Research Article

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Field evaluation of systemic imidacloprid for the management of avocado thrips and avocado lace bug in California avocado groves

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Abstract

BACKGROUND: The efficacy of systemic applications of imidacloprid for the management of avocado thrips and avocado lace bug was determined in field trials. Following insecticide treatment by chemigation, leaves of appropriate age for each insect were sampled over a 6 month period and used for bioassays. Imidacloprid residues were measured by ELISA in leaves used for bioassays to determine concentrations of insecticide that were toxic to both pests.

RESULTS: The uptake of imidacloprid into treated trees was extremely slow, peaking in the current year's leaf flush at only 8 ng cm^{-2} leaf tissue after 15 weeks. Avocado thrips mortality in bioassays with young flush leaves, the preferred feeding substrate for this insect, was minimal, indicating that imidacloprid concentrations were below threshold levels needed for effective control. Residues present in older leaves, which are preferred by the avocado lace bug, were higher than in young flush leaves, and provided good control of this pest. Probit analysis of bioassay data showed that the avocado lace bug (LC_{50} = 6.1 ng imidacloprid cm^{-2} leaf tissue) was more susceptible to imidacloprid than the avocado thrips (LC_{50} = 73 ng imidacloprid cm^{-2} leaf tissue).

CONCLUSIONS: In spite of the slow uptake of imidacloprid into avocado trees, the levels of imidacloprid would be sufficient to control avocado lace bug infestations. In contrast, the slow uptake would be problematic for avocado thrips control because inadequate levels of insecticide accumulate in new flush foliage and would allow avocado thrips populations to build to levels that would subsequently damage developing avocado fruit.

Keywords: *Scirtothrips perseae*; avocado thrips; *Pseudacysta perseae*; avocado lace bug; imidacloprid; systemic insecticide; *Persea americana*; pest control

1 INTRODUCTION

The California avocado industry is under increasing threat from the introduction of arthropod pests.[1,2] The avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), was first detected in California avocado groves in June 1996,[3] and it has since spread to most of the major production areas within the state where it has become the primary insect pest.[4] The main source of economic loss arises from feeding damage that causes scarring of immature fruit, leading to a reduction in fruit quality at harvest.[4] However, avocado thrips will also feed and oviposit on immature leaf tissue,[5] and high populations can develop on this growth. This is normally a lesser concern for growers, but high pest densities can cause premature leaf drop, with a subsequent decline in tree health.[6] The avocado lace bug, *Pseudacysta perseae* (Heidemann) (Hemiptera: Tingidae), was first detected on avocado trees located in homeowner gardens in the city of San Diego, California, in 2004.[7] This insect feeds on the undersides of leaves, resulting in the formation of brown necrotic areas of dead leaf tissue. Heavy feeding damage lowers photosynthetic capacity and can result in significant leaf drop.[8] While the distribution of the avocado thrips is widespread among commercial groves in southern California, the avocado lace bug has thus far been confined to the southern coastal areas of San Diego County, and has not been detected in commercial groves.[9]

In California avocado groves, the use of foliar insecticides is the predominant tactic adopted by growers for the management of arthropod pests, including the avocado thrips. Aerial applications by helicopter are needed for the majority of California avocado groves because most are grown on steep hillsides. However, helicopter applications are expensive, are not always immediately available when pest outbreaks occur[10] and may not provide complete coverage of infested trees unless large volumes are applied.[11] The location of groves near urban areas increases the

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threat to human health from direct exposure to pesticide drift and from contamination of groundwater. While foliar treatments are often preferred because their contact activity can promote an immediate and rapid decline in a pest population, they can be problematic in their non-target impacts against natural enemies and other beneficials, particularly honey bees,[12] which are important pollinators in avocado groves. Abamectin, sabadilla and spinetoram insecticides are currently the most effective active ingredients in foliar treatments for thrips control.[13] In spite of the diversity of chemicals available to growers, the propensity of avocado thrips to develop insecticide resistance was recently observed with the first detection of resistance to sabadilla in a commercial grove in Ventura County, California.[13]

In an effort to improve the management of arthropod pests threatening avocado production in California, the authors are evaluating the use of neonicotinoid insecticides as a management option. Although neonicotinoids can be applied as foliar treatments, they are particularly attractive to the industry as potential systemic treatments because they could be applied via irrigation systems already established within groves. Neonicotinoids have a different mode of action than the foliar treatments already in use within the industry, and could therefore contribute to the management of insecticide resistance.

The most promising neonicotinoid for use against avocado thrips is imidacloprid. In a previous study,[10] the systemic activity of four neonicotinoid insecticides in nursery avocado trees was evaluated against avocado thrips. Imidacloprid was the most effective insecticide in terms of its persistence at lethal concentrations within foliage that was present on the trees at the time of the application, and in its capacity to provide additional protection to a subsequent leaf flush that developed on the trees at about 7 weeks after the trees had been treated. While the results of that study provided important information on the activity of systemic imidacloprid treatments on nursery stock, the next phase of the program was to evaluate the activity of the insecticide in much larger trees that were typical of much of the commercial avocado industry in California. An important outcome of the nursery study was the establishment of the imidacloprid concentration within a leaf that was necessary to kill avocado thrips. A concentration of 100 ng imidacloprid cm^{-2} leaf tissue was effective in killing at least 70% of avocado thrips in bioassays, and this value has served as a useful target threshold for assessing the efficacy of imidacloprid treatments on avocado trees of different sizes.[10] No such data are available for the avocado lace bug, and thus an important objective of this study was to derive concentrations of systemic imidacloprid that were effective against this pest.

