

Citrus Trunk Injection Research Update

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Thank You

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CRDF 22-001

Grower Collaborators



OUTLINE

- **Background Information**
- **Preliminary Research**
- **Field Trials**
- **Tree Injury**



✓ **Background Information**

- Preliminary Research
- Field Trials
- Tree Injury



“Trunk injection”

- First evidence (12th century) → Arabic horticulturists applied perfumes, spices, dyes, and other things to wounds to affect the smell, color, or other attributes of flowers and fruits (**solid “injection”**)
- First documented experimentation (15th century) → Leonardo da Vinci injected arsenic and other poisonous solutions through bore holes into apple trees to make the fruit poisonous (**liquid injection**)

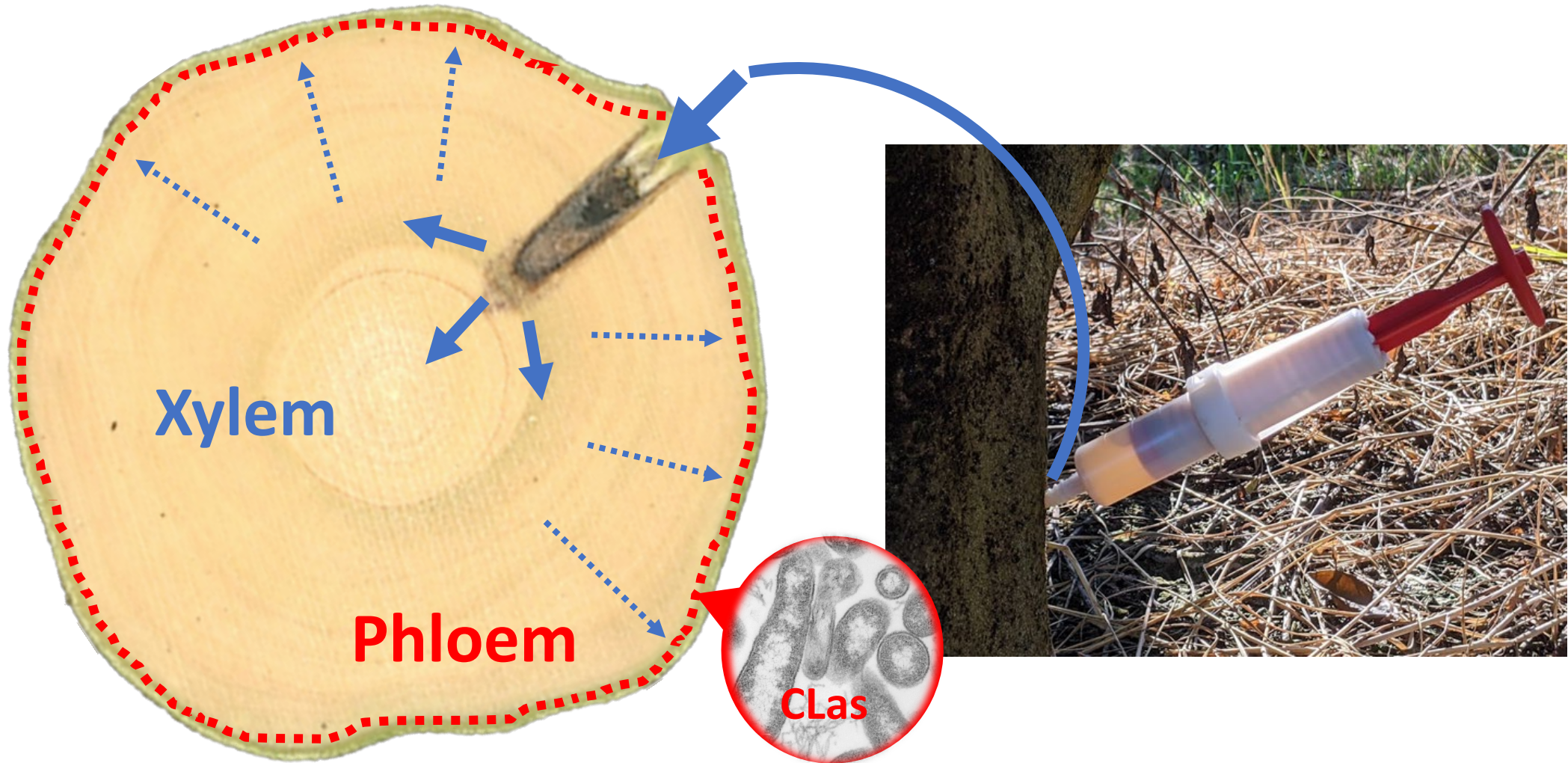


Trunk injection – modern definition

- The targeted delivery of crop protection materials into the stem or trunk of a woody plant as an alternative to spraying or soil drenching (“endotherapy”)
- Injection occurs into the **xylem** (not phloem) from where the materials are then distributed throughout the plant with the transpiration stream



Trunk injection in citrus trees



Modern areas of use

- Forest trees, non-crop-bearing ornamental trees, large woody shrubs, and palms in residential and commercial landscapes
- Some crop-bearing agricultural crops (e.g., avocado, peach, pear)
 - *20% of the commercial avocado acreage (~6000 acres) in Florida is managed for laurel wilt by trunk injections*



Targets

- *Insects* (stem and leaf feeding, bark boring)
- *Nematodes* (wood nematodes)
- *Fungi* (e.g., vascular wilt, powdery mildew, phytophthora)
- *Bacteria* (e.g., fire blight, blossom blight, bacterial blast)
- *Phytoplasmas* (lethal yellows/bronzing)
- *Other* (delivery of nutrients, growth regulators, etc.)



