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Final Report: Submitted 20 October 2016

Project contract number: CAC 65116

Total funds approved: $276,591 (2012-13: $89,786; 2013-14: $92,173; 2014-15: $47,316; 2015-16: $47,316)

Project title: Sustained Chemical Control of Avocado Arthropod Pests

Project start date: November 1, 2012

Project end date: October 31, 2016 (early termination by the CAC based on 8-18-16 letter; agreed on termination date had been 10-31-20, i.e. for 4 additional years, 2016-2020)

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Executive Summary

- We report below on research done 2012-2016 on avocado thrips and persea mite under CAC 65116. Note, however, that over the period Oct. 2014 – Mar. 2015 all Kuroshio shot hole borer research was funded from 65116 (i.e. until CAC 65123 was funded 3-23-15 – all SHB research is reported in the final report for that project as both projects terminated at the same time, i.e. 10-31-16).

- As background, based largely on past CAC funded research, the following products were registered for use on California avocados, in part due to research trials we conducted in cooperation with the relevant chemical company:
    - Veratran D avocado thrips [AT] Special Local Needs approved 2/97 (due to effort largely by Phil Phillips); registered in 1998
    - Abamectin Section 18 Emergency Exemption approved for use only against AT for 6 years, 1999-2004; Registered for use against both AT and persea mite [PM] 3-2-05
    - Delegate registered in 2007 for AT
    - Danitol in 2010 for both AT and PM
    - Envidor for PM in 2010
    - Movento for AT May, 2011
    - Zeal for PM May, 2012
    - Miteus (FujiMite) for PM July, 2013

    - Note that all materials are in different classes of chemistry from one another and from abamectin except Movento and Envidor are both in IRAC class 23 (IRAC = Insecticide Resistance Action Committee) – this was not by chance but instead, by design. We intentionally chose pesticides with different chemistries and with good efficacy so that they could be used in rotations that would minimize the chances for the evolution of pesticide resistance.

- We are extremely concerned about abamectin resistance developing in populations of one or both of avocado thrips and persea mite. We have "gotten the word" out regarding the danger of resistance but to date, we are still quite concerned that most PCAs are overusing abamectin and not trialing/ using the above alternatives to the degree they should (i.e. practicing proactive resistance management). If abamectin resistance develops, the concern is that the next most popular one or two materials would then be overused, also leading to the eventual loss of those products to resistance. For example, we believe fenpropathrin (Danitol) would fail due to resistance quite quickly.

- We list below what we consider the key accomplishments of this project over 2012-2016.
    - We have further refined methods for evaluating field populations of avocado thrips and persea mite for resistance to abamectin, spinetoram, and fenprothrin. It appears to us that abamectin resistance is evolving with both avocado thrips and persea mite but it has not proceeded far enough such that field failures are common – instead, one sees a shortening of field efficacy (for example, spring avocado thrips treatments used to control persea mite through the summer and fall in the Ventura region; most years it no longer does and a second treatment with a miticide is needed). Unfortunately, PCAs and growers often retreat with abamectin (sometimes with an alternative) when thrips or persea mite control is inadequate with an initial treatment. While the lack of efficacy of the first application

may be due to abiotic and/or operational problems, the possibility that resistance could be involved is often not given enough consideration.

  - We have developed working biochemical assays that will detect esterase- and insensitive AChE-based resistance mechanisms in avocado thrips. These are valuable tools that will enable greater numbers of groves to be monitored in a more timely manner, particularly when there are insufficient numbers of insects available for full-scale bioassays.
  - We have defined a discriminating bioassay dose of abamectin for future monitoring of resistance in field populations; we also propose tentative rates for both spinetoram (Delegate) and fenpropathrin (Danitol). Rates for the latter two materials rely on 4 field assays done in 2011 and gathering additional data from susceptible field sites would be prudent in the future. We had intended to do this work gathering more abamectin field data/ developing more sensitive methods of detecting the beginnings of abamectin resistance but with the early termination of this project, this was not done.
  - We have evaluated the use of Movento against avocado thrips (good potential for activity if used optimally) and persea mite (had great hopes it would be active based on activity on other crops against other mite species – Trial 3 in 2013 shows this is not likely the case).
  - We continue to work on developing an ELISA system for use with Movento. Movento (spirotetramat) is an unusual product that might be of great use against a number of avocado pests. Spirotetramat is NOT active against insects or mites in its parent form; it is sprayed as a foliar material, taken up by the plant and converted to a highly systemic material, spirotetramat-enol, which IS highly active against a range of pest species. The enol moves to the roots and then is redistributed into the plant – for this reason, we believe it might be ideal for application by helicopter with low water volumes (ground spray trials on citrus show better uptake and movement throughout the plant with low volume applications). However, to optimize the use of this material, we need a means of measuring leavels of the active ingredient (the enol) that is not confounded by picking up the parent (spirotetramat itself). That is what we are attempting to develop (still in progress, as detailed in the report below).
  - We have developed a working ELISA system for a second new product, Sivanto. Sivanto is also sprayed and moves systemically in the plant but whereas preliminary lab trials showed it had activity against avocado thrips, field trials (Trials 1, 3 in 2013; Trial 2 in 2014) showed that it was not persistent enough or present at high enough levels in the types of young leaves that avocado thrips prefer to feed on. Due largely to our CAC research, however, Sivanto will be registered for use on avocados in California (it was initially planned that Bayer would not include it in the final submission to EPA, i.e. the opportunity for later registration would be lost) and it is quite likely it may be of use to CA avocado growers in the future, e.g., for control of pests such as the avocado gall psyllid (*Trioza* sp.) predicted by M.S. Hoddle as a likely future CA invader.
  - We responded in a timely manner to requests for information from the CAC, CDFA, PCAs, growers, and others as listed in the 4th primary bullet above.

- Over the period 2012-2016, we have responded to industry and pest control advisor requests for information and targeted research. We believe it is partially due to our research on chemical control of avocado thrips and persea mite that the industry has been able to deal with these two pests effectively and without excessive damage most (but not all) years over the recent past. We also made ourselves available to the CAC, CDFA, PCAs, growers, and others when other issues arose, i.e. review of Pest Risk Analyses, helping to deal with export issues, and requests for advice on various pest management problems. Some of the issues dealt with include the following.

- Provided comments to Dixon/ Bellamore 12-27-12 on a Columbian Pest Risk Assessment proposing importation of avocados into the U.S. (significant concerns regarding seed moth, seed and stem weevils, etc.).
- Responded to a PCA "urgent" request 1-4-13 on which pesticides might be used on avocados during bloom for control of avocado thrips.
- Meeting with Gowan 1-13-13 to push for evaluation and registration of fenazaquin (Magister) on California avocados. Gowan agreed to run research trials to evaluate this material against persea mite.
- Worked with MGK Corporation 2-3-14 to evaluate their new organic formulation of Veratran D for use against avocado thrips (MGK purchased rights to this material from Dunhill which had not provided an organic formulation). Our intial trials showed the sabadilla seed grind was not fine enough and resulted in extreme sprayer plugging. This was simple to resolve by finer grinding and they provided the improved product to growers in time for the spring 2014 avocado thrips field season. Our trials showed that the new MGK formulation was at least as effective as the old Dunhill product.
- Responded to PCA request 3-3-13 for advice on addition of oil to Danitol when applied by air and concerns over past worker exposure (dermatitis) problems (see item C5 in the final report for CAC 65108 – 2010 problem). Worked with Valent to determine that their advice is to use a maximum of 1% oil added to low volume Danitol (not a binding restriction based on label language).
- Responded to PCA request 3-10-13 regarding Bayer advisory allowing use of Movento during bloom for control of avocado thrips and its limited impact on pollinators.
- Responded to PCA request 4-4-13 regarding use of Veratran D for control of avocado thrips and its limited impact on pollinators.
- Responded to avocado grower 4-8-13 concerned about several beetles found on his property.
- Responded to PCA request 4-13-13 for information regarding use of Biomite for control of persea mite.
- Responded to request for comments 4-20-14 from Bellamore on the proposed expansion of regions of Mexico shipping avocados to the U.S. (high risk of seed moth, easy to miss the insect via fruit cutting unless late instar larvae are present).
- Responded to PCA request 5-7-13 regarding how to best use Movento on avocados for control of avocado thrips.
- Responded to PCA request 6-1-13 on the impact of dry weather and heat on avocado thrips (looking for an explanation regarding why thrips levels were initially high and then declined precipitously).
- Avocado pest list reviewed for the CAC and comments sent to Melban/ Dixon/ Bellamore 6-25-13 and 1-31-15 (revised questions) in relation to CAC efforts to open the New Zealand market to California avocados and CDFA request to review the combined 2005 and 2013 pests of concern list provided by New Zealand.
- Sent report on two field trials to Bayer 3-3-14 to support a proposed label for Sivanto on California avocados.
- Response to a grower inquiry 4-15-14 on how to deal with persea mite found on young avocado trees received from a nursery and given the nursery had been using abamectin, how he might use registered alternatives to this material to avoid resistance problems.

- Worked with Bayer 8-12-14 to suggest a modification of their Movento label – based on our trials, suggested they remove label language indicating Movento suppresses persea mite populations.
- Multiple emails with a grower starting 8-12-14 about several species of beetles found boring into avocados. Corresponded with CDFA taxonomic experts and determined that these species had been reported in California previously and almost always were associated only with fallen fruit (rarely but occasionally attacked fruit in a tree but only after high levels had built up on fallen fruit).
- Responded to Melban request 10-8-14 for information regarding proposed IR-4 activity on the use of acetamiprid on avocados. Suggested he support the FL registration request due to activity against several pests and potential pests in California.
- Resonded to the CAC 12-6-14 on a request to review an Ecuador Pest Risk Assessment proposing avocado shipments into the U.S. (major concerns dealt with various avocado weevil species).
- Responded to CDFA request 1-18-15 for evaluation of California avocado pest list prepared by China. Conference call with Melban and Spann 1-21-15 to discuss the evaluation. Provided input to Melban 5-7-16 on the final version of the Chinese pest list after consulting with Eskalen, Hoddle, Phillips, and Walker on various issues, and after discussing several key questions with 17 PCAs at an AAIE meeting in Ventura, which fortuitously was held 5-6-16.
- Responded to PCA request 2-9-15 on use of electrostatic sprayers to improve avocado pest management. Consulted with UC Davis ag engineer to provide input regarding the advantages and disadvantages of this approach.
- Worked with the UC Statewide IPM Program 2-15-15 and 3-13-15 to modify UC Pest Management Guidelines (PMG) for Avocados – added Miteus to the list of materials recommended for use against persea mite, Esteem for use against armored scales, revised the pest list of primary concern, uncommon, or rarely managed pests based on PCA input, revised avocado thrips and persea mite listings, etc.; Second round of Avocado PMG review and revision done 5-31-16.
- Multiple emails starting 4-8-15 with an Irvine homeowner concerned about the potential impact of avocado treatments in a commercial grove near his residence (concerned about potential impacts on his children). Situation resolved to the homeowner's satisfaction in our opinion (once we determined what materials had been used and evaluated their safety, he appeared happy).
- Multiple emails starting 5-31-14 with people interested in developing the use of drones for aerial sprays on avocados in California.
- Worked with Bayer 7-1-15 to modify their draft Sivanto label for use on avocados so that it would include early morning helicopter applications. The draft label indicated Sivanto could be used during bloom because of its limited impact on pollinators but specified treatments should be applied late in the afternoon, evening, or at night outside of peak pollinator foraging periods. Bayer agreed to add early morning applications on avocados based on helicopter pilots indicating that was the ideal time to treat avocados (less windy).
- Emails with R&D Director at Brooks Tropical LLC in Florida 10-8-15 regarding joint FL-CA efforts on registration of new pesticides for use on avocados. Suggested he coordinate with Melban and Spann, which he did.
- Advice provided to Ben Faber 3-2-16 on what appeared to be a fly larva found in an avocado canker in a commercial grove.
- Advice to a grower 3-10-16 on beetle grubs found feeding on avocado roots in his grove.

- Provided an evaluation 5-9-16 to Melban, Spann, and Bellamore of a draft Pest Risk Analysis for importation of avocados from South Africa into the U.S.
- Email to Bellamore 6-10-16 about avocado weevils intercepted on mangos shipped to California from Guam (suggestions regarding the need for more research on avocado weevils).
- Several emails starting 9-3-16 with an avocado grower having problems with whiteflies (asked Hoddle to provide identification, which he did, and Morse provided control recommendations).

▪ Morse and Hoddle (2012) and Hoddle and Morse (2013) published comprehensive manuscripts in the California Avocado Society Yearbook detailing the history, biology, and best management practices for avocado thrips and persea mite, respectively. Morse viewed this as the best way to pass on most of what had been done on pest management of these two pests to the industry in a form that is readily accessible.

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Introduction and Background

Discovery and Establishment of Avocado Thrips in California

Avocado thrips (*Scirtothrips perseae* Nakahara [Thysanoptera: Thripidae]) was first discovered in California in June of 1996 when fruit scarring and the presence of a thrips that appeared similar to citrus thrips (*Scirtothrips citri* Moulton) were noticed on avocados. Pest control advisor (PCA) Charlie Gribble (Biospectrum Inc., Ventura, CA) took samples from an avocado grove near Port Hueneme (Saticoy, Ventura County) to Phil Phillips (Area IPM Advisor, UC Cooperative Extension, Ventura Co.) at about the same time that PCA Joe Barcinas (Entomological Services Inc., Corona, CA) took samples from a grove at the Irvine Ranch (Irvine, Orange County) to Joseph Morse at UC Riverside. Citrus thrips (*Scirtothrips citri* [Moulton]), which is very similar in appearance to the thrips attacking avocados, has rarely been observed on avocados. Thus, the presence of this thrips on avocados causing both leaf and fruit scarring was surprising. Phillips sent thrips samples to the California Department of Food and Agriculture (CDFA) Plant Pest Diagnostics Center (Sacramento, CA) and they were determined to be an unknown species of thrips similar to but different from citrus thrips. In 1997, Steve Nakahara (USDA Systematic Entomology Laboratory, Beltsville, MD) gave this insect a name, calling it avocado thrips, *Scirtothrips perseae* (Nakahara 1997).

