Grafted citrus stocks raised by the research team of Mikeal Roose, Ph.D., in a greenhouse at the University of California, Riverside.

DEVELOPING A PLAN TO PROTECT CITRUS NURSERY STOCK FROM ACP

Evaluating Uptake and Retention of Systemic Neonicotinoid Insecticides in Containerized Citrus

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PROJECT SUMMARY

In an effort to minimize the passive spread of Asian citrus psyllid (ACP) on citrus nursery stock, the California Department of Food and Agriculture (CDFA) established insecticide treatment protocols for containerized citrus trees being shipped from production nurseries within designated quarantine zones to retail outlets located both inside and outside the quarantine zones. For destinations outside quarantine zones, production nurseries are required to apply approved systemic and foliar treatments under regulatory supervision no more than 90 days, and no less than 30 days, prior to shipment. This study showed that under a variety of conditions that included different citrus varieties, potting media and watering amounts, the threshold concentration of imidacloprid required for the protection of trees against the establishment of ACP was reached within one week following treatment. Furthermore, the uptake and retention of thiamethoxam was as effective as imidacloprid at protecting trees from ACP infestation.

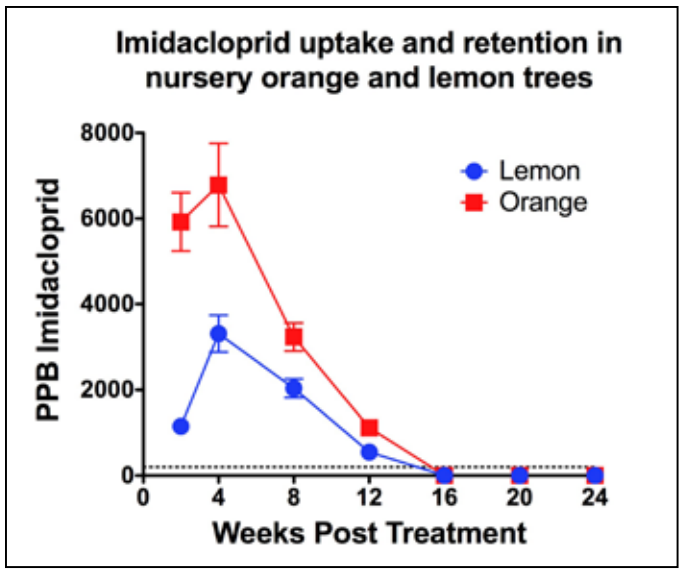

Figure 1. Uptake of imidacloprid in one-year old containerized lemon and orange trees; levels above 200-250 ppb (y-axis dotted line) are needed to prevent the survival of first instar ACP. The dotted line indicates the minimum amount of imidacloprid required to prevent the establishment of ACP on trees.

ACP was detected in southern California in 2008 on citrus at a residential property in San Diego County (Grafton-Cardwell 2010) and is now widespread in southern California on both residential and commercial citrus. The psyllid is an efficient vector of '*Candidatus* Liberibacter' species associated with huanglongbing (HLB or citrus greening disease). In 2012, the first HLB-positive tree in southern California was discovered in a residential neighborhood in Los Angeles County. Since that initial discovery, there have been further detections in Los Angeles County, all of them at private residences.

One of the primary responses to the detection of ACP in California was the implementation by the CDFA of quarantine restrictions on the movement of citrus nursery stock. In Florida, the unregulated movement of infested nursery stock is believed to have played a pivotal role in the spread of both ACP and HLB-associated '*Ca.* Liberibacter asiaticus' throughout the state and also has been implicated in the interstate spread of ACP. California production nurseries are now required to treat all citrus nursery stock prior to shipment to retail outlets. All plants must be treated under regulatory supervision with both an approved foliar insecticide and a systemic neonicotinoid insecticide, and must then ship within 90 days of treatment.

Table 1. Solubility of neonicotinoid insecticides in water.

Insecticide	Solubility in water
Imidacloprid	0.51 g/liter
Thiamethoxam	4.1 g/liter
Dinotefuran	39.8 g/liter

In addition, for shipments destined for locations outside the quarantine zones, nurseries must treat plants at least 30 days prior to shipment. There is concern within the nursery industry that this 30-day post-treatment shipping restriction is prohibitive, with the main argument being that if the systemic treatment has established well in advance of the 30-day limit, then trees would become vulnerable to infestation sooner at retail outlets once the original 90-day post-treatment certification period expires.

