Imidacloprid Fate and Transport in Immokalee Fine Sand

During the Control of the Asian Citrus Psyllid

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INTRODUCTION

Imidacloprid is a commonly used insecticide in agriculture, home lawns and gardens, and pets (Fishel, 2013). The pesticide is labeled for citrus, tomatoes, grapes, potatoes, and lettuce, just to name a few (Bayer, 2013), and is a control method for the vector of a serious disease to citrus. The Asian Citrus Psyllid (ACP) can vector the disease citrus Huanglongbing (HLB), Candidatus *Liberabacter asiaticus*, also known as citrus greening, the bacterial disease responsible for the devastation to the citrus industry (Brlansky et al, 2008). Citrus Greening originates in China (Graca and Korsten, 2004), and has spread to nearly all citrus producing areas of the world including the United States, South America, China, South Africa, South Korea, and Brazil. The disease was discovered in Miami-Dade county in 2005 in a commercial grove, and has crept up as far north as Putnam county; confirming 33 counties in Florida by February of 2009 (University of Florida, 2013).

Imidacloprid (or IMD for future reference) is one of the most commonly applied systemic pesticides on young, nonbearing citrus trees (Rogers et al., 2013). Imidacloprid is a nicotinic acetylcholine receptor stimulator (Elbert, 1991), which works by attacking a neurological pathway only found in arthropods (Tomizawa, 2005), which is why it is effective on insects without harming fish or mammals. The neonicotinoid blocks the pathway resulting in paralysis, and eventually death. Neonicatinoids are naturally found in the derivatives of many plants like tobacco (NPIC, 2012). Imidacloprid will act systemically when soil applied as a diluted solution. It travels up the xylem and throughout the plant to tissues such as the leaves and pollen (Fossen, 2000). Imidacloprid should be soil-applied around the plant’s drip line to ensure proper root uptake. When systemic insecticides are drenched on the soil, young trees are protected for up to 11 weeks (Se´Tamou et al., 2010).

With imidacloprid controlling pests on a wide variety of crops, and being an effective method of control of ACP, it was determined that studies should be initiated to determine if the chemical remains in Florida's sandy soil, or is more likely to leach out into the environment. Imidacloprid is currently effective on young, non-bearing citrus trees, but since HLB affects citrus of all ages, it has also been determined that the uptake efficiency of the chemical should be determined in multiple age groups, while monitoring the populations of the ACP. Also, little is known of the effects of irrigation rates on the leaching of imidacloprid in soil, or more specifically, Immokalee fine sand where citrus is commonly grown. With this study, the most effective rate of imidacloprid and irrigation will be determined, along with appropriate ages of citrus trees when using the insecticide, and most importantly, assist citrus growers in controlling the disease vector in the most environmentally conscious procedure.

LITERATURE REVIEW

INTRODUCTION OF ASIAN CITRUS PSYLLID TO FLORIDA

The Asian Citrus Psyllid was considered a pest in Florida when it arrived in 1998 (Halbert and Manjunath, 2004). At the time, it was mostly a backyard pest and infested jasmine, but then spread its movement to nursery plants. It is suspected that the pest was introduced via imported plants (Grafton-Cardwell, 2006). It was not known at the time that the ACP carried citrus greening.

ASIAN CITRUS PSYLLID LIFE CYCLE AND FEEDING PATTERN

The ACP has a hemimetabolous, or incomplete life cycle. It transforms from an egg to a nymph to an adult. It begins as a dark yellow egg; about 0.04mm long (Hall, 2012), which are laid by the adult female on the new flush of citrus leaf and stem growth in the spring. The ACP tends to host on all citrus species, and some other genera in the Rutaceae family (Mead and Fasulo, 2011). The total time of the life cycle depends on temperature (Halbert and Manjunath, 2004). *D. citri* eggs grow best in conditions of around 80 degrees Fahrenheit with high humidity, which is why Florida is a convenient location (Liu and Tsai, 2005). A female ACP will lay 500-800 eggs over a period of two months (Hall, 2012). After hatching two to four days later, the ACP morphs through five nymphal stages, remaining in the same dark yellow color. The instars are about 0.25 to 1.7 mm in length, increasing in size with each molt (Grafton-Cardwell et al., 2005). Once the insect has become an adult, its appearance changes to a brown color, about 3 mm in length, with red eyes (Grafton-Cardwell and Daugherty, 2013). Its wings are outlined in dark brown, and its body has a splotchy appearance with the tips of the short antennae being black.

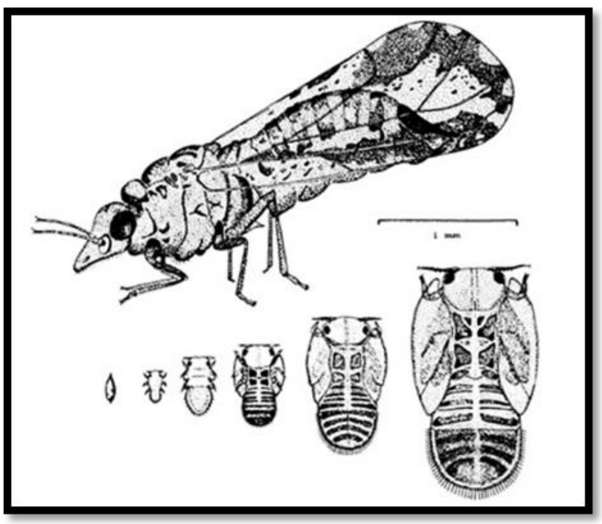

Fig 1. Life Cycle of ACP from egg to an adult (University of California, 2014).

Development to full maturity takes about 16 days (Grafton-Cardwell et al., 2005) with the adult life span being no more than two months (Mead and Fasulo, 2011). The adult female will lay more eggs as the temperature and humidity increases. In one year, a single female insect could be responsible for creating 30 generations of ACP (Grafton-Cardwell et al., 2005).

The insect's migration parameters are poorly known, but it has been observed that they do not fly far from their original habitat (Halbert and Manjunath, 2004). As mentioned previously, it is likely that they came to the Americas via imported plants, since the distance would not have been possible from Asia, especially considering their life span. A gradual transition from South America to Florida would be more likely if the psyllid was not accidentally introduced (Halbert and Manjunath, 2004).

PSYLLID AND CITRUS GREENING INTERACTION

The HLB pathogen cannot directly penetrate the plant, so it relies on the ACP, *Diaphorina citri*, to vector the disease as it feeds on the citrus tissue as a nymph and in its adult stage. The citrus greening pathogen is obtained by the nymph, and then spread as an adult, carrying the bacterium with it for the remainder of its life. Currently, HLB itself has no cure, but there are methods being used to fight the vector mostly in a chemical and biological sense. Since there is no antibiotic available for the trees to control the disease, insecticides and natural predators are being used to kill the ACP at this time with commercial groves and in dooryards (Boina et al., 2009).

The vector process works in two ways, one by the nymphal instars feeding on already diseased citrus, and carrying the bacteria internally as it develops into an adult. The other method is by the adult feeding on diseased trees, and carrying it from tree to tree as it feeds (Brlansky et al., 2008). The nymphs strictly feed on the new, soft growth in the spring and summer months. Fourth and fifth instar nymphs can acquire the pathogen which enables the emerging adult to vector the disease. The psyllid is very recognizable by the way it transitions its body as it feeds.

Once the ACP begins to suck on the leaf tissues, it angles it body at 45 degrees, which is unlike any other insect (Grafton-Cardwell and Daugherty, 2013).

The vector only require one hour of feeding on the tissues to acquire the bacterium and spread the disease (Mead and Fasulo, 2011). Once obtained, the psyllid retains the bacteria and the ability to spread it throughout its life (Gottwald et al, 2007). The adults will preferably feed on the new tissue growth, but will also eat the hardened leaves during the fall and winter to survive. The ACPs do not go dormant in the winter, but decrease in population density (Grafton-Cardwell et al., 2005). This creates a serious, year-round dilemma for citrus growers. It is being considered that targeting the adult psyllids in the winter months when they are most sensitive might be another way to prevent the spread of the disease (Brlansky et al., 2008).

Fig 2. ACP feeding at 45 degree angle (Photo: Caldwell, 2011).

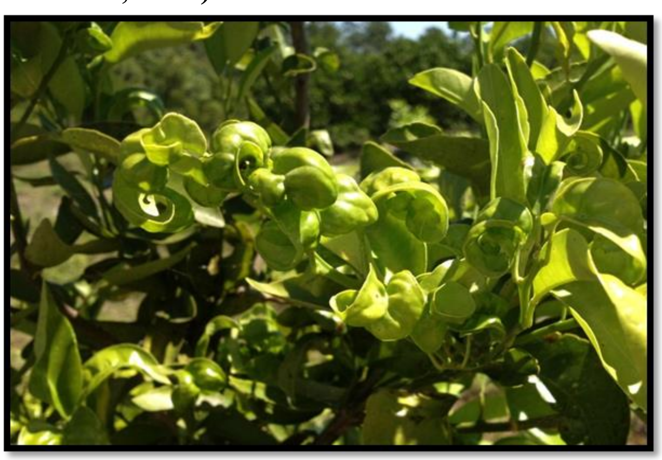

Fig 3. Leaf curling from ACP damage (Photo: Evelyn Fletcher, 2012).

As an infected psyllid feeds, a gram-negative bacteria, *Candidatus Liberabacter asiaticus*, enters the citrus tree and begins to clog the phloem and disrupt the plant's physiology.

Once a tree becomes infected by a vectoring ACP, citrus greening produces a variety of symptoms and intensity depending on the degree of the disease. Foliage is likely to be reduced, but those left intact will display a chlorotic, mottled or splotchy appearance, along with curling, especially in areas where the pest has fed on leaves (University of Florida, 2013). Chlorotic leaves are the most common symptom, and should not be confused with nutrient deficiencies since there is no pattern. Leaf veins become more pronounced, or "vein corking." Fruits become bitter from the starch packing and aborted of seeds (Gottwald et al., 2007). There is commonly a reduction of fruits, with many being small and green. Fruits can also become lopsided, with a yellow stain beneath the calyx. Twig die-back may occur and yellowing of shoots, especially on those that experience larval feeding. Overall tree decline can be seen in advanced infection stages.

Citrus trees have shallow root systems that can be found within 71 to 102 cm of the topsoil in flatwoods, with ¾ of the feeder roots existing in the first 30 cm of soil (Noling, 2011), such as in southwest Florida. Roots of infected trees will become less dense, and more susceptible to diseases like Phytophthera (Graham, personal communication). Roots are actually the first portion of the tree to exhibit symptoms (Johnson et al., 2013), but growers do not typically know that their trees are infected until symptoms are shown in the canopy. This has proven that insect feeding causes the bacteria to translocate through the phloem to the roots before reaching the limbs. Since plants receive the bulk of their nutrients through feeder roots, the damage done to roots also inhibits water and nutrients from being taken up by the plant, further weakening the tree (Johnson et al., 2013).

IMPACT OF CG ON FLORIDA CITRUS PRODUCTION AND ECONOMY

Florida is the top state in the USA for citrus and juice production with about 490,000 acres and accounting for 63% of production acreage, followed by California with 34%, and Texas and Arizona with a combined 3% (USDA, 2013). The value for the 2012-2013 season totaled $3.15 billion for the USA as the packinghouse equivalent. Oranges have the highest production value of all the citrus varieties.

The total cost of care for citrus tree groves in Florida has increased 41% or more due to citrus greening control (Morris and Muraro, 2008). Once a grove becomes economically unproductive it is assumed that it will need to be replanted, which consists of the removal of diseased trees, leveling the land, and modifying the irrigation all before planting the new trees (Morris and Muraro, 2008). After being infected with citrus greening, a citrus tree's life expectancy is five years (Grafton-Cardwell and Daugherty, 2013), and yield reductions ranges from 30-100% depending on tree size and number of infected limbs (Gottwald et al., 2007). One of the most difficult aspects in planning for the management of citrus greening is that it takes at least one year or more before obvious symptoms emerge (Grafton-Cardwell and Daugherty, 2013). The cost of spraying and monitoring citrus groves for psyllids has added an extra $381 per acre (Morris and Muraro, 2008). The need to scout and spray insecticides like imidacloprid with the addition of nutritional foliar sprays were expected to increase production costs to $1,848 per acre (Muraro, 2012), in order to manage Florida citrus groves infected with citrus greening. Imidacloprid is mostly soil and foliar applied, or used as seed treatments.

It is important for the sake of Florida's citrus industry that this insect is controlled quickly. Florida relies heavily on agriculture for its economy, along with tourism (reference). If these pests and diseases are not controlled, the citrus industry could fail in Florida with production increases in other states and countries supplying the demand for citrus products.

IMMOKALEE FINE SAND

Immokalee fine sand (Fig. 4), a spodosol, is commonly found in south Florida, especially among flatwoods. This series is formed from marine sedimentation with slopes generally in the 0-2% range. Its taxonomic class is described as sandy, siliceous, hyperthemic Arenic Alaquod. This translates to a sandy soil that is predominantly composed of silicate sand belonging to the spodosol order with a mineral horizon to approximately one meter depth followed by a spodic horizon (USDA-NRCS, 2013). These soil types occur in a temperature regime of 22 °C or higher. A typical pedon contains the following horizons: A, E1, E2, Bh1, Bh2, BC.

Fig 4. Immokalee fine sand profile (Photo: UF/IFAS, 2014).

During the rainy season, the water table can be as high as 6 inches (15 cm) below the soil surface, and as deep as 60 inches (150 cm) during the dry season. This makes Immokalee fine sand (IFS) difficult to grow citrus without constant water management, but the acidic conditions are favorable when growing this particular crop. These soil qualities make the possibility of imidacloprid leaching into the groundwater a possible threat, which is why a tracer in the form of bromide will be included in the solution to trace the movement of water, and determine if there are differences in irrigation rates.

The soil properties of IFS are listed in the table below where OC refers to the organic carbon content in the soil, and Koc is the K_D that has been normalized to the total organic carbon content. K_D is the ratio of chemical content sorbed in the soil to the amount of chemical in solution. Higher K_D values reflect a greater tendency to be adsorbed in the soil. Bulk density, or Pb, is the relationship between a soil's dry mass and its bulk volume. Sandy soils like Immokalee fine sand tend to have higher bulk densities than clayey soils since they have less organic matter and pore spaces.

	Texture	OC (g/g)	Koc	KD (mL/g)	K (cm/hr)	ρb (g/cm3)	pH
0-15cm	98% Fine sand	0.008	208	1.66	16	1.55	5.5
15-30cm	97% Fine sand	0.002	163	0.31	14	1.58	5.8
30-45cm	95% Fine sand	0.001	230	0.23	13	1.55	5.8

Fig 5. IFS Soil Properties and Imidacloprid Sorption Properties.

IMIDACLOPRID

Currently, little is known about the movement and environmental fate of imidacloprid under Florida's sandy soil conditions. Due to its aqueous solubility of 510 mg/L, imidacloprid is expected to have a high soil leaching potential (Cox et al., 1997). Imidacloprid is also known as Admire®, Advantage®, Confidor®, Gaucho®, Merit®, Premise®, or Touchstone®. It also goes by the IUPAC name: 1-(6-chloro-3-pyridinyl)methyl-4,5-dihydro-N-nitro-1H-imid azol-2-amine. Immidicloprid is an insecticide that was formulated in 1985 by Bayer® (Elbert, 1991), but did not become available in the United States until 1992 (Fishel, 2013). The chemical is a colorless crystal with a slight odor, but contains a blue dye in the product Admire. According to the EPA, neonictoinoids are toxicity Class II and III which are labeled with "Caution" and "Warning," and are especially toxic when ingested rather than through dermal contact or inhalation (Fishel, 2010).

Strategies to control leaching of imidacloprid when used as a soil drench must be studied. It is also not understood how irrigation and rainfall just after application affects plant uptake. Irrigation is expected to have little effect on the uptake of imidacloprid in the trees, but over irrigation can potentially leach significant amounts of the pesticide. Specific objectives of this study consist of the following: 1) document application of imidacloprid over time with different irrigation application rates on selected tree sizes; 2) determine how long imidacloprid lingers in the soil; and 3) determine efficacy on larger citrus trees. These results will assist in improved control of ACP and thus reduce incidence of Citrus Greening with reduced environmental impact by the pesticide through improved post application irrigation scheduling. It is expected that imidacloprid will be more effective on younger trees, since the label states that imidacloprid is not to be used on established, adult trees. The study will also prove that lower irrigation rates are better at maintaining higher concentrations of imidacloprid in soil.

MATERIALS AND METHODS

A split block experiment of three blocks consisting of "Hamlin" orange trees of three ages with twelve trees per block was established at the University of Florida/IFAS South West Florida Research and Education Center in Immokalee, Florida. Trial 1 began with an imidicloprid application in March of 2012 and Trial 2 was initiated in May of 2012. The experiments were designed to demonstrate how seasonal differences in irrigation amounts affect soil imidacloprid concentration with time after application. One year old ‘Hamlin’ sweet orange (*Citrus sinensis*) trees (B1), two to four years old ‘Hamlin’ (B2), and eight years old ‘Hamlin’(B3) were used to determine the effect of tree age and resulting size on imadicloprid efficacy.

Fig 6. Aerial map of UF/IFAS SWFREC with research blocks outlined (Photo: Google Maps, 2014).

Imidacloprid rates were based on current label recommended concentrations. The concentrated solution contained 43.7% active ingredient, with the common industry name of Admire Pro®. Imidacloprid has shown to have insect control at rates as low as 0.3 mg/L (Baskaran et al., 1997). The rates for Trial 1 representing 1x and 2x recommended AI rates were 7.0 and 14.0 ounces per acre, or 70 and 140 g/hectare in May and June of 2012. Trial 2, representing 0.5x and 1x recommended rates, were 3.5 and 7.0 ounces per acre, or 35 and 70 g/hectare in March and April of 2013. These rates were chosen based on the season, given that in the summer while the psyllids are more active and breeding is increased, a higher concentration would be needed. Also, summer rain may leach out the chemical if applied at too low of a rate.

Fig 7. Admire Pro soluble liquid (Photo: Evelyn Fletcher, 2013).

Fig 8. 38 LPG Microsprinkler (Photo: Evelyn Fletcher, 2012).

The irrigation was applied with microsprinklers situated in between every other tree. Irrigation timing was based on climate data from FAWN (Florida Automated Weather Network, fawn.ifas.edu) for an average of 1.5 hours twice per week. Each emitter covers an area of 18 inches (46 cm) in diameter. Water was applied at rate of 6 and 10 gallons per hour, or 23 and 38 liters, representing two treatments: 1y and 2y, respectively and combined with the two imidacloprid rates (1x and 2x) for a total of four factorial treatments: 1x, 1y (Treatment 1); 1x, 2y (Treatment 2); 2x, 1y (Treatment 3); 2x, 2y (Treatment 4). Background concentrations of imidacloprid were measured to determine presence of the insecticide prior to application, along with moisture content. Each treatment was applied to four trees of each block for two seasons in two years (i.e. 2012 and 2013). The imidacloprid solution was soil-drenched applied with 250 mL of solution within the drip line of each tree using a motorized sprayer. The solution also contains bromide in the form of sodium bromide at a rate of 1g/tree. Bromide is being used to trace water movement. This tracer will be appropriate because it will not be adsorbed in soil, since Br-is an ion, and has a retardation factor of 1, allowing it to move with water. After application, the trees were irrigated as suggested by the label. In the second trial, the volume applied to the trees was adjusted for the diameter of the canopy, and thus block two and three received 750 mL of solution at the time of application.

<u>Soil Sampling Method</u>

Soil samples were collected at a 3-interval depth of 0-45cm collecting 15cm of soil at a time from several locations within the tree canopy. Collections were made with a 2” bucket auger, except with the 1 year old trees in Block 1 that required a 0.5” push probe to prevent disturbing the roots. Soil samples were frozen until analysis.

Leaf Tissue Sampling Method

Leaves of new flush were used for the tissue collection. New flush consists of young non-fully expanded leaves. Samples consisted of 15-20 leaves per tree, and were collected from the lower branches of the canopy.