It is interesting that prior to avocado thrips showing up in California in 1996, there were no published reports of its presence or the damage it causes elsewhere in the world. This lack of information was surprising, as avocado thrips feeding damage is readily observable on young fruit at certain times of the year in areas where this pest is native. This feeding damage by avocado thrips may have been confused with something else, perhaps wind scarring, bacterial/fungal diseases affecting the skin of these fruit, or even feeding damage from other species of thrips known to be present in avocado orchards (e.g., *Frankliniella* spp.). Interestingly, after *S. perseae* was described, reports from Mexico were published that this thrips was present and causing damage to Hass fruit (González Hernández et al. 2000). This entrance into the literature is curious given Mexico is the world's largest producer and exporter of Hass avocados and this pest was apparently unknown until after its discovery in California. Consequently, we think it is very likely there are additional pest species attacking avocados in Mexico that await discovery, but whose identity and presence in Mexico are poorly documented, if at all, which could be unintentionally moved into new areas.

By July of 1997, avocado thrips had spread north to San Luis Obispo County and south to San Diego County (Hoddle et al. 2002b). Heavily infested groves in Ventura County showed 50-80% crop damage in 1997 (Hoddle & Morse 1997, 1998). By May 1998, avocado thrips infested 80% of California avocado acreage (Hoddle et al. 1999) and this reached 95% by 2002 (Hoddle et al. 2002b). An economic analysis by Hoddle et al. (2003a) estimated that thrips feeding in untreated infested avocado groves reduced industry revenues by 12% in 1998 and that short run (i.e., over the time period that the industry adapted to managing this new pest) costs were an estimated $8.65 million per year.

Given the speed with which avocado thrips spread (in part likely due to movement of contaminated picking bins containing leaves) and the type of scarring it caused on fruit, the UC Hansen Trust (July, 1997) and the California Avocado Commission (CAC) (Nov., 1997) funded a team of researchers (Morse, Hoddle, Phillips, and Faber) to study various aspects of avocado thrips biology: where the insect originated, whether biological control could be implemented, what pesticides were effective in chemical control, how to best monitor thrips levels, and how treatment decisions should be made. In addition, PCAs were critical to learning how to deal with this new pest, as were many growers who allowed research trials to be conducted in their avocado groves. Steve Peirce (CAC Field Coordinator) was instrumental to the avocado thrips project in coordinating research done at UC Riverside by Hoddle and

Morse with that done by UC Cooperative Extension in Ventura County by Phillips, Faber, John Rogers, and SRAs Lynn Wunderlich, Wee Yee, and Eve Oevering, who were hired (sequentially) to work on the project by helping to arrange field study sites, and in disseminating information about avocado thrips research to growers and PCAs. A second pivotal decision the CAC made was to invite Laurence Mound, a world-renowned authority on thrips identification, biology, and ecology to visit southern California in June 1997, tour field sites infested with avocado thrips, and give us his views on the situation. We clearly remember sitting down with Mound on June 23, his looking at slide mounted thrips specimens Hoddle had prepared, and telling us only five minutes later that this thrips likely originated from Mexico and/or Central America based on specimens he had viewed in past years. This helped set the stage for foreign exploration to determine the likely area of origin of avocado thrips and the potential for classical biological control (i.e., returning to the area of origin to search for natural enemies that co-evolved over millennia with the introduced pest species). Mound was especially critical of tree management in California, and made it clear in meetings where he was presenting his assessment of the avocado thrips situation, that the vast majority of avocado trees in commercial orchards were simply too tall. He viewed this size problem as greatly hampering pest monitoring, pesticide applications, and fruit harvesting, especially on steep hillsides in San Diego County.

Life Cycle

Avocado thrips has a life cycle typical of thrips in the Suborder Terebrantia; females use their ovipositor to lay eggs individually inside suitable plant material; for avocado thrips, young leaves and small fruit are highly preferred oviposition substrates. Following egg hatch, there are two larval instars, the first and second instar, that are mobile and whose feeding activities cause economic damage to fruit (such feeding can also result in significant scarring to the undersides of leaves). Following the larval stage, there are two non-feeding and non-motile stages, the propupa and pupa. It is during these two "pupal" stages that larvae undergo metamorphosis into the winged adult form. When ready to pupate, second instar larvae enter a walking phase and they will often look for pupation sites in cracks and crevices in twigs or bark, and sometimes pupation may occur inside the webbed nests of persea mite, another invasive avocado pest. Combined field and laboratory studies have indicated that approximately 77% of *S. perseae* larvae drop from avocado trees to pupate in the upper 2" of leaf duff beneath the tree canopy before emerging as winged adults that fly back up into the canopy to commence feeding and reproduction.

Damage

Numerous field surveys have clearly indicated that young fruit are vulnerable to avocado thrips feeding damage. As young foliage from the spring growth hardens in late May and early June during or after fruit set, adult female avocado thrips move from foliage to oviposit into young fruit. It is the feeding by emerged larvae that results in damage to the skin of developing fruit. Laboratory observation of field-collected fruit indicated that females lay eggs in fruit ranging from 0.16 - 3.0" in length. The majority of larvae (> 95%) emerged from fruit 0.6 - 2.5" in length. Once fruit exceed 2" in length, avocado thrips are found primarily on young leaves (Hoddle 2002b). These observations strongly suggest that fruit < 2" in length are quite vulnerable to attack by avocado thrips. This relationship between fruit size and attack by avocado thrips was investigated in commercial avocado orchards by Wee Yee in Ventura who was working with Phil Phillips and Ben Faber at the time (Yee et al. 2001a).

Field biology studies conducted over three years at three sites with different temperatures in Ventura and Santa Barbara counties indicated that fruit may be most susceptible to damage over a two-week growing period just after fruit set, when fruit are 0.2 - 0.6" in length (Yee et al. 2001a). These studies also indicated that when approximately 3-5 thrips were consistently found per leaf during fruit set, feeding caused 6-15% economic scarring damage on fruit. Furthermore, young fruit 0.5" long or less infested with an average of 0.5-1.5 larvae per fruit in May and June resulted in 22-51% economic scarring. Over all years and sites, thrips were generally more abundant on young leaves than on fruit from early to mid June when fruit were setting. When leaves aged and hardened from late June through August (depending on region), equal or higher numbers of thrips were generally found on fruit, although overall numbers of thrips declined during this period with increasing summer temperatures (Yee et al. 2003). These results suggest that thrips numbers on leaves prior to or during fruit set may be used to predict scarring damage on fruit, and that the economic injury level may be less than 5 larvae/leaf during this time (Yee et al. 2001a).

Specific Rationale for this Research Project

The options available to growers for the control of avocado thrips are limited. Biological control is generally not effective at reducing thrips populations to levels below economic thresholds (which vary from PCA to PCA), and growers have therefore, become reliant on the use of chemical pesticides to achieve satisfactory pest control. Abamectin is the most frequently used insecticide for the control of avocado thrips and persea mite, and the dependency of growers and pest management professionals on this product in the past has largely stemmed from the lack of availability of effective alternatives. Multiple treatments within a season elevate the likelihood that either pest could develop resistance. Humeres and Morse (2005) documented that persea mite susceptibility to abamectin dropped significantly below baseline levels in a commercial avocado grove that had been treated with abamectin seven times over four years (4 spring thrips treatments, 3 fall persea mite sprays). Unfortunately, growers and PCAs throughout the industry have become dependent on the use of abamectin for the management of both pests despite the availability now of alternative chemistries that have been shown by UC researchers to be highly effective. There are significant challenges associated with trying to convince growers and PCAs to change from their current reliance on the use of abamectin to using the alternatives. First, they feel secure in relying on abamectin and are confident that it will result in excellent control, even when applied using the challenging conditions of helicopter application (Yee et al, 2001a); second, abamectin is relatively selective and does not cause pest upsets because it is relatively “soft” on natural enemies; and third, because abamectin has gone off patent, there are a number of generic products now registered for use on avocados. Lower-priced products increase the likelihood that growers will favor their use over the alternatives, and potentially use them as an insurance treatment when pests are present, even if levels are below economic thresholds.

As long ago as 2004, UC researchers and the California Avocado Commission (CAC) were concerned with the dependency of the industry on abamectin. Realizing the potential for resistance evolution, they coordinated efforts with the federal IR-4 program and several chemical companies to evaluate and register alternatives to abamectin. This lead to the registration of several effective products: Delegate (2007), Danitol (2010) and Movento (2011) for avocado thrips management, and Envidor (2010), Zeal (2012) and Miteus (2013) for persea mite. The registrations met three important criteria: (1) efficacy against the target pests, (2) pest-specific products differed in mode of action, thereby minimizing the potential for cross resistance (IRAC, 2012); and (3) where there was a commonality of modes of action in products registered for different pests (e.g., Movento-thrips and Envidor-persea mite), there would be within-season separation of their use, thereby partially mitigating the threat of cross-resistance.

This project was intended largely as a service to the California avocado industry. Growers have been/ are overusing abamectin, largely because it is so inexpensive and they are comfortable with its strengths and weaknesses (Morse and Hoddle 2012, Hoddle and Morse 2013). If this continues, abamectin resistance will develop to the point where this material is of little use. There is then the danger that the next most popular material(s) will also be overused, eventually resulting in resistance to these products. It takes significant time to develop new pest control materials. A second avocado thrips material that would likely fail quickly if overused is fenpropathrin (Danitol) – we have seen this clearly from research with citrus thrips. A significant problem is that the two most effective persea mite control materials are abamectin and fenpropathrin – as avocado thrips resistance develops to these two products, it is likely that persea mite resistance will also develop (before, at the same time, or later is not clear).

For a number of years, we have used citrus thrips (*Scirtothrips citri*) as a model for pesticide efficacy research with avocado thrips (*Scirtothrips perseae*), i.e. substantial information was already available regarding citrus thrips chemical control and most of that experience has translated well to the avocado thrips situation. We strongly believe the same is true with resistance – i.e. what has happened with citrus thrips **will happen** with avocado thrips unless growers and PCAs change their practices. Morse has personally experienced 4 different "cycles" of resistance with citrus thrips populations on citrus (summarized in Morse & Grafton-Cardwell 2012). These include resistance appearing against dimethoate in 1981 (heavy use started in 1969; Morse & Brawner 1986), Carzol in 1986 (1980; Immaraju et al. 1989), Baythroid in 1996 (1991; Morse & Schweizer 1996), and the beginnings of Delegate resistance in 2011 (2007; but the related Success was the major pesticide used against citrus thrips starting in 1998; Morse & Grafton-Cardwell 2012). The pattern in each case has been disturbingly similar. Because relatively few effective products were available for rotation (or growers chose not to use them to a significant degree), the listed pesticide was the main material used each year for citrus thrips control.

Based on experience with many pests on several different commodities, a real problem with pesticide resistance is that once it clearly appears, it is difficult to recover pest baseline susceptibility to that entire class of chemistry. In the very early stages of resistance (loss of susceptibility, or tolerance, are perhaps better terms), resistance genes are typically non-adaptive and if the selection pressure is relaxed, evolution decreases the frequency of those genes to very low levels (i.e. the population returns to baseline susceptibility levels due to selection against the R genes). Progressively, as resistance evolves, this reversion is less pronounced (there is selection for "background" genes, which make resistance genes less detrimental in the absence of selection pressure).

Abamectin has been the major pesticide used for control of avocado thrips since 1999. Several PCAs have reported less effective thrips control with abamectin starting in 2010. However, some growers and PCAs are not convinced that abamectin resistance evolution is in progress and refuse to use alternatives until this is clearly demonstrated. On the other hand, by the time we have clear field failures, it will likely be too late to recover susceptibility to abamectin, even with several years of abstinence.

We believe it is essential that we augment our resistance monitoring efforts by developing biochemical and/or molecular methods to evaluate avocado thrips susceptibility to abamectin and other key pesticides so that we can present quantitative proof of what we are positive is happening, i.e. reliance on products like abamectin will lead to a decline in susceptibility.

Concurrent with this, we need to learn how to use the alternative chemistries more effectively. One product that shows real potential is spirotetramat (Movento). However, the formulation that is applied has no efficacy; it must be taken up by the plant and converted to the active spirotetramat enol to be effective. We struggled for several years trying to obtain good plant uptake on avocados until field trials in 2010-11 showed that this could be done effectively using bloom applications (this product has minimal impact on honey bees and Bayer's label allows applications during bloom). To really make progress in understanding how to best use this product, we must have a means of measuring levels of the toxic enol inside the plant. Bioassays are problematic – it takes 7-12 days for second instar avocado thrips to be killed due to the mode of action of the chemical. Key questions are what surfactants would best assist in uptake, what is the best timing for treatments during bloom or when flush is present, and because the product is highly systemic, how effective are low volume helicopter applications (perhaps the savings in water volume would somewhat offset the higher cost of this product in comparison with abamectin – field trials would answer this question)?

<u>Research Objectives (as stated in CAC-65116 Amendment 1 agreed on between the CAC and the Regents of the University of California effective 29 January 2015)</u>

- Evaluate new chemistries for the control of avocado pests and develop efficacy data for use in product registration efforts.

- Develop sensitive biochemical/molecular methods for measuring avocado thrips resistance to insecticides, and alert growers to current resistance levels in thrips populations.

- Develop an ELISA method to assay for spirotetramat enol in plant tissue.

- Conduct field trials demonstrating the efficacy of abamectin alternatives for the control of persea mite and avocado thrips, and evaluate the gallonage necessary for effective control by helicopter application.