OBJECTIVE I: OPTIMIZE UPTAKE AND RETENTION OF IMIDACLOPRID IN CONTAINERIZED CITRUS

CITRUS VARIETY

ACP population dynamics are inextricably linked with the flushing cycles of citrus hosts. The effective utilization of systemic insecticides requires that the chemicals be distributed within the newly developing tissue where the insects feed. The preponderance of newly flushing foliage on lemon trees is challenging because the rate of growth of the flush often outpaces the rate of movement of the insecticide into the new leaf tissue. In contrast, concentrations of imidacloprid can reach considerably higher levels in navel oranges because of the less aggressive flushing pattern. However, despite a significant difference in the concentrations of imidacloprid at peak uptake in lemons and oranges, the threshold concentrations of 200-250 ppb imidacloprid required to prevent the survival of young nymphs on trees were exceeded in both varieties **(Figure 1**). In addition, residues within the trees persisted above those thresholds for up to 12 weeks.

WATERING LEVEL

Water is the driving force behind the movement of systemic insecticides into a plant. The water solubility of neonicotinoids varies considerably (**Table 1**); therefore, the uptake of these insecticides can differ quite substantially under similar watering schedules.

One of our concerns with imidacloprid treatments was the potential for leaching of insecticide from the container during irrigation, especially under conditions where excessive amounts of water are used. At retail outlets, watering is often done by hand and is, therefore, more indiscriminate compared with the automated systems in place at production facilities. We evaluated three different watering levels that were experimentally defined as "replacement watering," in which water levels were maintained at 100-120 percent ET (evapotranspiration), "overwatering" (240 percent ET), and "severe overwatering" (480 percent ET).

Watering level had a significant effect on the concentrations of imidacloprid,

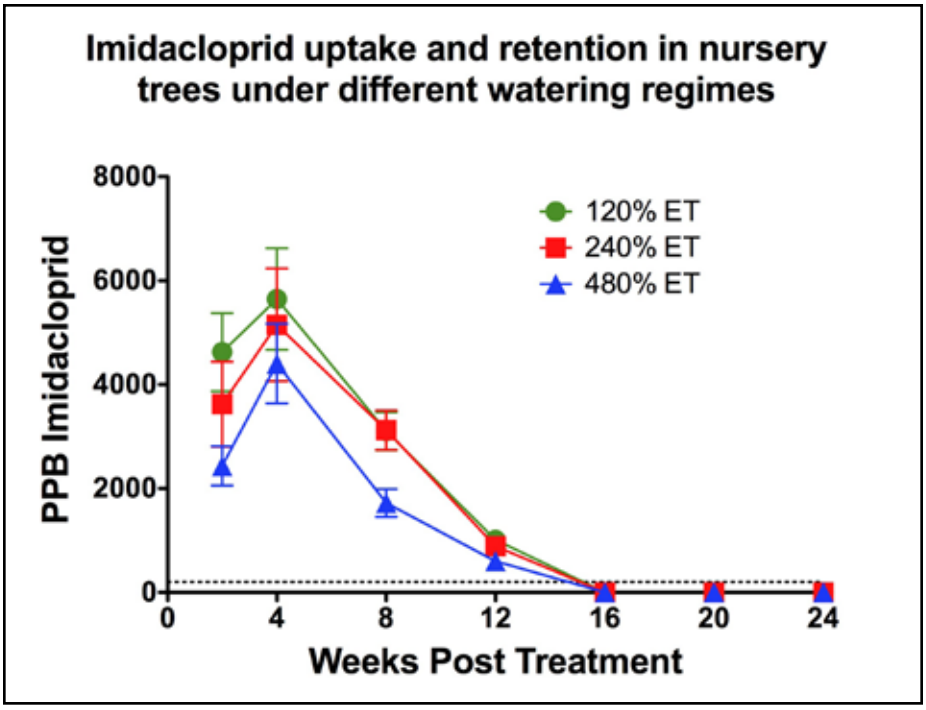

Figure 2. The uptake of imidacloprid by one-year old containerized citrus trees receiving different watering volumes during irrigation. Each point is the mean (± SEM) concentration for both lemon and orange. The dotted line indicates the minimum amount of imidacloprid required to prevent the establishment of ACP on trees.

with lower peak titers in trees receiving excessive amounts of water (**Figure 2**). However, regardless of watering amount, the 200-250 ppb imidacloprid threshold for ACP was reached within two weeks (when the first leaf tissue was tested from the trees) and was maintained for up to 12 weeks after the initial insecticide treatment.

