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Evaluation of Systemic Chemicals for Avocado Thrips and Avocado Lace Bug Management

New Project: Year 1

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Benefit to the Industry

Insecticides are an important component of pest management in California avocado groves. Our research is aimed at optimizing the use of available products, and evaluating new compounds that might overcome some of the difficulties encountered by growers with available products. In this study, we are evaluating the efficacy of two systemic neonicotinoid insecticides (Admire Pro® and Platinum®) against an established pest, the avocado thrips (AT), and a newly introduced pest, the avocado lace bug (ALB). The potential benefits to the industry from the use of neonicotinoids are numerous. This class of insecticide has a novel mode of action, thereby minimizing the risk of cross-resistance between the neonicotinoids and those chemicals currently being used for avocado pest management. Both of these materials can be applied through the irrigation system, thereby avoiding the need for expensive and environmentally contentious aerial applications of foliar insecticides. This will also allow for better timing of applications to deal with incipient outbreaks. The persistence of the neonicotinoids could provide prolonged protection against key pests during vulnerable periods of fruit and leaf development.

Summary of Results to Date

During the initial phase of this study, we evaluated the uptake of imidacloprid by first quantifying titers in both xylem fluid and leaf extracts, and then relating the residue levels with insect mortality in bioassays. Our strategy was to determine the initial mobilization of imidacloprid within trees by monitoring titers within the xylem vascular system. Ultimately, the xylem system deposits insecticide within leaves, the feeding sites for both the ALB and the AT. Therefore, quantification of titers within leaf extracts was undertaken to determine whether levels of imidacloprid being distributed by the xylem fluid could reach critical levels necessary for pest management. To measure the efficacy of imidacloprid residues within avocado leaves, bioassays were conducted on the same leaves for which residue levels were measured. In this way, we could make a direct correlation between insecticide levels and insect mortality.

Field site

The location of our trial site was in Fallbrook in a commercial avocado grove. At this site, we were able to evaluate the impact of tree size on imidacloprid uptake. The tree sizes chosen were designated as "Small", 6-8 year old trees, typical of the many new plantings in the industry, and "Large", 18-25 year old trees, 30-40 feet in height, typical of those with minimal thinning and pruning in San Diego and Riverside counties. The availability of trees of both sizes would minimize variation that might arise from using different geographical locations for uptake studies with the two tree sizes. Each tree was served by a single emitter (0.06" nozzle) that delivered 23.25 gal/hour with a 25 ft diameter.

Insecticide Applications

We used a new formulation of imidacloprid – Admire Pro®. The product was mixed with water and then administered to trees (0.5 gallons/tree) using a watering can. The applications were made during the normal irrigation schedule, which was 8 hours for the small trees and 16 hours for the large trees. Prior to application, trees received a minimum of 5 hours of irrigation. The irrigation was suspended during the application process. Following application, the irrigation was run for a further 3 hours for both the small and large trees to deliver the insecticide below the surface of the soil to the tree roots. The irrigation of the trees was done on a weekly basis.

Quantification of Imidacloprid in Xylem Fluid

Xylem fluid was extracted from individual stems of avocado trees using a pressure bomb device. Terminal cuttings were taken at two locations on each tree. The phloem was carefully peeled away and the stem inserted through the grommet in the lid of the pressure chamber. Under pressures of 30 psi, the xylem fluid (200 µl) was extruded through the severed end of the stem where it was collected by pipette and transferred to 1.5 ml microcentrifuge tubes. The tubes were stored in a container of dry ice in the field to await transfer to the laboratory for imidacloprid measurements.