ACP Sampling Method

ACP and their predators were monitored with several different scouting or monitoring techniques. One of which is a new method developed by Arevalo et al. (2011). It is described as the "tap" sampling method, where a branch is tapped three times repeatedly by the hand or use of a PVC pipe to sample a 38.9 cm^3 volume of large tree canopies. The insects land on a piece of white paper held in the other hand, and counted. The square frame was placed randomly on the outer tree canopy; roughly 1-2m aboveground and flush was counted. Shoot populations were estimated by using 10 randomly selected shoots per tree. A hand lens was used to identify eggs, nymphs and their abundance.

Soil Imidacloprid Extraction Method

Soil samples were thawed at 40° F in a refrigerator overnight before use. 20g of each soil sample was weighed in a 50mL polypropylene centrifuge tube with 20mL of 80:20 $MeOH:H_2O\cdot$ 0.01 M $CaCl_2$ added, and shaken for two hours (Baskaran et al., 1997). The tubes are then left to stand at least for two hours, or centrifuged at 8000 rpm for 15 minutes if containing noticeable amounts of organic matter based on color. Samples are then filtered using Whatman 42 filter paper into 20 mL scintillation vials. Each vial contained 10-20mL of extractant. Extractants are stored at 10° F until prepared for HPLC-UV analysis.

Soil Bromide Extraction Method:

Soil samples were thawed at 40° F in a refrigerator overnight before extraction. 20g of each soil sample was weighed in a 50mL polypropylene centrifuge tube with 20mL of HPLC grade water. The tubes are then left to stand at least for two hours, or centrifuged at 8000 rpm for 15 minutes if needed. Samples are then filtered using Whatman 42 filter paper into 20 mL scintillation vials. Each vial contained 10-20mL of extractant. Extractants are stored at 10° F until prepared for ICP analysis.

Leaf Imidacloprid Extraction Method

Leaves were stored at 10° F until ready for extraction. Using a ceramic mortar and pestle, leaves combined by treatment were ground with liquid nitrogen until flakes were less than 1 square mm. The amount of liquid nitrogen used was dependent on the leaf size and quantity.

An average of 3g of ground leaf tissue was combined with 20 mL of methanol then placed on a vortex stirrer for one minute with a speed of 1. The sample was allowed to sit for 30 minutes to allow for diffusion. Using a syringe, 2mL of supernatant was removed and filtered using Whatman 0.2 um pore size syringe filters before being added to the HPLC vial which held 1mL of extracts. The sample was then diluted with 1mL of HPLC grade water and placed on the vortex at a speed of 1 for five seconds.

Moisture Analysis Method

Gravimetric soil water content was determined by drying the soil at 105 degrees C for 24 hours (Carter and Gregorich, 2007) and noting the differences in weight. The bulk density was used to convert the water from gravimetric to volumetric water content.

Soil pH

The soil pH was measured in distilled water and 0.01M $CaCl_2$. A mass of 10g of soil and 20mL of solution were mixed for 30 minutes for each soil depth. After that time, the pH was measured using a Fisher Scientific Benchtop pH meter using a 4.0 and 7.0 buffer solution in the Soil Pedology Laboratory at the University of Florida in Gainesville, FL.

Soil Bromide Analysis Method

Soil bromide was analyzed using a colorimetric method that is also referred as “flow injection analysis” with equipment from Lachat Instruments and is part of the QuikChem series. Phenol red produced bromophenol blue when in the presence of bromide. The absorbance was measured at 590nm (Nikolic et al., 1992). The system is located at the University of Florida SWFREC Soil and Water Laboratory in Immokalee, FL.

Soil Imidacloprid Analysis Method

The HPLC system available to the project is manufactured by Agilent™ and the model used is the Infiniti 1260 with UV detector. The column used for the mobile phase was the SUPELCOSIL™ LC-18 HPLC Column, 15cm x 4.6 mm, commonly used for small molecules. Before each analysis, standards containing 15, 7.5, 3.75, and 1.88 ppm were developed using a 50% serial dilution method from a stock solution of 99.4% imidacloprid. It was used to create or confirm the standard curve. Area under the curve observed as milliauto units (mAU) were used to create the line of regression to determine the concentration of each sample, and an R squared to determine the precision.

Mobile phase consists of 60% HPLC grade distilled water and 40% methanol (Samnani and Vishwakarma, 2011). Retention time for the peak came at roughly 3.8 minutes with a 5% buffer time. The wavelength was set at 272 nm. An aliquot of 2mL of the supernatant was put into an HPLC specific vial for analysis. A total of 30 uL was injected into the HPLC-UV at a rate of 20 uL/minute. The HPLC system was located in the Soil Physics Laboratory at the University of Florida in Gainesville, FL.

Leaf Tissue Analysis Method

Leaf extracts were examined using HPLC-MS/MS, specifically the Thermo Finnigan Liquid Surveyor, Model TSQ Quantum. A ZORBAX Eclipse XDB-C8 Narrow Bore Rapid Resolution, 2.1 x 100mm, 3.5 µm column was used for the mobile phase. Standards for analysis consisted of 100, 50, 25, 10, 5, 1, 0.25 and 0.1 concentrations (ng/mL).

The mobile phase consists of 95:5 methanol:water and 0.1% formic acid. Retention time for the peak came at roughly 9.6 minutes. The time was longer with mass spectrophotometry because the device has to convert the molecules into ions so that the detector can measure it by mass and charge. An aliquot of 2mL of the supernatant was put into an HPLC specific vial for analysis. A total of 5 uL were injected into the HPLC-APCI-MS/MS at a rate of 400 uL/minute. The Scan Mode was on Scanning Mode Microscopy (SPM) with a range of 255.9 to 209.1 eV. In this range, the scanner can detect the molecular structure of imidacloprid. The equipment for this procedure was located in the Food and Environmental Toxicology Laboratory at the University of Florida, which is part of the IR4 program in Gainesville, FL.

RESULTS AND DISCUSSION

The results have been organized by time after application, treatments and by tree ages and soil depth. The soil moisture tables were used to correspond with rain activity given from FAWN to explain sudden decreases in imidacloprid concentrations. Soil sampling occurred at least 12 hours after application do to the REI (restricted entry interval) on the label.

SOIL MOISTURE CONTENT

Trial 1

Regarding the one year old trees, the moisture content on average had a volumetric water content of 0.04 in the 0-15cm depth for the 23LPH irrigation treatment (1y) with 0.05 in the 15-30cm depth, and 0.04 in the 30-45cm depth. For the same group of trees, the 38 LPH irrigation treatment (2y) had an average water of 0.05 for all depths. The error bars showed no significant difference between the two and neither reached soil field capacity (0.10), as seen below in Figures 9-11.

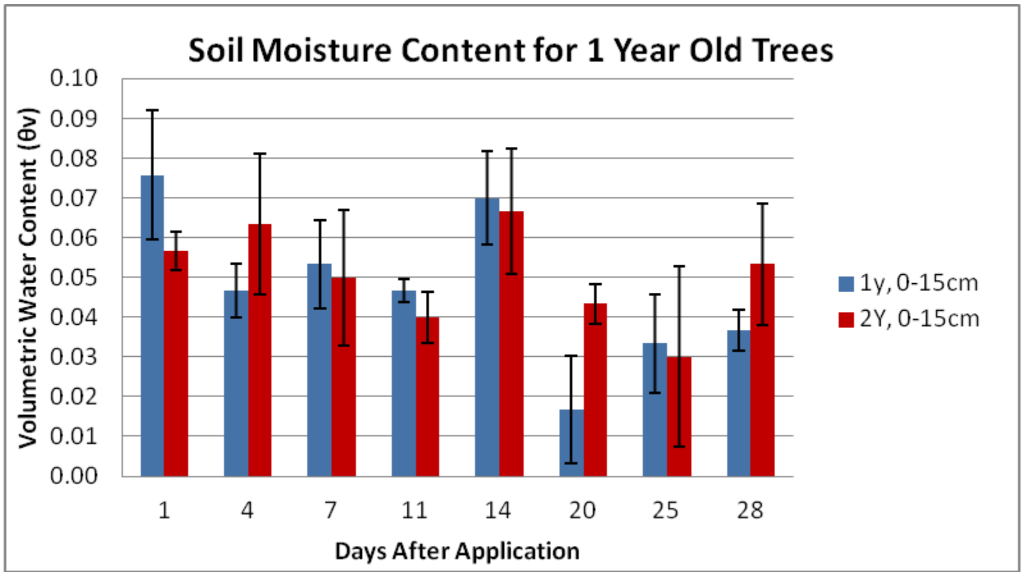

Fig 9. Soil Moisture in 0-15cm for 1 Year Old Trees.

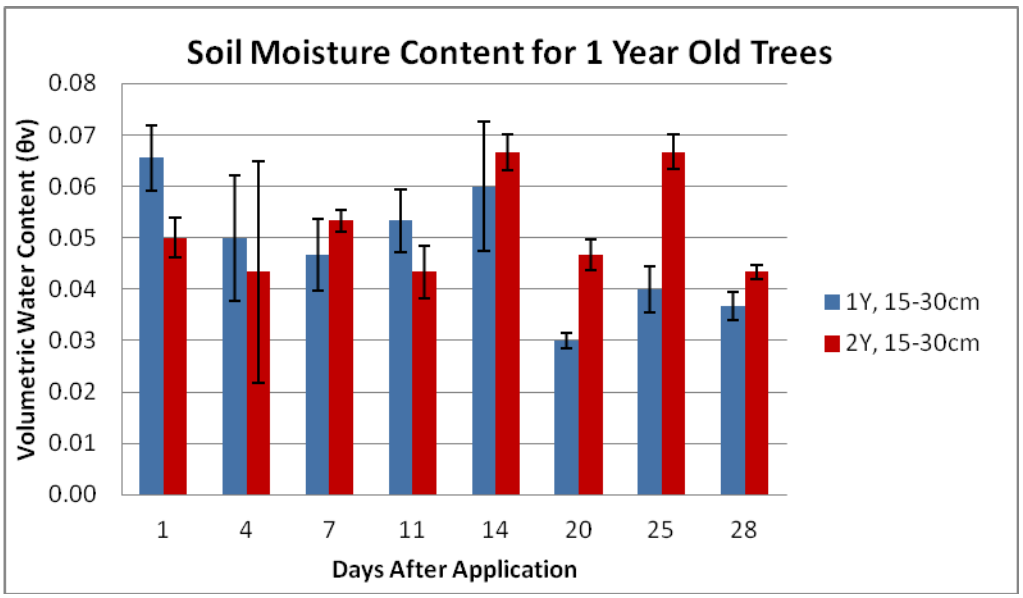

Fig 10. Soil Moisture in 15-30cm for 1 Year Old Trees.

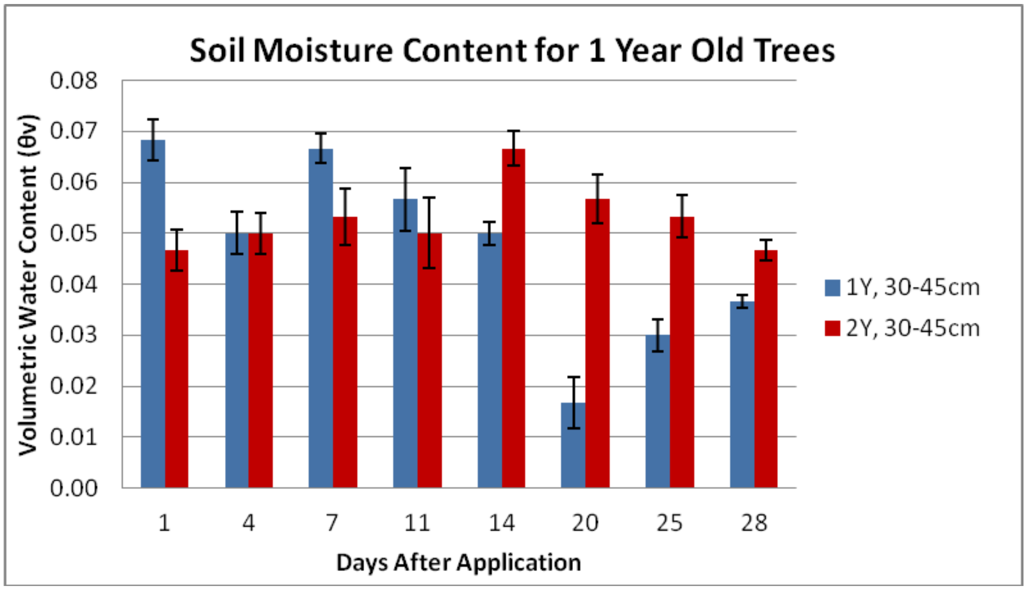

Fig 11. Soil Moisture in 30-45cm for 1 Year Old Trees.

In the 3-5 year old trees, the moisture content on average had a volumetric water content of 0.07 in the 0-15cm depth for the 23 LPH irrigation treatment (1y) with 0.07 in the 15-30cm depth, and 0.05 in the 30-45cm depth. For the same group of trees, the 38 LPH irrigation treatment (2y) had a water content of 0.08 in the 0-15cm depth, 0.08 in the 15-30 depth and 0.06 in the 30-45cm depth. This block of trees also showed no significant difference between the irrigation rates, and the water content was below field capacity as shown (Figs.12-14).

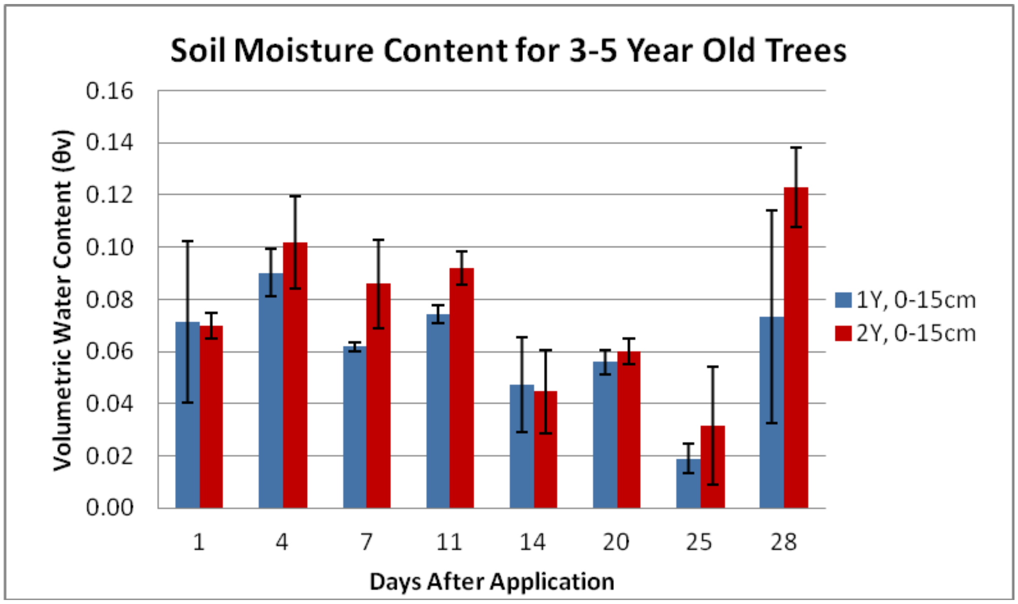

Fig 12. Soil Moisture in 0-15cm of 3-5 Year Old Trees.

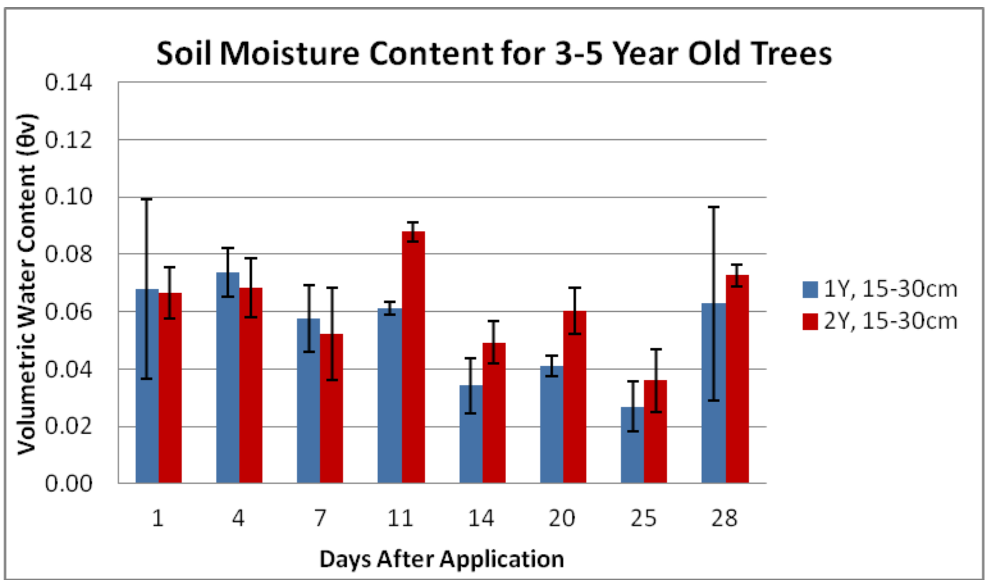

Fig 13. Soil Moisture in 15-30cm of 3-5 Year Old Trees.

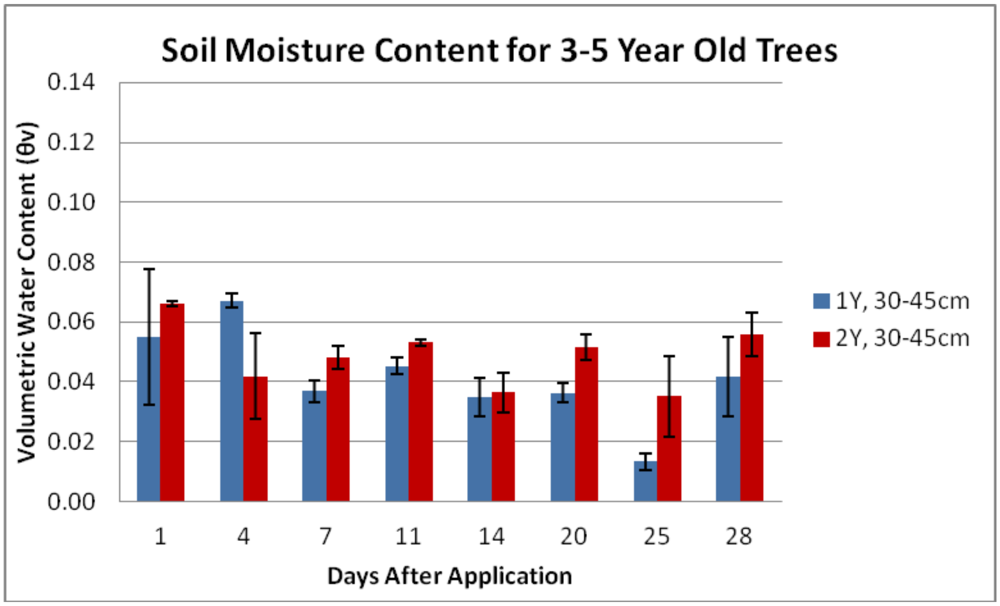

Fig 14. Soil Moisture in 30-45cm of 3-5 Year Old Trees.

The oldest trees at 8 years old had an average volumetric water content of 0.08 in the 0-15cm depth with the 23 LPH irrigation treatment (1y), 0.06 in the 15-30cm depth and 0.07 in the 30-45cm depth. With the 38 LPH irrigation treatment (2y), the top depth of 0-15cm contained 0.09, 0.06 in the 30-45cm depth and 0.07 in the 30-45cm depth, as shown below (Figs.15-17).

The water content was greater with the 38 LPH is in the bottom depth, where water is less likely to evaporate than in the top layer that is exposed to sunlight.