2 MATERIALS AND METHODS

2.1 Insects

Avocado thrips were collected from commercial avocado groves 1–2 days prior to conducting each series of bioassays. Field sites were chosen on the basis of thrips availability, and collections were limited to groves with no history of imidacloprid use.

A colony of avocado lace bugs was established in a dedicated quarantine unit in Chula Vista, San Diego, which was located within the quarantine zone for that pest. Colonies of avocado lace bugs were established from field-collected adults (five sites from around San Diego County) and were reared on potted avocado trees (Bacon variety scions grafted to either Duke 7 or Toro Canyon rootstock), approximately 1.5 years old, in a greenhouse at 25 ± 5 °C, 60% RH and 14 : 10 h light : dark photoperiod.

2.2 Field trial – 2005

2.2.1 *Description of the field site*

In June 2005, a field study was conducted in a commercial Hass avocado grove located in Fallbrook, California, where the availability of two tree sizes in adjacent blocks made it possible to evaluate the impact of tree size on imidacloprid uptake. At this location, the soil type was classified as a Las Posas stony fine sandy loam with a clay content of 15–25% between 0 and 10.2 cm and 35–45% between 10.2 and 83.8 cm, and an organic matter content of 1% [USDA web soil survey (http://websoilsurvey.nrcs.usda.gov/)]. However, there was a thick layer of organic matter beneath the loose leaf litter under the large trees that had accumulated since the grove was established. The large trees (0.1 ha block of 32 trees) were 25 years old and 9–12 m in height. The small trees (0.2 ha block of 55 trees) were 6 years old and 3–4 m in height. The trees were irrigated by microsprinklers consisting of a rotospray microspinner (RS-15) that delivered 100 L h^{-1} for either 8 h (small trees) or 16 h (large trees) during once-weekly irrigations.

2.2.2 *Insecticide applications*

Prior to insecticide treatments (9 June), the leaf litter within the sprinkler patterns was removed with a garden rake to enable easier access to the feeder roots. Imidacloprid 550 g L^{-1} SC (Admire Pro; Bayer CropScience, Research Triangle, Raleigh, NC) was then applied during the regular irrigation to individual trees using a watering can. Half and full label pesticide rates [maximum rate of 1.02 L formulation ha^{-1} (560 g AI ha^{-1})] were applied. The insecticide was diluted in water and applied uniformly within the sprinkler pattern of each tree in a final volume of 1.9 L. Final application rates were 280 g AI ha^{-1} and 560 g AI ha^{-1} to separate sets of small trees ($n = 5$ trees for each treatment rate) and 560 g ha^{-1} to the large trees ($n = 5$ trees). Because imidacloprid was not labeled for prebloom or mid-bloom use on avocados at the time of this study, it was necessary to destroy all of the fruit on the trees at the end of the experiment, which limited the number of trees available for replication of treatments. Prior to treatments, trees within each block were numbered, and then experimental trees were chosen using a random number generator.

2.2.3 *Measurement of imidacloprid uptake*

The rate of uptake of imidacloprid was determined from measurements of insecticide in samples of xylem fluid taken at 1, 2, 4, 6, 9, 12, 15 and 18 weeks after treatments. Xylem fluid was extracted from terminal shoots using a pressure bomb,[14] and the imidacloprid was quantified by ELISA.[15,16] Imidacloprid concentrations in leaves at different stages of flush were quantified at 4 and 8 weeks after treatments. At the time of the treatments, the summer leaf flush had begun, and leaves chosen for the age comparison represented fully expanded leaves from the spring (mature) and summer flushes ($n = 20$ leaves for each age class on each sampling date). The seasonal bud-scale scars were used to distinguish leaves arising from different leaf flushes.

2.3 Field trial – 2006

2.3.1 *Description of the field sites*

In 2006, a second study was conducted at three commercial Hass avocado groves located west of Temecula, Riverside County, California, where the trees were 20–25 years old. At this time, a 24(c) supplemental label (EPA SLN No. CA-06 005) permitted the application of imidacloprid at prebloom and during bloom,

enabling the authors to conduct a much larger trial than in 2005 and to provide a more comprehensive evaluation of the effectiveness of soil applications of imidacloprid for the protection of trees against avocado thrips and avocado lace bugs. The sites were designated P37, P68 and GGG. The soil type for each site was obtained from the USDA web soil survey (http://websoilsurvey.nrcs.usda.gov/). At site P37, the soil type was classified as a Fallbrook rocky sandy loam with 10–20% clay and 0.5–1% organic matter to a depth of 20 cm. The soil types at site P68 and site GGG were both classified as Lodo rocky loam with 18–35% clay and 1–6% organic matter to a depth of 20 cm. There was also a heavy layer of leaf litter under the trees at each of these sites, which would have contributed substantial increases to the organic matter content of the soils since the original soil surveys were conducted. Trees were irrigated using microsprinklers that delivered 34 (site GGG), 38 (site P68) and 57 L h^{-1} (site P37) during once-weekly irrigations that were scheduled according to tensiometer readings.