- Continue to develop and evaluate trunk injection and trunk sprays of systemic chemicals for the control of a variety of avocado pests.

- Determine the impact of new pesticides on the natural enemies important to the suppression of secondary avocado pests.

In order to deliver on these objectives, a series of milestones were developed during discussions between the CAC and UCR researchers Morse and Byrne. Specific dates were established for each milestone and upon completion, activity reports were submitted to show progress. However, an e-mail dated 8-18-16 from the CAC Research Director Tim Spann indicated that this project would be terminated 4 years earlier than anticipated. With no advance notice we have not been able to complete all objectives to the degree we would have otherwise. All milestones to date have been completed, and we hope that as funds become available in the future, the CAC will prioritize some of the objectives we were unable to address due to the early termination.

We list below the major research that was conducted each of the 4 years of this project, 2012-13 through 2015-16 (project terminated by the CAC as of 10-31-16).

<u>A. 2012-13 RESEARCH (Year 1 of the project)</u>

<u>A1. Year 1 Summary:</u> This project started on 11-1-12. By the end of Year 1 (activity report filed 10-15-13), we had made excellent progress. Three spring 2013 avocado thrips field trials were put on. We obtained interesting data from all 3 studies. We made good progress in developing an ELISA system for Movento and rapid progress on a system for Sivanto. Unfortunately, the results from an avocado thrips trial in Ventura were disappointing, as it did not appear that Sivanto had as much avocado thrips activity as limited previous data had suggested. Also, although Movento appeared very effective against avocado thrips, it did not have the persea mite efficacy we had hoped for. Evaluations of esterase and cytochrome P450 activities were completed as planned. It was possible to measure esterase activity in a fraction of a single thrips adult; as a major cause of pyrethroid resistance, modified esterases, and their associated changes in activity, could potentially be used as a marker for pyrethroid resistance. This would be a very cheap tool for detecting resistance in field populations. It was not possible to quantify cytochrome P450 in an individual thrips due to very low levels of activity (typical of other thrips species also). We recommend the development of a PCR-based assay for this enzyme system. An assay for acetylcholinesterase (AChE) insensitivity was also developed for avocado thrips. The colorimetric assay measures the biochemical response of thrips AChE to the OP paraoxon. Although not used for field control, paraoxon is an ideal OP for use in diagnostic testing, and when insensitivity is detected at the biochemical level, there is usually an associated change in toxicological response.

A2. Year 1 Milestone Table

Milestone	Activities for Year 1
1a	• Initial evaluation of enzyme assays for thrips resistance mechanisms completed
1b	• Year 1 pesticide efficacy and/or application methodology trial(s) established
2a	• Initial research on the development of an ELISA test method for spirotetramat enol completed
2b	• Year 1 evaluation of pesticide efficacy and/or application methodology completed

A3. Summary of Year 1 Milestone Accomplishments (as of 10-15-13)

A3.1 Milestone 1a -- Initial evaluation of enzyme assays for thrips resistance mechanisms

Introduction

As the first step in doing resistance work with avocado thrips, we evaluated 3 enzyme systems that could be potentially activated/enhanced in resistant thrips. The purpose of the evaluations was to determine whether the specific enzyme systems could be detected in an individual insect, and therefore would be amenable to use in field monitoring.

Materials and Methods

Esterases. Esterase activity was determined in individual thrips using 1-naphthyl acetate as the substrate. Esterases cleave the 1-naphthyl ester, releasing 1-naphthol, which can then be quantified by the use of an azo dye such as Fast Blue B salt. Mass homogenates of adult thrips were prepared in 0.1 M phosphate buffer, pH 7.5, containing 0.1% Triton X-100. Each homogenate was incubated directly with 0.3 mM 1-naphthyl acetate for 30 minutes at room temperature (25 C), after which the reaction was terminated by the addition of DBLS (a solution of Fast Blue B salt and lauryl sulphate). After a further 15 minutes, the absorbance (A620 nm) was measured. The activities in different amounts of thrips homogenates were compared to determine the amount of enzyme required for an effective field assay.

Cytochrome P_{450}. Based on a literature review, the cytochrome P_{450} activity was the most likely system to signal changes in an arthropod's response to insecticides, and we believed that it would be the prime source of abamectin resistance should it arise. We evaluated CytP_{450} activity using 7-ethoxycoumarin (7-EC) as substrate, which is the most common substrate used for resistance studies. The substrate is oxidized to produce 7-hydroxycoumarin (7-HC; umbelliferone), which is fluorescent at 465 nm.

The final assay incubation consisted of enzyme prep, 0.1 M Na phosphate buffer, pH 7.6, 100 µM 7-EC, and 2 mM NADPH. The reaction was allowed to proceed for 30 minutes before it was terminated by the addition of Trizma Base/acetonitrile. The 7-HC was quantified in a spectrofluorimeter with A_{390}/A_{465}. We included rat microsomes and housefly and ACP microsomal preparations for comparison.

Insensitive AChE. We evaluated a colorimetric assay for AChE activity that utilizes the substrate acetylthiocholine iodide (ATChI). A mass homogenate of avocado thrips was prepared in 0.1 M phosphate buffer, pH 7.5 and 0.1% Triton X-100, and then the equivalents of 0.25, 0.5, 0.75 and 1 insects were assayed separately using a concentration of 0.5 mM ATChI. Activity was monitored continuously over a 30-min incubation period.

Results and Discussion

Esterases. Esterase activity was detected in individual thrips. In fact, activity could be measured in as little as 0.1 insect equivalents and there was a linear relationship between insect amount and activity up to 1 insect (Table 1).

Table 1. Total esterase activity in avocado thrips using 1-naphthyl acetate as substrate. The esterase activity was quantified after 30-min incubation and is expressed as absorbance units at 620 nm.

Amount of Thrips (insect equivalents)	Esterase activity (A_{620})
10	3.4
5	3.2
2	1.9
1	1.1
0.5	0.64
0.1**	0.24
0.05	0.18
Control	0.13

**Recommended dilution for field monitoring

Our assay can now be used to monitor esterase levels in thrips populations. The esterase assay will be especially important in monitoring for pyrethroid (e.g. Danitol) resistance, because esterases have been shown in many insect species to be important in the hydrolysis of these insecticides. The assay has the potential to detect the early stages of resistance well before a standard bioassay method, and requires fewer insects to do so. We recommend using 0.1 insect equivalents per assay because this would allow for the detection of elevated levels of esterase. If changes in esterase levels are detected it is still important to confirm that the change in enzyme activity is related to a change in response to an insecticide.

Cytochrome P_{450}. The $CytP_{450}$ assay yielded a result of 28 pmoles 7-HC/mg protein/min for rat purified microsomes. The housefly abdominal prep yielded a result of 15 pmoles/ mg protein/ min. Both the ACP and the thrips preparations did not yield any activity. This result indicates that, at least for the 7-EC substrate, it will not be possible to quantify $CytP_{450}$ activity in an individual avocado thrips due to insufficient levels of protein. This is not a surprising result given the amount of protein required to generate activity with rat microsomes. Mass homeogenates of avocado thrips could be used to provide sufficient protein for the assays, but we feel that is not really the best path forward for studying this mechanism. We recommend a molecular approach – the development of a PCR method could be used to screen individual insects, enabling frequencies of resistant insects within field populations to be determined.

Insensitive AChE. AChE activity was detected in an individual insect at a level of 4.7 mOD/min (Abs 405 nm) (Figure 1). However, this amount of activity is not high enough to set up multiple assays with a single insect that would allow a comparison of AChE activity in the presence and absence of diagnostic concentrations of an organophosphate. Interestingly, the activity was higher in citrus thrips, but it too would be a difficult insect to assay. Although it was not possible to use a single insect for assays, it would be possible to use mass homogenates of insects to generate sufficient activity. Mass homogenates could be used to screen different OPs and carbamates for their ability to inhibit enzyme activity (a measure of efficacy), and, with an appropriate diagnostic concentration of insecticide, could be used to compare populations that differed in their responses to field treatments with these pesticides. Future efforts should also evaluate fluorimetric assays that are generally more sensitive than ATChI, although these assays are also more difficult to execute. With proper calibration, fluorimetric assays could be a promising approach to monitoring for AChE insensitivity.

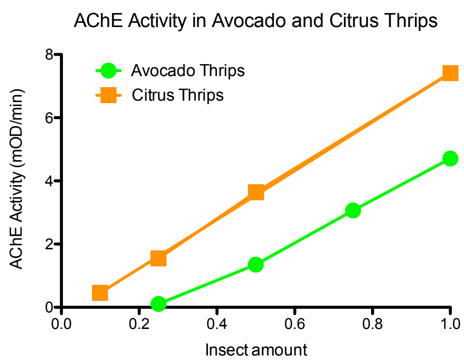


Figure 1. AChE activity in avocado thrips and citrus thrips.

A3.2 Milestone 2a -- Progress in developing ELISA systems for Movento and Sivanto

<u>Introduction</u>

Movento is an unusual product because the material that is applied as a foliar spray (spirotetramat) is inactive against insects and mites and it is only after the material is taken up by the plant and converted to the active spirotetramat enol that it moves systemically in the plant via the phloem. In trials by us on avocado and by others on citrus, Movento showed variable efficacy. In order to figure out what is going on, we embarked on the development of an ELISA system that could quantify the amount of active material in plant tissue. Other reasons to develop a Movento ELISA include: (1) uptake of the material on avocados appears more difficult compared to experience on other crops such as citrus; on avocados uptake appears best either during bloom or possibly when young flush is present; (2) Movento shows very slow activity against avocado thrips (bioassays need to go 7-12 days); and (3) it may be possible to improve Movento uptake using different surfactants but this is quite difficult to evaluate without a method of quantification like ELISA.

Sivanto is a neonicotinoid that is applied as a foliar spray and is systemic. We are very interested in this product because of its minimal impact on honey bees. Bayer representatives have told us that Sivanto will be registered for use DURING BLOOM, even on extremely attractive crops like citrus. With all the current bad press related to pesticide impacts on honey bees, having a material that is effective against avocado thrips (we do not know this for sure yet) and that could be applied during bloom by helicopter would help a great deal to alleviate the spray queues that arise some years both before and after bloom.

There is an usual situation with Sivanto that warrants inclusion in this report. Bayer CropScience has already submitted their Tier 1 registration package for Sivanto to EPA and they plan only a single later Tier 2 submission that is planned for the first quarter of 2015. Bayer says they will not support any registrations after Tier 2 and we presume that without their support, this material could not be registered on other crops, even by going through the IR-4 program (i.e. IR-4 requires company support). Thus, avocados are either in the Tier 2 package or it is likely this material will never be registered on avocados. We came close to this being a reality as the Sivanto registration process has moved more quickly than was expected and for some reason, Bayer thought that an avocado submission of Sivanto to IR-4 had already occurred (there was a major change in Bayer administration and communication on this was not what it could have been). For this reason, we have made Sivanto research a very high priority for 2013 (both ELISA work and field trials). We believe we have a firm commitment that Bayer will do the residue work required to include avocados in the Tier 2 Sivanto submission but positive field data could only enhance this likelihood.

Materials and Methods

Movento. Bayer provided us with two spirotetramat metabolites that were used in the synthesis of the Movento hapten. Due to the conditions of an MTA (material transfer agreement) signed between UC Riverside and Bayer CropScience, we are not permitted to publish the protocol used in the synthesis.

Sivanto. We determined that there was sufficient similarity between the flupyradifurone and imidacloprid molecules that antibodies raised to one chemical might cross-react with the other. We have been using a commercially available imidacloprid ELISA for several years and we tested that system with formulated flupyradifurone provided by Bayer CropScience, since we did not have technical flupyradifurone at the time. For the test, we incubated flupyradifurone at concentrations of 0.1 – 1,000 ng/ml (ppb) using the standard protocol we developed for the imidacloprid ELISA.

Results and Discussion

Movento. After well over a year of time and effort, we finally were able to develop a Material Transfer Agreement (MTA) between UC Riverside, Bayer CropScience, and the private antibody lab. We received two metabolites of spirotetramat that Dr Jocelyn Millar's group at UCR has been working with to develop a synthesis pathway for the hapten. They have synthesized a compound that is awaiting NMR identification. The latter will confirm if the linker group (needed to attach the hapten to the carrier protein) is located at the optimal position on the metabolite. If this is the case, then we will be able to move on to the next phase of the development process – conjugating the hapten to the protein and injecting the rabbits. Anticipating this, we already have a signed MTA with Sargent Laboratories that are licensed to do this work.

Sivanto. The ELISA we use for imidacloprid cross-reacts with the Sivanto molecule (Figure 2). We were able to confirm the cross-reactivity using formulated Sivanto and found that the imidacloprid ELISA would be adequate for our work with a detection range of 3 – 100 ppb.

After we had completed this work for the 2013 report, we received the technical material from the project manager (Amanda Beaudoin). We used the technical to quantify more accurately the levels of cross-reactivity between the two molecules and then converted all our Sivanto data (expressed as imidacloprid equivalents because we used imidacloprid calibrators to generate the standard curve for the ELISA) into meaningful flupyradifurone units.

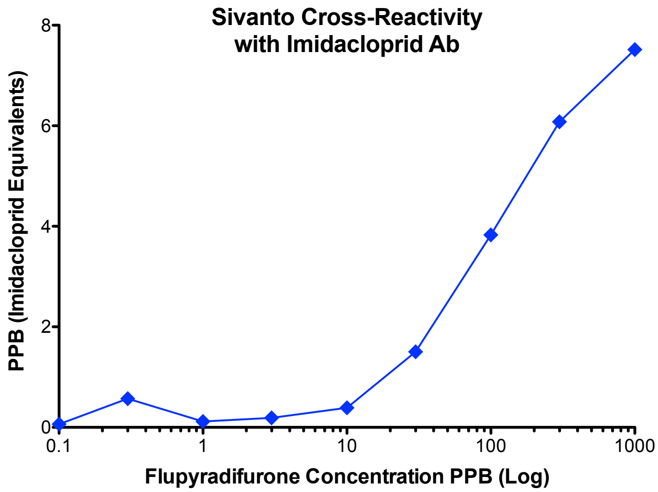


Figure 2. Cross-reactivity test for fupyradifurone with imidacloprid ELISA. Normal imidacloprid ELISA conditions were used and flupyradifurone was included as a test substance at different concentrations to test for reactivity. Flupyradifurone began to react with the imidacloprid antibody at concentrations aboce 10 ppb (ng per ml).