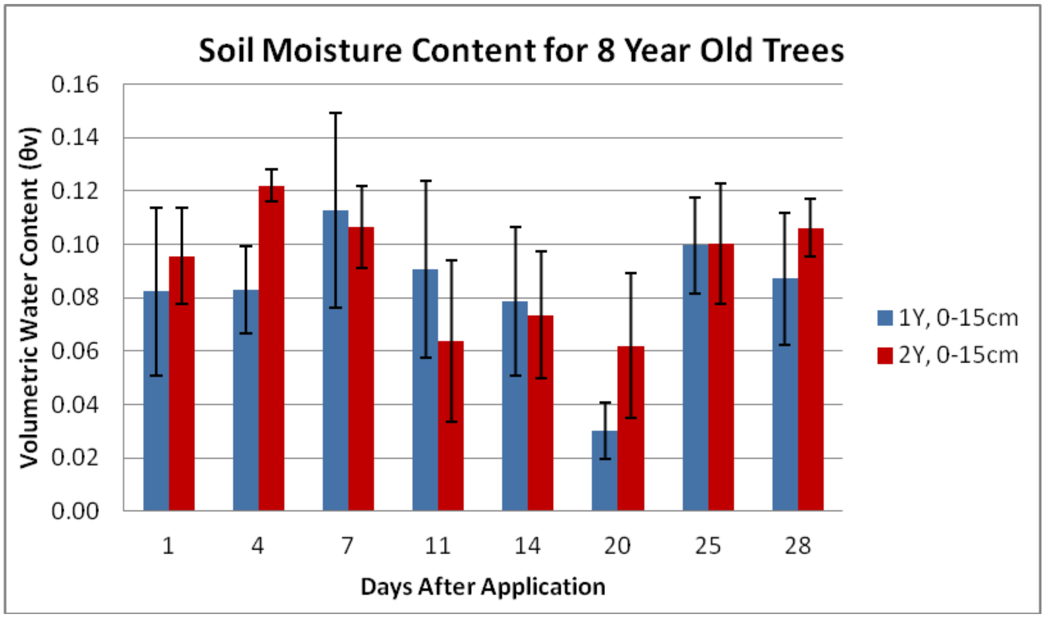

Fig 15. Soil Moisture in 0-15cm of 8 Year Old Trees.

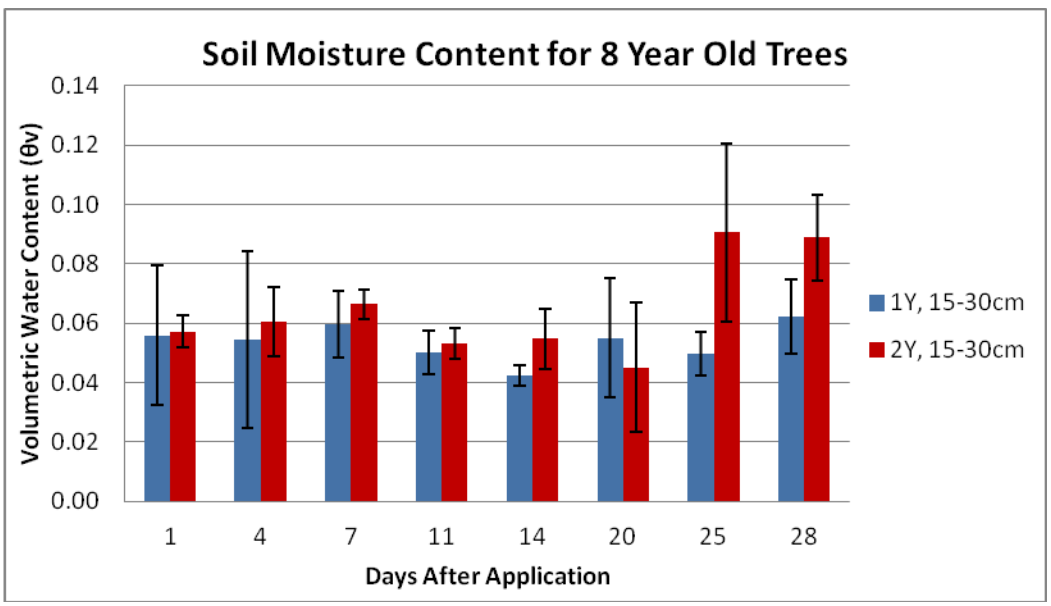

Fig 16. Soil Moisture in 15-30cm of 8 Year Old Trees.

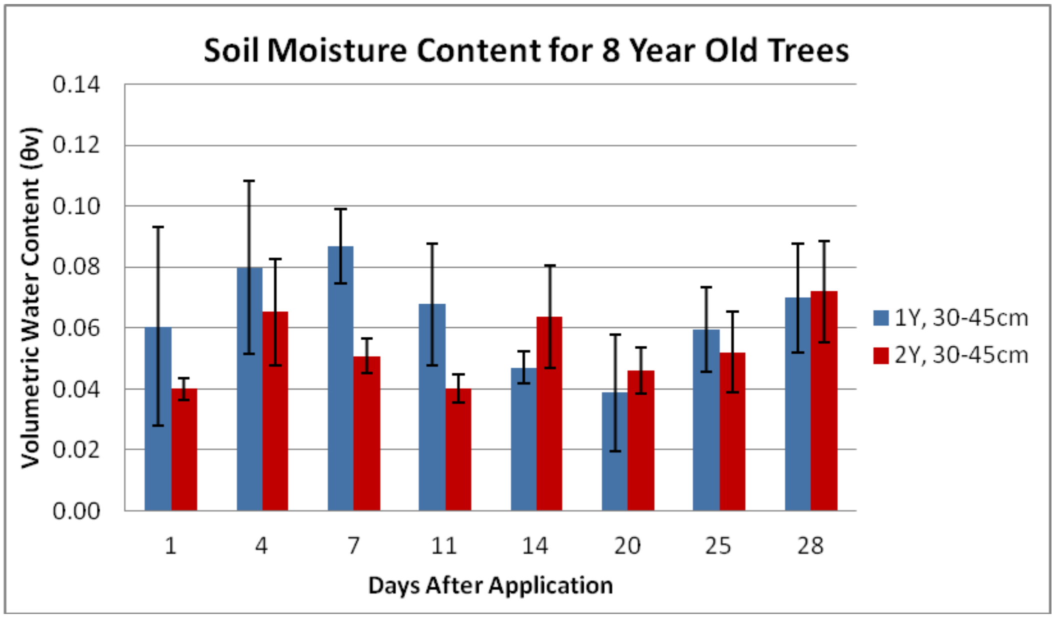

Fig 17. Soil Moisture in 30-45cm of 8 Year Old Trees.

Trial 2

The one year old trees, on average, had a volumetric water content of 0.09 in the 0-15cm depth (Fig. 18) for the 23 LPH irrigation treatment (1y) with 0.08 in the 15-30cm depth (Fig. 19), and 0.08 in the 30-45cm depth (Fig. 20). For this block of trees, the 38 LPH irrigation treatment (2y) had the same values for all depths; 0.09, 0.08 and 0.08. The values of water content are not significantly different for any of the depths. The soil achieves field capacity on multiple dates with both irrigation rates most likely due to rainfall.

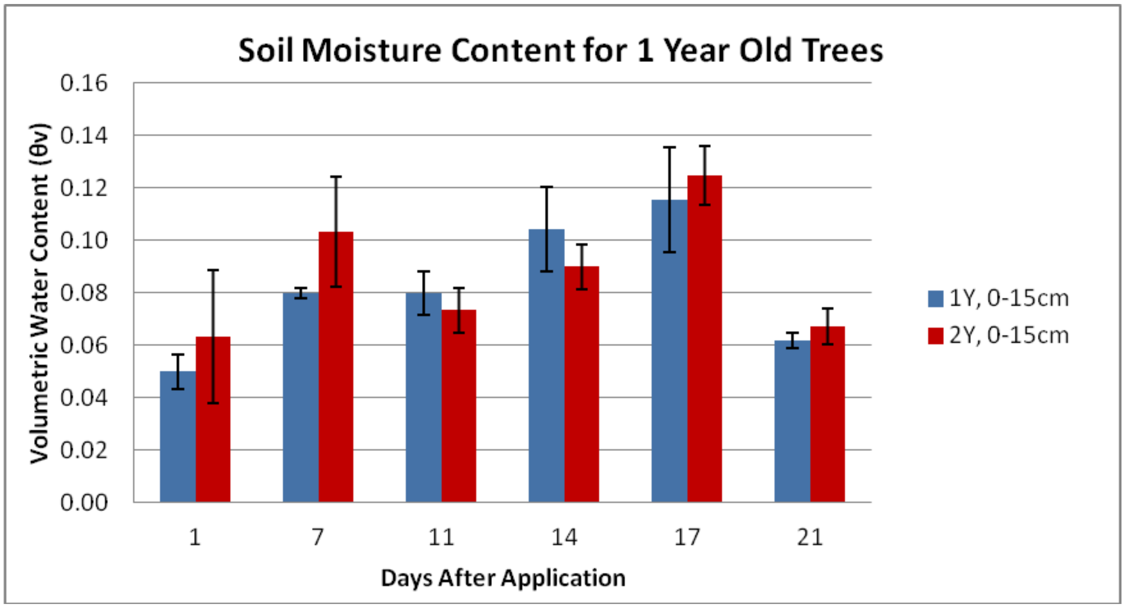

Fig 18. Soil Moisture in 0-15cm of 1 Year Old Trees.

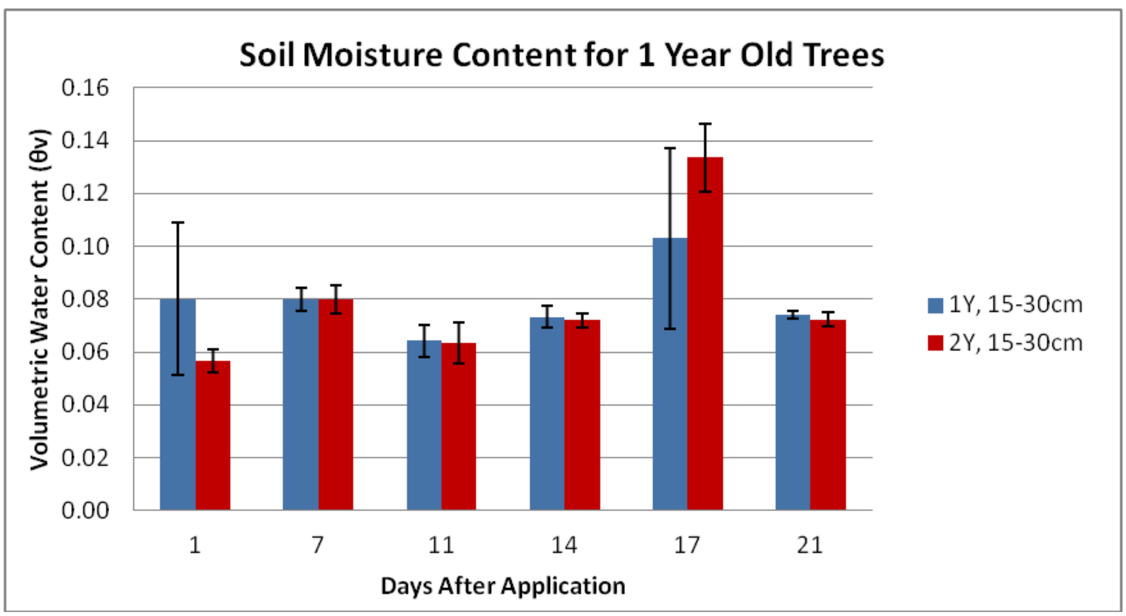

Fig 19. Soil Moisture in 15-30cm of 1 Year Old Trees.

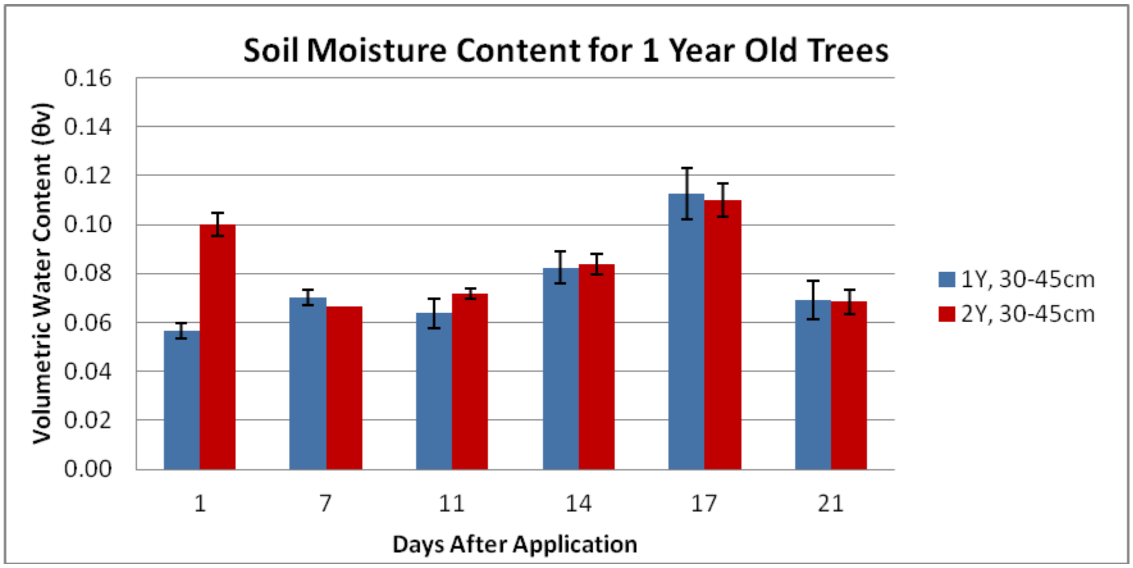

Fig 20. Soil Moisture in 30-45cm of 1 Year Old Trees.

In the 3-5 year old trees, the moisture content on average had a volumetric water content of 0.13 in the 0-15cm depth (Figure 21) for the 23 LPH irrigation treatment (1y) with 0.13 in the 15-30cm depth, and 0.10 in the 30-45cm depth. For the same group of trees, the 38 LPH irrigationtreatment (2y) had a water content of 0.12 in the 0-15cm depth (Fig. 22), 0.13 in the 15-30 depth and 0.10 in the 30-45cm depth (Fig. 23). Water content over time was not consistent for either irrigation rate in the 0-15cm depth, while the irrigation rates showed no differences in the 15-45cm depths.

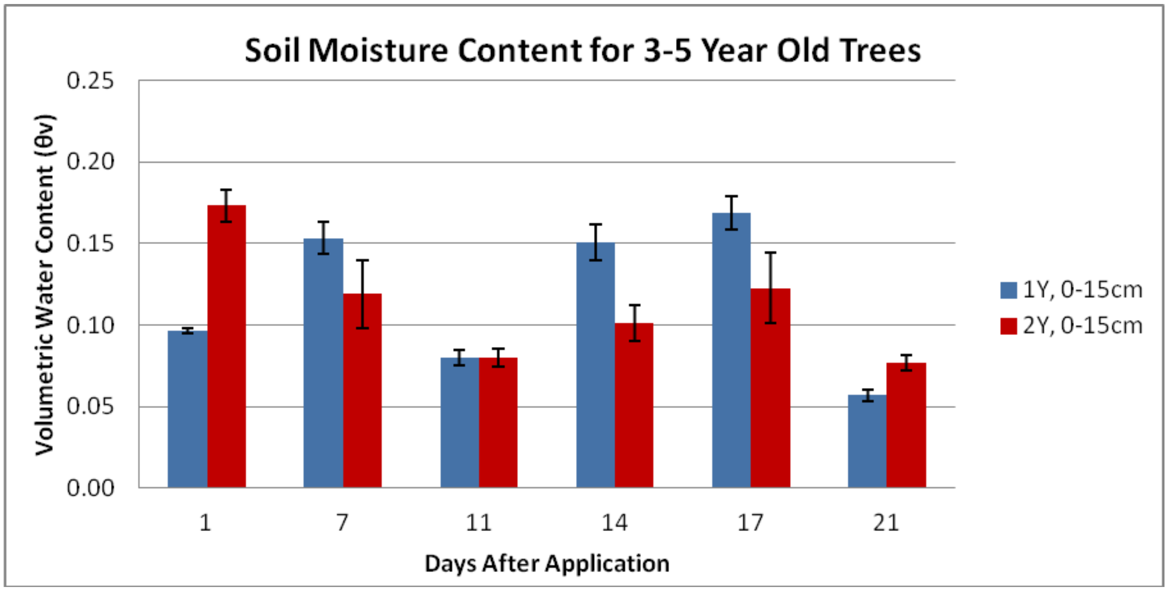

Fig 21. Soil Moisture in 0-15cm of 3-5 Year Old Trees.

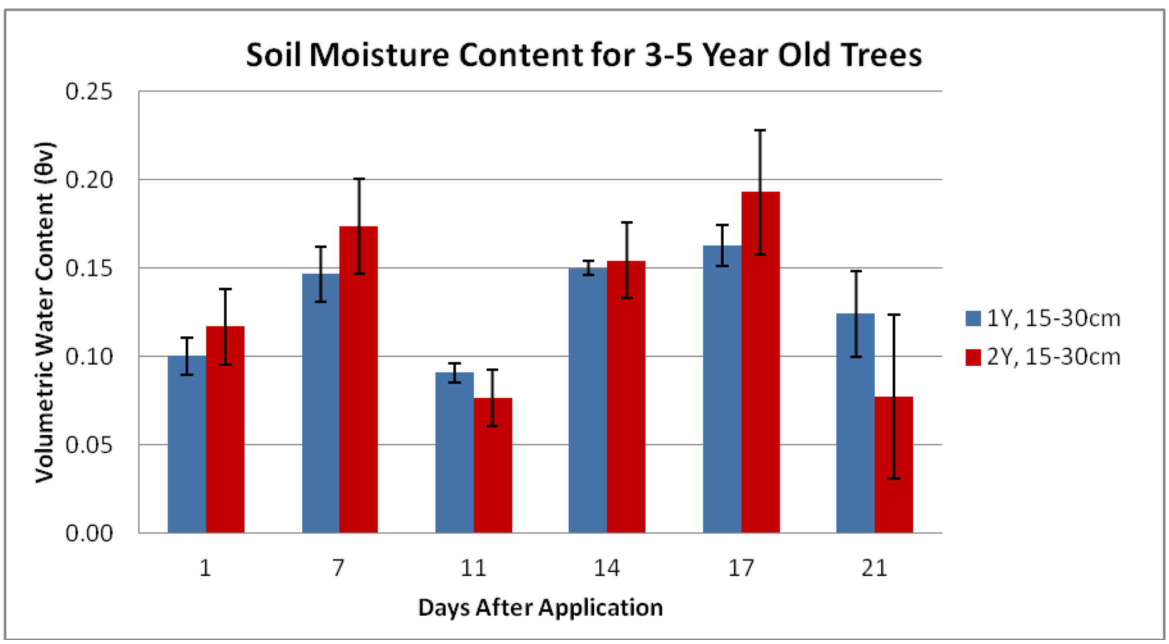

Fig 22. Soil Moisture in 15-30cm of 3-5 Year Old Trees.

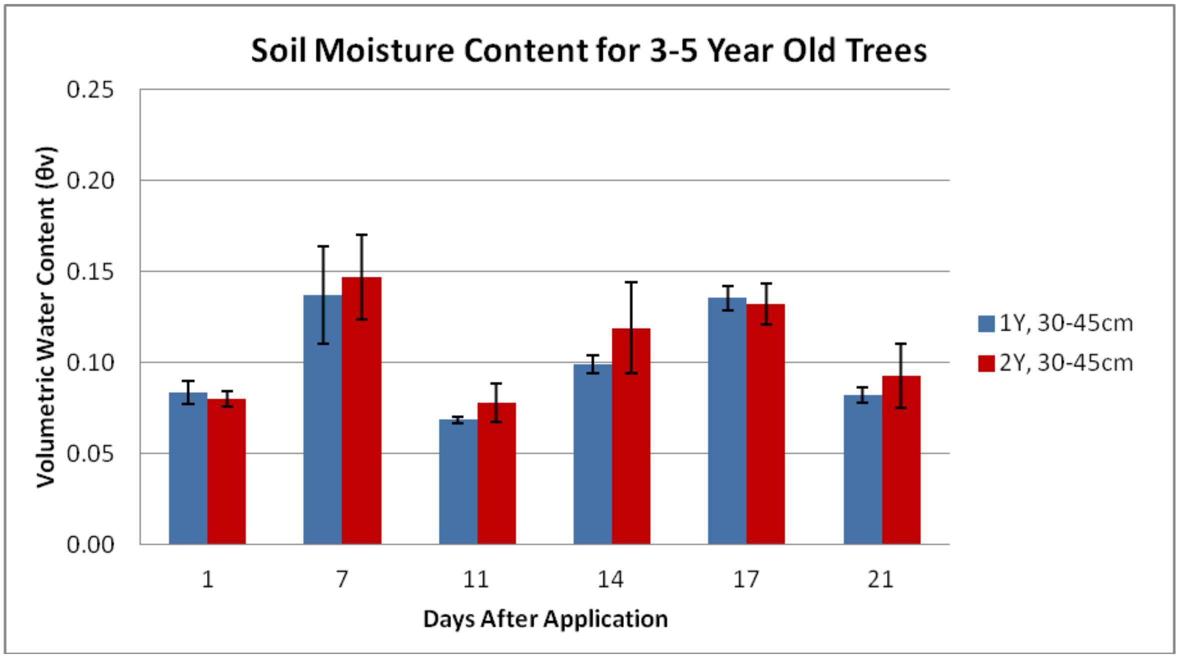

Fig 23. Soil Moisture in 30-45cm of 3-5 Year Old Trees.