Advantages

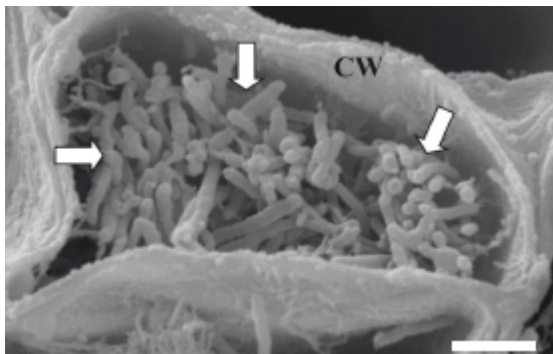
- Precise delivery of materials
- Elimination of spray drift
- Reduced risk for worker exposure
- Reduced risk for non-target organisms
- Reduced pesticide load into the environment
- Potentially longer residual activity of materials



Trunk injection – HLB



CLas is
phloem-
limited



Martinez et al. 1970
Schwarz and Van Vuren 1970
Moll and Van Vuuren 1977
Chiu et al. 1979
Aubert and Bove 1980
Cheema et al. 1986

Hu and Wang 2016
Hu et al. 2018
Zhang et al. 2019
Li et al. 2019
Li et al. 2021

✓ Background
Information

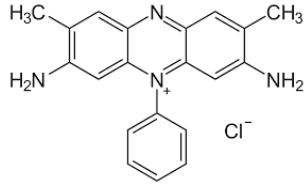
✓ **Preliminary
Research**

- Field Trials
- Tree Injury

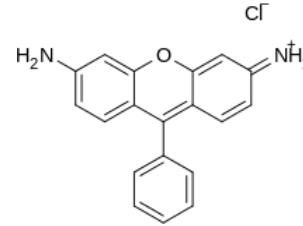


Compound mobility

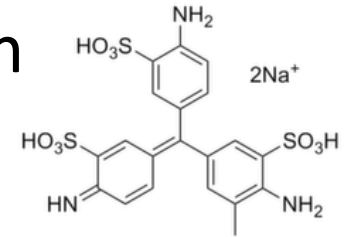
Safranin



Rhodamine



Acid Fuchsin



Formulation matters!

Methods of injection



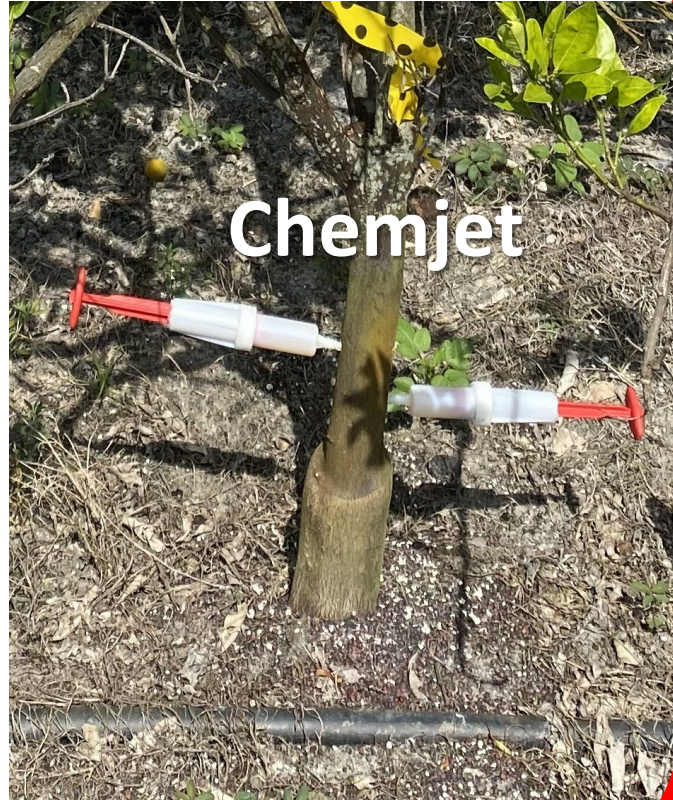
Most technologies are drill-based. Few are no-drill (needle)-based.
All require relatively large injection holes.