A3.3 Milestones 1b and 2b -- Year 1 pesticide efficacy and/or application methodology trials

<u>Introduction</u>
3 spring 2013 avocado thrips field trials were established and completed. During Trial 3, there were good populations of persea mite available that permitted us to conduct evaluations against both arthropod pests.

We used the newly optimized Sivanto ELISA to determine levels of flupyradifurone (active ingredient [AI] in Sivanto) in leaf tissues sampled from treated avocado trees. We plan to conduct similar evaluations with spirotetramat-enol (AI resulting from Movento application), for which we are currently developing an ELISA. An important objective on avocados is to determine when to best time insecticide treatments in relation to bloom, in order to minimize fruit scarring from avocado thrips feeding, and the ELISAs will be a valuable tool for quantifying residues over time.

<u>Materials and Methods</u>
Sivanto Bioassay. In order to determine what level of Sivanto present in leaves will likely result in effective avocado thrips control, we ran a laboratory bioassay using second instar avocado thrips collected from a field site in Fallbrook. Individual fully expanded but tender avocado leaves were picked from an untreated grove on the UCR campus. The leaf petioles were placed in solutions of various concentrations of Sivanto 200SL. After 24 hours, the volume of solution taken up by each leaf was determined and the leaves then placed in Munger cells with 11-19 second instar avocado thrips. After 48 hours exposure, thrips mortality was assessed. The area of each leaf was then measured, and based on the volume of solution taken up (and assuming uniform distribution throughout the leaf), the ng AI/cm^2 was calculated and regressed against thrips mortality using probit analysis.

Spring 2013 Field Trial #1 – Optimizing the Timing of Movento and Sivanto Applications. We conducted a field trial at a commercial avocado grove in Fallbrook to evaluate foliar applications of Sivanto (14 fl oz per acre) and Movento (10 fl oz per acre); spray concentrations were based on per acre dilutions of 750 ml to spray each tree (76 gallons per acre, trees were relatively small; tree spacing of

7.5’ x 15’, 387 trees per acre). The intent was to determine how the persistence of both materials varied depending on the timing of application and status of bloom and flush. The trees were clonal 2 year-old Hass on clonal Dusa rootstock that were planted in April 2011. Each treatment was applied to 8 single-tree replicates using a Stihl 400 backpack mist-blower (setting 3 of 5 on the machine) with 1% NR-415 oil. Treatments were applied at 4 stages during bloom (4 treatment timings x 2 materials = 8 treatments) beginning when flowering had reached the extended inflorescence (candle) stage. At our experimental site, all treatments were applied when there were newly flushing leaves on the trees. The treatment dates and flowering status are summarized in Figure – and were selected with the assistance of Dr. Mary Lu Arpaia. Sivanto was not registered at the time of the study, and was applied, therefore, on a crop destruct basis after filing a UC Appendix I form (similar to an NOI).

Leaf samples were collected regularly (1, 2, 3, 4, 6, 7, 10, 12, 16, 20, and 24 weeks after treatment) from treated trees for residue measurements, beginning at 1 week post-treatment. Sivanto residues were measured using the optimized ELISA. The method for Movento is under development, so the results from these samples will not be available until the Movento ELISA is available. The leaves are being stored at -20C.

Spring 2013 Field Trial #2 – Screening Avocado Thrips Products. The second field study was done in cooperation with a second Fallbrook grower cooperator who was interested in seeing how some of the registered/ pesticides nearing registration would perform against avocado thrips. This was an unusual trial in that 12 treatments (oil alone at 1%, Movento 10 fl oz/a + 1% oil, Entrust SC at 10 fl oz/a + 1% oil, Veratran D 20 lb/a + 10 lb sugar [new formulation from MGK], Delegate 4 oz/a + 1% oil, Danitol 16 fl oz/a, water control, Epi-Mek 20 fl oz/a + 1% oil, Sivanto 14 fl oz/a + 1% oil, Bexar 27 fl oz/a + 1% oil, Closer 8.5 fl oz/a + 1% oil, Azera 3.5 pt/a + 1% oil) were applied to individual pallets of small trees (ca. 5 feet high; 4 replicate pallets per treatment; ca. 36 trees per pallet (Figure 3)) showing a fair amount of flush. Although we provided chemicals and provided oversight on treatments and counts, treatments and counts were done by the grower cooperator. Treatment rates were calculated based on a per acre dilution rate of 300 GPA and treatments were applied with a small backpack sprayer which provided quite thorough coverage of these small trees. Adjacent pallets treated with different products were shielded from drift using plastic sheeting held in place by PVC pipe (Figure 3).

Figure 3. Pallet of small potted trees (36 trees per pallet) being sprayed with 1 of the 12 avocado thrips treatments. Note the plastic sheeting that was used to shield adjacent pallets from drift.

Spring 2013 Field Trial #3 – Impact of Movento and Sivanto on Avocado Thrips and Persea Mite. Our third field trial was conducted in Moorpark with applications applied 13 June 2013. Trial trees were 3-year-old Hass avocados and tree spacing was 12.5 feet down the row, 23.5 feet between rows; thus, there were 148 trees per acre. Tree height was 6-9 feet at the time of treatment; average height was ca. 7.5 feet.

Thirty-five trees were selected for pre-counts on 7 June 2013 based on trees being at least 6 feet in height and having a fair amount of flush leaves present. Five fully expanded flush leaves were selected on each tree by walking around the periphery of the tree and the number of adult avocado thrips, immature avocado thrips (first and second instars combined), and motile persea mites (all stages except eggs) were counted on each leaf. In addition, 30 inside leaves were selected and the number of predaceous mites (mostly *Euseius hibisci*) and predaceous thrips were counted.

Trees were ranked from high to low based on the total number of thrips per leaf (including adults) and because 5 treatments and 6 single-tree replicates were planned, they were separated into 6 blocks of 5 trees. The first block had the trees with the 5 highest pre-counts, the next had the next 5 highest counts, etc. Trees with the 5 lowest counts were not used in the trial. One tree from each block was randomly selected for assignment to each of the 5 treatments. Following initial assignment, a few trees were reassigned to treatments to equalize pre-treatment mean thrips levels per treatment.

Sprays were applied 13 June 2013 using a Stihl SR-400 backpack mist-blower using setting #3 (of 5 on the sprayer). A little less than 0.5 gallons was applied per tree using outside coverage; rates were calculated on the basis of 100 GPA although because of tree size, this amount of spray was not used (instead, ca. 74 gpa). At the time of the spray, fruit were BB size "in jackets" (still covered by the sepals), exposed BB's, pea size, or small olive size and there was no more bloom present; most trees still had some new leaf flush and expanding leaves. Treatments were (1) untreated control; (2) Epi-Mek 0.15EC at 20 fl oz/a + 1% NR-415 oil, which was considered the "standard"; (3) Sivanto 200SL at 14 fl oz/a + 1% NR-415 oil applied on a crop destruct basis; (4) Movento 2 lbs ai/gal at 10 fl oz/a + 1% NR-415 oil; and (5) Movento at the same rate + 0.25% S-200 surfactant (Evonik Goldschmidt Corp., Hopewell, VA; same as Breakthrough S-200 or Widespread Max). Post-treatment counts were taken in the same way as the pre-counts at +6, 13, 19, 28, 40, 54, 68, and 82 days post-treatment.

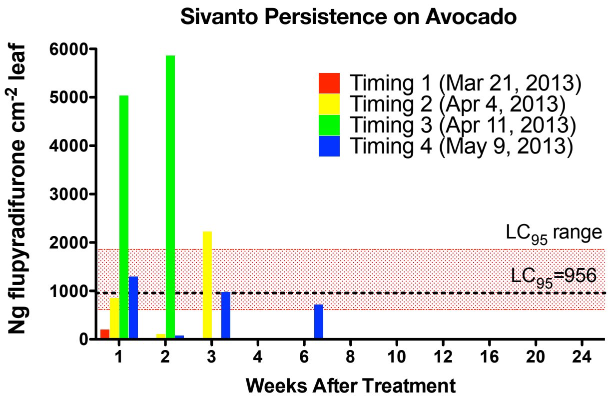
This study was initially intended mainly as an avocado thrips trial to evaluate Sivanto and Movento against the Epi-Mek standard and to see if the S-200 surfactant would improve Movento performance over using 1% NR-415 spray oil. However, we also decided to take persea mite counts to see how Sivanto and Movento would perform against this pest; counts were also taken to evaluate impacts against predaceous mites and thrips.

<u>Results and Discussion</u>

Sivanto bioassay. The Sivanto LC_{50} was 138.5 ng AI/cm^2 of leaf tissue (95% fiducial limits of 103.5-186.6) and the LC_{95} was 956.2 ng AI/cm^2 (956.2-1,856); chi-square probability was strong (0.8293) indicating an excellent regression relationship.

Spring 2013 Field Trial #1 – Optimizing the Timing of Movento and Sivanto Applications. We completed scar counts for both the Movento and Sivanto treatments on August 27 (Table 2 below). We normally consider 10% of the fruit surface area scarred to be economic scarring in a normal year. Although scarring was low throughout the test plot (avocado thrips populations were low in the grove where our experimental trees were situated), it is difficult to attribute the lack of scarring entirely to the Movento and Sivanto treatments. At the site, thrips and persea mite activities increased in late May and the grower applied abamectin to all trees in our grove and the surrounding groves on June 4. At that time, we had already completed our 4 treatment timings, so both the Movento/Sivanto treatments and the abamectin treatment could have contributed to the low incidence of initial scarring. There is some suggestion of control with both Movento and Sivanto and that the first 3 timings were more effective at preventing scarring than the late timing. Nevertheless, the important aspect of this trial was to determine the level of persistence of the new insecticides so that we could optimize their use for thrips control as an alternative to abamectin.

Sivanto bioassay data were determined from exposure of thrips to avocado leaves treated systemically with Sivanto, whereas all the field data were based on foliar applications. All we can do with the field data was measure the concentration of flupyradifurone in the leaf extracts – the ELISA test does not distinguish between material that was inside or on the surface of the leaf. So, in terms of interpreting the data from the different application timings (see the graph above), there were concentrations of flupyradifurone in the leaf extracts at levels above the LC_{95} at all timings except the first one. The main value of the ELISA work was to determine the persistence of the insecticides, and then take that information into consideration when evaluating the scarring data. Our data show that Sivanto can persist for 2-3 weeks (Figure 4) at or above the LC_{95}, suggesting that applications for thrips control would need to be timed according to when economic thresholds (ET) were either reached or when the PCA determined that the ET was approaching. The level of protection to the trees at the Fallbrook ranch would probably be good because of the excess leaf flush present throughout our experimental period. However, in older trees with less abundance of flush, the efficacy might not be as good, so timing would be more critical. In this trial, the highest levels of flupyradifurone were detected at Timing 3 (40-60% fruit set) when fruit would potentially be quite vulnerable to thrips feeding. However, the amount of flush present on avocados in relation to when fruit set occurs varies greatly from year to year. At Timing 3 at this site in 2013, flush was well advanced but there were still plenty of leaves present for thrips to feed on. Despite the presence of vulnerable fruit, thrips would be unlikely to cause much damage because of the abundance of flush that they would continue to feed on. From this perspective the thrips would be exposed to high levels of Sivanto and control would be effective. Because thrips populations did not increase until much later (June) in the season, it is difficult to credit the Sivanto or Movento treatments with the low scarring. The main objective of this trial was to establish the levels of persistence of the Sivanto treatments, and this goal was achieved.



Application Date	Flush Status	Flowering Status
March 21	• Flush appearing on most trees (1" x 0.5" sized leaves)	• Very few flowers open (<1%) • Past cauliflower stage (100% candles)
April 4	• Flush was noticeably more advanced • Older leaves nearing full expansion • Flush leaves ranged in size from 10-50% expansion	• 90% candles/10% fruit set
April 11	• Flush more advanced • Oldest leaves at full expansion	• 40-60% fruit set (very few BBs)
May 9	• Still a lot of flush on the trees • a lot of small red leaves to keep the thrips off the fruitlets	• Flowering complete (lot of fruitlets had fallen from the trees) • Most fruitlets at about 2 mm (some at 5 mm) and very vulnerable to thrips damage

Figure 4. Persistence of Sivanto on avocado leaves at the 2013 Fallbrook trial site and the status of flush and flowers for each of the four application timings.