Imidacloprid titers within the xylem fluid were quantified using a commercially available ELISA kit. For each assay, an aliquot of xylem fluid was diluted in water (at least 10-fold) to eliminate matrix effects. This dilution is necessary to ensure accurate imidacloprid measurements within samples. Samples are then mixed with an imidacloprid-enzyme conjugate before addition to an antibody-coated microplate. Imidacloprid in the xylem extract competes with the imidacloprid-enzyme conjugate for a finite number of antibody binding sites on the wells of the microplate. Samples containing large titers of imidacloprid will displace more of the conjugate. Therefore, in subsequent enzyme assays that quantify the bound conjugate colorimetrically, the concentrations of imidacloprid present in the original xylem fluid can be determined by comparing the level of color development with a standard curve prepared from known imidacloprid calibrators.

Quantification of Imidacloprid in Leaves

This component of the study was done in conjunction with the Munger cell bioassays. Four leaf discs (0.39 cm^2) were taken from avocado leaves prior to their insertion into Munger cells. The discs were located immediately adjacent to the bioassay arena. The discs were extracted in methanol and the imidacloprid titers then quantified by ELISA (as described above). We are

currently quantifying imidacloprid metabolites within the extracts; for this report, however, the data provided represent a composite measurement of imidacloprid and its metabolites.

Results

Profiles of Imidacloprid Uptake in Small and Large Avocado Trees - Xylem

Imidacloprid was detected in the xylem fluid of small trees within 1 week of treatment with either the 14 or 28 fl oz Admire Pro® rate (***Figure 1***). During subsequent sampling dates, the titers of imidacloprid measured within the xylem system increased steadily. Peak levels were reached in the 14 fl oz treatment on Aug 12 (9 weeks after treatment), and in the 28 fl oz treatment on July 22 (6 weeks after treatment). Thereafter, levels began to decline. Although levels in the 28 fl oz treatment were usually higher than in the 14 fl oz treatment, the differences between the two treatments were not significantly different.

There were noticeable differences in the titers of imidacloprid measured between trees within each treatment. However, on most sampling dates, there was overlap in the xylem levels of imidacloprid in the two treatments.

Uptake into the large trees was very poor. Despite the lower levels, the profile of uptake and decline for these trees was similar in shape to those determined for the smaller trees at the two treatment rates.