The oldest trees at 8 years old had an average volumetric water content of 0.10 in the 0-15cm depth with the 23 LPH irrigation treatment (1y), 0.12 in the 15-30cm depth and 0.10 in the 30-45cm depth. With the 38 LPH irrigation treatment (2y), the top depth of 0-15cm contained 0.12, 0.12 in the 15-30cm depth and 0.11 in the 30-45cm depth. The 38 LPH rate exhibited higher water content initially in the 0-15cm depth, but proved to be similar to the 23 LPH rate in all depths by day 14. Data are are presented below (Figs. 24-26).

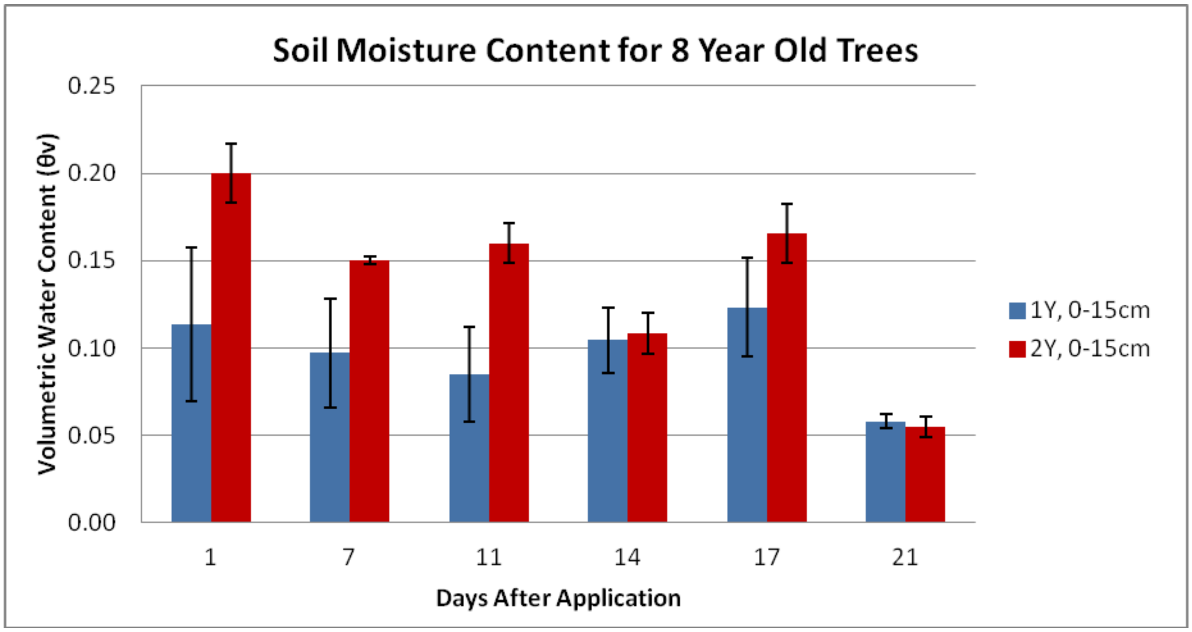

Fig 24. Soil Moisture in 0-15cm of 8 Year Old Trees.

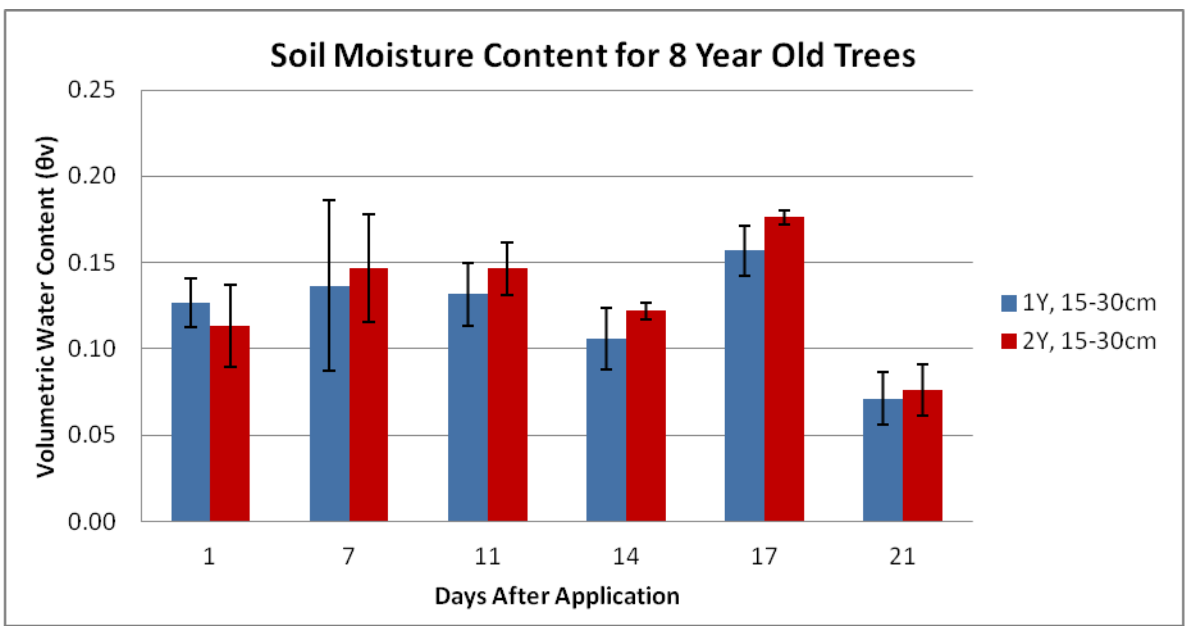

Fig 25. Soil Moisture in 15-30cm of 8 Year Old Trees.

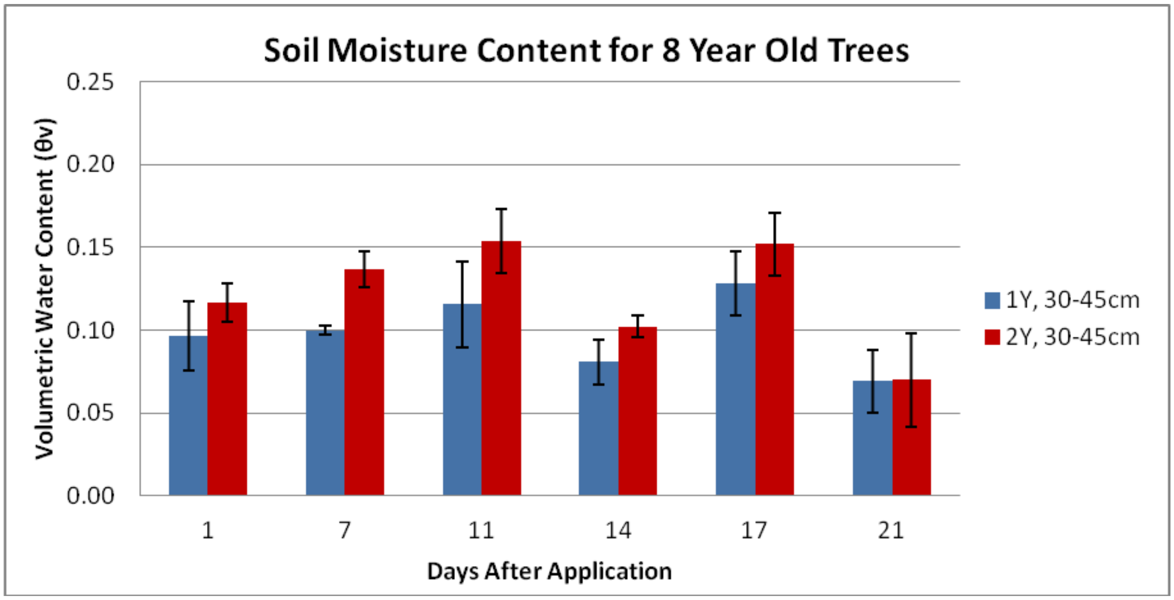

Fig 26. Soil Moisture in 30-45cm of 8 Year Old Trees.

The Immokalee Fine Sand at the research center has a field capacity of 0.1, for all three depths which shows that on average, the soil moisture content did not reach field capacity during the time of sampling in all three blocks for the first trial. Refer to Appendix 8 to review the total depth of water including irrigation and rain events over time. With neither of the treatments achieving this mark, the soil was experiencing unsaturated flow, with the exception of rain events. Unsaturated flow is when water is moving through soil that has not met field capacity, or dry soil. The macropores as typically filled with air, meaning that what water is present is in the meso- and micropores. This leads to a drastic decline the hydraulic conductivity for a sandy soil, making vertical water movement very slow.

The second trial experienced higher values of soil moisture throughout the season due to more rainfall events. The research center received 6.9cm of rain during the span of the second trial, while only 1.6cm of rain occurred during the first trial (FAWN, 2014). The depth of water represents the two irrigation treatments, while showing rain events in early and mid-April.

There was little variation between the two irrigation rates for either treatment, and in either season. The irrigation applied was enough to replenish ET (evapotranspiration), but not enough to create saturated flow. During unsaturated flow, matric potential (energy due to absorptive forces) of the soil is what predominantly drives the movement of water and solutes. The hydraulic conductivity (K) is not constant in these situations, but is equal to the flux (volumetric flow per unit area), where $K = f(\theta v)$ since the volumetric water content does not significantly change with depth, and K is proportional to water filled pore space. The application of bromide can further explain the movement of water, which will essentially be used to explain the movement of imidacloprid.

SOIL BROMIDE

Bromide data collected from the second trial explained the trend of water with time. After 11 days, small traces of bromide remain in the 15-30cm depth, while in the top and bottom depths were essentially 0, indicating the bromide had leached out of the soil. Concentrations per gram of soil are displayed in Appendix #5. In the youngest trees, about 15% of bromide remained in the soil after 17 days with the 23 LPH irrigation rate (1y), while about 29% remains with 38 LPH irrigation rate (2y). The greatest difference between the two rates is on the first day of sampling when the amount in soil is nearly three times greater in the 0-15cm depth with the 23 LPG irrigation rate, as shown below (Figs 27-29). In the 3-5 year old trees, 13% of bromide remains in the 23 LPH irrigated trees, and 14% remains in the 38 LPH irrigated trees. The oldest trees at 8 years old showed 9% of bromide remaining after 17 days with 23 LPH irrigation, while almost 29% remained in the higher irrigation rate.

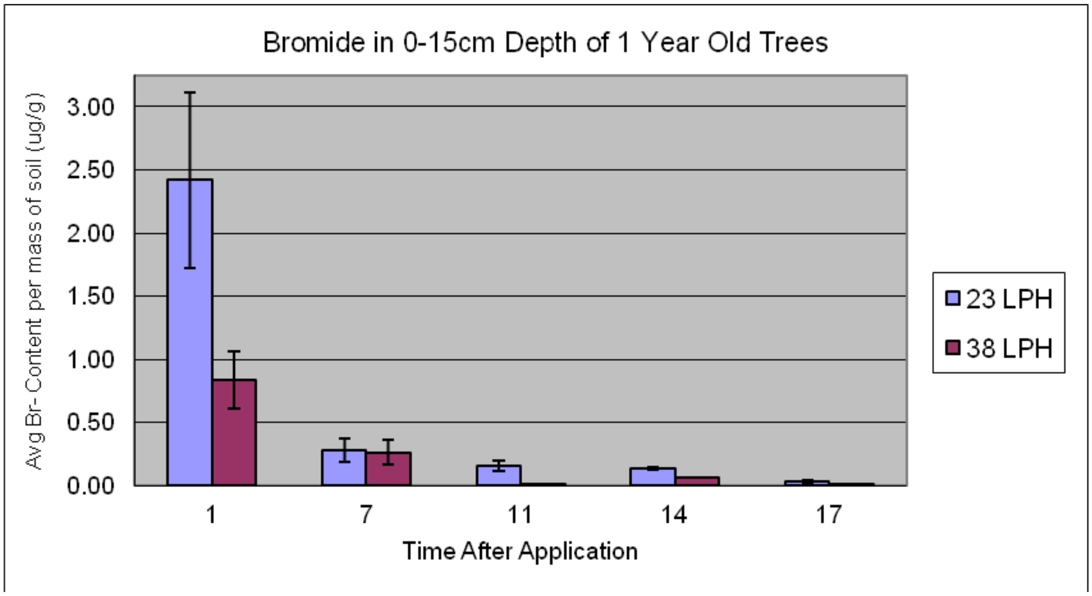

Fig 27. Bromide in 0-15cm of 1 Year Old Trees.

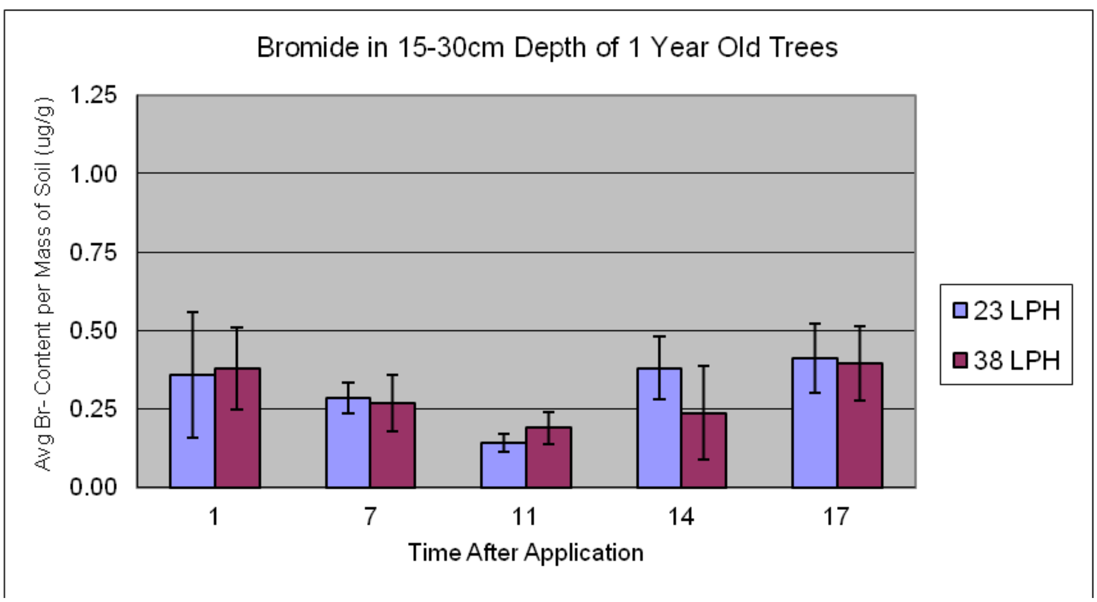

Fig 28. Bromide in 15-30cm of 1 Year Old Trees.

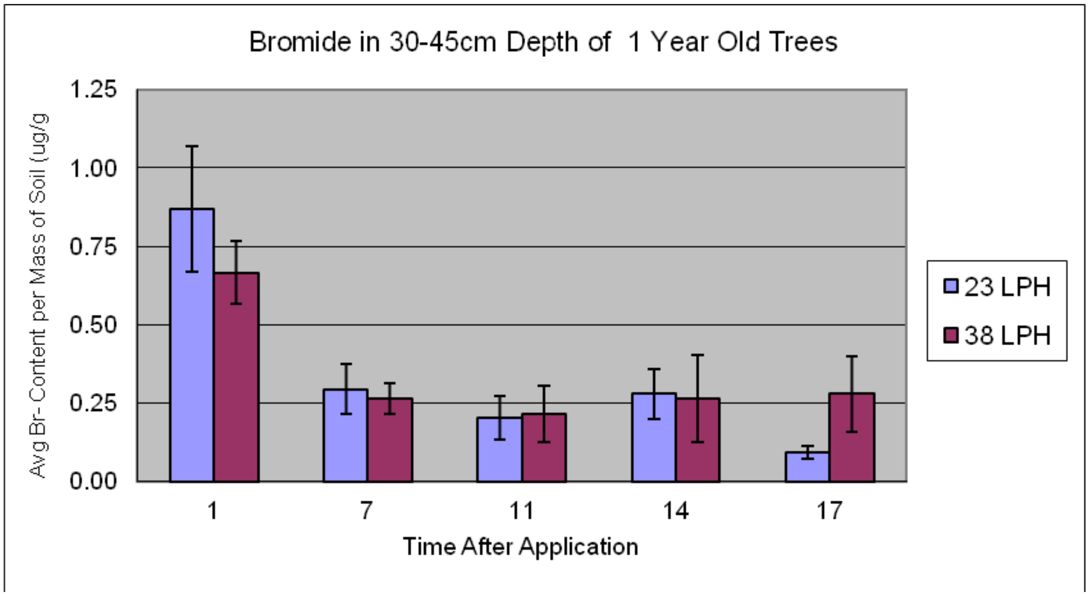

Fig 29. Bromide in 30-45cm of 1 Year Old Trees.

Over time, bromide left the 0-15cm depth and increased in concentration in the lower depths, particularly in 15-30cm. There are no traces of bromide in the top depth by 11 days with the 38 LPH, or by 17 days with the 23 LPH irrigation in the youngest trees.

Relative concentrations of bromide (Figs.30-32) show an exponential decline in soil over time through the entire soil sampling depth (citrus rooting zone). When looking at bromide concentrations by individual depths, the only apparent difference between the concentrations with different irrigation strategies is a higher concentration in the 0-15cm depth at the beginning of the soil sampling with 23 LPH. All depths below the topsoil are essentially the same, with bromide being almost 20% left after two weeks.

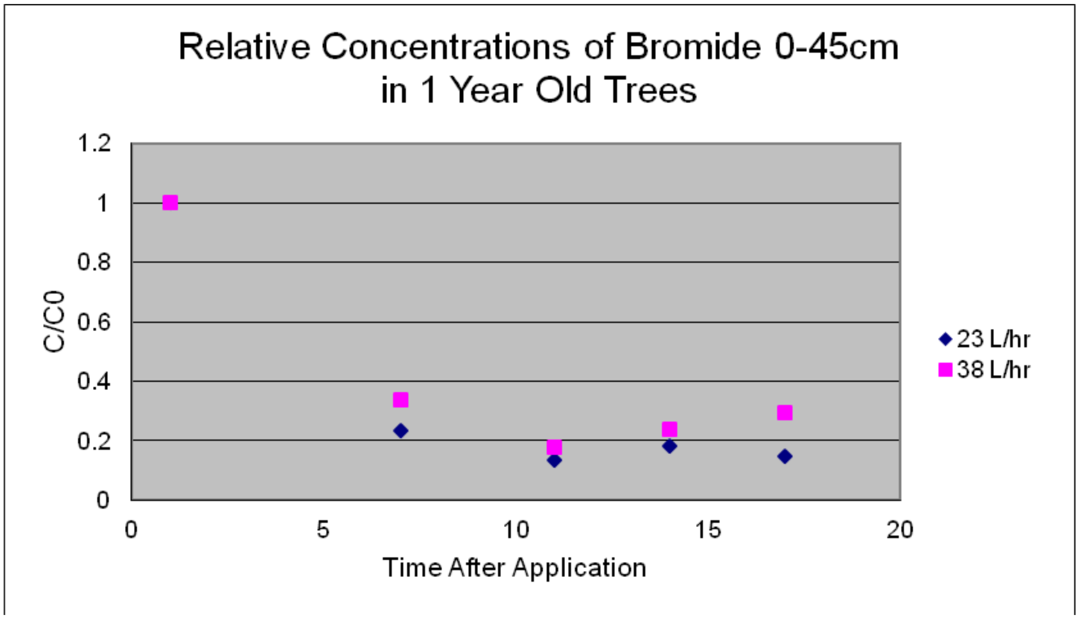

Fig 30. Relative Soil Bromide in 0-45cm of 1 Year Old Trees.