Table 2. Fruit scarring caused by avocado thrips at the 2013 Fallbrook Sivanto trial site

	March 21	April 4	April 11	May 9
Movento - # of fruit	311	311	230	324
% of fruit, any thrips scar	3.5%	4.2%	2.2%	4.6%
% of fruit 5% or more scarring	0.3%	0.3%	0.0%	0.6%
Fruit scarring if 5% or more	1 at 5%	1 at 5%	none	1 each at 5, 15%
Sivanto - # of fruit	301	291	384	268
% of fruit, any thrips scar	2.7%	3.1%	2.1%	5.2%
% of fruit 5% or more scarring	0.7%	0.3%	0.3%	0.7%
Fruit scarring if 5% or more	2 at 5%	1 at 5%	1 at 5%	1 each at 5, 10%

Spring 2013 Field Trial #2 – Screening Avocado Thrips Products. Treatments were assigned to pallets based on pre-counts; mean number of immature thrips per pallet after the pre-count varied from 8.9-11.4 per leaf. Post-counts were taken 2, 10, and 16 days after treatment because a hot spell on day 5 knocked down thrips levels and we waited until day 10 for them to come back. Ten days after treatment, all of the treatments were effective in keeping thrips well below (highest was 0.3 immatures per leaf) control levels (11.4 per leaf) except Azera (8.8 per leaf). Immature thrips levels per leaf on day 16 were Water Control 9.6, Azera 6.8, Bexar 2.9, Closer 1.1, Veratran D 0.8, Sivanto 0.8, 1% oil alone 0.5 (not surprising with excellent ground coverage), Epi-Mek 0.5, Movento 0.1, Entrust 0.1, Danitol 0, and Delegate 0. The trial was discontinued after day 16 due to very hot weather resulting in thrips levels being extremely low during each of 3 later partial post-counts. This started out as a very interesting study but the severe impact of a hot spell on avocado thrips resulted in our not learning as much about treatment efficacy as we had hoped.

Spring 2013 Field Trial #3 –Impact of Movento and Sivanto on Avocado Thrips and Persea Mite. Based on levels of immature avocado thrips per leaf, pre-counts taken 6 days before treatment varied from 6.4 to 7.5 per leaf. Thrips levels on control trees were quite high 6 and 13 days post-treatment but a hot spell June 29-30 reduced thrips levels somewhat (Figure 5). Movento with either surfactant and Epi-Mek + oil controlled avocado thrips effectively throughout the trial; Sivanto was not very effective against either avocado thrips or persea mite. Cumulative immature thrips-days from June 19 to Sept 3 (+6 to +82 days) were: Control= 571, Movento + oil= 142 (24.9% of control levels), Movento + S-200= 155 (27.1%), Epi-Mek + oil= 224 (39.2%), and Sivanto + oil= 532 (93.1%).

We were quite pleased to see persea mite levels increase on the control trees as the trial progressed as this allowed us to also evaluate the impact of the treatments on this mite. Although motile mite levels were low prior to treatment (0.5-1.1 motile mite per leaf), untreated control levels rose to 34.9 per leaf at +40 days, 63.9 at +68 days, and 166.2 per leaf at +82 days. Epi-Mek + oil was the only treatment that maintained persea mite at low levels throughout the trial. Cumulative persea mite-days from June 19 to Sept 3 (+6 to +82 days) were: Control= 3,282, Epi-Mek + oil= 437 (13.3% of control levels), Movento + oil= 2,634 (80.2%), Sivanto + oil= 2,827 (86.1%); and Movento + S-200= 3,355 (102.2%) (Figure 6).

All fruit that could be reached easily were assessed for levels of avocado thrips scarring on 19 November 2013 when fruit were full sized. Some trees had very few fruit. The percent of the surface area of each fruit was assessed for thrips scarring and we use roughly 10% as an "economic" level that might lead to fruit being downgraded from first to second grade in a normal year (i.e. this level varies some from year to year based on the availability of fruit in the marketplace). Thrips fruit scarring was relatively low in all plots (non-economic in all cases). The maximum fruit area scarred on any fruit was 5% (one control fruit). The percent of fruit with minor thrips scarring appeared similar between treatments except it was higher on Sivanto + oil trees.

The good news from this trial is that Movento appears quite effective against avocado thrips. The bad news is that Movento does not appear effective at reducing persea mite buildup.

Despite Sivanto not controlling avocado thrips or persea mite in this trial, it could be a very important material for future use on avocados. Trials in Florida and Texas have shown that Sivanto is quite effective when applied against Asian citrus psyllid (ACP, *Diaphorina citri)* on citrus. Dr. Mark Hoddle has surveyed for avocado pests in Mexico and Central America and has found four species of psyllids that are common (see http://biocontrol.ucr.edu/hoddle/trioza/trioza.html). Several of these psyllid species have been found on smuggled avocado plants in San Antonio and Brownsville in Texas and San Diego in California. Given the close proximity of Mexico to California, the high daily volume of tourism, traffic, and trade across the California-Mexico border, Dr. Hoddle speculates several of these psyllid species have high invasion and establishment potential in California. If so, and if Sivanto is as effective against some of these leaf galling psyllids as it is against ACP, then a systemic product like Sivanto might be a very important future control option. We also plan future Sivanto trials to determine if an earlier treatment timing might result in better control of avocado thrips.

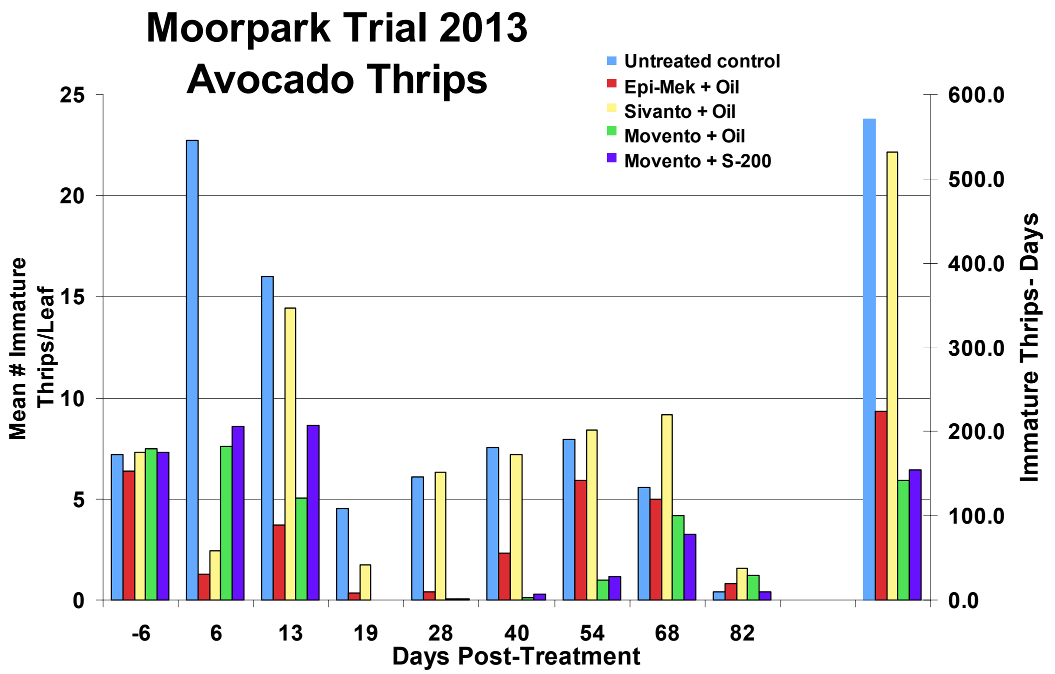


Figure 5. Levels of immature avocado thrips per leaf (first and second instars combined) at the 2013 field trial in Moorpark. Total immature thrips-days (cumulative sum of numbers x days present) were calculated from 6 to 82 days post-treatment and are shown on the right side of the graph.

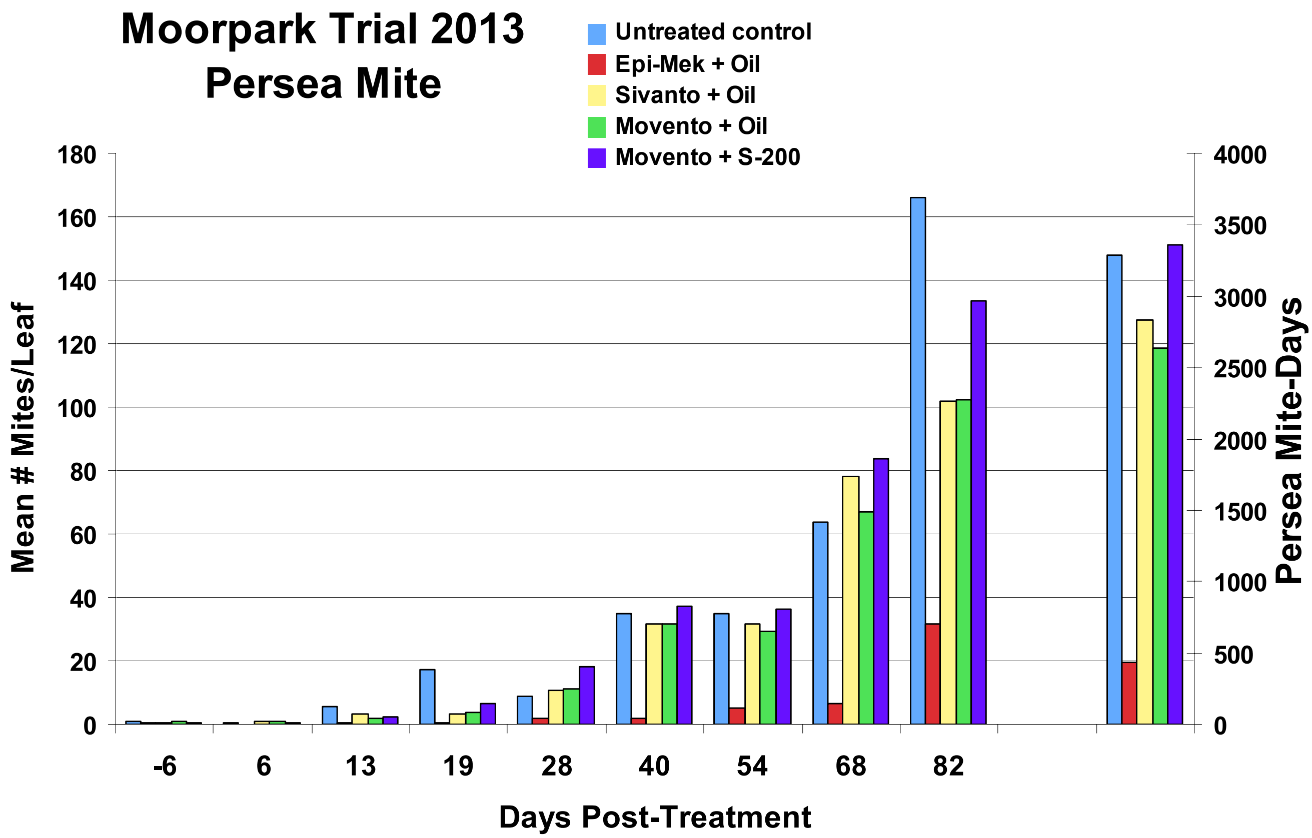


Figure 6. Levels of motile persea mites per leaf (all stages except eggs) at the 2013 field trial in Moorpark. Total immature mite-days were calculated from 6 to 82 days post-treatment and are shown on the right side of the graph.

B. 2013-14 RESEARCH (Year 2 of the project)

B1. Year 2 Summary: As of the end of Year 2, we had made good progress. Two avocado thrips field trials were conducted in 2014 and all that remained was to take fruit scarring assessments from the second of these (Moorpark). Ahead of when we had planned, we completed the optimization of a commercially available ELISA system for use in our Sivanto trials and we made good progress in developing our own Movento ELISA system with the immunization of 2 rabbits for antibody production. Research on monitoring avocado thrips resistance to abamectin continued, and the need for growers and pest control advisors to not overuse abamectin was stressed at each of the August CAC/UCCE/CAS grower seminars (Aug. 5 in SLO, 6 in Ventura, 7 in Fallbrook).

B2. Year 2 Milestone Table

Milestone	Activities for Year 2
3	• Update literature review to determine new developments in subjects relevant to this project • Year 2 pesticide efficacy and/or application methodology trial(s) established
4	• Thrips resistance detection methods refined and if necessary, additional methods explored • Evaluation of assay conditions for pesticide levels in plant tissue completed • Evaluation of alternative methods for pesticide residue detection completed • Year 2 evaluation of pesticide application methodology and/or efficacy completed

B3. Summary of Year 2 Milestone Accomplishments (as of 10-15-14)

B3.1 Milestone 3 -- Literature review and pesticide trials

Introduction

Both Byrne and Morse have stayed abreast of literature relevant to the chemical control of avocado thrips.

Two field trials were conducted during this reporting period as part of Milestone 3. After high avocado thrips levels were observed by an organic grower early in 2014, we set up a field trial in Fallbrook to evaluate various organic products that might be used for control of avocado thrips. The second field trial in 2014 was conducted in Moorpark with the dual objectives of (1) comparing the efficacy of alternative timings of Sivanto (the new experimental product from Bayer CropScience) and (2) comparing the new formulation of abamectin, Agri-Mek SC (0.7 lb ai/gal suspension concentrate) to the original formulation familiar to most growers and PCAs, Epi-Mek 0.15 EC.

Materials and methods

2014 Field Trial #1, Fallbrook – Organic Products. We looked at 3 possible field sites and settled on a hillside grove of top-worked trees that had good leaf flush, where trees were not too large for backpack sprayer application to single tree replicates (6-8 feet in height), and where thrips levels were moderately high. This grove was split into 4 "blocks" (areas) and the numbers of immature avocado thrips per leaf (first and second instars combined) were counted on 4 fully expanded but tender leaves per tree on a total of 72 trees, i.e. on 18 trees per block on 21 January 2014. Trees were ranked high to low based on these pre-counts and 2 trees per block were assigned to each of 9 treatments based on a random number table (thus, a total of 8 single tree replications per treatment). After some reassignment of trees to balance pre-counts, tree assignment to treatments was finalized.

Treatments evaluated are shown in the table below. Trees were sprayed on 24 January using an SP Systems Professional Backpack Sprayer equipped with a lever-action manual diaphragm pump. We estimated that 2 gallons of spray would be needed per tree and given 145 trees per acre, calculated spray dilution based on the listed per acre treatment rates. The manufacturer warned us in advance that the new organic Veratran D grind was not as fine as desired and thus, we put this treatment on last; this was an "early" batch of the Veratran D and we proceeded with the trial even though we suspected that the sabadilla seeds had not been ground finely enough. With the sprayer we used, the Veratran D + molasses mixture did not go through the sprayer well so we dropped this treatment from further evaluations. Post-treatment counts were taken in the same way as pre-counts (4 leaves per tree) at 3, 8, 15, and 22 days after treatment.