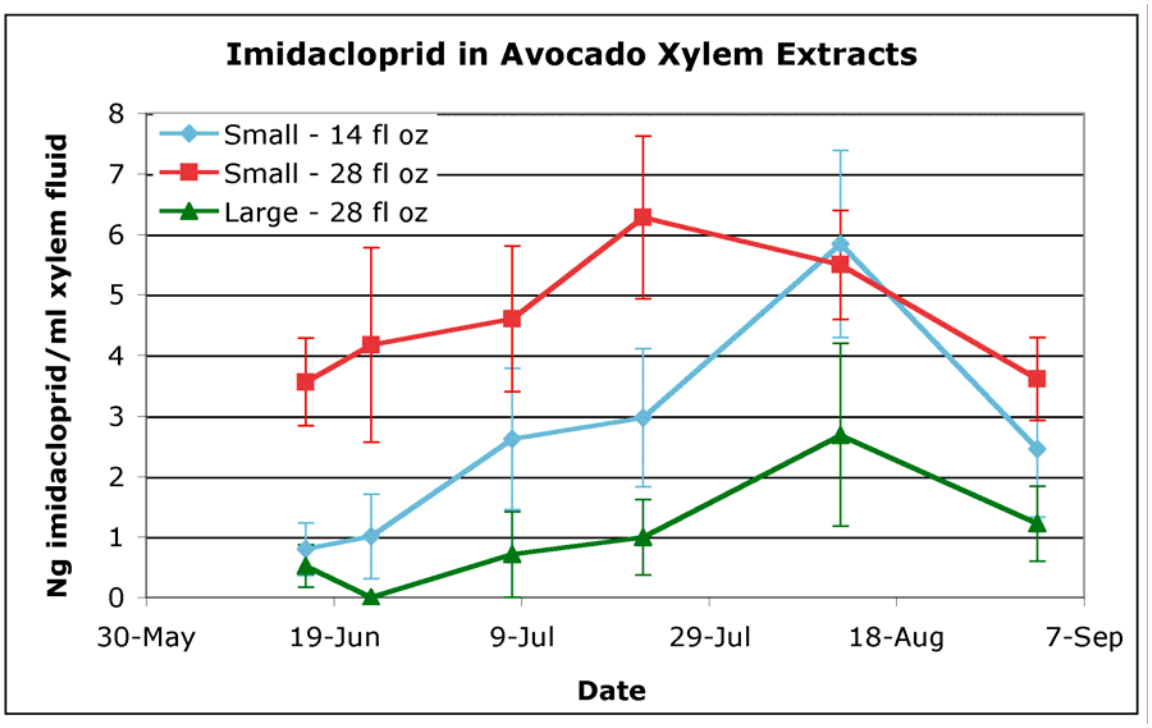

Figure 1. Imidacloprid levels in xylem fluid extracted from two tree sizes treated with Admire Pro®. Two samples were extracted from each of 5 trees for each treatment on each sampling date. The small trees were treated on June 9th, while the large trees were treated on June 10th.

Correlation of Imidacloprid Residues and Insect Bioassays

Bioassays of avocado thrips and ALB were conducted on several dates during the current trial. The bioassay data can be summarized as follows:

*Bioassay #1 was conducted on July 13 against the Avocado Lace Bug – **Figure 2***

Two trees, one each from both small tree treatments, were selected as the source of bioassay leaves based upon imidacloprid levels detected within their xylem systems.

There was excellent mortality of ALB on leaves collected from trees treated at both 14 (tree #3) and 28 fl oz/acre (tree #8). Residue analyses indicated that despite the apparently low insecticide levels in the xylem, deposition of imidacloprid and its potentially insecticidal metabolites was significant. Mortality of ALB was assessed at 48 and 72 hours and indicated a time-dependent response.