Injection pressure

INJECTION



High pressure



Medium pressure

INFUSION

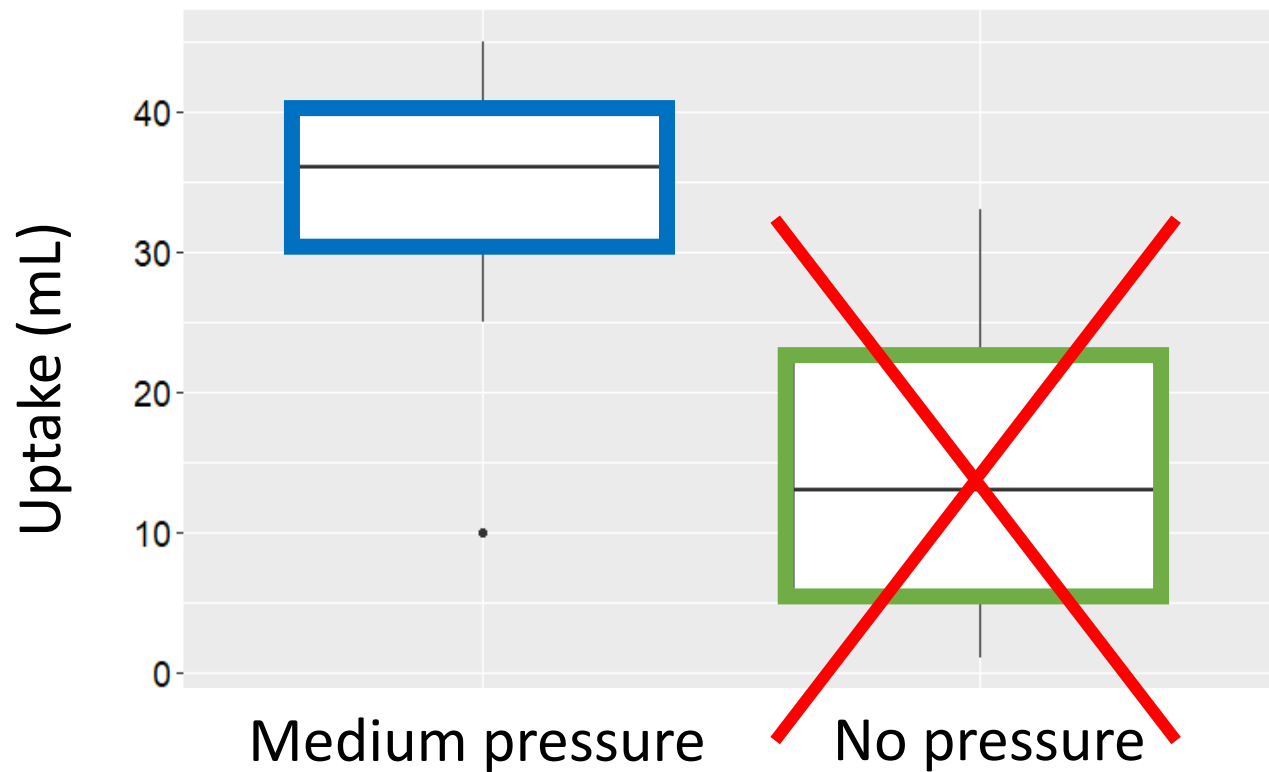


Low pressure



No pressure

Efficacy



High pressure vs. medium pressure

HIGH PRESSURE INJECTION



Water



Oxytetracycline



Imidacloprid

MEDIUM PRESSURE INJECTION



Water



Oxytetracycline

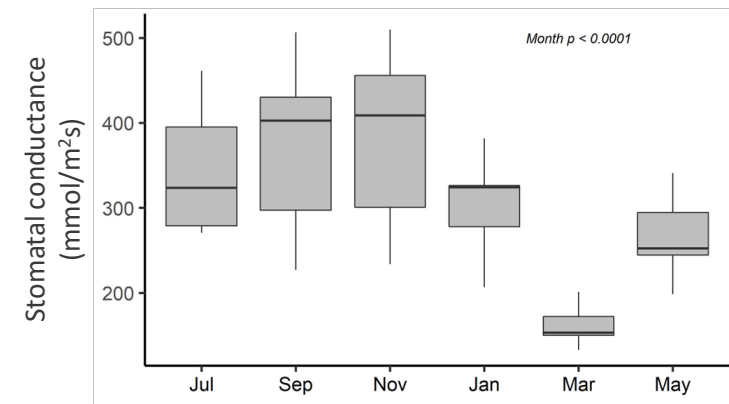
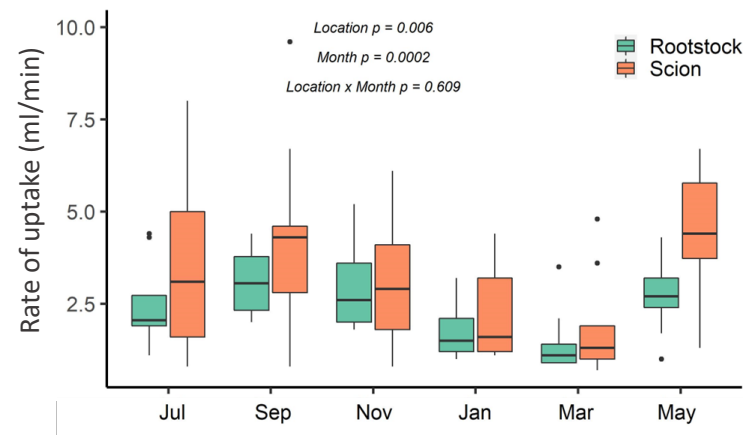


Imidacloprid

High pressure injection in combination with plugs causes greater injury

Other results

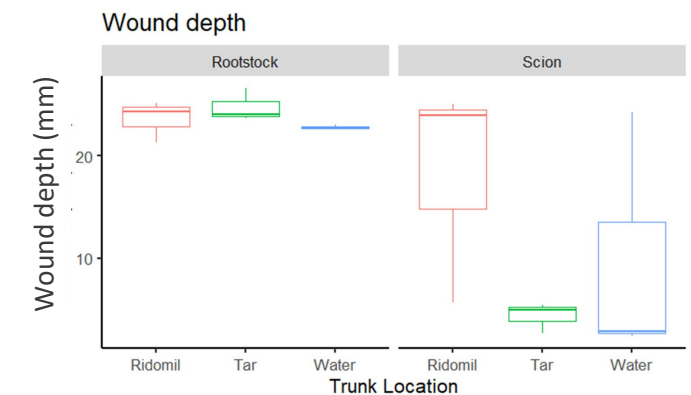
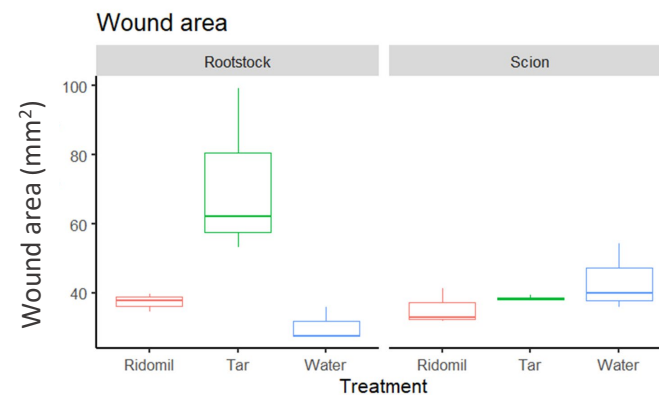
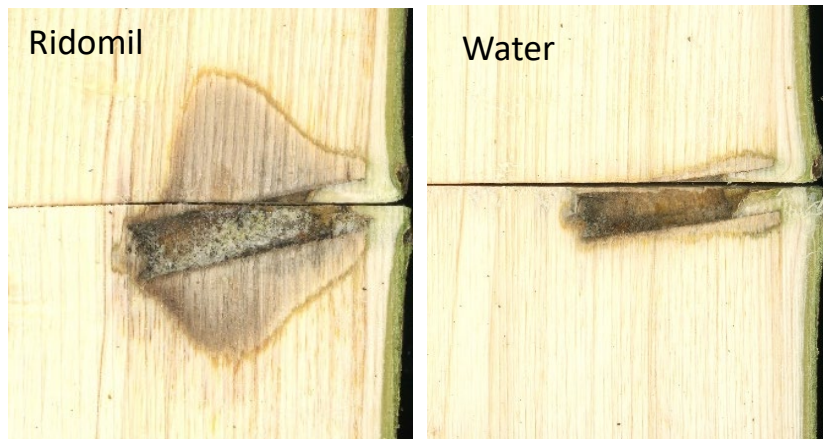
- The speed of uptake and distribution depends on the transpiration rate of the tree (which depends on weather, season, time of day, etc., and the physiological state of the tree)
- The uptake and distribution of injected compounds may vary between rootstock and scion



Other results



- It is better to leave wounds alone as post-injection treatments may interfere with wound healing
- Wound closure is faster when injections occur in the spring than in the fall
- Wound closure efficiency may differ in rootstock and scion



✓ Background Information

✓ Preliminary Research

✓ **Field Trials**

• Tree Injury



Valencia trial



Valencia/Kuharske
(5-year-old)

Injections performed in **Oct 2020** and **April 2021**:

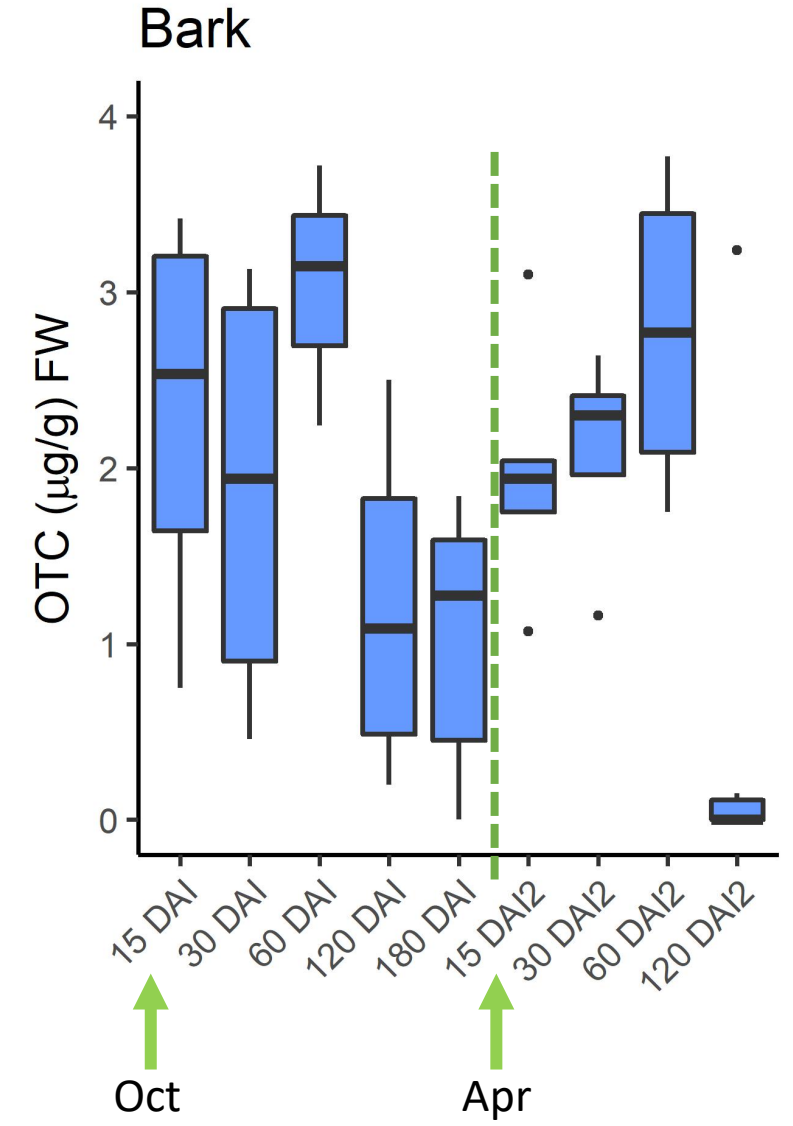
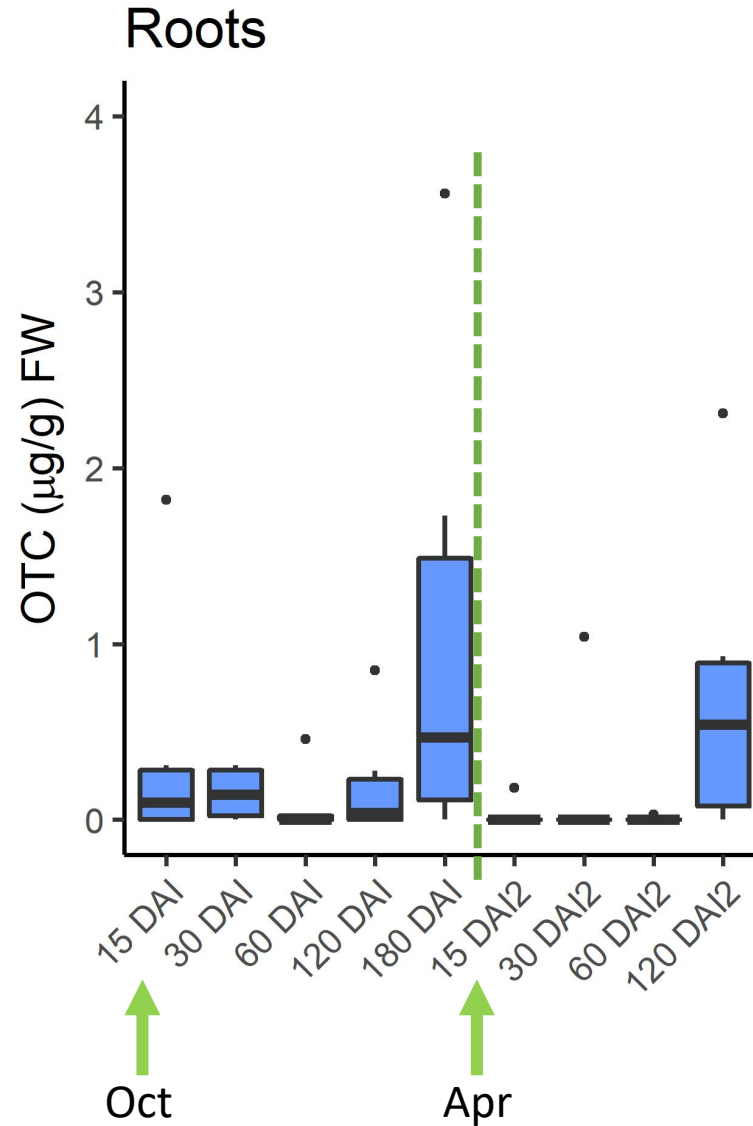
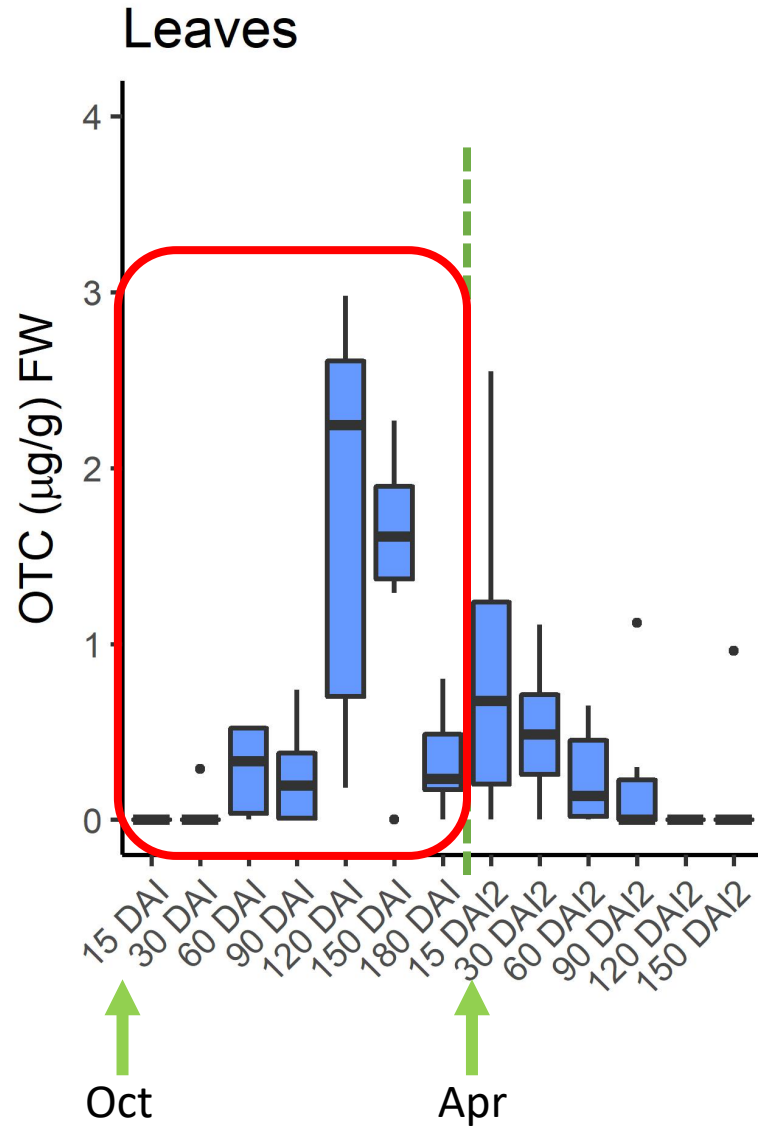
1. Oxytetracycline (OTC)
 - ✓ Arbor-OTC (Arborjet Inc.) @ 0.79g a.i./tree
2. Imidacloprid (IMI)
 - ✓ Xytect (Rainbow Ecoscience) @ 0.4 g a.i./tree
3. Water injection
4. No Injection