2014 Field Trial #2, Moorpark – Conventional Products. Applications were applied 6 May (Sivanto timing 1), 21 May (Sivanto timing 2, Agri-Mek, Epi-Mek), or 3 June 2014 (Sivanto 3). Trial trees were 4-year-old Hass avocados and tree spacing was 12.5 feet down the row, 23.5 feet between rows; thus, there were 148 trees per acre. Tree height was 8-10 feet at the time of treatment. Forty trees were selected for pre-counts on 30 April 2014 based on trees being at least 8 feet in height and having a fair amount of flush leaves present. Five fully expanded flush leaves were selected on each tree by walking around the periphery of the tree and the numbers of immature avocado thrips (first and second instars combined) were counted on each leaf. Trees were ranked from high to low based on the total number of thrips per leaf and because 6 treatments and 6 single-tree replicates were planned, they were separated into 6 blocks of 6 trees. The first block had the trees with the 6 highest counts, the next had the next 6 highest counts, etc. Trees with the 4 lowest counts were not used in the trial. One tree from each block was randomly selected for assignment to each of the 6 treatments. Following initial assignment, a few trees were reassigned to treatments to equalize pre-treatment mean thrips levels per treatment. Pre-treatment levels varied from 2.78 to 2.86 immature avocado thrips per leaf.

Sprays were applied using a Stihl SR-450 backpack mist-blower using setting #3 (of 5 on the sprayer). A little less than 0.5 gallons was applied per tree using outside coverage; rates were calculated on the basis of 100 gpa although because of tree size, this amount of spray was not used (instead, ca. 74 gpa). Treatments were (1) untreated control; (2) Epi-Mek 0.15 EC at 20 fl oz/a + 1% NR-415 oil, which was considered the "standard"; (3) Agri-Mek SC at 4.25 fl oz/a (same amount of active ingredient as with the Epi-Mek) + 1% NR-415 oil; and (4)-(6) Sivanto 200 SL at 14 fl oz/a + 1% NR-415 oil applied on a crop destruct basis at 3 different timings. Post-treatment counts were taken in the same way as the pre-counts at +8 (Sivanto 1 timing trees only), 15, 23, 30, 38, and 52 days after the first treatment. However, with these post-counts, the number of predatory mites (*Euseius hibisci*) and adult avocado thrips were also noted on the 5 sample leaves.

<u>Results and Discussion</u>

Literature review. There have been very few contributions to the scientific press on the subject of chemical control of avocado thrips, apart from the studies conducted by UCR researchers and their collaborators. A literature review of avocado thrips publications between 1996 (when the thrips was first detected in California) and 2016 showed the following. Zahn and Morse (2013) evaluated *Bacillus thuringiensis* (Bt) subsp. *israelensis* proteins (Cyt 1A and Cry 11A, activated and inactivated) and multiple strains (GHA, 1741ss, SFBb1, S44ss, NI1ss, and 3769ss) of *Beauveria bassiana* (Balsamo) Vuillemin against avocado thrips. Avocado thrips were not susceptible to either Bt protein tested, regardless of activation status. All strains of *B. bassiana* were able to infect avocado thrips. However, the commercially available GHA strain was the most effective strain and had a faster rate of infection then the other strains tested. Yee et al. (2001a,b) described the effects of sabadilla and abamectin spray volume and coverage on the control of avocado thrips; this study represents important work that was done to understand how these chemicals were performing in commercial groves. Humeres and Morse (2006) would later report the first case of resistance to sabadilla, highlighting the need to monitor for resistance in chemicals that are favored by growers. There are many insecticides registered for use on avocados, and improving their efficacy under field conditions was the focus of a Tollerup and Morse (2005) study that evaluated the effect of horticultural spray oil and surfactants on the residual efficacy of spinosad. Several publications by Byrne et al. (2005, 2007, 2010, 2012 and 2014) evaluated the efficacy of systemic insecticides, both as soil drenches and as trunk injections. In terms of resistance work, there has been no reported work for avocado thrips other than the sabadilla resistance study published by Humeres and Morse (2006). Resistance to abamectin has been reported in other thrips species, primarily the western flower thrips, *Frankliniella occidentalis*. Chen et al (2011) selected for 46-fold resistance under laboratory conditions, and measured a 7-fold increase in cytochrome P_{450} activity. Field-evolved resistance was reported from China (Wang et al, 2014), and cytochrome P_{450}s were implicated as the major mechanism through the suppression of resistance by the incorporation of piperonyl butoxide into the bioassays. A BIOSIS search of abamectin resistance yielded 325 hits, and included studies on abamectin resistance in diamondback moth, various thrips and mite species; both target-site and metabolic mechanisms were found to play a role, with the latter being more prevalent. The key point to note from the available literature is that thrips species have the potential to develop resistance; of further significance is the potential for resistance to one insecticide to confer cross-resistance to a hitherto unused product. Cross-resistance is far less likely to occur between two pesticides with different modes of action when they are used in rotation.

2014 Field Trial #1, Fallbrook – Organic Products. Results in Table 3 are listed from most effective (lowest cumulative thrips-day over 3 to 22 days after treatment) to least effective. Thrips levels declined from pre-treatment levels on Jan. 21 to Jan. 27 and then Feb. 1 probably because of about a week of cold nighttime temperatures. In contrast to levels seen with the water control, both oils alone, Grandevo, and PFR-97 all seemed to elevate thrips levels, likely because these materials reduced natural enemy levels (although levels were not high enough to take counts and document this). The Trilogy and Entrust, however, appear to be good organic treatments for avocado thrips and would be worthwhile including in a future trial. Since this trial, MGK has starting grinding the sadadilla seed more finely and when we tried applying this product using the same sprayer, the spray went on without any trouble. So that material would also be worthwhile evaluating in a future trial.

Table 3. Results of the January 2014 Fallbrook spray trial to evaluate the efficacy of organic products for avocado thrips control.

Material	Rate/acre	Pre-counts 1/21/14 (-3 d)	Counts 1/27/14 (+3 d)	Counts 2/1/14 (+ 8 d)	Counts 2/8/14 (+15 d)	Counts 2/15/14 (+22 d)	Immature Thrips-days +3d to +22 d
Entrust SC + Omni 6E	10 fl oz/a + 1%	14.8	0.8	0.3	0.7	0.7	12.5
Trilogy	2%	14.2	1.2	0.2	1.0	0.9	15.6
Entrust SC + Oroboost	10 fl oz/a + 0.5%	14.5	0.5	0.5	1.5	0.1	18.3
Water control		14.6	3.3	0.9	0.8	0.4	24.0
PureSpray Green Oil	1%	14.6	6.5	0.8	2.8	1.0	49.4
Omni Oil 6E	1%	14.7	4.1	0.7	4.0	1.2	53.6
Grandevo + Oroboost	3 lbs + 0.5%	14.9	10.9	1.1	3.0	1.3	65.7
PFR-97	2 lbs/a	14.6	7.0	1.1	5.3	1.9	77.5

2014 Field Trial 2, Moorpark - Conventional Products. Fruit scarring levels have not been taken yet but our initial impression is that fruit scarring by avocado thrips was light this year. Based on post-treatment counts of avocado thrips on 13 May (Sivanto timing 1 only), 20 May (all 30 data trees were assessed on this and later dates), 28 May, 4 June, 12 June, and 26 June, immature avocado thrips-days from 20 May to 26 June were 92.1 on untreated control trees. Thrips-days were similar to each other with both abamectin treatments (35.7% of control levels with Agri-Mek, 36.3% with Epi-Mek) but none of the Sivanto treatment timings were highly effective (74.8% of control thrips-days with timing 2 at 21 May; 124.8% with timing 1 at 5 May; and 208.7% with timing 3 at 3 June). In fact, the higher thrips levels with Sivanto timing 1 and 3 versus the untreated control suggest that the material was either reducing natural enemy levels or stimulating the avocado thrips, or both.

B3.2 Milestone 4 -- Progress in developing ELISA systems and resistance detection.

Introduction
See corresponding section for Year 1 milestone 2b

Materials and Methods
See corresponding section for Year 1 milestone 2b

Results and Discussion
Progress in developing an ELISA system for Movento. Professor Jocelyn Millar's group at UC Riverside synthesized a carboxyl ester derivative of spirotetramat enol. We conjugated this compound to a carrier protein and have immunized 2 rabbits. In June, we will receive the first serum from the rabbits and should be able to determine if there has been an immune response. Typically, at 7 weeks after immunization, we should get some response. At that time, the antibody titer is likely to be highest. However, we will continue to evaluate serum until August. Over time, the antibody titer is likely to decline, but as this happens the affinity of the antibodies increase. We are interested in producing an ELISA with high affinity for the enol derivative, so the development of the assay will take some time.

Progress in developing an ELISA system for Sivanto. We obtained technical flupyradifurone from Bayer CropScience and used it to generate an accurate standard curve for the ELISA. In addition, we optimized the extraction procedure for avocado leaves to ensure that matrix effects were eliminated, thereby minimizing the likelihood of false negatives. This part of Milestone 4 was completed well ahead of when we planned, giving us a workable ELISA system that is already being used in field studies.

Avocado thrips resistance detection system. Our initial plan was to start an avocado thrips greenhouse colony from field sites reporting problems with control using abamectin and then subject that colony to further abamectin selection. However, based on lack of high avocado thrips levels in the field in 2014 (persea mite appears to be of greater concern instead) and lack of reported avocado thrips control problems with abamectin in the field, we decided instead to do an initial screening of avocado thrips for resistance to abamectin using field populations in discriminating dose bioassays. Based on baseline susceptibility testing with abamectin, we know what discriminating doses of abamectin to use. In conjunction with the bioassays, we had hoped to be able to test for elevated levels of cytochrome P450, the most likely source of metabolic resistance to abamectin. However, testing with 7-ethoxycoumarin as a potential substrate for the measurement of monooxygenase activity in avocado thrips proved fruitless, despite a good experimental system being developed; initial tests were done with microsomes prepared from rat liver (available commercially) and housefly abdomens (prepared by Frank Byrne in-house from Alec Gerry's colony at UCR), known sources of high enzyme activity. Having established the lower limit of detection for this substrate (~30 pmoles 7-hydroxycoumarin), it was clear that this level of sensitivity was not attainable for avocado thrips.

C. 2014-15 RESEARCH (Year 3 of the project)

C1. Year 3 summary: Major Project Revision Approved 2-26-15. Soon after the Kuroshio strain of the shot hole borer (KSHB) became problematic in the Escondido area, we were approached by the CAC and asked to shift most of our research effort to working on that problem, cutting back on work for this project (65116). Seeing it as the best thing for the industry, we agreed to do so. Morse participated in a field meeting 11-6-14 in Escondido, a field meeting at 3 groves 12-1-14, and at grower meetings in

Escondido 12-16-14 and Ventura 12-17-14. In fact, a great percentage of our time and effort Oct. 2014 – 2015 was spent on KSHB and Fusarium dieback – see the final report for that project for details.

The CAC asked what funding we would need to aggressively start research on KSHB. We indicated we would initially work off funds from this project (65116) and then once it became clear what was needed with KSBH, we would put in a proposal to do that work. A proposal to work on KSHB was submitted 2-14-15 but due to communication problems between the UCR contracts office and the CAC, it was not signed until 3-23-15 (CAC 65123).

With Morse planning to retire 6-30-17, most of the work on 65116 and 65123 falls to Dr. Frank Byrne, although Morse has agreed to continue spearheading efforts coordinating research with IR-4, Syngenta, and several other companies involved in this work, as well as assisting with other aspects of the project as needed (grower meetings, Section 18 applications, etc.). In part to make the funding request for 65123 palatable to the CAC, but also so that we could put a substantial portion of our efforts on KSBH, we proposed reducing scheduled funding for 65116 to ca. half each year of what had been planned over 2014-2017 (original Years 3-5 of what had been a 5-year approved project) and the project was extended an additional 3 years (to run until Oct. 31, 2020). Thus, the total funding from the CAC was the same as agreed on in 2012 but as of 2-26-15, instead of running for 3 more years (2014-2017) with a budget of $291,570 in total and a mean per year budget of $97,190, the revised 65116 ran for 6 more years (2014-2029) for a total of $291,570 and a mean budget of $48,595 for those 6 years. In fact, the project was terminated 10-31-16 so funding for 2014-2016 was $94,632. With the cut in annual budget to 65116 to about half of what it had been, we revised project milestones to retain the most essential items as listed below.

However, we did not stop working on this project (65116) in the interim. In fact, all 2015 milestones were completed (details are provided below). Dr. Byrne hired a full time Junior Specialist (Ivan Tellez) to work in combination on this project (65116) and on KSHB (65123). Ivan Tellez was trained on both avocado thrips and persea mite bioassays by SRA Alan Urena. Both Mr. Urena and Ms. Lindsay Robinson (second key SRA in Morse's lab) retired 6-30-15 and thus, it was important Ivan know how to do these bioassays before then.

The second milestone for 2015 was to test for spirotetramat-enol (active metabolite in Movento) antibodies in serum from the first rabbit immunizations. Dr. Byrne has done this and unfortunately, results were negative. We plan a new round of rabbit immunizations, given that we still feel that optimization of Movento use on avocado is important, especially if it could be used effectively with helicopter application at low gallonage (because it is highly systemic, low gallonage might work nicely).