2.3.2 *Insecticide applications*

In contrast to the 2005 study, imidacloprid was applied through the irrigation system (chemigation) on 30 March when the spring leaf flush had begun. Flow rates were determined for the sprinklers at each site, based upon meter readings taken at 15 min intervals during a regular irrigation. The maximum field rate of 560 g AI ha^{-1} was applied at each location. At site P68, two injection strategies were evaluated in which the insecticide was injected into irrigation lines over 2 h, beginning at either 6 or 16 h after the water was initially turned on during a 24 h irrigation cycle. At site P37 and site GGG, the insecticide was injected over 2 h, beginning 6 h after irrigation was initiated.

At site P37 and site GGG, the leaf litter was removed from beneath five trees using a garden rake to determine whether exposure of the feeder roots during chemigation improved uptake (in subsequent discussions, these trees will be referred to as the raked treatment and the remaining trees as the unraked treatment).

2.3.3 *Measurement of imidacloprid uptake*

The concentrations of imidacloprid were quantified in leaves collected at 6, 8, 11 and 15 weeks after treatment. On sampling days, six leaves were sampled from each of 16 trees. Leaf discs (0.39 cm^2) cut from each set of six leaves (one leaf disc from each leaf cut to one side of the mid-vein) were then combined to prepare composite extracts for each tree.

2.4 Leaf sampling for bioassays and residue analysis

Bioassays were conducted during the 2005 and 2006 trials. Leaves used in avocado thrips bioassays were sampled from the most recent leaf flush when they were at least 3.8 cm wide (to fit in Munger cells, see below), whereas leaves used for avocado lace bug bioassays were the oldest available leaves from the previous flush growth. In southern California Hass avocado groves there are normally two seasonal leaf flushes that occur in spring and summer. At the time the bioassays were conducted during the 2005 trial (July and August), the leaves from the spring flush were hardened off and were chosen for avocado lace bug bioassays. Leaves from the summer flush were already developing, and these were chosen for avocado thrips bioassays. In 2006, because of the earlier application timing, bioassay leaves for the avocado lace bug and avocado thrips were chosen from summer 2005 and spring 2006 leaf flushes respectively. Typically, a single leaf flush on a Hass avocado tree will have between 12 and 18 leaves when complete, and so leaves within the mid-range (leaf numbers 6 to 10, where leaf 6 is oldest and was initiated approximately 10 days prior to leaf 10, based upon a Plastochron model developed for avocados)[17] were chosen for the tests. Bioassays for both insects were conducted using Munger cells.[18,19] Imidacloprid residues were measured in discs cut from the leaf area immediately outside the insect chamber enclosed by the Munger cell using a 0.39 cm^2 cork borer. Four discs were cut from each leaf before the leaves were inserted into the Munger cells. A minimum of ten insects (second-instar avocado thrips and third-instar avocado lace bugs) were added to each Munger cell chamber. For each set of bioassays, ten replicate cells were run on each date for each insecticide treatment, including ten control cells using leaves collected from insecticide-free Hass avocado trees. For mortality assessments, the Munger cells were disassembled after 48 h (avocado thrips) and 72 h (avocado lace bug), and the leaves were examined under a light microscope. Insects that failed to move one body length following probing were scored as dead. In addition, avocado thrips rapidly desiccate once dead, making the mortality assessments relatively easy. Unaffected insects from both species dispersed rapidly from view once probed and were easily distinguished from those insects killed by the insecticide.

2.5 Chemical quantification

Concentrations of imidacloprid were determined according to the method of Byrne *et al.*[15] using a commercially available competitive ELISA kit (QuantiPlate kit for imidacloprid, cat. no. EP-006; EnviroLogix, Inc., Portland, ME) with a reported 0.2–6 µg imidacloprid L^{-1} sensitivity range.

For the measurement of imidacloprid residues, the leaf discs were homogenized in 100% methanol (1 disc 200 μL^{-1}) in 1.5 mL Eppendorf tubes using Kontes® pellet pestles. The homogenates were shaken vigorously overnight and then centrifuged at 10 000 × *g* for 5 min to pellet the particulate matter. An aliquot (100 µL) of each extract was loaded onto individual lanes of TLC plates and chromatographed in methylene chloride + methanol + ammonium hydroxide (45 + 5 + 1). The position of imidacloprid was determined by cochromatographing an imidacloprid standard with the avocado samples. Bands were scraped from the plates at the imidacloprid position and eluted from the silica in 1 mL methanol. Aliquots (150 µL) of each wash were dried completely in a TurboVap (Caliper Life Sciences, Hopkinton, MA) at 35 °C and 4 psi, resuspended in water containing 0.05% Triton X100 and quantified by ELISA. The purification step using TLC was necessary to remove imidacloprid metabolites that cross-react with the imidacloprid antibody in the ELISA.[15]

2.6 Statistical analysis

All statistical analyses of field data for imidacloprid uptake were performed using JMP Statistical Discovery Software, v.8.0.[20] A repeated-measures multivariate analysis of variance (MANOVA) was used to test for significant effects of insecticide application rates and conditions on the uptake of imidacloprid. The Tukey HSD test was used to test for the significance of means at a 5% significance level.