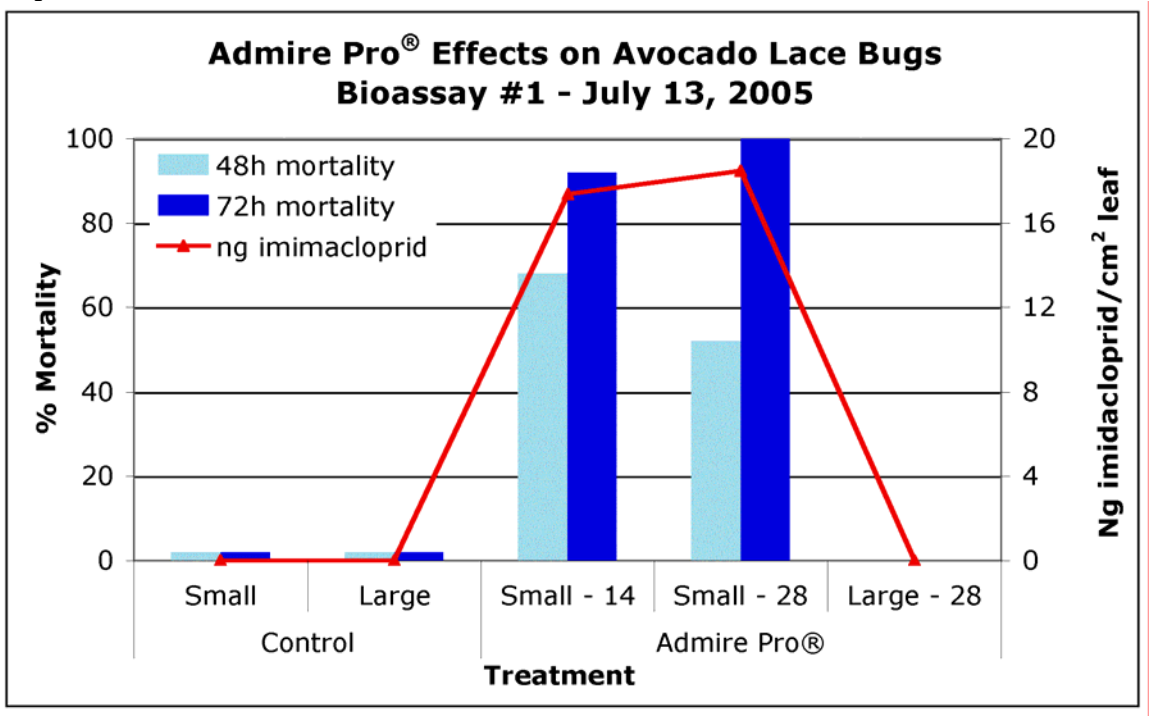

Figure 2. Correlation of ALB mortality with imidacloprid residues. Imidacloprid levels were quantified in the same leaves used in Munger cell bioassays.

Bioassay #2 was conducted on July 18 against Avocado Thrips

Young leaves from the same trees used for the ALB bioassay described above (Figure 2) were used in this bioassay. No mortality was recorded in the bioassays, despite low levels of imidacloprid. Clearly, the threshold levels of toxicant had not been reached in these young leaves.

*Bioassay #3 was conducted on August 3 against both ALB and Avocado Thrips – **Figures 3 & 4***

For this bioassay, 10 leaves were collected from each of two small trees (one from each treatment rate). ALB (***Figure 3***) and avocado thrips (***Figure 4***) were exposed to both mature and young leaves from each tree in Munger cell bioassays in order to assess whether the different

levels of insecticide detected in these two leaf ages could account for differential mortality in the previous two bioassays.

There was approximately 40% mortality of avocado thrips exposed to the treated mature leaves. Taking into account the moderate control mortality (avocado thrips do not prefer to feed on mature leaves), these levels of mortality indicate an insecticidal effect with the mature leaves. No mortality of avocado thrips was detected on the younger leaves, corroborating results from our earlier bioassay. In these leaves, the 'apparent' imidacloprid titers were close to threshold levels of insecticide for avocado thrips control (6 ng imidacloprid/cm^2 leaf). But, as we have since learned from our studies of metabolites, part of this residue value will be contributed by metabolites that lack toxicity towards avocado thrips. Therefore, the lack of avocado thrips mortality was not unexpected in these bioassays.

The bioassay results for the ALB were very encouraging. As expected, we obtained excellent mortality of ALB on the mature leaves. There was also excellent mortality of ALB on young leaves, despite the lower titers of insecticide. This indicates the greater susceptibility to imidacloprid of the ALB compared to the avocado thrips. Even at apparent imidacloprid levels of 6 ng/cm^2 leaf, 80% mortality of ALB was achieved compared with no mortality of avocado thrips at a similar dose.