C2. Year 3 Milestone Table (as revised and approved 2-26-15)

Milestone	Activities for Year 3
5	• Begin abamectin resistance monitoring in thrips populations collected from north and south avocado growing regions
6	• Test for spirotetramat-enol antibodies in serum from first rabbit immunizations

C3. Summary of Year 3 Milestone Accomplishments (as of 10-15-15)

C3.1 Milestone 5 -- Abamectin Resistance Monitoring

Introduction

The priority for avocado thrips resistance monitoring in 2015 was to train a new hire (Ivan Tellez started work at UCR in April 2015) to conduct the avocado thrips field collections and run the bioassays in the laboratory; a second objective was training him in running persea mite resistance bioassays (after Humeres and Morse 2005). With the retirement of 2 key personnel in the Morse lab (Alan Urena and Lindsay Robinson), who were hitherto responsible for running the thrips and persea mite bioassays, it was necessary to train new personnel in how to conduct the bioassay so that future monitoring efforts could be continued after their retirement. Alan Urena supervised the initial training that Ivan undertook, and Ivan is now proficient at running the bioassays. He made contact with PCAs in the major avocado growing regions, and they coordinated with him on the initial collections during 2015.

Materials and Methods

Avocado thrips were sampled by new hire Ivan Tellez from several groves in San Diego and Ventura counties. Sample sites were chosen in 2015 on the basis of frequent past use (annual) of abamectin. Leaves were collected with live thrips (preferably second instars) and stored in a refrigerator overnight. Leaves from untreated Hass avocado trees on the campus at UC Riverside were used for bioassays and were collected fresh on the day of the bioassay. The leaves were completely immersed for 15 seconds in solutions of abamectin (prepared using the Agri-Mek 0.7 SC Insecticide/miticide formulation from Syngenta) to provide a range of test concentrations. Leaves were placed on absorbent paper and then wedged in “Munger cells”: plexiglass cells with a modified ‘arena’ area on leaf underside (Figure 7; method modified from Morse et al. 1986). Second instar thrips were transferred from the sampled groves into cells with a fine paint brush. Final mortality was assessed after 72 hours of exposure.

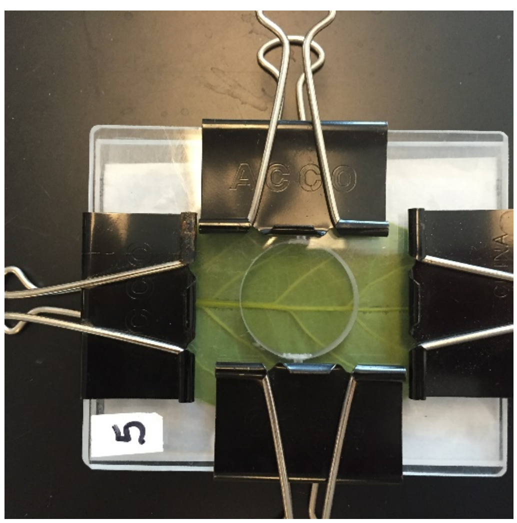


Figure 7. Avocado thrips bioassays are conducted using Munger cells. An avocado leaf, either treated or untreated (control), is sandwiched between layers of plexiglass. Insects are confined within a circular arena cut into the central plexiglass layer, which exposes them to the underside of the leaf. Leaves are laid adaxial side down on a layer of moist filter paper to maintain the integrity of the leaf for the duration of the bioassay (72 hours).

Results and Discussion

Abamectin bioassay data are summarized in Figure 8 and include test results from 2005 and 2007 with populations from San Diego, Santa Barbara, and Ventura counties for comparison. There are 2 noteworthy conclusions that can be drawn from 2015 bioassay data. First, compared with data generated in the earlier studies in 2005 and 2007, there was a general increase in tolerance in the two populations collected from groves where abamectin is regularly used for thrips management (San Diego 2015_1 and Ventura 2015). Note from the plots in Figure 8 that the point at which 50% of the test insects were killed by the abamectin (the LD_{50}) has increased, indicating that the insects at these sites were not as susceptible as those from previous bioassays. The thrips population collected from an organic grove in San Diego County (San Diego 2015_2) showed no such increase in tolerance relative to earlier data. And second, 100% mortality was never achieved in bioassays with the abamectin-treated populations, suggesting that a proportion of avocado thrips in those populations was expressing resistance to the abamectin. The survival of resistant insects following a treatment in a bioassay is akin to what happens under field conditions. Applications of insecticide eliminate the susceptible component of the population, and where there is minimal survival of susceptible insects, a population dominated by resistant individuals results. Subsequent applications of the same insecticide at a similar rate to that which was used originally is unlikely to have any major impact on the resistant population, and the product will be ineffective in that situation until the levels of resistance decline (this is the basis for rotating products with different modes of action so that all chemicals remain effective over time with no disruptions to the use of any one chemical due to resistance).

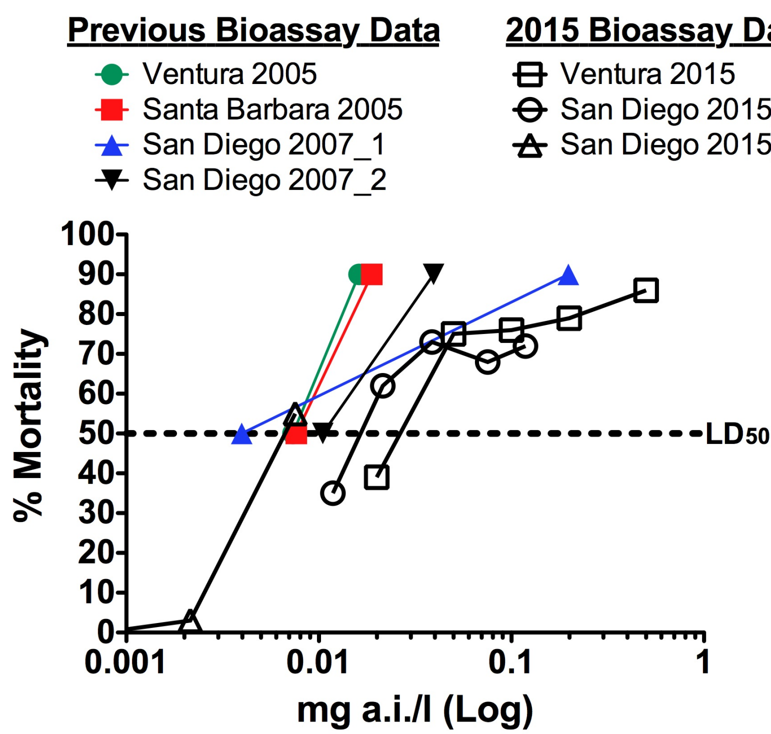


Figure 8. Abamectin dose-response data for avocado thrips populations collected from avocado groves in 2015. Data from bioassays conducted in 2005 and 2007 are included for comparison. The insects were exposed to avocado leaves treated with a range of insecticide concentrations to generate the dose-response curves. The dose is represented on a log scale and is expressed in units of mg active ingredient (abamectin) per liter.

C3.2 Milestone 6 -- Development of an ELISA system for Movento

Introduction
The second milestone for 2015 was to test for spirotetramat-enol (active metabolite in Movento) antibodies in serum from the first rabbit immunizations. Dr. Byrne has completed this milestone using the serum supplied from the two immunized rabbits.

Materials and Methods
The rabbit immunizations were initiated in April 17, 2014 using a commercial laboratory to conduct all animal handling under USDA regulations. The hapten conjugation to the carrier protein was performed by Dr. Byrne and provided to the laboratory for immunizations. Booster immunizations were conducted for the next 8 months according to established protocols, and serum was collected on 2 occasions each month up until the final bleed in January 2015. Dr. Byrne performed checkerboard titration assays on the serum from both rabbits.

Results and Discussion
The checkerboard titration assays did not show detectable levels of antibodies to the insecticide, despite quite a lengthy period of immunizations, and very low serum dilutions. Our next step regarding this milestone is to run a second set of immunizations, but with modifications to the protocol used for conjugating the hapten to the carrier protein in the hope that an increase in conjugation efficiency will improve the immunogenic response in the rabbits (two rabbits will be used for the second series of tests also).

D. 2015-16 RESEARCH (Year 4 of the project)

D1. Year 4 Summary: As part of our resistance monitoring program, Ivan Tellez conducted 7 bioassays with abamectin to determine susceptibility of avocado thrips populations from north and south growing regions. We have sufficient data now to define diagnostic concentrations of abamectin that could be used for future resistance monitoring. As in 2015, there is considerable variability in response to abamectin, ranging from highly effective to overall trend in the abamectin data is

D2. Year 4 Milestone Table

Milestone	**Activities for Year 4**
7	• Continue abamectin resistance monitoring in thrips populations collected from north and south avocado growing regions • Define diagnostic doses for future large-scale monitoring of field populations
8	• Continue work with spirotetramat-enol ELISA development

D3. Summary of Year 4 Milestone Accomplishments (as of 9-30-16)

D3.1 Milestone 7 -- Abamectin resistance monitoring and defining diagnostic doses

Introduction
Abamectin resistance monitoring in avocado thrips was continued during 2016. When persea mites were available, we availed of the opportunity to conduct abamectin bioassays on this arthropod also.

One of the goals of the avocado thrips resistance testing was to define discriminating doses that could be used in preliminary evaluations of populations. With discriminating doses, fewer insects are required to complete the assessment, thereby enabling testing of populations from a larger number of groves.

<u>Materials and Methods</u>

Leaves were collected with live thrips (preferably second instars) from sites in Ventura and San Diego Counties. All trees were Hass avocado. The Munger Cell bioassay was run as described previously, with control leaves sampled from untreated trees on the campus at UC Riverside. Agri-Mek 0.7 SC Insecticide/miticide formulation from Syngenta was used to prepare the range of test concentrations. Second instar thrips were transferred to the Munger Cells and final mortality was assessed after 72 hours of exposure. One site was chosen in Ventura County and was defined as a conventional grove, with yearly abamectin usage. Two sites were selected for sampling in San Diego County – a conventional grove and an organic grove.

<u>Results and Discussion</u>

Ventura County. The data for the thrips populations tested in 2015 and 2016 show that a proportion of the populations are more tolerant of abamectin than previously determined in 2005 (Figure 9). Some variation in response is to be expected from year to year, but the shallow slope of the dose-response lines (particularly in the 2015 data) at higher doses is usually an indication of a change in susceptibility. This is something that needs to be monitored carefully over time. If resistance is indeed developing in this area, then we would expect the dose response line to become shallower as the proportion of resistant insects increases. Rotation of abamectin with a product having an alternate mode of action would help mitigate the development of a serious resistance problem in this grove.

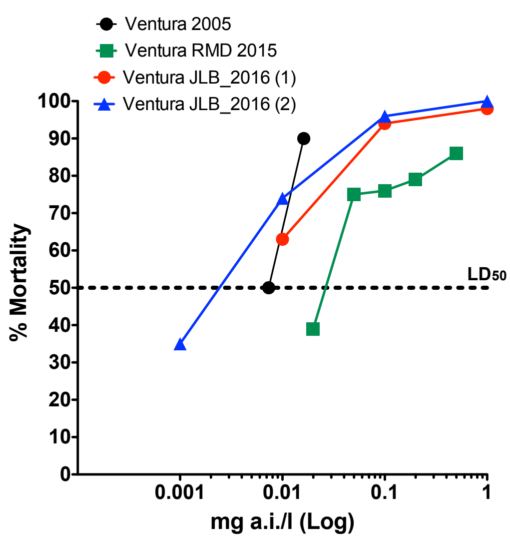
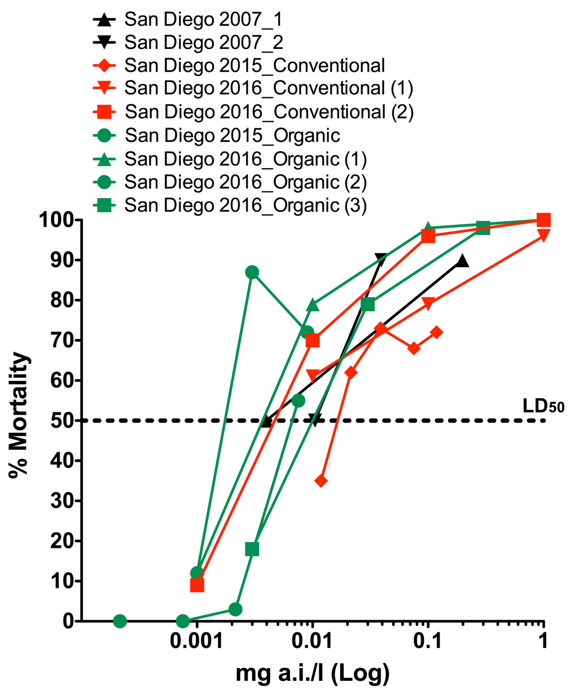


Figure 9. Abamectin dose-response data for Ventura County avocado thrips populations collected from avocado groves in 2015 and 2016. Data from bioassays conducted in 2005 are included for comparison. The insects were exposed to avocado leaves treated with a range of insecticide concentrations to generate the dose-response curves. The dose is represented on a log scale and is expressed in units of mg active ingredient (abamectin) per liter.

San Diego County. The situation in San Diego County is similar to that in Ventura County (Figure 10), although there are marked differences between the organic and several of the conventional samples. We observed the same shift in susceptibility to abamectin in conventional groves, with shallow slopes at higher doses. There is not a great deal of difference at the LC50 levels, perhaps a 10-fold difference at most between the extremes. The most disconcerting aspect to the data is the survival of thrips at high doses, an indicaton that resistance may be evolving in these populations.

Discriminating concentrations for resistance monitoring. We have evaluated our bioassay data and have determined discriminating concentrations that should be used for resistance testing in avocado



thrips Munger cell bioassays (Table 4). Five-ten Munger cells should be used at each concentration with at least 10 thrips per cell (50 thrips per rate minimum, more is better). Significant survival of thrips at these concentrations should trigger a full dose-response bioassay. In the event that insufficient insects are available to run tests at both concentrations, the higher concentration should be used, as significant survival at the higher concentration is a greater indication that resistance is evolving. The low rate should give at least 90% mortality in a susceptible population; the higher rate 100%, even if there is low levels resistance. Note that selection of the higher test dose varies with the slope of each regression line as well as with other factors (with fenpropathrin, the likelihood resistance may move quickly).