Avocado thrips and avocado lace bug bioassay data were analyzed by probit analysis[21] using the POLO-PC program,[22,23] with correction for control mortality where necessary.[24]

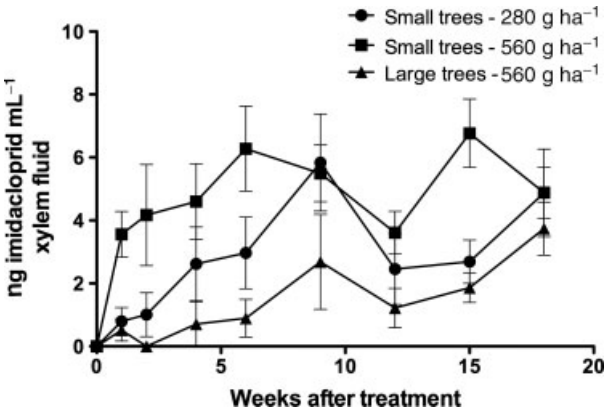

Figure 1. Concentrations of imidacloprid in xylem fluid of avocado trees. The small trees were treated at two rates of imidacloprid (280 and 560 g ha^{-1}) and the large trees were treated at the higher rate only. Each point is the mean imidacloprid concentration for five trees (± SE).

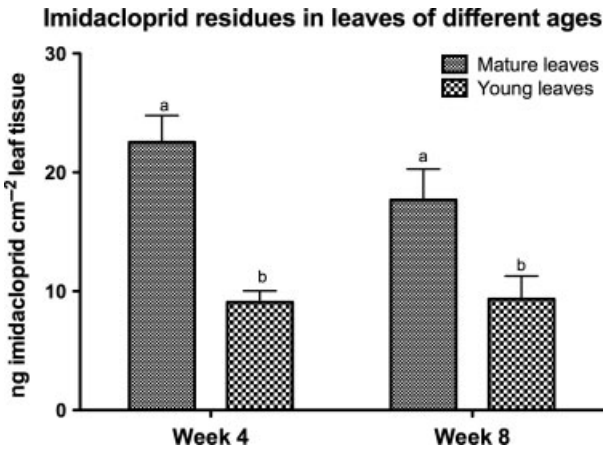

Figure 2. Comparison of imidacloprid concentrations in leaves sampled from small avocado trees treated with imidacloprid. Samples were collected at 4 and 8 weeks after the trees were treated. Each bar is the mean imidacloprid concentration (± SE) for 20 leaves. Means that are significantly different ($\alpha = 0.05$) are indicated by a different letter above the bar.

3 RESULTS

3.1 Field trial – 2005

3.1.1 Imidacloprid uptake into xylem

Imidacloprid was detected in the xylem fluid of all tree sizes within 1 week of treatment (Fig. 1). The levels of imidacloprid increased steadily, peaking in the small trees at 9 and 15 weeks for the 280 g ha^{-1} and 560 g ha^{-1} rates respectively, and at 18 weeks for the large trees treated with the 560 g ha^{-1} rate. During the first 2 weeks of monitoring, the rate of uptake of imidacloprid into the larger trees was considerably slower than uptake into the small trees. Although imidacloprid concentrations in the large trees were still increasing at 18 weeks, the mean titers never exceeded those measured in the smaller trees treated at either of the two treatment rates. There was an unexplained decline in imidacloprid concentrations at 12 weeks for all treatments, after which levels began to increase again. This decline may be temperature related – in the week preceding the week 12 samples (taken on 2 September) there was a sharp increase in temperature (33.6 °C average between 25 and 29 August), perhaps causing heat stress to the trees, resulting in reduced transpiration. This effect would have been more pronounced on the smaller trees because they were more exposed to sunlight.

A repeated-measures MANOVA indicated that there was an overall significant treatment effect ($F_{2,27} = 8.05$; $P = 0.002$). Pairwise contrasts indicated that the general treatment effect was the result of significantly lower imidacloprid titers in the large trees compared with the small trees treated at the 560 g ha^{-1} rate ($F_{1,18} = 26.29$; $P < 0.0001$). There was no significant treatment effect detected among the small trees treated at different rates ($F_{1,18} = 3.80$; $P = 0.07$) or the small trees treated at 280 g ha^{-1} and the large trees treated at 560 g ha^{-1} ($F_{1,18} = 2.87$; $P = 0.11$).