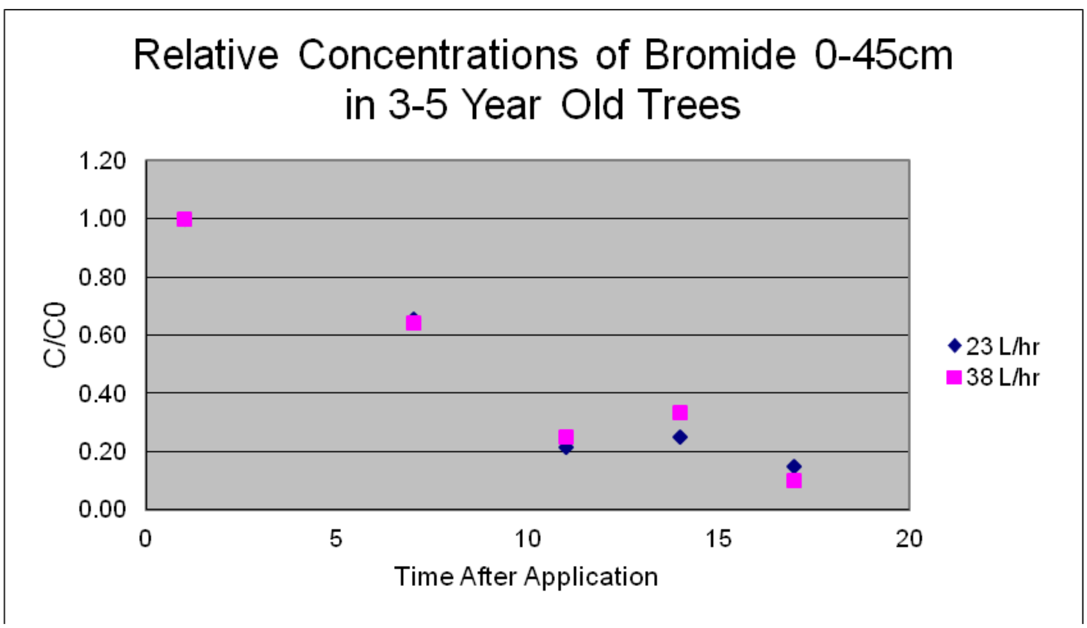

Fig 31. Relative Soil Bromide in 0-45cm of 3-5 Year Old Trees.

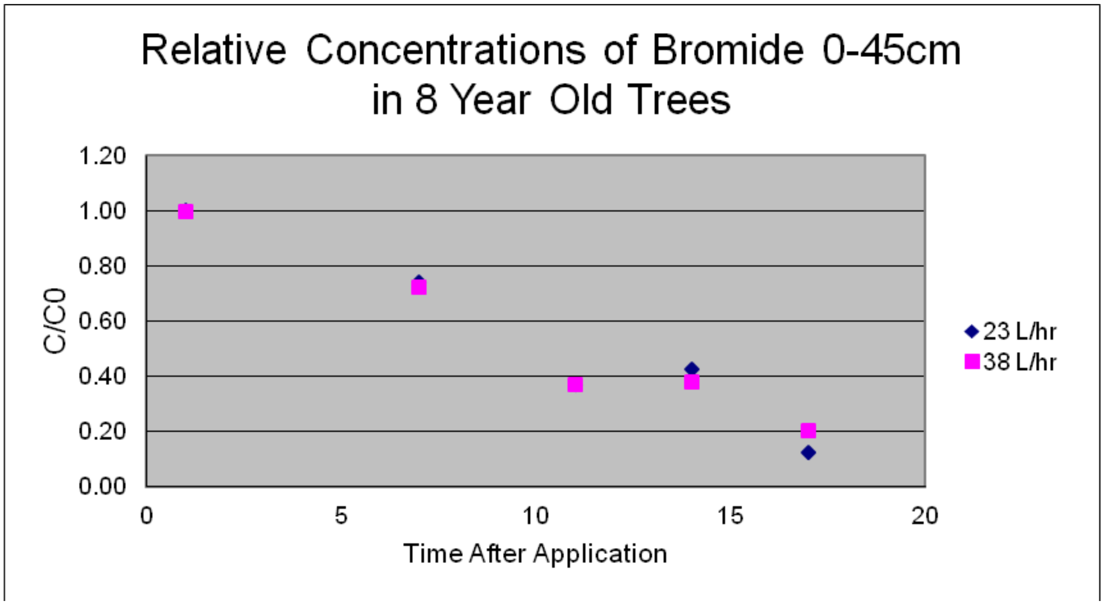

Fig 32. Relative Soil Bromide in 0-45cm of 8 Year Old Trees.

It is expected that the different irrigation rates have not impact the presence of imidacloprid at a given rate, given the similarities in soil water content between the two rates of 23 and 38 liters per hour (6 and 10 gallons per hour). Solutes move with water, preventing the movement or uptake of imidacloprid during unsaturated conditions except when rain or irrigation is applied. Therefore, the rest of the discussion will only compare differences in treatments of imidacloprid rates without the acknowledgement of differing irrigation rates. Since the retardation factor is greater for IMD versus Br, it should theoretically take much longer to leave the system.

SOIL IMIDACLOPRID CONCENTRATION

Trial 1

Imidacloprid in soil displayed the differences in imidacloprid rates. Initially, the one year old trees contained almost double the concentration, 6 ug/cm^3 of imidacloprid in soil in the 414 mL/Acre application (Fig. 33) in comparison with the 207 mL/Acre rate of 3 ug/cm^3 in soil. However, at the end of the trial 32% of the 414 mL/Acre rate remained in soil, while the 1x rate had 22% left behind. Most of the imidacloprid remained in the top 0-15cm throughout the trial in the 2x application rate. However, in the 1x rate (Fig. 34), after application, there was an even distribution through all three depths. In all tree ages, imidacloprid content increased on the 25th day, which may have been caused by an increasing water table due to the summer rain. IFS has a very shallow water table during the rainy season.

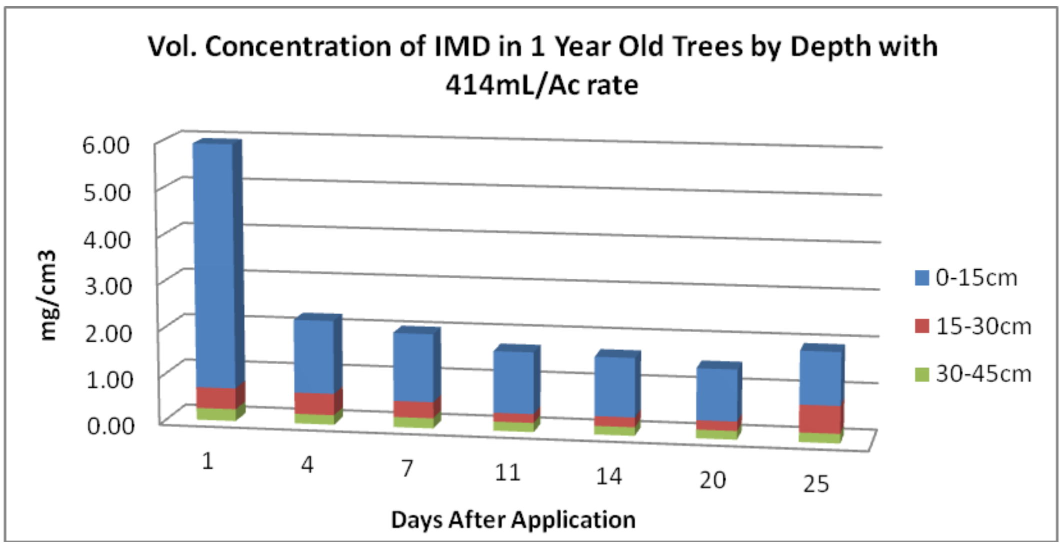

Fig 33. Volumetric Concentration of IMD in 1 Year Old Trees.

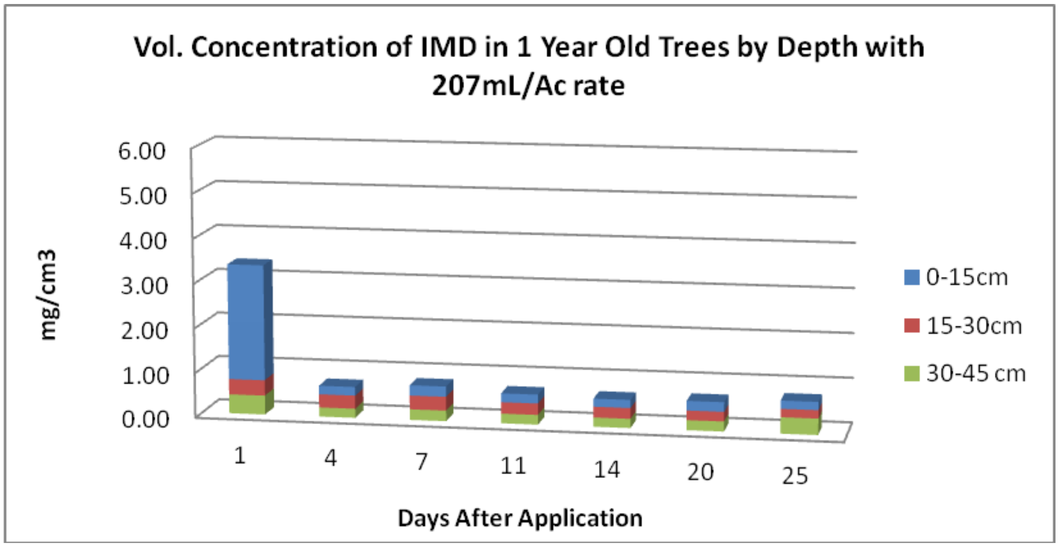

Fig 34. Volumetric Concentration of IMD in 1 Year Old Trees.

Similar results were found in the block of 3-5 year old trees. Initially, the soil contained almost double the concentration, 2 ug/cm^3 of imidacloprid in the 414 mL/Acre application (Fig. 35) in comparison with the 207 mL/Acre rate (Fig. 36) of 1 ug/cm^3 in soil. However, at the end of the trial 48% of the 414 mL/Acre rate remained in soil, while the 1x rate of 104 mL/Acre had 58% left behind. Imidacloprid remained in the top 0-15cm throughout the trial, and by the majority, in the 414 mL/Acre application rate. The concentration was evenly distributed at lower depth with the 1x application rate.

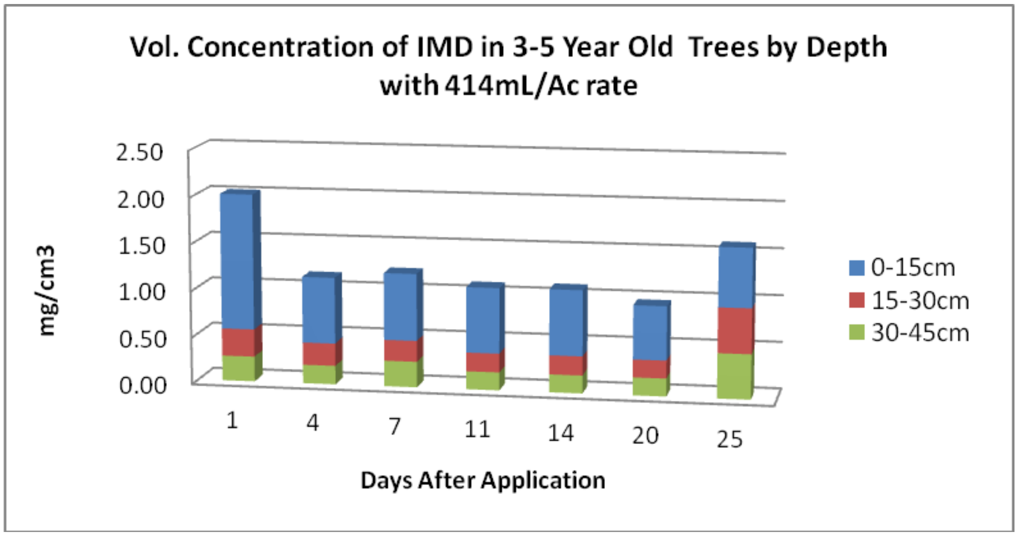

Fig 35. Volumetric Concentration of IMD in 3-5 Year Old Trees.

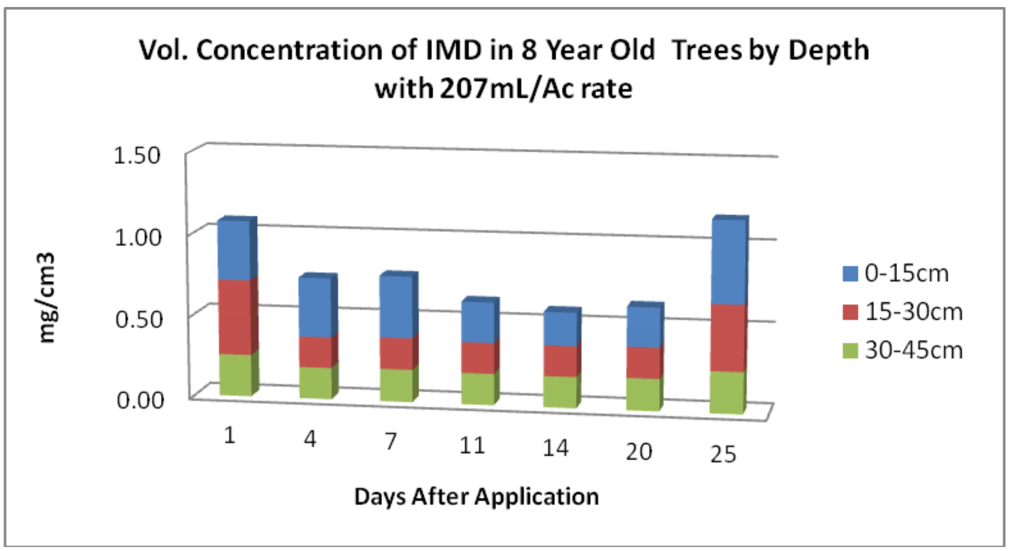

Fig 36. Volumetric Concentration of IMD in 3-5 Year Old Trees.

The oldest trees at 8 years old had the lowest concentration of imidacloprid in soil of all the tree ages, initially with 1 mg/cm^3 in the 2x rate (Fig. 37) and 1x rate (Fig. 38). By the end of the trial, 56% was left in the soil of 414 mL/Acre rate, while 57% was left in the 1x rate. The majority of imidacloprid stayed in the 0-15cm depth, especially with the 2x rate.

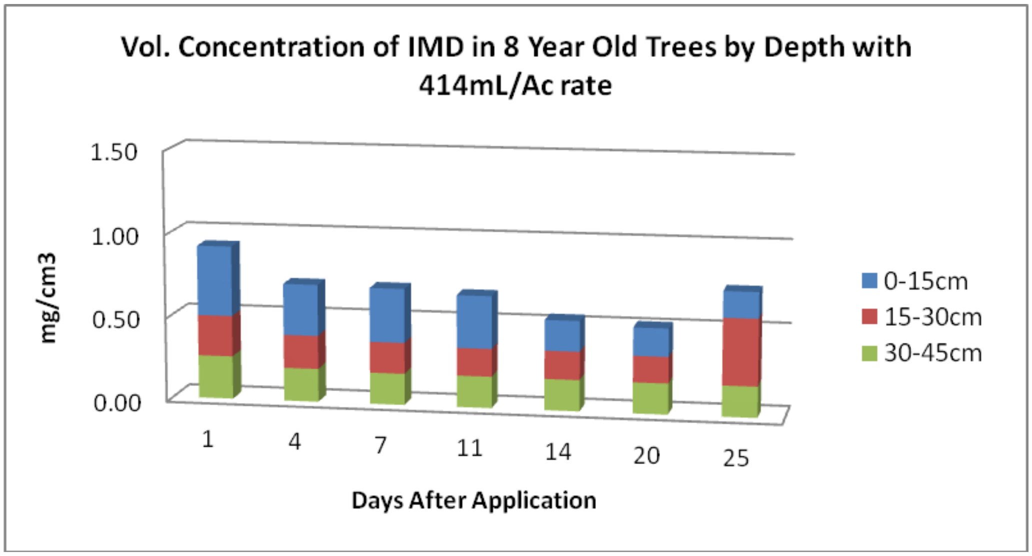

Fig 37. Volumetric Concentration of IMD in 8 Year Old Trees.

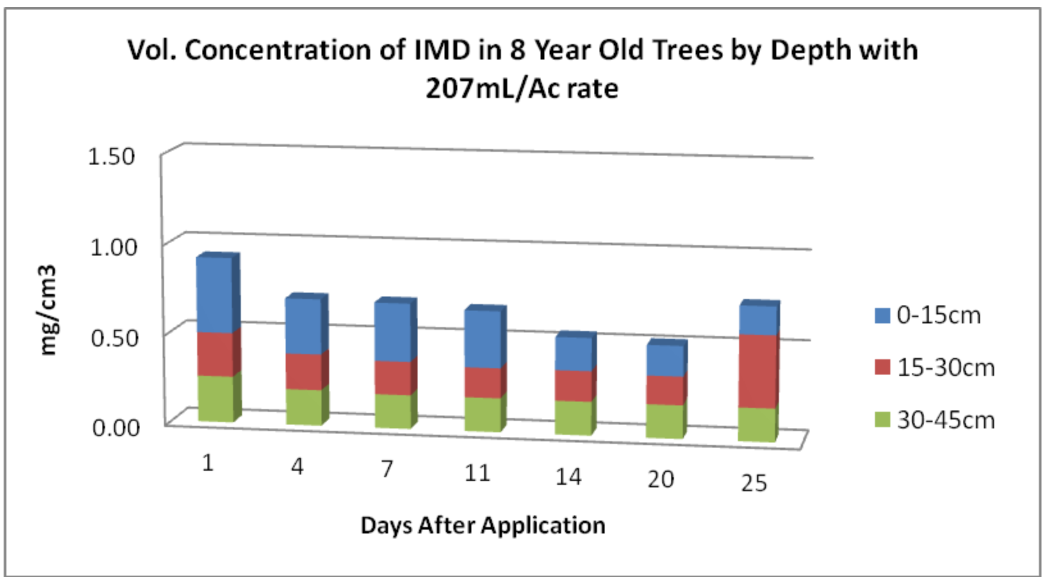

Fig 38. Volumetric Concentration of IMD in 8 Year Old Trees.

Trial 2

The concentrations of imidacloprid in soil show higher retention in the 0-15cm depth for all tree ages. The one year old trees had an initial concentration of 1 mg/cm^3 in the 0.5x application rate, and ending with .5 mg/cm^3 or 41%. The 0.5x (104 mL/Ac) treatment maintained a concentration below 1 ppm throughout the trial with little movement in the 15-45cm depths in the 1 year old tres. The 1x application rate showed 4 mg/cm^3 initially with 1 mg/cm^3 remaining, or 33%. More movement in the lower layers was observed in the 1x application rate than with the 0.5x rate. Volumetric concentration over time is shown below (Figs. 39 and 40).