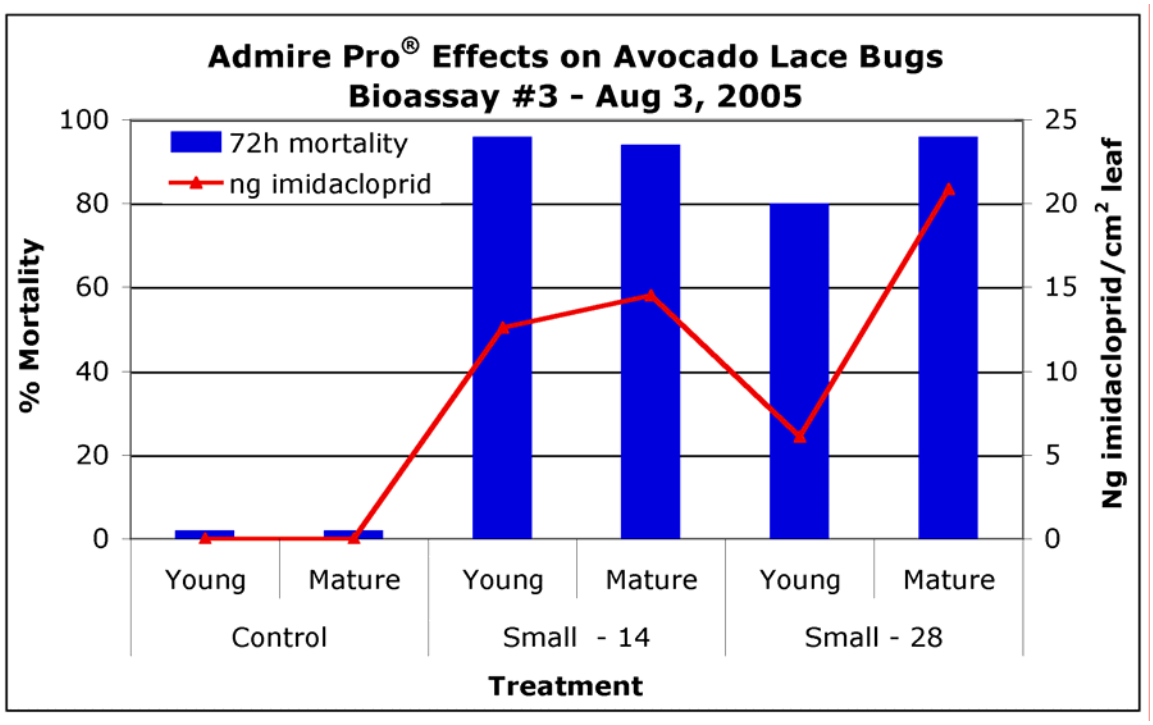

Figure 3. Correlation of ALB mortality with imidacloprid residues in young and mature leaves. The mature leaves were present on the trees at the time the insecticide applications were administered. The young leaves developed on the trees in the weeks following the applications, and would, therefore, not be expected to have the same insecticide levels. Imidacloprid levels were quantified in the same leaves used in Munger cell bioassays.