3.1.2 Imidacloprid titers in leaf tissue – young versus mature leaves

In young leaves, the concentrations of imidacloprid were significantly lower than in the mature leaves ($F_{1,38} = 25.79$; $P < 0.0001$) (Fig. 2). For the 2005 study, mature leaves were defined as those arising from the spring flush immediately preceding that from which the young leaves were chosen on the same branch. Within the two age classes there was no significant difference between the imidacloprid concentrations measured at 4 and 8 weeks after treatments ($F_{1,38} = 1.46$; $P = 0.23$).

3.1.3 Avocado thrips bioassays

Avocado thrips bioassays were conducted at 2 (22 June), 4 (8 July) and 8 (3 August) weeks after treatments. In the bioassays conducted at weeks 2 and 4, mortality levels never exceeded 10% (results not shown), reflecting the low concentrations of imidacloprid measured by ELISA in young leaves (Fig. 2). The bioassay on week 8 was modified, therefore, to include both young and mature leaves from the same two trees that were used to compare the imidacloprid levels in leaves arising from successive flushes (Fig. 2). Although avocado thrips do not normally feed on older foliage, older leaves were included in the bioassay to determine if higher imidacloprid concentrations in these leaves would have a toxic effect on insects that attempted to feed on them. In control bioassays, mortality of avocado thrips was higher on mature foliage, although not significantly so (Fig. 3). However, after correction for control mortality using Abbott's[24] formula (corrected mortalities are indicated numerically above the bars in Fig. 3), there was evidence of significantly ($\alpha = 0.05\%$) greater insecticidal activity when insects were confined on mature leaves.

Data generated from this study and a nursery study[10] were analyzed using probit analysis in order to generate target thresholds for imidacloprid activity against avocado thrips (Table 1). The LC_5, LC_{50} and LC_{95} values were 14, 73 and 392 ng imidacloprid cm^{-2} leaf tissue respectively.

3.1.4 Avocado lace bug bioassays

Avocado lace bug bioassays were conducted at weeks 4, 8, 12, 18, 24 and 28 after treatments (Fig. 4). On each bioassay date, the imidacloprid concentrations were measured in the bioassay leaves. Mortality was assessed at 48 h and 72 h during bioassays. The levels of mortality were always higher at 72 h, and in some bioassays the mortality had increased by as much as twofold over the initial 48 h reading. It was difficult to maintain the quality of excised leaves beyond the 72 h reading, so bioassays were terminated at 72 h.

In the bioassays that were conducted up to 24 weeks, the levels of mortality were high when insects were exposed to leaves sampled from small trees treated with either the low or high rates of imidacloprid. Mortality levels were much lower when leaves from the large trees were used, although, by week 18, mortality

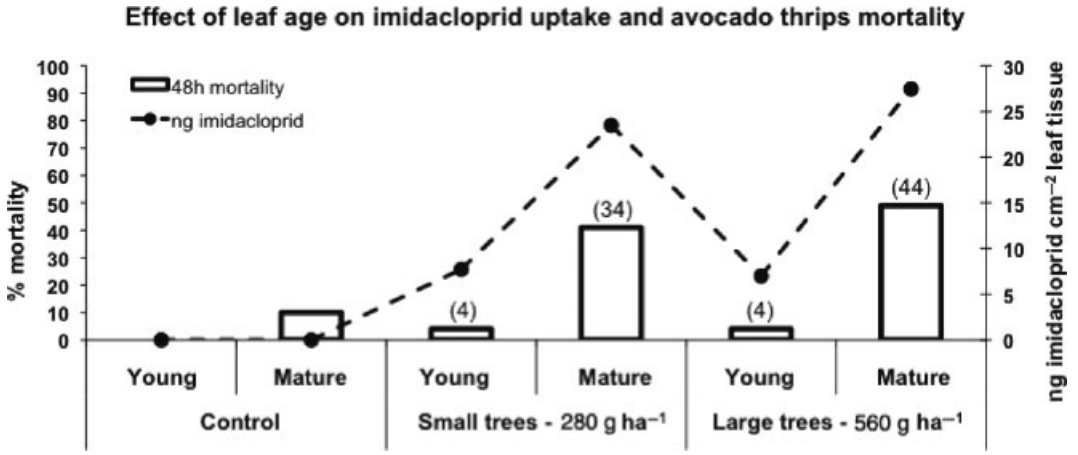

Figure 3. Effect of leaf age and imidacloprid concentration on avocado thrips mortality in Munger cell bioassays. Each bar is the uncorrected mean % mortality (left-hand *y*-axis) for ten bioassay cells. Values for corrected mortality are indicated above each bar. The corresponding concentrations of imidacloprid in the bioassay leaves (right-hand *y*-axis) are shown as individual points. Although the axis for leaf age is a nominal variable, the points are joined by a dashed line to emphasize the increased mortality associated with the higher residues in the mature leaves.