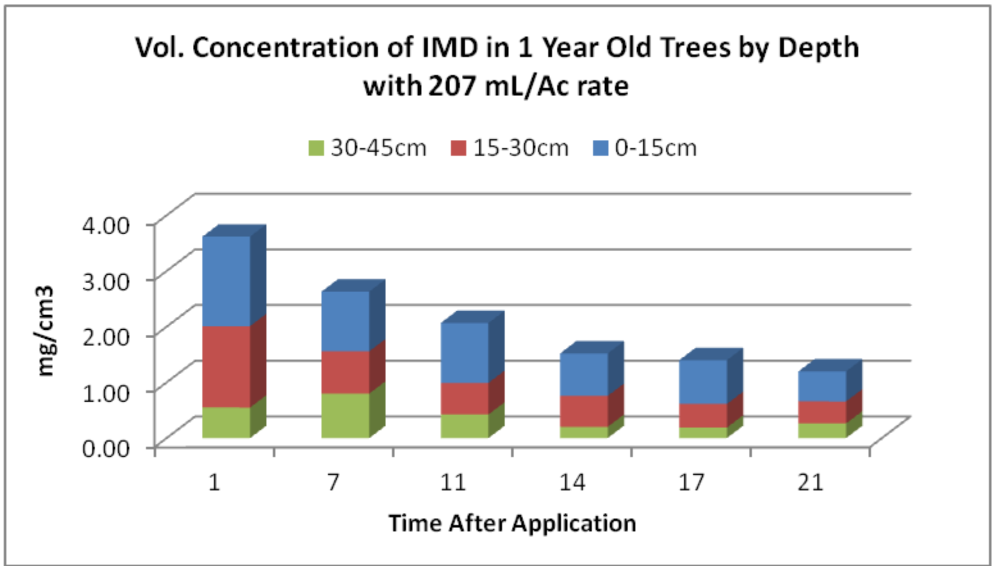

Fig 39. Volumetric Concentration (1x) of IMD in 1 Year Old Trees.

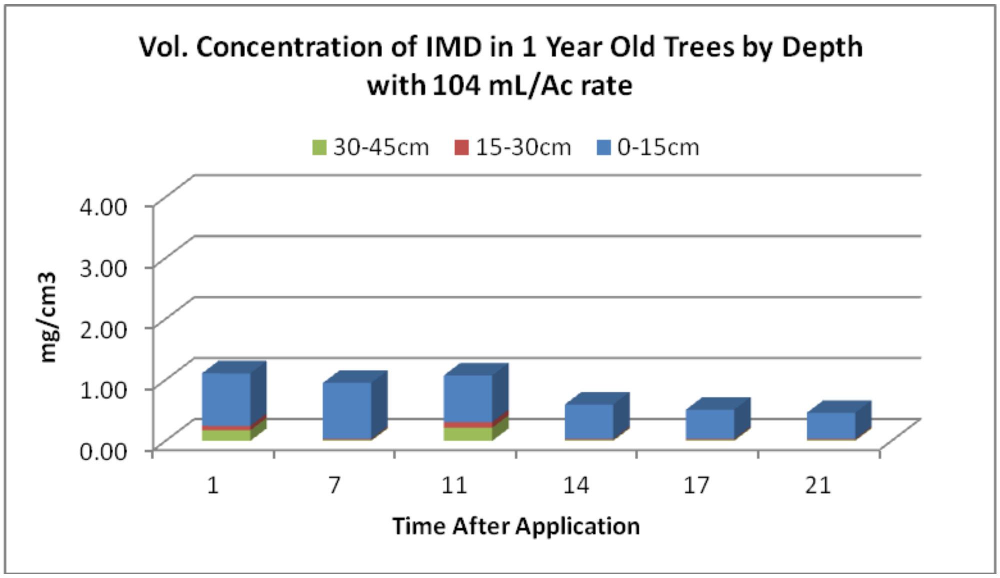

Fig 40. Volumetric Concentration (0.5x) of IMD in 1 Year Old Trees.

The 3-5 year old trees had an initial concentration of 0.6 mg/cm^3 in the 0.5x application rate, and 0.5 mg/cm^3 or 59% by the end of the trial. The 1x application rate showed 4 mg/cm^3 initially with 1 mg/cm^3 remaining, or 42%. Imidacloprid maintained the majority of its presence in the 0-15cm depth for both rates, but did show a slight increase in the lower depths with time, especially in the 1x rate. Volumetric concentrations over time are shown below in Figures 41-42.

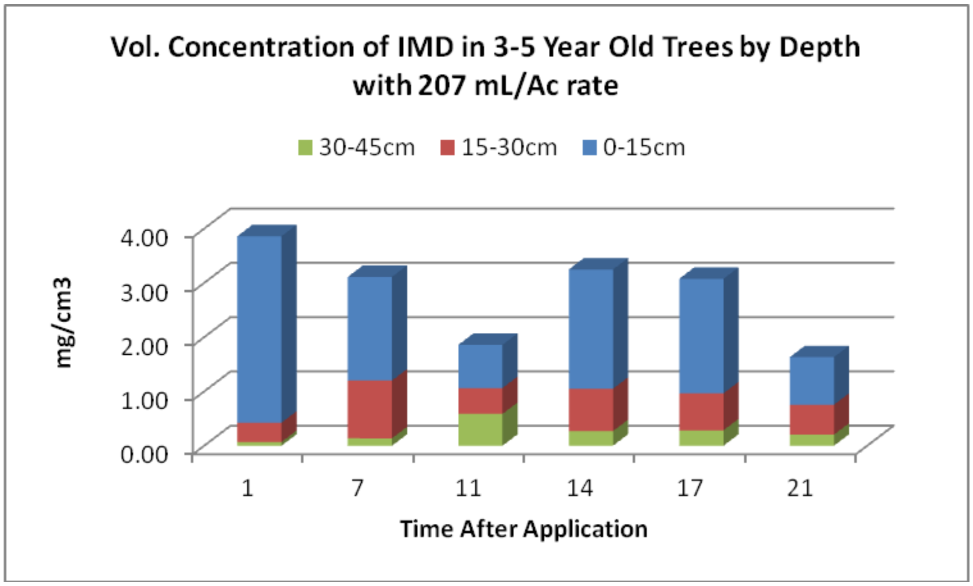

Fig 41. Volumetric Concentration (1x) of IMD in 3-5 Year Old Trees.

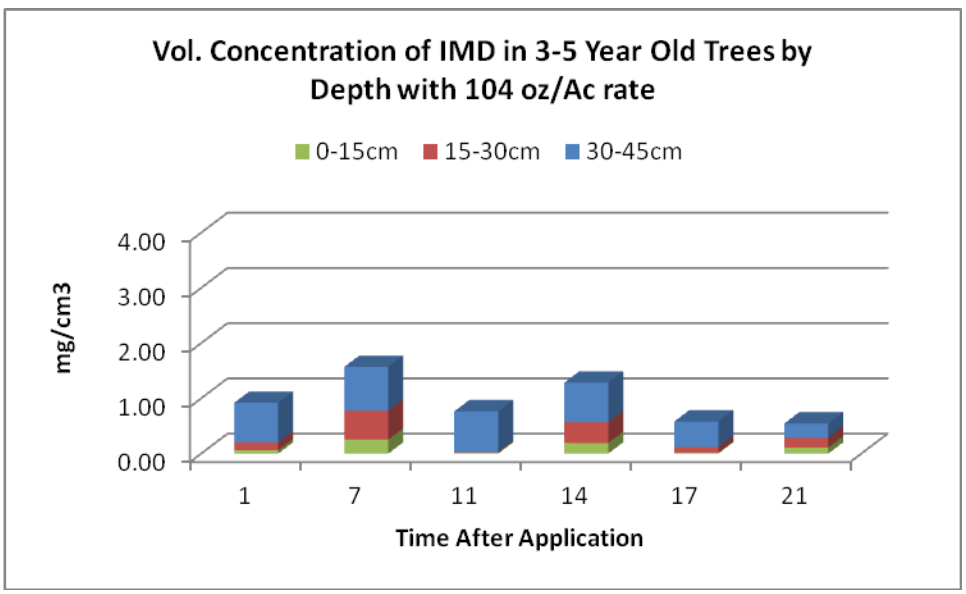

Fig 42. Volumetric Concentration (0.5x) of IMD in 3-5 Year Old Trees.

The eight year old trees had an initial concentration of 2 mg/cm^3 in the 0.5x application rate (Fig. 43), and 0.50 mg/cm^3 or 26% by the end of the trial. The 1x application rate (Fig. 44) showed 5 mg/cm^3 initially with 1 mg/cm^3 remaining, or 27%. Imidacloprid maintained the majority of its presence in the 0-15cm depth for both rates, but did show a slight increase in the lower depths with time, especially in the 1x rate.

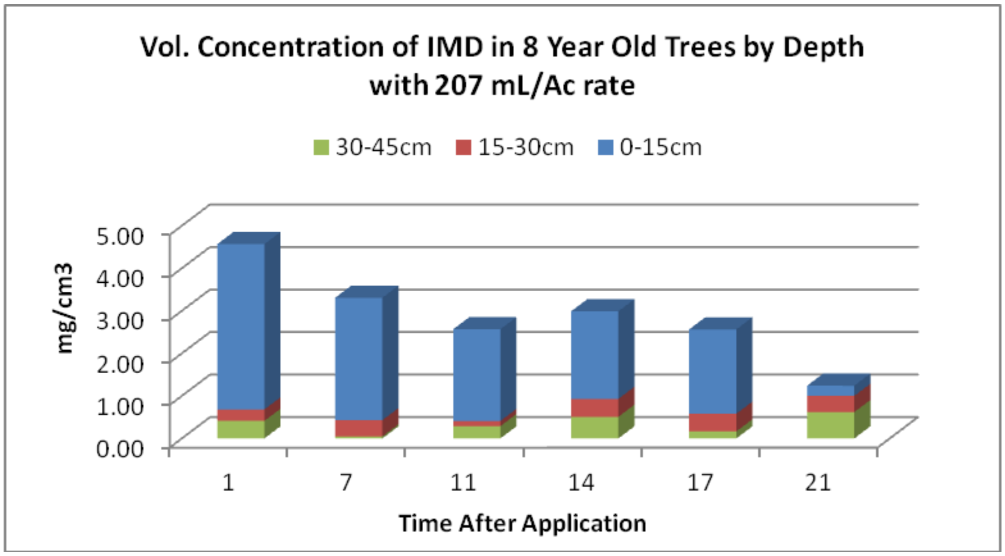

Fig 43. Volumetric Concentration (1x) of IMD in 8 Year Old Trees.

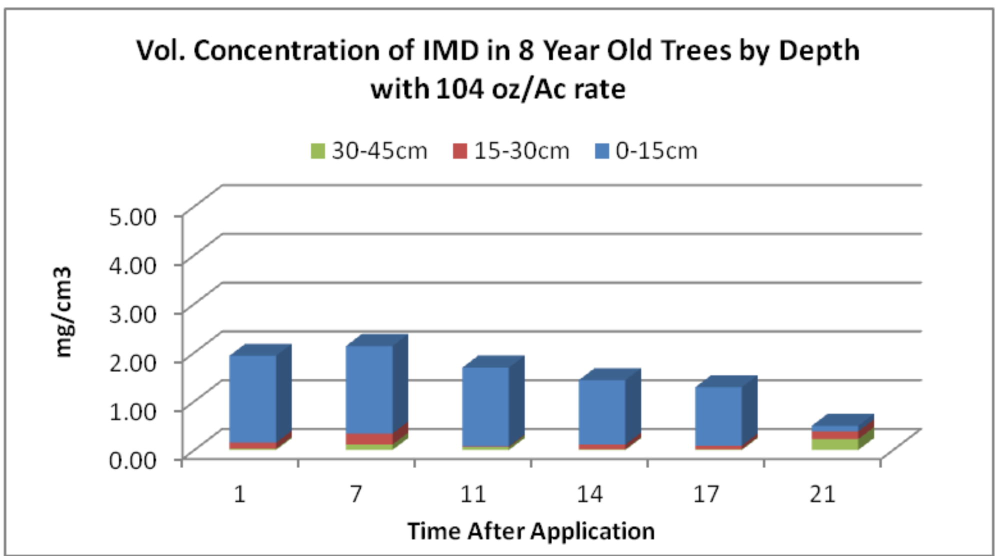

Fig 44. Volumetric Concentration (0.5x) of IMD in 8 Year Old Trees.

Less imidacloprid was remaining in the soil in the second trial versus the first, which shows how different application rates (104 and 207 mL/Ac compared to 207 and 414 mL/Ac) will behave differently over time in the soil. For example, the block of eight year old trees had 1x – 26% and 2x – 27% imidacloprid remaining in the soil after 21 days in trial two, while the same block of trees had 1x – 57% and 2x – 56% remaining after 21 days in trial one, or an average of 30% between the two studies. Another explanation of the difference is that trial two experienced a rain event of 2.4cm on the 20th day after application of imidacloprid. The sudden loss of imidacloprid concentration can be explained by the increased depth of the wetting front from 0.08cm to 1.02cm due to rainfall in that block of trees (Appendix 4). At this time, the chemical was either leached out, or taken up by the plant.

Having a low K_D in IFS makes imidacloprid likely to leach when in saturated soils. In this study, the soil is unsaturated, causing the chemical to behave differently. Imidacloprid is retained in the soil due to increase in the retardation factor, where:

$$R = \text{Velocity of Water/Velocity of Solute or } R = 1 + (\rho b * K_D)/\theta v$$

For example, in Trial 1, the youngest trees have R values as follows, using the values from Figure 5 and average moisture values:

- 0-15cm, R = 65
- 15-30cm, R = 10
- 30-45cm, R = 10

Imidacloprid is more strongly retained in the A horizon (0-15cm) due to slightly higher organic matter, so the residence time is longer. The 0-15cm depth has 4x more organic carbon than the 15-30cm depth. This can be explained with the retardation factor above, which decreases with depth. As R decreases, the chemical is less sorbed in soil, making it more available for root uptake. The value of R will change over time with water content. The movement is very slow from the top depth, so the likelihood of leaching is low since a high retardation factor slows mobility as the water content goes from saturation to unsaturated flow. The water content in both trials did not approach saturated water content (0.43). During unsaturated flow the hydraulic conductivity exponentially decreases with decrease in water content in addition to increasing the retardation factor of IMD. What was originally considered a burden to overcome is actually a blessing in disguise for keeping IMD in the root zone.

LEAF IMIDACLOPRID CONCENTRATION

Trial 1

The concentration of imidacloprid in leaves showed great differences between the two imidacloprid rates, especially between trials. Since the effects of irrigation did not impact soil moisture or soil imidacloprid, it is presumed to not effect IMD concentration in citrus leaf tissue either, and therefore only the IMD rates will be discussed. Concentrations are expressed in ppb.

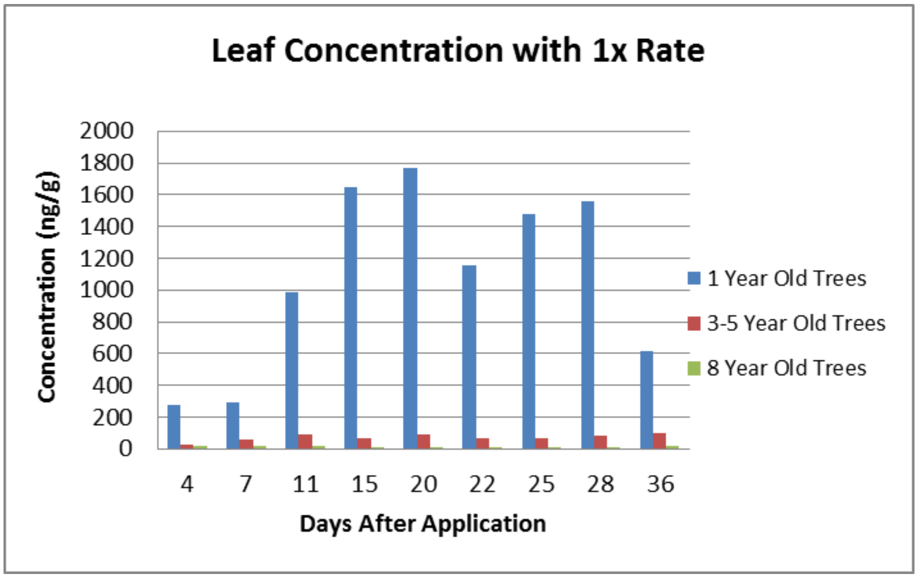

Fig 45. IMD Leaf Concentration with 1x Rate.

The 1x rate of 207 mL/Ac (Figure 45) achieved its highest concentration of 1772 ng/g by day 20 in the tissues of the youngest trees. There was a steady incline of IMD content up until that day. The three to five year old trees never broke 100 ng/g, but had a consistent concentration throughout the trial. The eight year old trees maintained concentrations below 21 ng/g, with the lowest concentration among tree ages. The concentration of imidacloprid in leaf tissue begins to decline by day 36 in the youngest trees, giving a bell shaped curve.

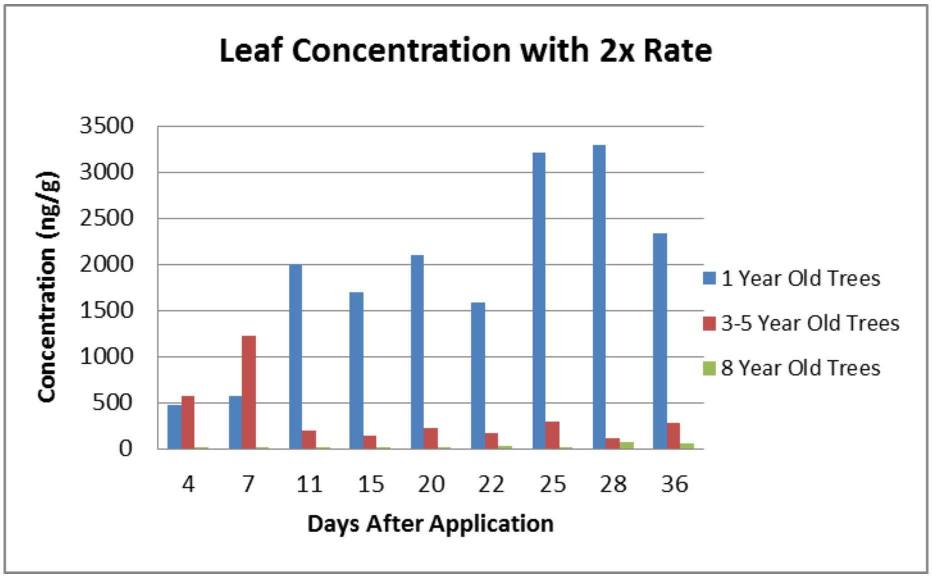

Fig 46. IMD Leaf Concentration with 2x Rate.

The one year old trees reached concentrations into the 3000s with 3208 ng/g at day 25 and 3298 ng/g by day 28 (Figure 46), having the highest concentration among tree ages and rates for the entire first trial. The 3-5 year old trees finally show significant content of IMD in the first two sampling days, and actually are greater in value than the 1 year old trees! On day 7, the IMD content in the 3-5 year old trees is 1232 ng/g. It will be interesting to see if the psyllids are controlled in this tree age during that time. There is a slow increase of IMD concentration in the 8 year old trees over time with the highest concentration is 70 ng/g on day 28.

Trial 2

Initially, leaf concentrations in the 0.5x rate of 104 mL/Ac had the highest concentration on day 1 with 804 ng/g (Figure 47). However, over time, the concentration had levels below 100 ng/g for the rest of the trial. The three to five year old trees started with 635 ng/g in treatment one, and also stayed below 100 ng/g for the remainder of the trial. The initial concentration of the oldest trees at eight years started was 11 ng/g, then it slightly increases before leveling off by day 17.

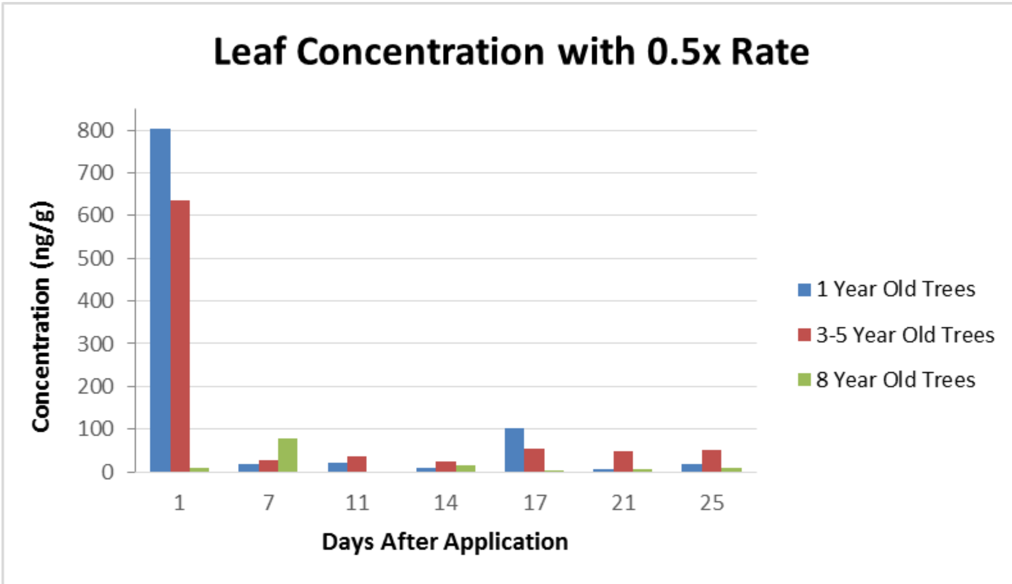

Fig 47. IMD Leaf Concentration with 0.5x Rate.