Figure 10. Abamectin dose-response data for San Diego County avocado thrips populations collected from avocado groves in 2015 and 2016. Data from bioassays conducted in 2007 are included for comparison. The insects were exposed to avocado leaves treated with a range of insecticide concentrations to generate the dose-response curves. The dose is represented on a log scale and is expressed in units of mg active ingredient (abamectin) per liter.

Table 4. Discriminating concentrations of abamectin, insecticide 2, insecticide 3 for avocado thrips resistance monitoring.

Insecticide	Discriminating Concentrations	
	Low rate (mg AI/liter)	High rate (mg AI/liter)
Abamectin	0.02	0.3
Spinetoram	0.045	0.3
Fenpropathrin	1.0	10

The discriminating concentrations for abamectin were obtained using a series of field avocado thrips bioassays in groves where little abamectin had been used and control was excellent (susceptible sites) compared with data from groves where we suspected there was the beginning of resistance.

In contrast, discriminating concentrations with spinetoram (Delegate) and fenpropathrin (Danitol) were based only on data from sites where these materials had not been used. Spinetoram data were from the South Coast Research and Extension Center on tests done 5-27-11 (0.05 mg AI/liter, 51/52 dead thrips at this rate, controls 0/53 dead), 6-8-11 (0.0005, 1/70; 0.005, 19/70; controls 1/75), 6-15-11 (0.02, 47/64, 0/63), and 6-29-11 (0.01, 25/61; 0.03, 52/56, controls 0/56). The probit regression line from these spinetoram data results in an estimated LC_{50} and LC_{90} of 0.01019 (95% fiducial limits 0.00862-0.01182) and 0.04377 (0.03416-0.06221) mg AI/liter, slope of 2.5980 ± 0.01019 and a chi-square value of 0.3089, i.e. this is a solid regression (well above the threshold of 0.05) and with future tests, we use non-overlap of fiducal limits as our criteria for LC values being different. Fenpropathrin data were also from the SCREC on 6-15-11 (0.05 mg AI/liter, 10/65 dead thrips, controls 0/63 dead), 6-21-11 (0.2, 33/62; 0.6, 58/64; 2.0, 63/63; same control mortality of 0/63 applies to all 3 rates), and 6-29-11 (0.4, 46/60, 0/56). The probit regression for these data resulted in an estimated LC_{50} and LC_{90} of 0.16575 (95% fiducial limits 0.13333-0.20047) and 0.95019 (0.71064-1.42525) mg AI/liter, slope of 2.169 ± 0.2190 and a chi-square value of 0.5983.

We had intended to gather more baseline data on spinetoram and fenpropathrin from groves with minimal use of these materials in future years of CAC project 65116. This came down to a matter of resources and what was urgent at the time. For two reasons, this was not done: (1) we did not see much field use of spinetoram and fenpropathrin by growers and instead, they appeared to continue to overuse abamectin – thus, we thought it more important to work on collecting more abamectin field data and developing more sensitive methods of detecting abamectin resistance in the field; (2) once the shot hole borers became problematic, we decided to focus more of our collective energies on this pest complex (with extension of 65116 to 2020, we thought we would have plenty of time to do this work later once spinetoram and fenpropathrin field use increased). There is nothing wrong with the spinetoram and fenpropathrin data we have collected, in the interim it can be used, but in the future, additional data from field sites with minimal use of these products would be prudent for confirmation.

Persea mite. Although not included in the milestones for 2015/16, Ivan Tellez initiated abamectin bioassays on persea mites sampled from 3 groves. The data showed similar trends to data generated in the Morse lab over several years of monitoring. Based on the current data, there does not appear to be a shift in susceptibility to abamectin in persea mite populations.

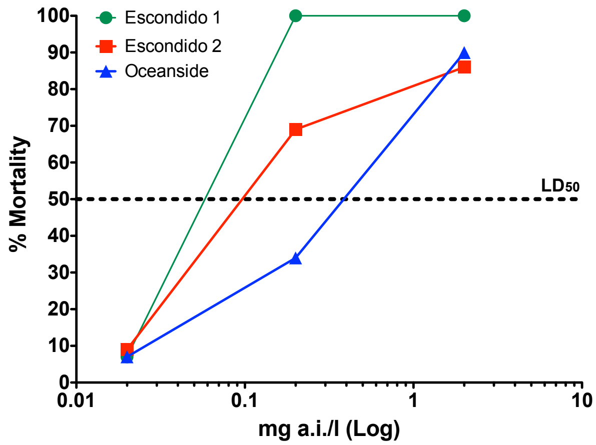


Figure 11. Abamectin dose-response data for San Diego County persea mite populations collected from avocado groves in 2016. The mites were exposed to avocado leaves treated with a range of insecticide concentrations to generate the dose-response curves. The dose is represented on a log scale and is expressed in units of mg active ingredient (abamectin) per liter.

D.3.2 Milestone 8 -- Continue work with Spirotetramat-Enol ELISA development

Introduction

Despite the disappointment of the first set of immunizations, we plan to pursue our goal of developing an ELISA detection method for Movento. A new conjugation protocol has been developed and tested, with improved yield of hapten-protein conjugate.

Materials and Methods

For the first set of immunizations, Thermo Scientific Imject protein and EDC conjugation kits were used to conjugate the hapent to mcKLH (for immunization) and BSA (coating antigen). The conjugation efficiency was low with this kit, so we have decided to dispense with using the kit and proceed with the traditional method used before the kits were developed. The essential difference between using a kit and the conventional method is that the conventional method allows for better control over the buffering conditions throughout the conjugation process. We will use dimethylformamide (DMF), an organic compound that is a commonly used solvent for chemical reactions.

Results and Discussion

The DMF protocol worked well with improved conjugation efficiency between BSA and the hapten. Using two different protocols for quantification (both use TNBSA, but at different substrate dilutions), the percentage "loss" of active primary amines (the group on the protein that the hapten links to) was 6.5% (mean of two measurements) based on color differences between the hapten and control reactions. The DMF system was clearly better than the Imject kit, and will be the method used for the next hapten-mcKLH conjugations.

E. Overall Conclusions

We are very concerned that abamectin resistance is developing in both avocado thrips and persea mite field populations and that the majority of PCAs and growers are not aware of how serious this would be are not using alternative chemistries that are available and effective. The real danger with pesticide resistance is that it can be a fairly "silent killer" i.e. once one knows it is present and it has progressed far enough, it may be too late to do much about it. Our bioassay data indicate that there is a change in response of thrips populations to abamectin. Although the changes are not dramatic at this stage, the fact that there is increasing survival of insects at higher doses should sound a warning that tolerant insects are present in the avocado groves.

We think it would be prudent to continue developing methods to better define levels of avocado thrips and persea mite resistance, including more sensitive molecular methods. If we could show growers and PCAs what to do before it is too late, many of them would likely start trialing the alternative chemistries and rotating materials. The shame of this is that abamectin is a remarkably effective and fairly unique product. With proper rotation of other chemistries, it could remain effective against persea mite and avocado thrips indefinitely. We have developed assays that can monitor for changes in important resistant markers – these can be used now to monitor for changes in resistance to OPs, carbamates and pyrethroids. Further work is required to define effective markers for abamectin resistance.

We will continue trying to develop an ELISA method that can detect spirotetramat-enol because we think Movento could be a very important chemical for use against a number of pest species on avocados and citrus. Movento has a different mode of action than abamectin and these two chemicals could thus be great rotational partners for avocado pest management.

We are disappointed that the CAC chose to terminate our project as of 10-31-16. We would have appreciated some discussion of alternatives to this (e.g., no funding for 2016-17, then continuing the project in 2017-18) but instead, we were notified of termination by email 8-18-16, with no advance warning or discussion. At the same time we were notified that our shot hole borer funding would not be continued. We are in the process of deciding how to wrap up the projects we have so that the data we have collected is not lost and can be published to the degree that is possible. Thus, we anticipate much of this research continuing in to 2016-17 without additional funding from the CAC beyond the final report invoices we will submit with this final report for 65116 and the one for 65123.

F. Overall project summary (similar to the Executive Summary)

- We have reported above on research done 2012-2016, i.e. over a 4-year period, on avocado thrips and persea mite under CAC 65116. Note, however, that over the period Oct. 2014 – Mar. 2015 all Kuroshio shot hole borer research was funded from 65116 (i.e. until CAC 65123 was funded 3-23-15 – all SHB research is reported in the final report for that project as both projects terminated at the same time, i.e. 10-31-16).

- As background, based largely on past CAC funded research, the following products were registered for use on California avocados, largely due to research trials we conducted in cooperation with the relevant chemical company:
    - Veratran D avocado thrips [AT] Special Local Needs approved 2/97; registered in 1998
    - Abamectin Section 18 Emergency Exemption approved for use only against AT for 6 years, 1999-2004; Registered for use against both AT and persea mite [PM] 3-2-05
    - Delegate registered in 2007 for AT
    - Danitol in 2010 for both AT and PM
    - Envidor for PM in 2010
    - Movento for AT May, 2011
    - Zeal for PM May, 2012

- Miteus (FujiMite) for PM July, 2013

- Note that all materials are in different classes of chemistry from one another and from abamectin except Movento and Envidor are both in IRAC class 23 (IRAC = Insecticide Resistance Action Committee) – this was not by chance but instead, by design. We intentionally chose pesticides with different chemistries and with good efficacy.

▪ We are extremely concerned about abamectin resistance developing in populations of one or both of avocado thrips and persea mite. We have "gotten the word" out regarding the danger of resistance but to date, we are still very concerned that most PCAs are overusing abamectin and not trialing the above alternatives to the degree they should (i.e. practicing proactive resistance management). If abamectin resistance develops, the concern is that the next most popular one or two materials would then be overused, also leading to the eventual loss of those products to resistance. For example, we believe fenpropathrin (Danitol) would fail due to resistance quite quickly.

▪ We list below what we consider the key accomplishments of this project over 2012-2016.
  - We have further refined methods for evaluating field populations of avocado thrips and persea mite for resistance to abamectin and other products. It appears to us that abamectin resistance is evolving with both avocado thrips and persea mite but it has not proceeded far enough such that field failures are common – instead, one sees a shortening of field efficacy (for example, spring avocado thrips treatments used to control persea mite through the summer and fall in the Ventura region; most years it no longer does and a second treatment with a miticide is needed). Unfortunately, PCAs and growers often retreat with abamectin (sometimes with an alternative) when thrips or persea mite control is inadequate with an initial treatment. While the lack of efficacy of the first application may be due to abiotic and/or operational problems, the possibility that resistance could be involved is often not given enough consideration.
  - We have developed working biochemical assays that will detect esterase- and insensitive AChE-based resistance mechanisms in avocado thrips. These are valuable tools that will enable greater numbers of groves to be monitored in a more timely manner, particularly when there are insufficient numbers of insects available for full-scale bioassays.
  - We have defined a discriminating bioassay dose of abamectin for future monitoring of resistance in field populations; we also propose tentative rates for both spinetoram (Delegate) and fenpropathrin (Danitol).
  - We have evaluated the use of Movento against avocado thrips (good potential for activity if used optimally) and persea mite (had great hopes it would be active based on activity on other crops against other mite species – Trial 3 in 2013 shows this is not likely the case).
  - We continue to work on developing an ELISA system for use with Movento. Movento (spirotetramat) is an unusual product that might be of great use against a number of avocado pests. Spirotetramat is NOT active against insects or mites in its parent form; it is sprayed as a foliar material, taken up by the plant and converted to a highly systemic material, spirotetramat-enol, which IS highly active against a range of pest species. The enol moves to the roots and then is redistributed into the plant – for this reason, we believe it might be ideal for application by helicopter with low water volumes (ground spray trials on citrus show better uptake and movement throughout the plant with low volume applications). However, to optimize the use of this material, we need a means of measuring levels of the active ingredient (the enol) that is not confounded by picking up

the parent (spirotetramat itself). That is what we are attempting to develop (still in progress, as detailed in the report below).

- We have developed a working ELISA system for a second new product, Sivanto. Sivanto is also sprayed and moves systemically in the plant but whereas preliminary lab trials showed it had activity against avocado thrips, field trials (Trials 1, 3 in 2013; Trial 2 in 2014) showed that it was not persistent enough or present at high enough levels in the types of young leaves that avocado thrips prefer to feed on. Due largely to our CAC research, however, Sivanto will be registered for use on avocados in California (it was initially planned that Bayer would not include it in the final submission to EPA, i.e. the opportunity for later registration would be lost) and it is quite likely it may be of use to CA avocado growers in the future, e.g., for control of pests such as the avocado gall psyllid (*Trioza* sp.) predicted by M.S. Hoddle as a likely future CA invader.
- We responded in a timely manner to requests for information from the CAC, CDFA, PCAs, growers, and others as listed in the 4th primary bullet above.

▪ Over the period 2012-2016, we have responded to industry and pest control advisor requests for information and targeted research. We believe it is partially due to our research on chemical control of avocado thrips and persea mite that the industry has been able to deal with these two pests effectively and without excessive damage most (but not all) years over the recent past. We also made ourselves available to the CAC, CDFA, PCAs, growers, and others when other issues arose, i.e. review of Pest Risk Analyses, helping to deal with export issues, requests for advice on various pest management problems, etc. (partial listed under the Executive Summary at the beginning of this report).

▪ Morse and Hoddle (2012) and Hoddle and Morse (2013) published comprehensive manuscripts in the California Avocado Society Yearbook detailing the history, biology, and best management practices for avocado thrips and persea mite, respectively. Morse viewed this as the best way to pass on most of what had been done on pest management of these two pests to the industry in a form that is readily accessible.

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