Injections were performed into the scion using 2 chemjets (1 each on opposite sides). Each chemjet holds a volume of 20 ml. Trunk diameters at injection site were approximately 2.5 ".



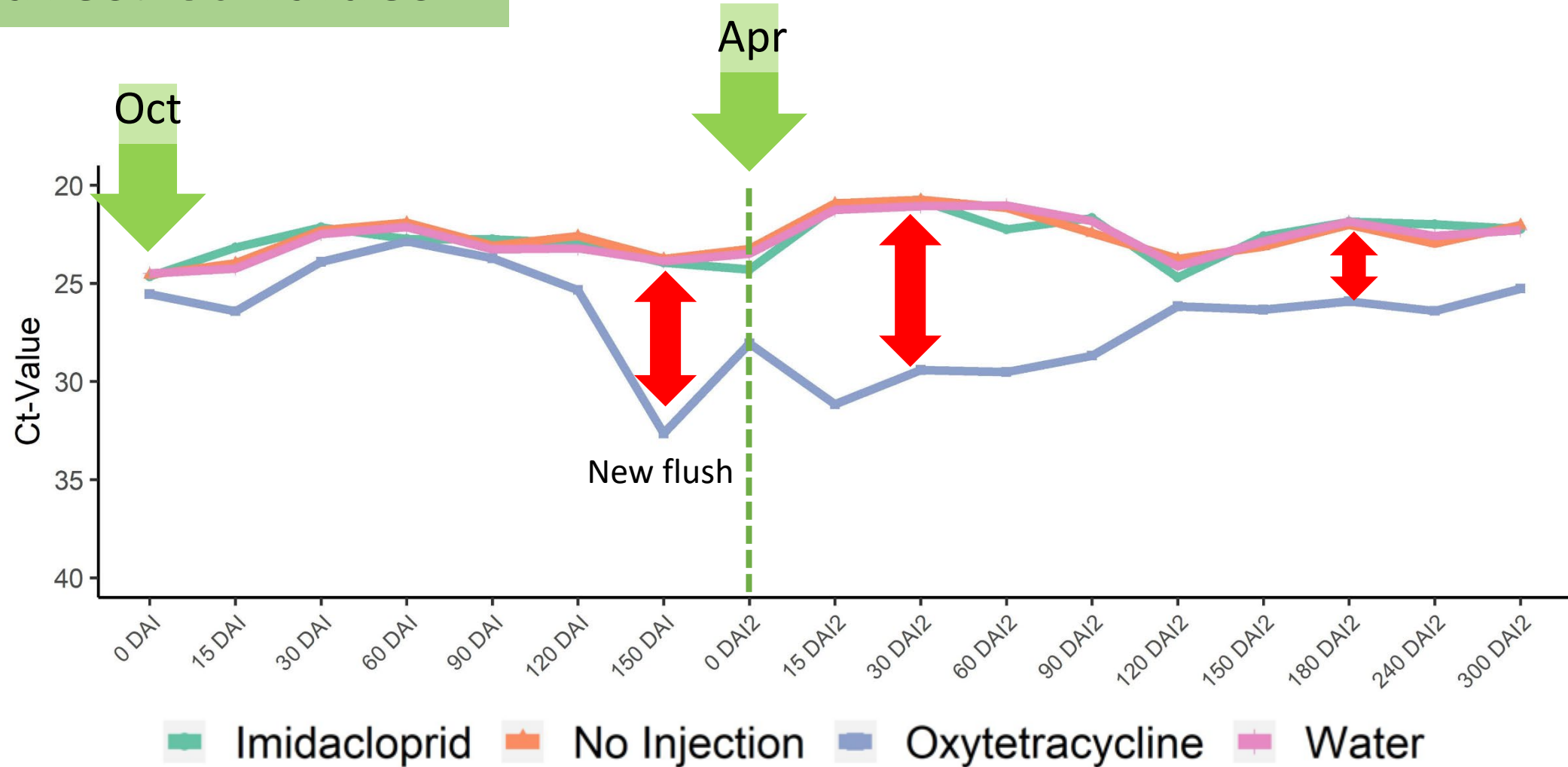


OTC Residues