Trees treated with the higher imidacloprid rate of 7 oz/Ac had initial concentrations of 1554 ng/g in the youngest trees (Figure 48). Concentrations increased slightly by day 17, and declined again for the remainder of the trial. The three to five year old trees showed concentrations of 141 ng/g and increased to 261 ng/g on day 11. The third block of trees at eight year of age began with 3 ng/g and ended the trial with 10 ng/g.

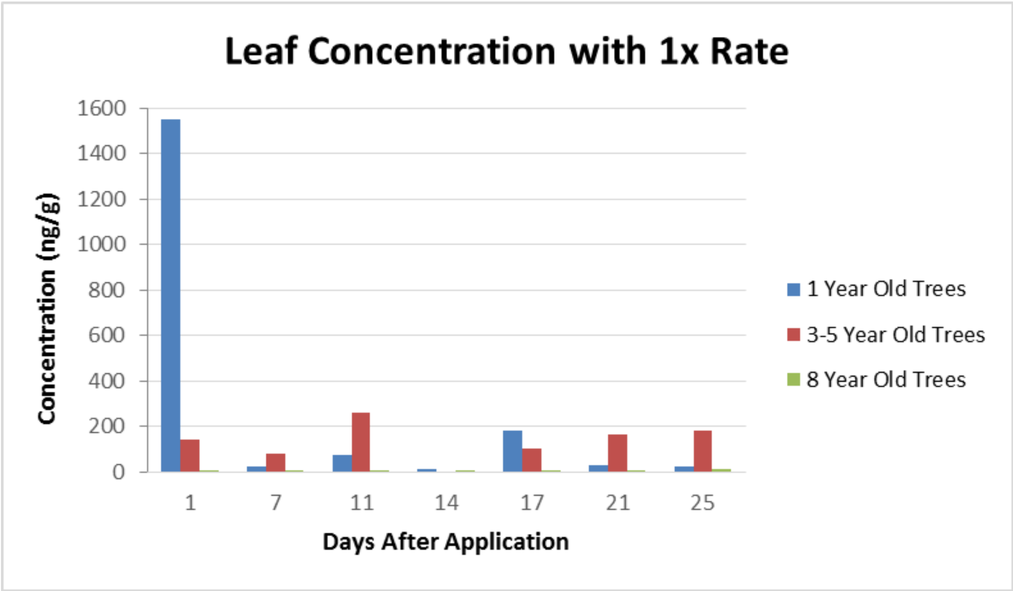

Fig 48. IMD Leaf Concentration with 1x Rate.

As imidacloprid in soil decreased, there was a steady increase in leaf tissue. What at first appeared to be a loss of the chemical is actually being taken up by the plant. When observing relative concentrations of IMD (Appendix 9), there was not a consistent percentage of what is left in the soil when comparing rates, however, the higher rates are steadily greater in the leaves. It was also noticed that more IMD, as a percentage, was left behind in the soil in the older trees, while little was shown in the tree tissue. This statement is more accurate with Trial 1 data.

PSYLLID POPULATION

Trial 1

Psyllid populations are averaged by two taps per limb, and total taps were dependent on the tree size. Populations are expressed on an average per acre, assuming 200 trees per acre. Totals of live and dead adult psyllids, and eggs and nymphs found on shoots can be viewed in Appendix 6 and 7 for both trials.

Average Live Adult Psyllids Per Acre								
Time:	7	14	20	27	34	41	48	60
1 Y.O. Trees	33	0	67	0	33	0	33	33
3-5 Y.O. Trees	167	0	167	0	367	133	367	200
8 Y.O. Trees	0	0	0	33	100	67	133	567

Fig 49. Average Live Psyllid Adults per Acre - 1x Rate.

Average Live Adult Psyllids Per Acre								
Time:	7	14	20	27	34	41	48	60
1 Y.O. Trees	0	0	33	0	33	0	0	0
3-5 Y.O. Trees	0	0	67	233	233	433	67	300
8 Y.O. Trees	0	0	0	0	67	0	67	433

Fig 50. Average Live Psyllids per Acre - 2x Rate.

The average population of adult psyllids were lowest for the one year old trees in trial one when compared to older trees. Initially, fewer psyllids were observed in the 2x rate (Figure 50) with the higher imidacloprid rate of 414 mL/Ac than with the lower rate (Figure 49). Psyllids appeared sooner, and in equal or greater numbers with the lower IMD rate.

Adult psyllids in the three to five year old trees were more prominent in the trees treated with lower imidacloprid rates (1x). No psyllids were observed in the 2x rate until day 20, which is interesting because the 3-5 year old trees had its highest IMD concentration in leaves during the time. This means that IMD at a rate of 414 mL/Ac could control psyllids in mature trees, even if only temporarily. As of day 34, adult psyllids were noticed in both IMD treatments for the remainder of the trial.

In the oldest trees, psyllids were not present until day 27 with the 1x rate. No psyllids were counted in the 2x rate until day 34, where treatment it had 67 psyllids per acre.

Psyllids were best controlled in trees that had leaf concentration values in the 1000 ppbs. Even the 3-5 year old trees showed good control when the concentration peaked in the mentioned range.

Nymphs and eggs were controlled better in the older trees than the youngest trees, but showed control for several weeks in all ages.

Trial 2

Adult psyllids were observed on all dates in the one year old trees, except for day 25 in the 0.5x rate. Neither rate had effective control of the ACP. Psyllids were present more often in the higher treatments of imidacloprid with this group of trees. The 207 mL/Ac application rate was more effective in Trial 1 than in Trial 2. It cannot be determined at this time if the ACP are resistant to the chemical, but the same rate applied at two different trials showed different levels of control. While the control in trial 1 was not consistent, it did show a smaller population with the 207 mL/Ac rate. Psyllids were observed regardless of irrigation rate, and did not show trends for any of the treatments in this age group.

Average Live Adult Psyllids Per Acre							
Time:	1	18	25	33	41	48	55
1 Y.O. Trees	100	200	100	133	100	100	33
3-5 Y.O. Trees	100	133	0	0	0	0	67
8 Y.O. Trees	167	167	0	33	600	67	200

Fig 51. Average Live Adult Psyllids per Acre - 0.5x Rate.

Average Live Adult Psyllids Per Acre							
Time:	1	18	25	33	41	48	55
1 Y.O. Trees	200	433	0	33	167	67	300
3-5 Y.O. Trees	0	0	0	0	0	0	100
8 Y.O. Trees	200	433	0	33	167	67	300

Fig 52. Average Live Adult Psyllids per Acre - 1x Rate.

Treatment 1x showed no presence of adult psyllids until day 55 in the three to five year old trees. The 0.5x treatment had adult psyllids initially present for the first 18 days, but none were observed again until day 55. There is not consistent control with this rate. .

Neither the 1x nor the 0.5x had effective control of the adult ACP for this trial.

Eggs and nymphs were present during the entire trial in all tree ages.

CONCLUSIONS

In conclusion, imidacloprid has proven to be a safer chemical for the environment than the hypothesis presumed. As mentioned before, imidacloprid will move with water, and since water from irrigation and rain was enough to replenish ET, and not cause saturated conditions, imidacloprid is more likely to be retained in soil than originally suggested.

Irrigation at the rate of 23 or 38 liters per hour will not cause leaching unless a heavy rainstorm occurs, since they are practically the same in the 0-45cm depth. Thanks to the results of bromide, citrus growers can apply the lower irrigation rate and maintain adequate moisture in the soil for the trees, while not being concerned with leaching potential. However, it will depend on the ET of the local climate, bulk density, rainfall and volumetric water content of the soil, or in a nutshell, water! In general these concepts can be applied to other groves with Immokalee fine sand.

Imidacloprid is an effective insecticide to control the Asian citrus psyllid on young citrus trees for almost 2 months with the possibility of being effective with 3-5 year old trees for a short period of time. The label legally allows up to 14 oz (or 414 mL) per acre, which controlled psyllids in the 1 year age group for 14 days. This is not promising enough to recommend, but does give optimism to the possibility of spreading the range of imidacloprid, and avoiding the use of broad spectrum pesticides. However, to avoid pesticide resistance, it is always important to rotate the active ingredient with crops.

It was also determined that imidacloprid can be recovered in the leaf tissue, and will show concentrations that correlate to the application rate. With further research, concentrations of IMD in leaf tissue can give hints regarding the minimum concentration for controlling ACP in multiple tree ages.

Future research suggestions include attempting the study in vitro to determine how much imidacloprid is actually lost to leaching when exposed to different levels of soil moisture, or simulations of heavy rainfall. Future research that would be valuable to the grower would include knowing what irrigation limits are in sandy soil conditions, and how many seasons Admire Pro can be used before sucking insects will develop a resistance. In all, citrus growers will be pleased to know that one of the most popular insect control methods is effective at the legal rate, while being environmentally friendly when used in spodosols.

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Figure 4 – Immokalee Fine Sand Soil Profile. University of Florida IFAS. FAWN. http://fawn.ifas.ufl.edu/tools/irrigation/citrus/scheduler/help.html.

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Appendices

1. Average volumetric water content by irrigation treatment per age group. Trial 1

1 Y.O. Trees	6 GPH	10GPH		3-5 Y.O. Tr	6 GPH	10GPH		8 Y.O. Tre	6 GPH	10GPH
0-15cm	**0.04**	**0.05**		0-15cm	**0.07**	**0.08**		0-15cm	**0.08**	**0.09**
15-30cm	**0.05**	**0.05**		15-30cm	**0.07**	**0.08**		15-30cm	**0.06**	**0.06**
30-45cm	**0.04**	**0.05**		30-45cm	**0.05**	**0.06**		30-45cm	**0.07**	**0.07**

2. Average volumetric water content by irrigation treatment per age group. Trial 2

Avg water content										
1 Y.O. Tre	6 GPH	10GPH		3-5 Y.O. Tr	6 GPH	10GPH		8 Y.O. Tre	6 GPH	10GPH
0-15cm	**0.09**	**0.09**		0-15cm	**0.13**	**0.12**		0-15cm	**0.10**	**0.12**
15-30cm	**0.08**	**0.08**		15-30cm	**0.13**	**0.13**		15-30cm	**0.12**	**0.12**
30-45cm	**0.08**	**0.08**		30-45cm	**0.10**	**0.10**		30-45cm	**0.10**	**0.11**

3. Depth of water over time including irrigation and rainfall in Trial 1

Block 1		Depth of water from irrigation+rainfall (cm)											
	Time (Day	10-May	13-May	14-May	16-May	17-May	19-May	20-May	21-May	24-May	28-May	31-May	1-Jun
	6 GPH	5.99	5.99	5.77	5.87	6.17	6.48	5.87	5.77	5.77	6.1	5.77	6.02
	10GPH	9.65	9.65	9.62	9.75	10.02	10.33	9.72	9.62	9.62	9.95	9.62	9.87
Block 2		Depth of water from irrigation+rainfall (cm)											
	Time:	10-May	13-May	14-May	16-May	17-May	19-May	20-May	21-May	24-May	28-May	31-May	1-Jun
	6 GPH	0.18	0.18	0.15	0.25	0.55	0.86	0.25	0.15	0.15	0.48	0.15	0.40
	10 GPH	0.27	0.27	0.24	0.34	0.64	0.95	0.34	0.24	0.24	0.57	0.24	0.49
Block 3		Depth of water from irrigation+rainfall (cm)											
	Time (Day	10-May	13-May	14-May	16-May	17-May	19-May	20-May	21-May	24-May	28-May	31-May	1-Jun
	6 GPH	0.14	0.14	0.11	0.21	0.61	1.32	1.42	0.11	0.11	0.44	0.11	0.36
	10GPH	0.21	0.21	0.14	0.24	0.64	1.35	1.45	0.14	0.14	0.47	0.14	0.39

4. Depth of water over time including irrigation and rainfall in Trial 2

Block 1		Depth of water from irrigation+rainfall (cm)											
	Time (Days	28-Mar	4-Apr	5-Apr	7-Apr	11-Apr	12-Apr	14-Apr	15-Apr	18-Apr	19-Apr	20-Apr	21-Apr
	6 GPH	5.96	6.54	6.39	5.96	5.96	6.1	5.96	6.9	5.96	6	6.29	6.2
	10GPH	9.62	10.2	10.05	9.62	9.62	9.76	9.62	10.56	9.62	9.66	9.95	9.86

Block 2		Depth of water from irrigation+rainfall (cm)											
	Time:	28-Mar	4-Apr	5-Apr	7-Apr	11-Apr	12-Apr	14-Apr	15-Apr	18-Apr	19-Apr	20-Apr	21-Apr
	6 GPH	0.11	0.69	0.54	0.11	0.11	0.25	0.11	1.05	0.11	0.15	0.44	0.11
	10 GPH	0.16	0.74	0.59	0.16	0.16	0.3	0.16	1.1	0.16	0.2	0.49	0.16

Block 3		Depth of water from irrigation+rainfall (cm)											
	Time (Days	28-Mar	4-Apr	5-Apr	7-Apr	11-Apr	12-Apr	14-Apr	15-Apr	18-Apr	19-Apr	20-Apr	21-Apr
	6 GPH	0.08	0.66	0.51	0.08	0.08	0.22	0.08	1.02	0.08	0.12	0.41	0.08
	10GPH	0.12	0.7	0.55	0.12	0.12	0.26	0.12	1.06	0.12	0.16	0.45	0.12

5. Tables of Bromide Concentration by Volume in all tree ages.

Block 1		**Avg bromide content per volume of soil (ug/cm3)**				
	Time (Days	1	7	11	14	17
Treatment:						
6GPH						
0-15cm		2.42	0.28	0.16	0.14	0.03
15-30cm		0.36	0.28	0.14	0.38	0.41
30-45cm		0.87	0.29	0.20	0.16	0.09
10GPH						
0-15cm		0.84	0.26	0.02	0.06	0.02
15-30cm		0.85	0.27	0.19	0.24	0.40
30-45cm		0.67	0.26	0.22	0.26	0.28

Block 2		**Avg bromide content per volume of soil (ug/cm3)**				
	Time (Day	1	7	11	14	17
Treatment:						
6GPH						
0-15cm		1.87	0.77	0.30	0.26	0.04
15-30cm		0.93	0.83	0.33	0.44	0.09
30-45cm		0.96	0.86	0.18	0.24	0.42
10GPH						
0-15cm		1.20	0.67	0.51	0.20	0.03
15-30cm		1.02	0.72	0.25	0.57	0.00
30-45cm		1.09	0.74	0.06	0.34	0.30

Block 3		Avg bromide content per volume of soil (ug/cm3)				
	Time (Day	1	7	11	14	17
Treatment:						
6GPH						
0-15cm		1.08	0.71	0.28	0.40	0.05
15-30cm		0.99	0.78	0.53	0.46	0.09
30-45cm		1.11	0.86	0.37	0.51	0.25
10GPH						
0-15cm		1.24	0.74	0.25	0.28	0.20
15-30cm		0.90	0.78	0.55	0.38	0.26
30-45cm		1.16	0.86	0.42	0.60	0.22

6. Psyllid populations in Trial 1 – Treatments 1-4

	Treatment 1									
	Days After Application	7	14	20	27	34	41	48	60	74
B1	Avg Adults	33.3	0	66.7	0	33.3	0	33.3	33.3	33.3
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	1933.3	466.7	0	800.0	266.7
	Avg shoots w/ nymphs	0	0	0	0	1466.7	733.3	266.7	66.7	200.0
B2	Avg Adults	166.7	0	166.7	0	366.7	133.3	366.7	200	300
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	0	333.3	933.3
	Avg shoots w/ nymphs	0	0	0	0	0	0	0	333.3	933.3
B3	Avg Adults	0	0	0	33.3	100	66.7	133.3	566.7	100
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	600	466.7	133.3	0
	Avg shoots w/ nymphs	0	0	0	0	0	600	466.7	133.3	0

	Treatment 2									
	Days After Application	7	14	20	27	34	41	48	60	74
B1	Avg Adults	100	0	66.66667	0	0	0	33.33333	33.33333	66.66667
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	666.6667	666.6667	0
	Avg shoots w/ nymphs	0	0	0	0	0	0	666.6667	666.6667	0
B2	Avg Adults	600	0	233.3333	266.6667	366.6667	133.3333	366.6667	266.6667	466.6667
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	0	0	133.3333
	Avg shoots w/ nymphs	0	0	0	0	0	0	0	0	133.3333
B3	Avg Adults	0	0	233.3333	33.33333	133.3333	133.3333	66.66667	266.6667	100
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	66.66667	0	0
	Avg shoots w/ nymphs	0	0	0	0	0	0	66.66667	0	0

	Treatment 3								
	Days After Applicati	7	14	20	27	34	41	48	60	74
B1	Avg Adults	0	0	33.3	0	33.3	0	0	0	0
	Avg Dead	0	0	0	0	0	0	0	0	400
	Avg Shoots w/ eggs	0	0	0	0	400	0	0	0	0
	Avg shoots w/ nymp	0	0	0	0	266.7	0	0	0	0
B2	Avg Adults	0	0	66.7	233.3	233.3	433.3	66.7	300	133.3
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	0	0	133.3
	Avg shoots w/ nymp	0	0	0	0	0	0	0	0	133.3
B3	Avg Adults	0	0	0	0	66.7	0	66.7	433.3	66.7
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	200	0	0
	Avg shoots w/ nymp	0	0	0	0	0	0	333.3	0	333.3

		Treatment 4								
	Days After Applicati	7	14	20	27	34	41	48	60	74
B1	Avg Adults	0	0	0	0	0	0	0	33.33333	0
	Avg Dead	0	0	0	0	66.66667	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	400	266.6667	0	0	200	466.6667
	Avg shoots w/ nymp	0	0	0	400	0	0	0	0	466.6667
B2	Avg Adults	33.33333	0	133.3333	66.66667	300	166.6667	300	133.3333	66.66667
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	0	0	266.6667
	Avg shoots w/ nymp	0	0	0	0	0	0	0	0	266.6667
B3	Avg Adults	0	0	0	0	33.33333	66.66667	0	166.6667	66.66667
	Avg Dead	0	0	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	0	0	0	0	0	0	66.66667	0
	Avg shoots w/ nymp	0	0	0	0	0	0	0	66.66667	0

7. Psyllid populations in Trial 2 – Treatments 1-4

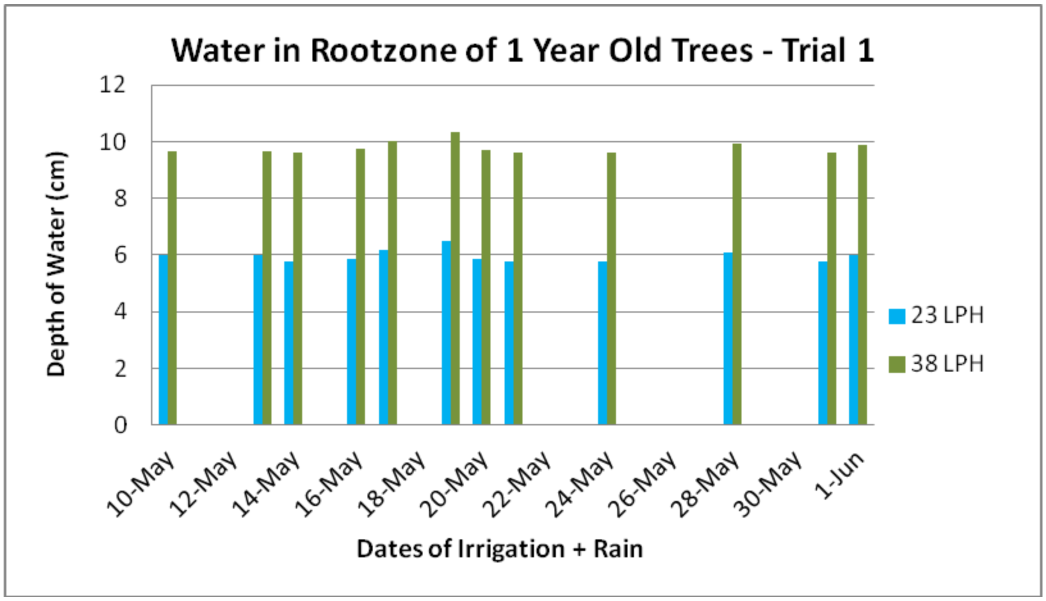
	Treatment 1							
	Days After Application	1	18	25	33	41	48	55
B1	Avg Adults	100	200	100	133.3	100	100	33.3
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	933.3	466.7	200	733.3	133.3	1400
	Avg shoots w/ nymphs	0	133.3	600	666.7	1066.7	466.7	200
B2	Avg Adults	100	133.3	0	0	0	0	66.7
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1333.3	0	0	200	200	533.3
	Avg shoots w/ nymphs	0	466.7	0	66.7	133.3	0	133.3
B3	Avg Adults	166.7	166.7	0	33.3	600	66.7	200
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1533.3	66.7	200	333.3	400	400
	Avg shoots w/ nymphs	0	666.7	0	600	200	333.3	400