OBJECTIVE II: COMPARE IMIDACLOPRID, DINOTEFURAN AND THIAMETHOXAM UPTAKE IN CONTAINERIZED CITRUS

Having determined conditions that were optimal for the uptake and retention of imidacloprid in one-year old containerized lemon and orange trees, the second phase of the study compared the relative efficacies of two additional neonicotinoids (dinotefuran and thiamethoxam) with imidacloprid. Dinotefuran and thiamethoxam are included on the treatment schedule for intra-quarantine shipments, while dinotefuran treatments are permitted on shipments from production facilities within quarantine to retail outlets located outside quarantine zones. Our Year Two investigations were conducted on one-year old containerized navel orange trees that were irrigated according to the "replacement watering" (120 percent ET) schedule for the duration of the study. Treatment rates for thiamethoxam and dinotefuran were determined from their respective labels (**Table 2**). The current label rate for Admire Pro is 0.33-0.5 ml/0.1 cubic ft. of potting media. However, we used a 10-fold lower rate because many of the generic imidacloprid formulations use the lower rate. We believed that it was important to test the uptake of imidacloprid under the lowest use rate, which would also harmonize the use rates of active ingredients for the three insecticides.

Table 2. Treatment rates for neonicotinoid insecticides.

Insecticide	Label Rate	Active Ingredient per Tree (based on label rate)
Imidacloprid	0.33 ml Admire Pro/cubic ft potting media	182 mg
Thiamethoxam	8.5 oz Flagship 25 WG/100 gallons: 25 fl oz/tree	118 mg
Dinotefuran	24 oz Safari 20 SG/100 gallons: 16 fl oz/tree	170 mg

NEONICOTINOID RESIDUES OVER TIME

Residues of the three neonicotinoids were detected in leaf tissues within one week of treatments, with peak residues of imidacloprid and dinotefuran occurring at that time (**Figure 3**). Despite very similar application rates (182 mg imidacloprid per pot, 170 mg dinotefuran per pot; **Table 2**), peak dinotefuran concentrations were 4.3-fold higher than peak levels of imidacloprid. Thiamethoxam residues peaked at two weeks after treatment, and concentrations were 1.7-fold higher than those of imidacloprid at peak uptake, despite a 1.5-fold lower application rate. The impact of water solubility on the uptake of the three insecticides is clearly evident and emphasizes the need to carefully control the amount of water that trees receive during irrigation.

CONCENTRATIONS OF NEONICOTINOIDS AT FIRST DETECTION OF ACP ON TREATED NAVEL ORANGE TREES

A comparison of insecticide residues in trees at the time of first presence of any ACP life stage provides some insight into threshold levels of activity for the three neonicotinoids (**Figure 4**). ACP established on imidacloprid-treated trees after residues had dropped below 75 nanograms/gram (75 ng/g = 75 billionth of a gram) of plant tissue. ACP established on thiamethoxam-treated trees at concentrations of 163 ng/g of plant tissue for adults and 121 ng/g for nymphs or eggs. On dinotefuran-treated trees, ACP (all life stages) established at concentrations as high as 900 ng/g of plant tissue.