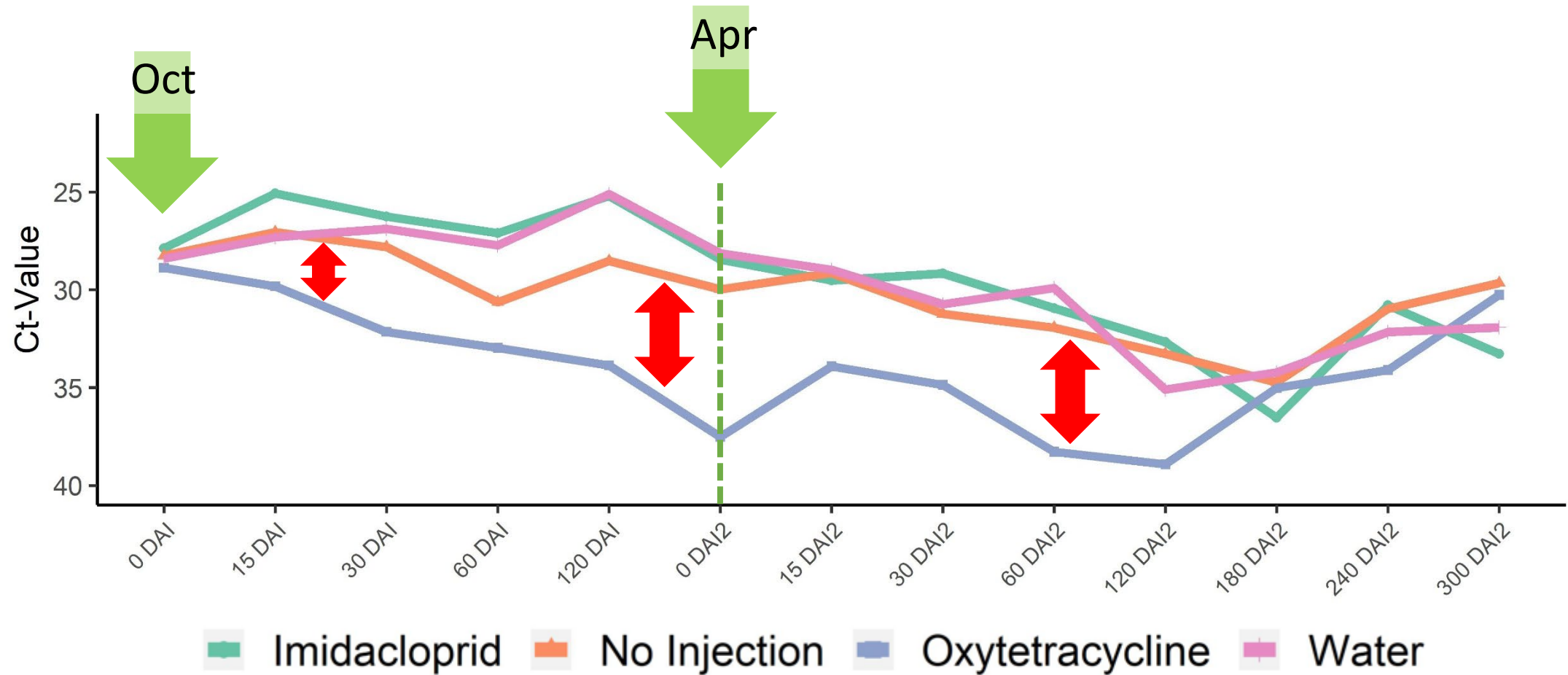
CLas titer

Leaves: Ct-values

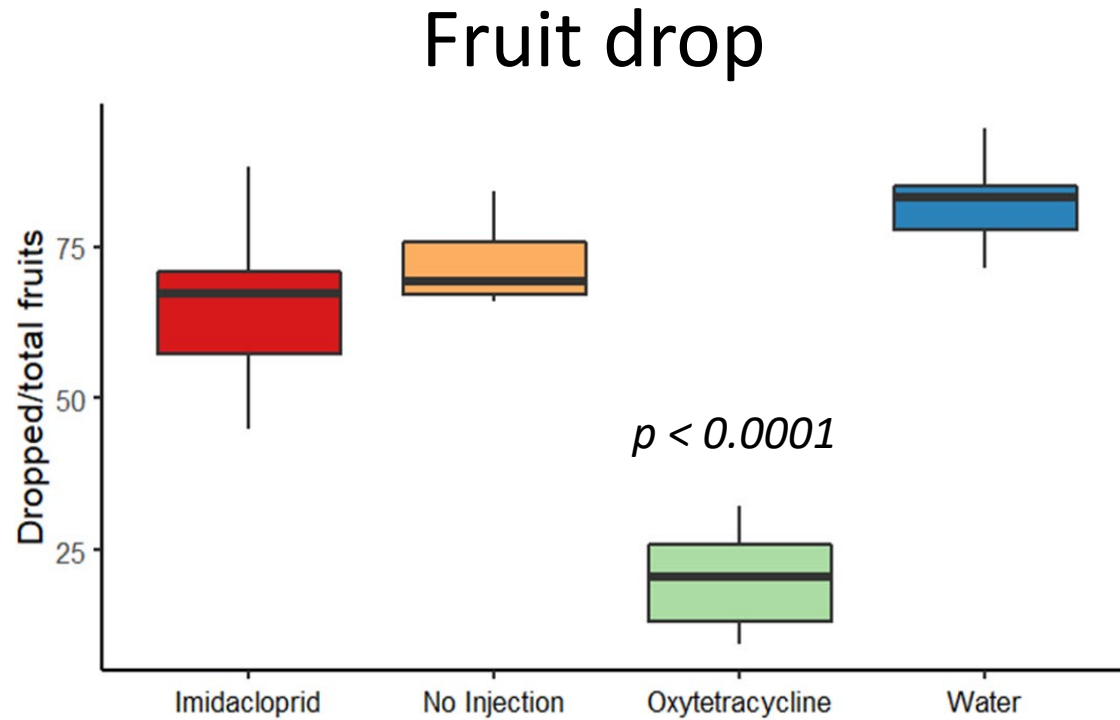


CLas titer

Roots: Ct-values



Fruit drop and yield

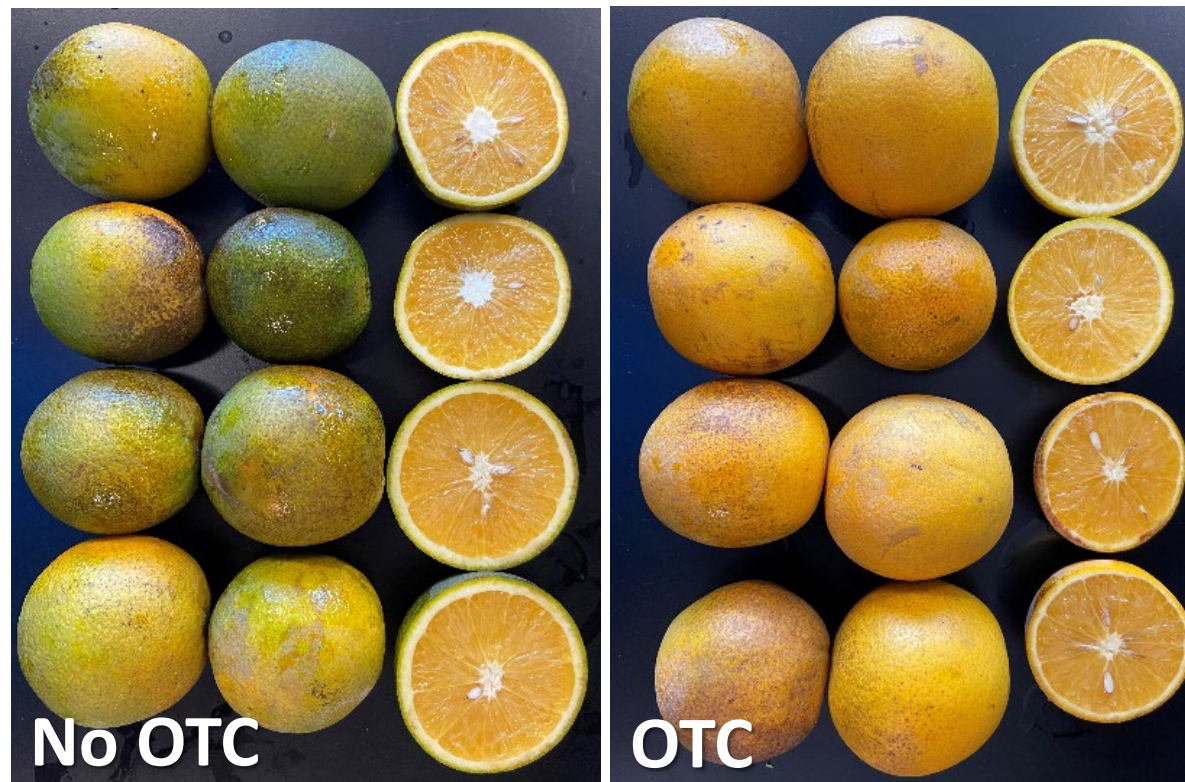
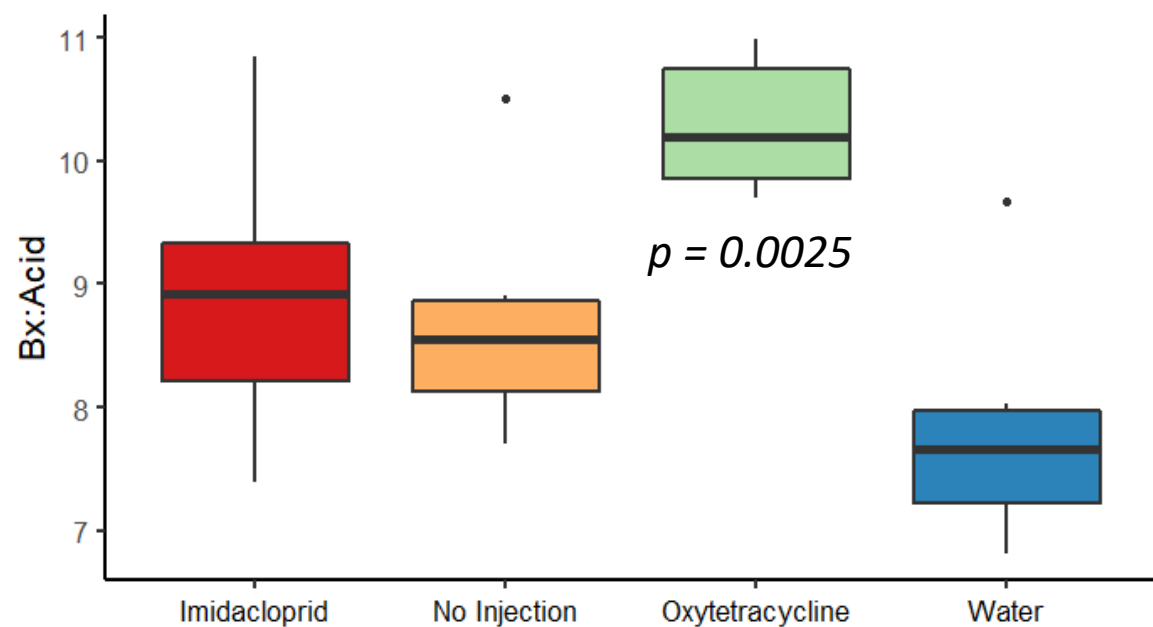