Table 1. Log dose–probit analysis of avocado thrips and avocado lace bug bioassay data (ng imidacloprid cm^{-2} leaf tissue)

Insect	*N*	Slope (± SEM)	LC_5 (95% FL)	LC_{50} (95% FL)	LC_{95} (95% FL)	df	χ^2
Avocado thrips	752	2.25 (±0.17)	13.5 (9.0–18.1)	72.7 (61.5–86.7)	391.6 (283.3–618)	8	9.0
Avocado lace bug	820	2.96 (±0.45)	1.7 (0.7–2.7)	6.1 (4.4–7.4)	22 (19.6–26.6)	9	2.7

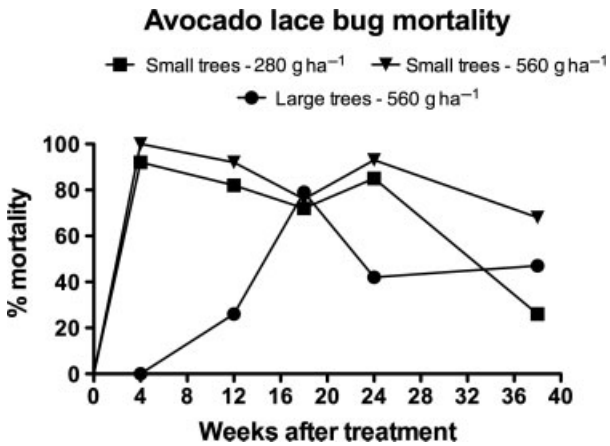

Figure 4. Avocado lace bug mortality in bioassays conducted using leaves sampled from avocado trees treated with imidacloprid. The small trees were treated at two rates of imidacloprid (280 and 560 g ha^{-1}) and the large trees were treated at the higher rate only. Each point is the mean % mortality for ten bioassay cells.

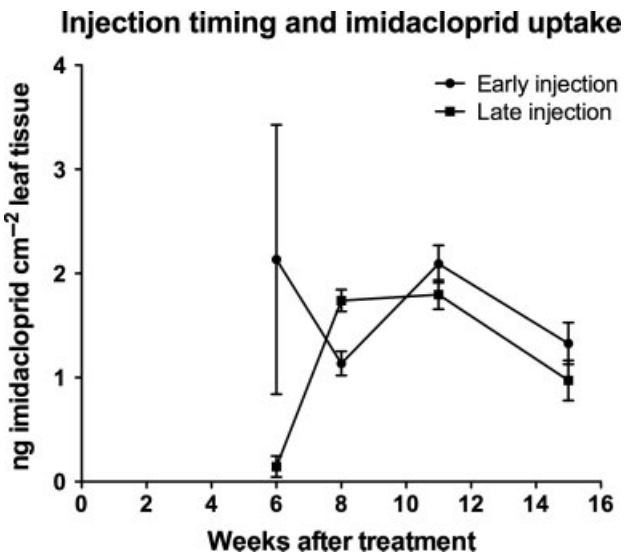

Figure 5. Effect of injection timing during chemigation on the uptake of imidacloprid. Imidacloprid was injected at either 6 h (early injection) or 18 h (late injection) after a 24 h irrigation cycle was begun. This study was conducted at site P68 in 2006. Each point is the mean imidacloprid concentration (± SE) in leaves sampled from 16 trees.

in all bioassays was at parity (Fig. 4). In bioassays on week 38 (conducted in March 2006 just prior to the initiation of the new spring flush) there was still good residual activity in the small trees treated with 560 g ha^{-1} (mortality = 68%), while mortality in the small trees treated with 280 g ha^{-1} and the large trees treated with 560 g ha^{-1} had dropped below 50%.

3.2 2006 trial – effect of application conditions on imidacloprid uptake

3.2.1 Timing of injection during irrigation

At site P68 there was a significant interaction between the injection timing during chemigation and time ($F_{3,28}$ = 3.96; P = 0.018). The high mean imidacloprid level measured at 6 weeks in the trees that were injected early in the chemigation cycle was due to exceptionally high readings in two of the 16 study trees, and this likely contributed to the significant interaction (Fig. 5). In subsequent samples, beginning 2 weeks later, imidacloprid concentrations in these two trees did not differ greatly from the levels in the other trees within the same treatment. Separate analyses indicated significant effects of time for both the

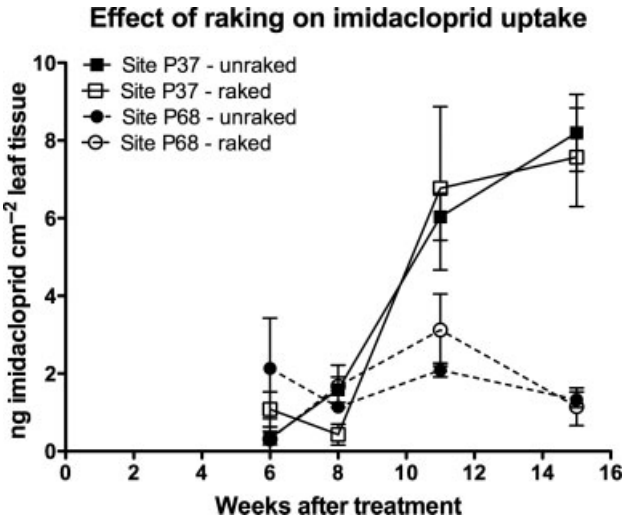

Figure 6. Effect of leaf removal on the uptake of imidacloprid in avocado trees treated by chemigation. Imidacloprid was injected early (6 h) during the 24 h irrigation cycle. Each point is the mean imidacloprid concentration (± SE) for five (leaves raked to expose feeder roots) or 16 (unraked) trees.