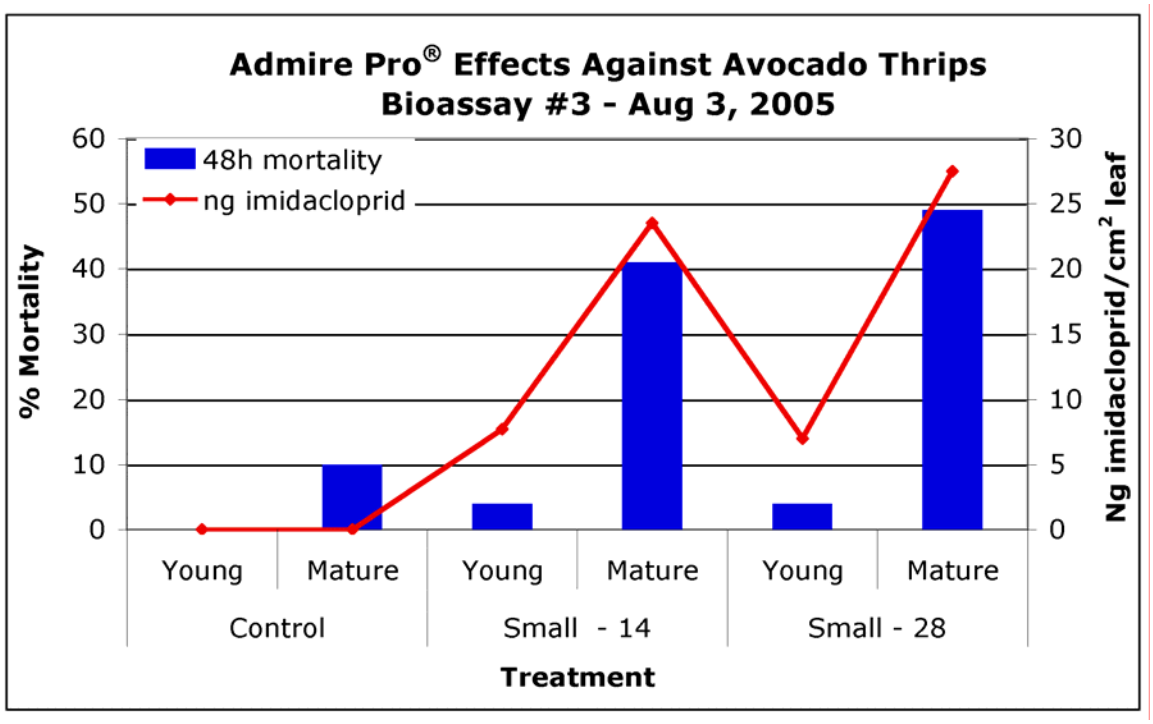

Figure 4. Correlation of avocado thrips mortality with imidacloprid residues in young and mature leaves. The mature leaves were present on the trees at the time the insecticide applications were administered. The young leaves developed on the trees in the weeks following the applications, and would, therefore, not be expected to have the same insecticide levels. Imidacloprid levels were quantified in the same leaves used in Munger cell bioassays.

Bioassay #4 was conducted on September 2 against ALB – ***Figure 5***

At this time, insecticide residues had declined significantly within the xylem fluid, indicating that the source of insecticide within the soil was diminishing. For Munger cell bioassays, leaves from all trees were tested. Four leaves were sampled from each tree (5 trees for each treatment, including the large trees that were treated with the 28 fl oz rate). We chose leaves from all trees to obtain a broader picture of the control potential of the treatments between trees, rather than focusing on individual trees that had the higher levels of insecticide.

Imidacloprid was detected in all leaves, including those sampled from the large trees. Once again, there was high mortality of ALB in the leaves collected from the small trees (at both application rates). The concentrations of imidacloprid appear to be close to threshold levels at this time, judging from the less than perfect mortality readings in bioassays. Despite the detection of imidacloprid within the large trees, the levels were inadequate to effect significant mortality of ALB.