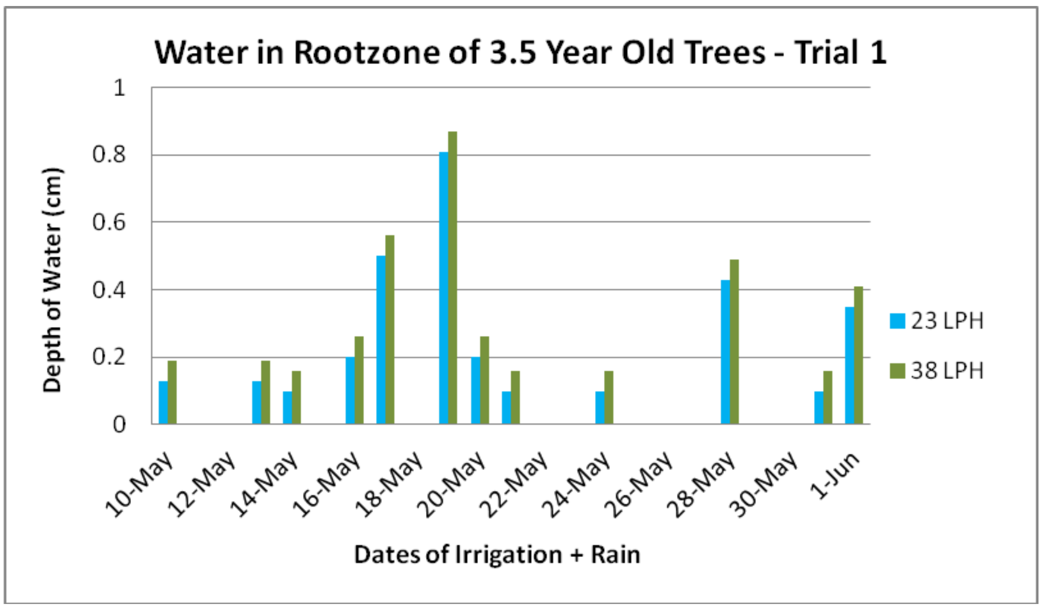
	Treatment 2							
	Days After Application	1	18	25	33	41	48	55
B1	Avg Adults	200	33.3	0	200	300	166.7	0
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1533.3	466.7	266.7	266.7	133.3	1200
	Avg shoots w/ nymphs	0	666.7	1066.7	1000	400	333.3	266.7
B2	Avg Adults	200	100	0	0	66.7	33.3	100
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1000	0	66.7	266.7	266.7	333.3
	Avg shoots w/ nymphs	0	1066.7	0	66.7	400	66.7	66.7
B3	Avg Adults	200	0	0	0	200	33.3	133.3
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1733.3	133.3	66.7	133.3	600	600
	Avg shoots w/ nymphs	0	200	133.3	866.7	733.3	1133.3	1200

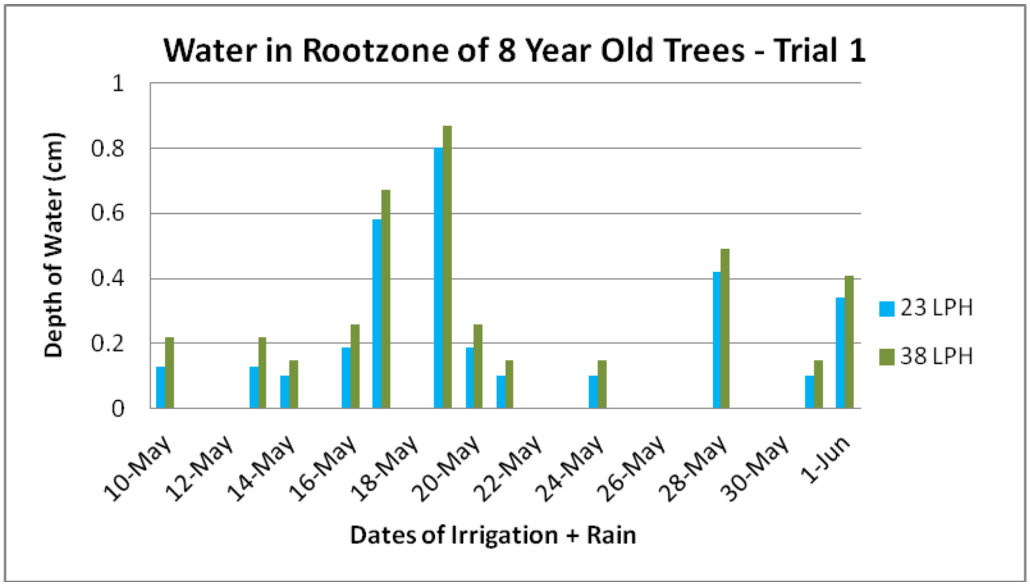
	Treatment 3							
	Days After Application	1	18	25	33	41	48	55
B1	Avg Adults	200	433.3	0	33.3	166.7	66.7	300
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	533.3	0	466.7	666.7	200	400
	Avg shoots w/ nymphs	0	1000	0	800	666.7	1400	2600
B2	Avg Adults	0	0	0	0	0	0	100
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1200	0	0	66.7	400	1200
	Avg shoots w/ nymphs	0	200	0	200	0	66.7	800
B3	Avg Adults	200	433.3	0	33.3	166.7	66.7	300
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	533.3	0	466.7	666.7	200	400
	Avg shoots w/ nymphs	0	1000	0	800	666.7	1400	2600

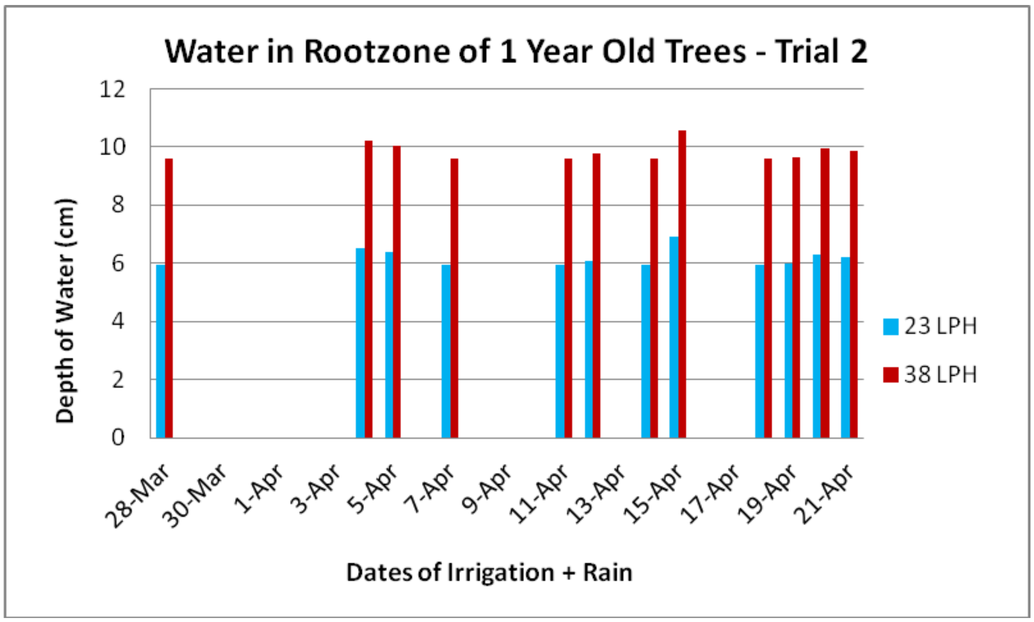
	Treatment 4							
	Days After Application	1	18	25	33	41	48	55
B1	Avg Adults	366.7	400	100	300	100	33.3	100
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1066.7	400	400	133.3	533.3	1466.7
	Avg shoots w/ nymphs	0	666.7	1266.7	1733.3	200	266.7	533.3
B2	Avg Adults	133.3	33.3	0	0	0	0	33.3
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1466.7	0	66.7	66.7	466.7	400
	Avg shoots w/ nymphs	0	200	0	200	66.7	66.7	133.3
B3	Avg Adults	200	266.7	0	0	33.3	0	66.7
	Avg Dead	0	0	0	0	0	0	0
	Avg Shoots w/ eggs	0	1466.7	0	200	66.7	200	466.7
	Avg shoots w/ nymphs	0	466.7	0	733.3	400	1200	1133.3

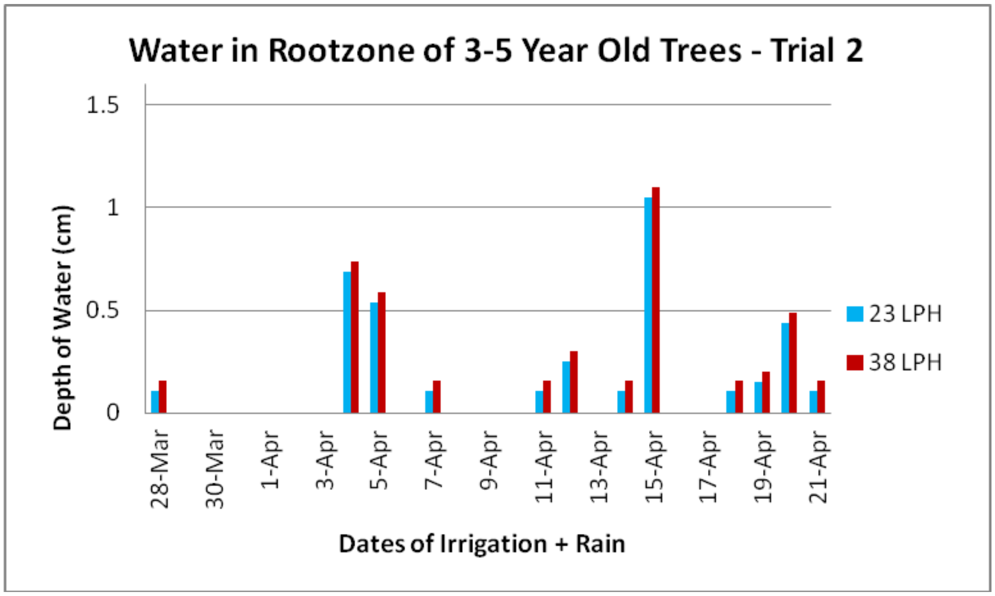
8. Depth of Water in Trials 1 and 2.

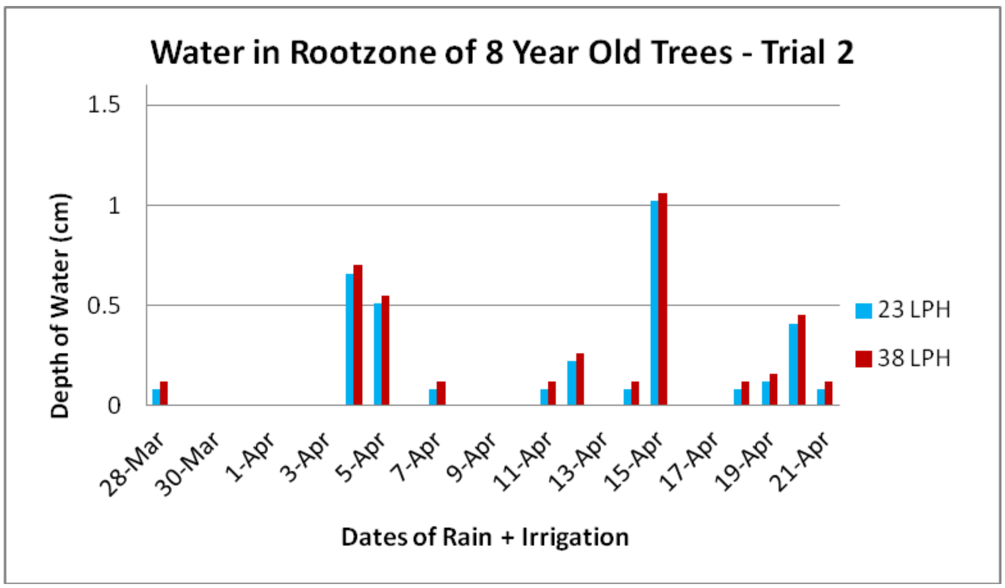

Water in Rootzone of 1 Year Old Trees - Trial 1
Depth of Water (cm)
0
2
4
6
8
10
12
10-May
12-May
14-May
16-May
18-May
20-May
22-May
24-May
26-May
28-May
30-May
1-Jun
Dates of Irrigation + Rain
23 LPH
38 LPH


Water in Rootzone of 3.5 Year Old Trees - Trial 1
Depth of Water (cm)
0
0.2
0.4
0.6
0.8
1
10-May
12-May
14-May
16-May
18-May
20-May
22-May
24-May
26-May
28-May
30-May
1-Jun
Dates of Irrigation + Rain
23 LPH
38 LPH

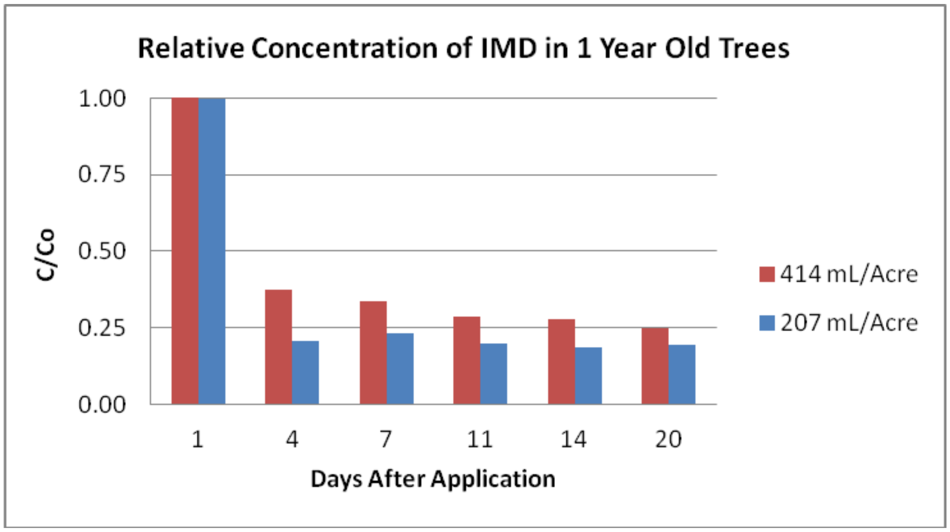

Water in Rootzone of 8 Year Old Trees - Trial 1
Depth of Water (cm)
1
0.8
0.6
0.4
0.2
0
10-May
12-May
14-May
16-May
18-May
20-May
22-May
24-May
26-May
28-May
30-May
1-Jun
Dates of Irrigation + Rain
23 LPH
38 LPH

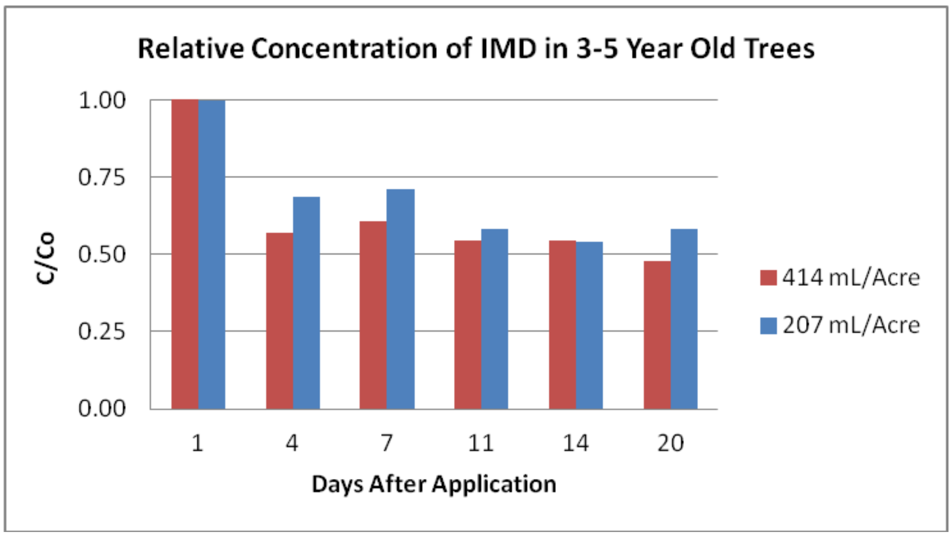

Water in Rootzone of 1 Year Old Trees - Trial 2
Depth of Water (cm)
12
10
8
6
4
2
0
28-Mar
30-Mar
1-Apr
3-Apr
5-Apr
7-Apr
9-Apr
11-Apr
13-Apr
15-Apr
17-Apr
19-Apr
21-Apr
Dates of Irrigation + Rain
23 LPH
38 LPH

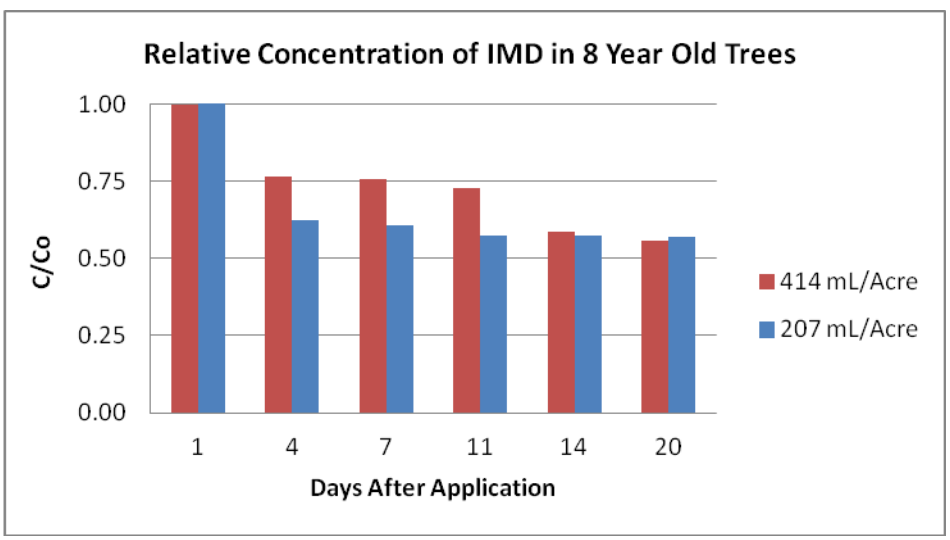




9. Relative concentrations of IMD in soil – Trial 1.


Relative Concentration of IMD in 1 Year Old Trees
C/Co
1.00
0.75
0.50
0.25
0.00
1
4
7
11
14
20
Days After Application
414 mL/Acre
207 mL/Acre


Relative Concentration of IMD in 3-5 Year Old Trees
C/Co
1.00
0.75
0.50
0.25
0.00
1
4
7
11
14
20
Days After Application
414 mL/Acre
207 mL/Acre


Relative Concentration of IMD in 8 Year Old Trees
C/Co
1.00
0.75
0.50
0.25
0.00
1
4
7
11
14
20
Days After Application
414 mL/Acre
207 mL/Acre

10. Relative concentrations of IMD in soil – Trial 2