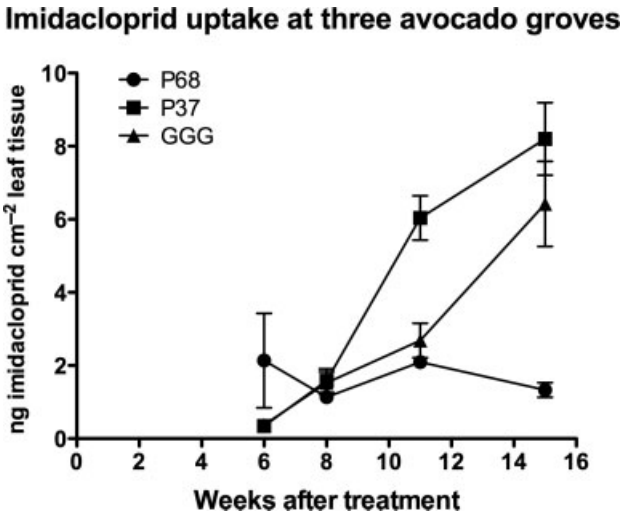

Figure 7. Concentrations of imidacloprid in leaves sampled from avocado trees treated by chemigation at three commercial groves. Each point is the mean imidacloprid concentration (± SE) for 16 trees.

early injection ($F_{3,13} = 8.79$; $P = 0.002$) and the late injection ($F_{3,13} = 5.56$; $P = 0.01$).

3.2.2 *Impact of leaf litter on imidacloprid uptake*

At site P37, under both raked and unraked conditions, the concentrations of imidacloprid in leaves peaked at approximately 8 ng cm^{-2} leaf tissue at 15 weeks after the treatments were applied, with no apparent impact on the pattern of uptake during the sampling period ($F_{1,19} = 0.02$; $P = 0.92$) (Fig. 6). At site P68 (data not shown), the highest concentrations of imidacloprid were detected at 11 weeks (3 ng cm^{-2} leaf tissue), with no significant differences between the raked and unraked conditions ($F_{1,19} = 0.03$; $P = 0.86$).

3.2.3 *Comparison of uptake at three commercial groves*

In order to compare the uptake in trees at the three study sites, only those trees that were treated early in the chemigation cycle (6 h after irrigation was begun) and whose leaf litter was left undisturbed prior to the injections (unraked treatment) were included in the analysis. The initial rate of uptake at all sites was slow (Fig. 7). After this time, imidacloprid levels continued to rise in trees at site P37 and site GGG, whereas no further increases were measured at site P68. There was a significant difference in the levels of imidacloprid measured between the three sites over the 15 weeks ($F_{2,45} = 10.10$; $P = 0.0002$). Contrasts between sites indicated significantly higher concentrations of imidacloprid in trees at site P37 compared with either site GGG ($F_{1,30} = 5.28$, $P = 0.029$) or site P68 ($F_{1,30} = 22.62$, $P < 0.0001$). Significantly lower concentrations of imidacloprid were measured in trees at site P68 than in trees at site GGG ($F_{1,30} = 4.28$, $P = 0.047$). There was a highly significant interaction effect between time and study site ($F_{6,86} = 10.60$, $P < 0.0001$), indicating that the dynamics of uptake was not consistent across the three sites.

3.2.4 *Impact of leaf age on imidacloprid uptake and efficacy against avocado thrips and avocado lace bugs*

In the 2006 study there were significant differences in the concentrations of imidacloprid measured in leaves of different ages, corroborating results from the 2005 trial (Fig. 2). At 19 weeks after treatment (18 August), the concentrations of imidacloprid in the mature leaves (spring 2006 flush) were significantly higher than in the younger leaves (summer 2006 flush) ($F_{3,84} = 35.62$, $P < 0.0001$), particularly in samples from site P37 and site GGG trees, which overall had higher levels compared with site P68 (Fig. 7). Avocado thrips and avocado lace bug bioassays were conducted with the same leaves used to compare leaf age and imidacloprid uptake. There was no mortality when avocado thrips were exposed to the younger leaves (data not shown), whereas mortality of avocado lace bugs exposed to mature leaves was high. Data generated from avocado lace bug bioassays conducted during both the 2005 and 2006 trials were combined and analyzed using POLO-PC[22] to generate dose–response values for LC_5, LC_{50} and LC_{95} of 1.7, 6.1 and 22.0 ng imidacloprid cm^{-2} leaf tissue respectively (Table 1).