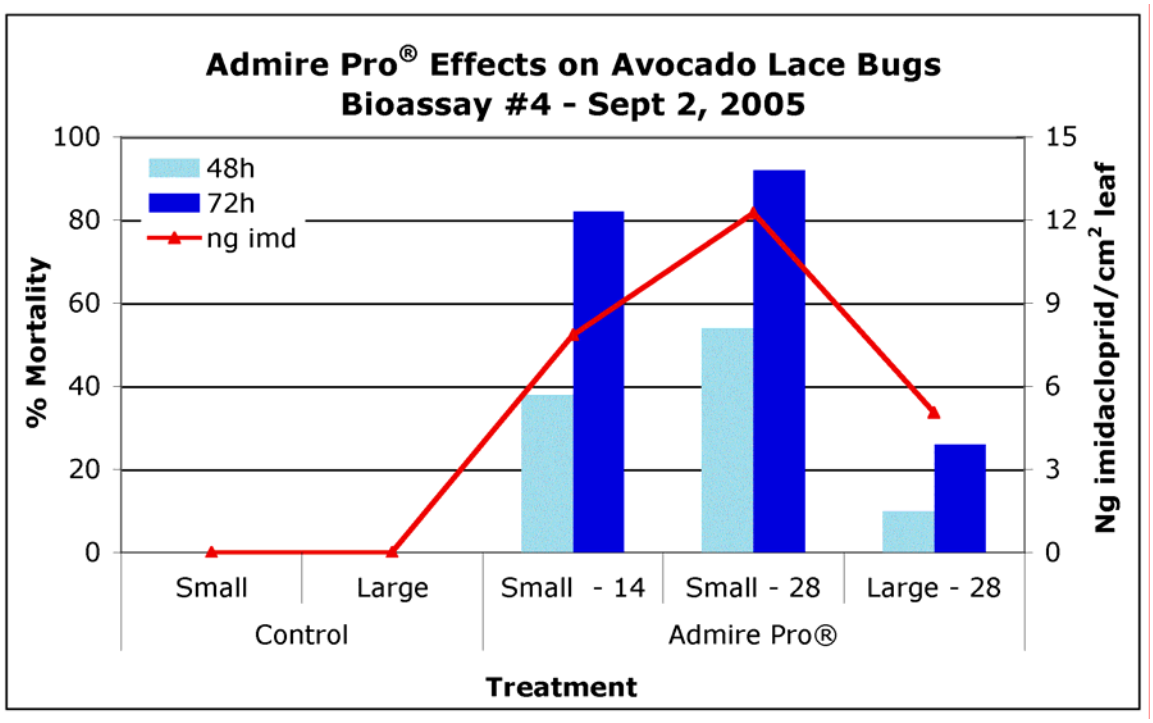

Figure 5. Correlation of ALB mortality with imidacloprid residues. Four leaves were sampled from each of five trees at each treatment. Imidacloprid levels were quantified in the same leaves used in Munger cell bioassays.

Imidacloprid Metabolism Within Avocado Leaves

We have completed a preliminary measurement of imidacloprid metabolism within avocado leaves, and will develop this aspect of the work during the forthcoming field trials. In the most recent nursery study (conducted at Fallbrook in 2004 – see 2004 annual report) with these materials, leaf discs taken from the Admire® bioassay leaves were used to assess the insecticide residues. In that way, we were able to define a threshold imidacloprid concentration within the leaves for avocado thrips control (6 ng imidacloprid/cm^2 leaf). By week 11 of that study, imidacloprid was still present at apparently high concentrations within the leaves. However, these apparent readings did not result in significant mortality of avocado thrips. In our most recent laboratory studies, we have shown that there was significant degradation of imidacloprid during the later phase of the trial period (***Figure 6***).

Two of the breakdown products have been identified as the olefin and mono-hydroxy metabolites. These are known to exhibit toxicity of their own against whiteflies, aphids, and glassy-winged sharpshooter. There was also a significant presence of highly polar metabolites. Based upon these data, it now seems likely that the loss of correlation between the "apparent' imidacloprid residue readings, generated by the ELISA, and avocado thrips bioassay mortality in bioassays was due to significant losses in imidacloprid due to the formation of metabolites that also cross-reacted with the antibody. Although imidacloprid was predominant throughout most of the study period, its metabolism increased over time and thus, it will have to be taken into account when future estimates of imidacloprid uptake are determined. We are currently evaluating the metabolite component within leaves used in the ALB and avocado thrips bioassays described in this report.