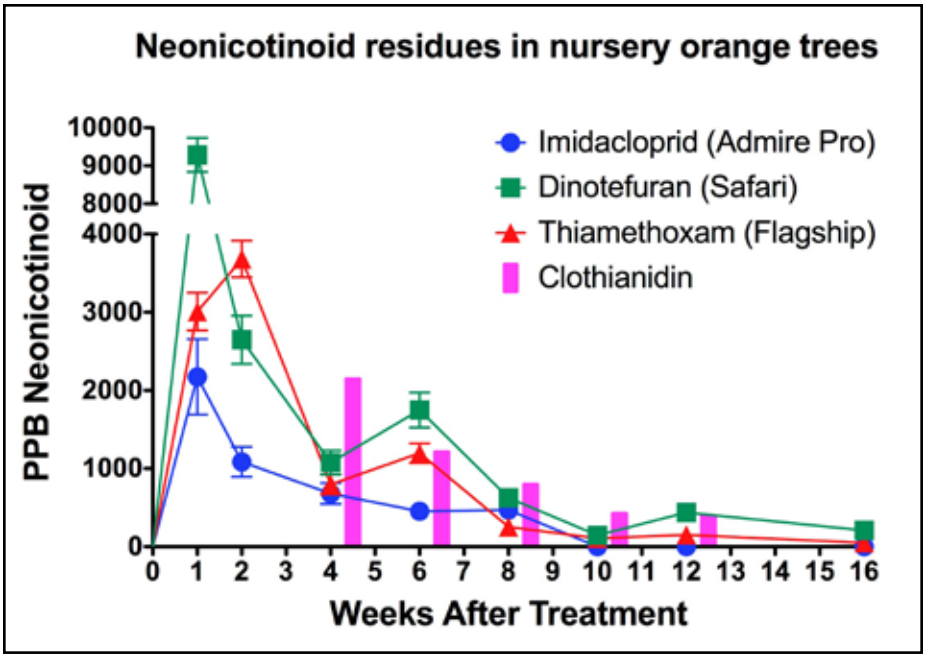

Figure 3. Uptake of imidacloprid, dinotefuran and thiamethoxam by one-year old containerized navel oranges. Each point is the mean (± SEM) for 25 trees. The pink bars represent the concentrations of clothianidin that were measured in trees treated with thiamethoxam.

EFFICACY OF NEONICOTINOID TREATMENTS OVER TIME

Laboratory bioassays were conducted using leaf tissue sampled from the treated trees to compare the efficacies of the three neonicotinoids against adult ACP (**Figure 5**). ACP mortality in the dinotefuran, imidacloprid and thiamethoxam treatments were simlarly high at one week after treatment, averaging 60-80 percent. Mortality in dinotefuran and imidacloprid treatments declined relatively quickly, however, and averaged levels similar to those in the control bioassays before 90 days had elapsed since the treatments. Exposure to leaves sampled from thiamethoxam-treated trees resulted in higher levels of mortality compared with imidacloprid and dinotefuran (**Figure 5**). We detected clothianidinin leaf samples taken from thiamethoxam-treated trees (**Figure 4**), and it is likely that both thiamethoxam and clothianidin are contributing to the toxic effect in bioassays.

DISCUSSION

The purpose of this two-year project was to better understand the dynamics of systemic neonicotinoid uptake and activity within containerized citrus. Under optimal watering conditions, frequently flushing varieties, such as lemons, could be effectively protected for up to 12 weeks. By determining the time to first detection of ACP on treated trees, we established activity thresholds for ACP in areas with low natural populations. Activity thresholds should be a key determinant in how soon after treatment treated trees could leave a production facility destined for areas outside of quarantine where ACP has not been detected. By minimizing the time between treatment and shipment, trees will remain protected for a greater period of time while awaiting sale at the retail outlets where further treatments are unlikely and impractical.

In this study, peak concentrations of three neonicotinoids occurred within two weeks of application, with imidacloprid and dinotefuran peaking at one week post-treatment. Thus, the 30-day post-treatment shipping restriction to non-quarantined areas could be shortened significantly, a measure that would greatly extend the period of protection for trees in retail.