4 DISCUSSION

A target threshold for imidacloprid activity against avocado thrips was previously determined to be 100 ng cm^{-2} leaf tissue.[10] This value was the lowest concentration in leaves for which 70% mortality of insects was recorded from bioassays. In this study, a more comprehensive probit analysis of data combining all avocado thrips bioassays, including those from the 2004 study,[10] generated LC_5, LC_{50} and LC_{95} values of 14, 73 and 392 ng cm^{-2} leaf tissue respectively. These data indicate that systemic uptake of imidacloprid into mature avocado trees is not sufficient to provide effective avocado thrips control, because the highest concentrations of imidacloprid measured at any site were below the LC_5 level. The feeding preference of avocado thrips for younger spring flush foliage is especially problematic, given the lower concentrations of insecticide in these leaves compared with the mature foliage of the preceding flush on the same tree. In southern California, the development of new leaves during the spring flush occurs over several weeks, and the data indicate that the rate of imidacloprid uptake is too slow to keep pace with the abundance of rapidly growing leaves. The higher residues of imidacloprid present in the mature foliage support the earlier conclusion[10] that the fully developed leaves present on the trees at the time of the

treatments received higher amounts of imidacloprid than rapidly developing younger leaves. Exposing avocado thrips in bioassays to older leaves where significantly higher imidacloprid residues occurred resulted in significantly higher mortality (in addition to increased control mortality likely due to starvation). Nevertheless, with concentrations rarely exceeding 10 ng cm^{-2} leaf tissue, a direct lethal impact of imidacloprid on avocado thrips is likely to be minimal in mature groves. Avocado thrips levels begin to build within groves when the new foliage begins to develop during spring. As the foliage matures, it becomes less attractive to the thrips and the insects begin to feed on immature fruit. Fruit is especially vulnerable to thrips attack up to July, when it is <2.5 cm in diameter.[5,25] Although the timing of the treatments in 2005 (9 June) was late in terms of when young fruit would typically need to be protected from avocado thrips attack, the earlier treatments conducted during the 2006 trial still would not have improved the level of protection owing to poor insecticide uptake. Without adequate residues of imidacloprid in the leaves at this critical stage, the fruit would be under extreme threat if a thrips outbreak occurred.

The 2006 trial was designed to investigate strategies for improving the use of imidacloprid as a systemic treatment for avocado thrips control. Overall, the uptake of insecticide was inefficient in terms of achieving effective concentrations. Removing leaf litter to permit easier penetration of insecticide to the root zone and varying the pre-injection and post-injection water amounts did not markedly improve imidacloprid uptake. At site P68, significant differences were detected between the early and late injection timings. However, the modest differences in imidacloprid uptake observed with the earlier injections (the maximum concentrations measured were only 2 ng cm^{-2} leaf tissue) would still have proved ineffective against avocado thrips given the requirement of at least 73 ng cm^{-2} leaf tissue for 50% control.

The avocado lace bug was only recently introduced into California, and has not been detected in commercial groves.[7] Avocado lace bug does not feed on fruit, so its pest status is less significant than that of avocado thrips. However, in sufficient numbers it can cause severe defoliation,[8,26] resulting in sunburn of fruit and exposed tree trunks. The results indicate that imidacloprid would be more effective against avocado lace bugs than against avocado thrips, given its greater sensitivity to the insecticide and the fact that it will feed on older leaves where concentrations of insecticide are higher. Concentrations of imidacloprid as low as 1.7 ng cm^{-2} leaf tissue would have some effect on an avocado lace bug population. In spite of the poor uptake of insecticide for avocado thrips management, the concentrations of imidacloprid observed in the two trials would be high enough to be effective against avocado lace bug. This pest feeds exclusively on leaves, and, as a consequence, this would allow a greater tolerance for damage while the imidacloprid levels reached effective concentrations.

The size of avocado trees affected the rate of uptake of imidacloprid. In the smaller trees, peak imidacloprid concentrations exceeded the LC_{95} for avocado lace bug within 4 weeks after treatment when the first measurements were taken. In bioassays with leaves from the larger trees, 100% mortality was never observed. Interestingly, the persistence of imidacloprid was high, with mortality of 38% recorded from bioassays 38 weeks after the treatments were applied.

The reasons for the poor uptake of imidacloprid are most likely related to the heavy layer of organic matter within the soil at each experimental site. The leaf litter on the ground beneath the avocado trees had accumulated over time, resulting in a thick layer that comprised leaf litter in various stages of decomposition. Sorption–desorption processes are important in determining the fate and distribution of pesticides within the soil/water environment,[27] and it has been well established that sorption to organic components within the soil represents the single most important factor reducing the effectiveness of imidacloprid as a soil treatment.[28,29] In this study, removal of the loose leaf litter from beneath the trees to expose the feeder roots to the insecticide did not significantly improve uptake, indicating a dominant sorptive effect of the soil organic matter rather than leaf litter on the soil surface. Additional post-injection watering was ineffective at overcoming these sorptive effects because the thresholds required for the avocado thrips were still not attained.

Options for the use of imidacloprid as a soil drench against avocado thrips in mature avocado groves appear to be limited. In the absence of a direct lethal effect of treatments on large trees, there may still be potential for sublethal effects[30] (e.g. reduced feeding or impacts on fecundity), although these were not assessed during this study. However, sublethal effects, should they exist, may be insufficient to prevent economic damage to immature avocado fruit. The authors are continuing to evaluate the potential use of trunk injections of imidacloprid for avocado thrips control because this would eliminate the impact of soil conditions on the uptake process.

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