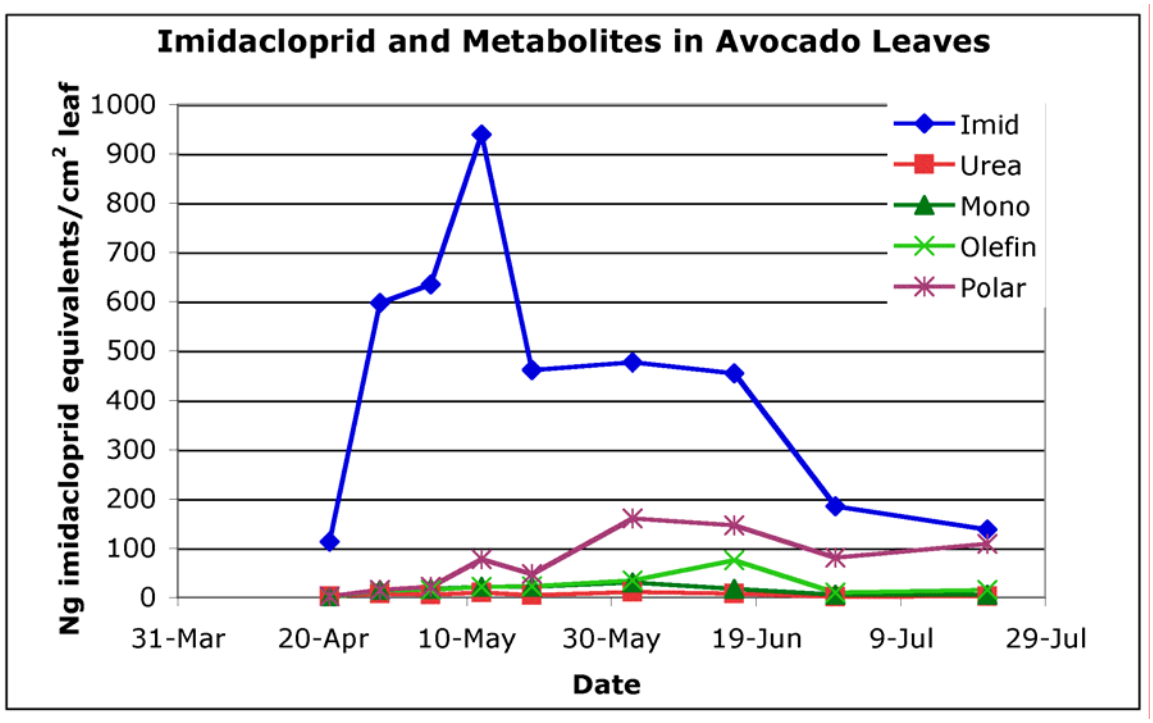

Figure 6. Detection of imidacloprid and its metabolites in avocado leaves. The leaves used for these analyses were collected from nursery trees during the Fallbrook 2004 trial – see 2004 annual report for details.

Discussion

The results from our first trial indicate several important points regarding the use of neonicotinoids within avocado groves. First, tree size is important. We observed better uptake into smaller trees, and levels of toxicant were reached that were effective against the ALB. While the insecticide was detected within the large trees, it will be necessary to conduct further evaluations on the larger tree size to determine the feasibility of using imidacloprid as a pest management tool. The insecticide was taken up into the trees and did afford some control, but satisfactory threshold levels were not reached. Second, quantitative differences will exist between mature leaves (i.e. those that are present on the tree at the time of insecticide application) and new leaves (i.e. those that develop after the materials have been applied). In our current trial, we believe that an earlier application would have provided better residues within the trees (it was as early as we could apply the treatment given the very late date we were notified of funding). And third, while the rate of application will have an impact on the overall levels of material that are present in the trees, the general uptake patterns (in terms of the ascending and declining phases of the uptake profiles) are the same. Thus, increasing the rate of application will ensure higher titers within the trees, but may not guarantee significantly extended protection beyond that afforded at lower rates.

The leaves that receive the best protection from the insecticide treatment are those that are present on the tree at the time of application. Leaves arising from subsequent flushes do not appear to receive sufficient toxicant for effective thrips management. This result concurs with that obtained from the potted avocado trial. Depletion of available material occurs before the

newer leaves can receive enough for protection. For this reason, the split application of 14/14 may be an important option for smaller trees by allowing growers to make an additional 14 fl oz/acre application later in the season when the newer leaves might be exposed to a thrips attack.

Future Research Plans

In the Spring of 2006, we will select different sites to assess the uptake of neonicotinoids following application by chemigation. Our main priorities will be to evaluate the effect of tree size and application rate on uptake. It is especially important to establish the utility of using neonicotinoids on very large trees. To perform a proper evaluation, we will conduct uptake studies at different sites that best reflect the range of conditions associated with the California avocado industry. We will continue to monitor the levels of insecticide within the xylem and leaves of treated trees to determine the effectiveness of different rates of application at attaining effective thresholds for ALB and avocado thrips control. The timing of treatments will be important if adequate insecticide is to be incorporated into fresh leaf flushes which are most attractive to thrips attack.

Relevant Recent Publications

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