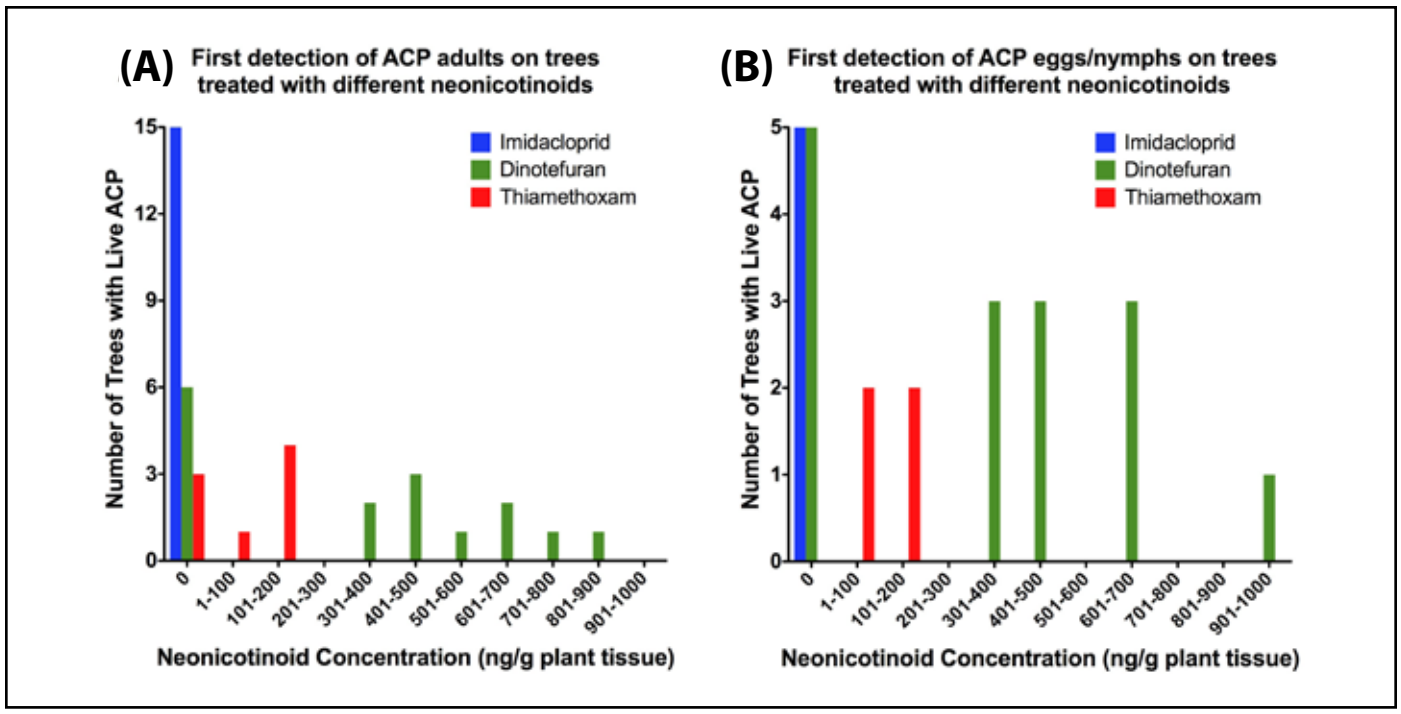

Figure 4. Frequency histograms of insecticide residues in Parent Washington navel orange trees first found with live (A) adult ACP present, and (B) ACP nymphs or eggs. Each bar represents the number of trees with live ACP at each concentration.

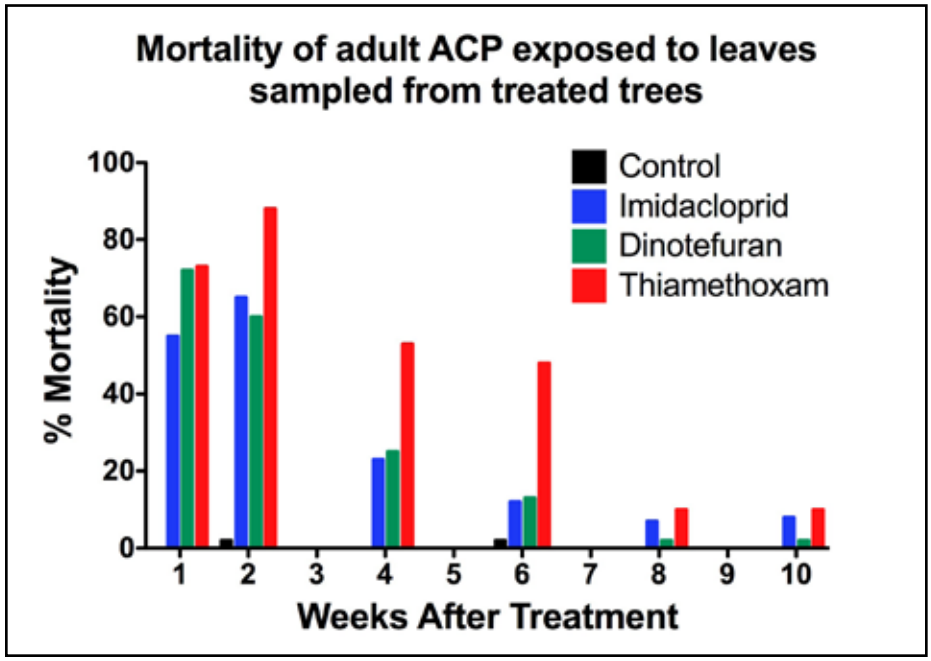

Figure 5. Mortality of adult ACP in bioassays following exposure to leaves sampled from trees (Parent Washington navel oranges) treated with neonicotinoid insecticides.

ACP adults survived extremely high concentrations of insecticide in bioassays, yet natural infestations on trees occurred when concentrations in leaf tissue were at least an order of magnitude lower. At the time ACPs were detected, imidacloprid residues were below detectable levels, suggesting that there may still have been a strong anti-feedant effect of the insecticide. The obvious anti-feedant effects are a clear indication that bioassay data may not be the most reliable indicator of imidacloprid efficacy against the ACP, and that tree colonization may be a more suitable indicator. This is an area that requires further investigation, especially now that HLB has been confirmed at several residential locations within Los Angeles County.

Literature

Grafton-Cardwell, E. 2010. How serious is the threat of Asian citrus psyllid and huanglongbing? *Citrograph* Jan/Feb 1(1):8-10.

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