however, to avoid phytotoxicity, especially during warm periods or going into winter. Again, it will be important to validate our work in field trials. Also, it will be important to test the idea that lower rates used more often can achieve the same level of control compared to a single application of a high rate.

We tested several new experimental products. The new product, MBI-206, shows promise causing high levels of mortality of ACP nymphs. A consistent mortality impact on the nymphal population will most certainly have an overall impact on the local ACP population.

As we face new invasive pests becoming established in the U.S., there is an ever-increasing need for effective and persistent organic pesticide alternatives in agriculture, especially if we are to continue to provide locally grown organic fruits and vegetables. The days of a single spray to control a pest on a calendar basis are over, and research projects like the one described here will be in greater demand as the challenges of controlling multiple pests and newly invasive pests continue to grow.

Initially, our intention was to find an effective organic pesticide to be used by organic citrus growers to control ACP. This, however, is only one of the objectives that we have for research at the Chula Vista site. Others include the verification of systemic insecticide thresholds for effective control of ACP nymphs, developing baseline data for ACP susceptibility to key pesticides before pesticide use against psyllids is widespread in California. As is common in scientific research, we will conduct other research studies on an opportunistic basis and in response to our results.

Acknowledgements

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James Bethke is the UC Cooperative Extension Floriculture and Nursery Farm Advisor for San Diego and Riverside counties; Marianne Whitehead is an Agricultural Technician for UC Cooperative Extension, San Diego County; Joseph Morse, Ph.D., is Professor of Entomology in the Department of Entomology, UC Riverside; Frank Byrne, Ph.D., is an Associate Researcher in the Department of Entomology, UC Riverside; Elizabeth Grafton-Cardwell, Ph.D., is an Extension Specialist at UC Riverside and Director of the Lindcove Research & Extension Center; Kris Godfrey, Ph.D., is a Project Scientist at the Contained Research Facility at UC Davis; and Mark Hoddle, Ph.D., is an Extension Specialist at UC Riverside.

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