OTC reduced fruit drop and increased yield significantly



Fruit quality

Brix/acid ratio



OTC improved internal and external fruit quality significantly

Tree health

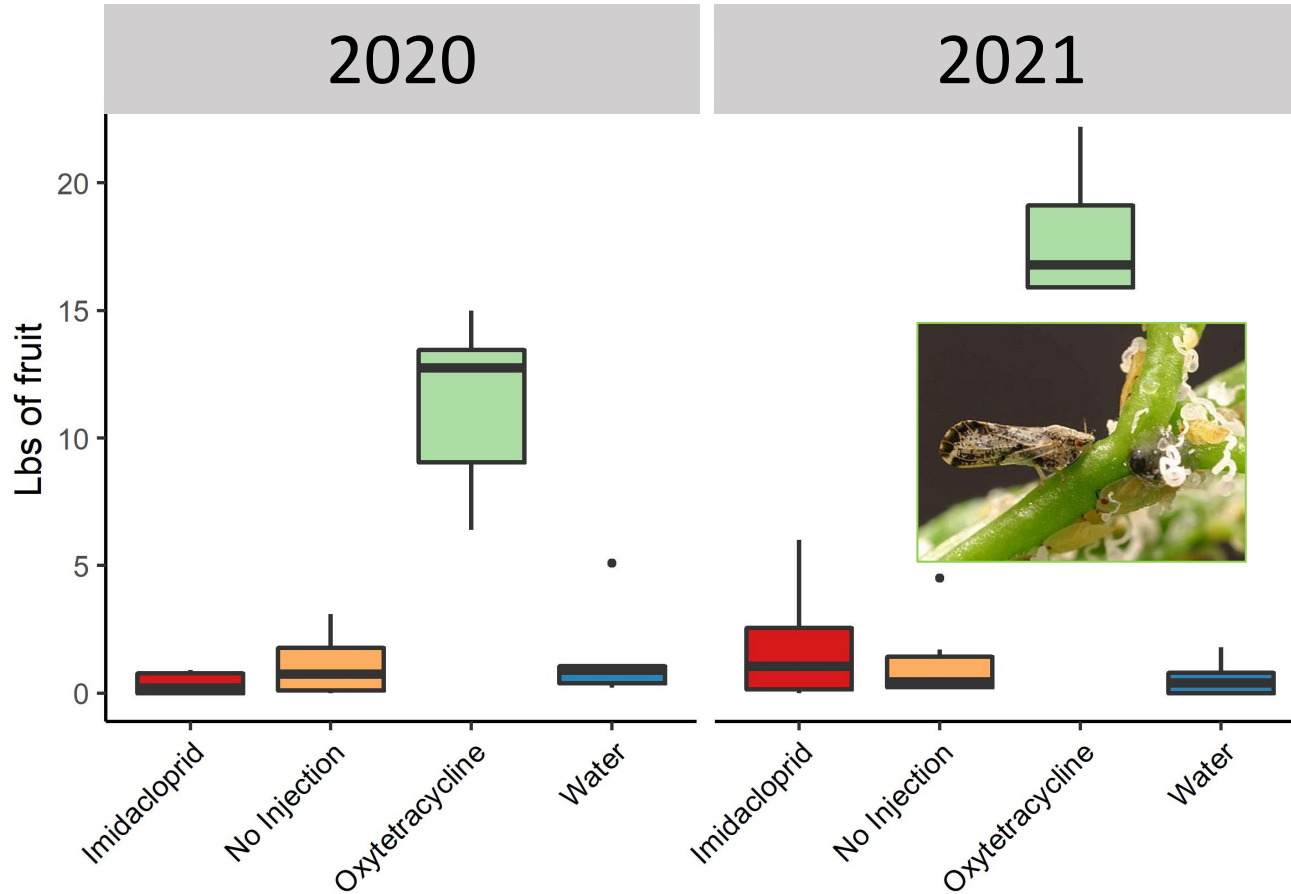
Oct 2021

Water

OTC



Midsweet study



Effect of OTC on fruit production remained in year 2 without any additional injection (but CLas titers increased again)

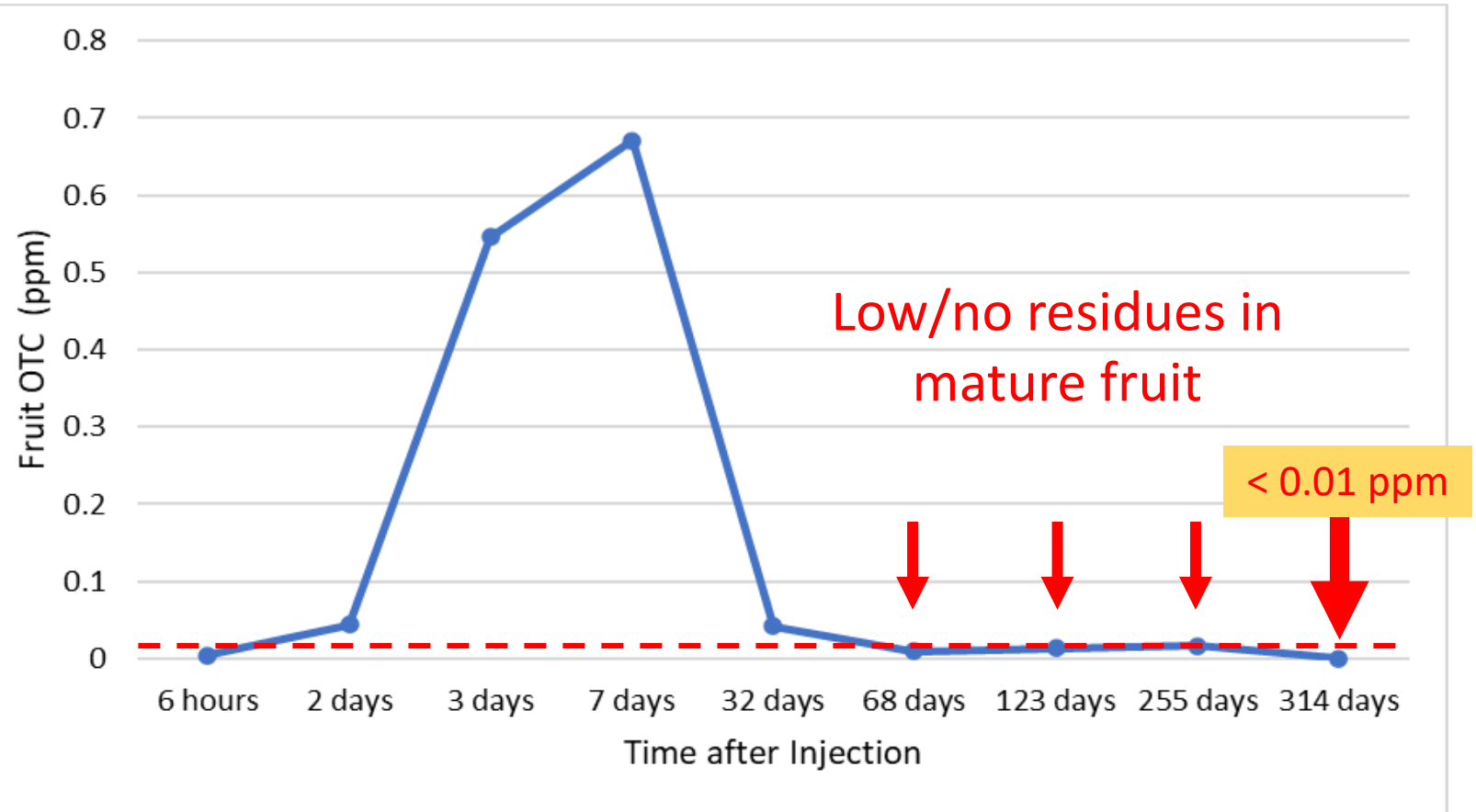
→ Injections may not need to be performed every year

Fruit size



Fruits are larger after spring injection than after fall injection

Fruit residues



OTC residues in fruit decreases dramatically within 30-60 days after injection

Flushing phenology

Stage 0: No Flush



Stage 1: Feather Flush



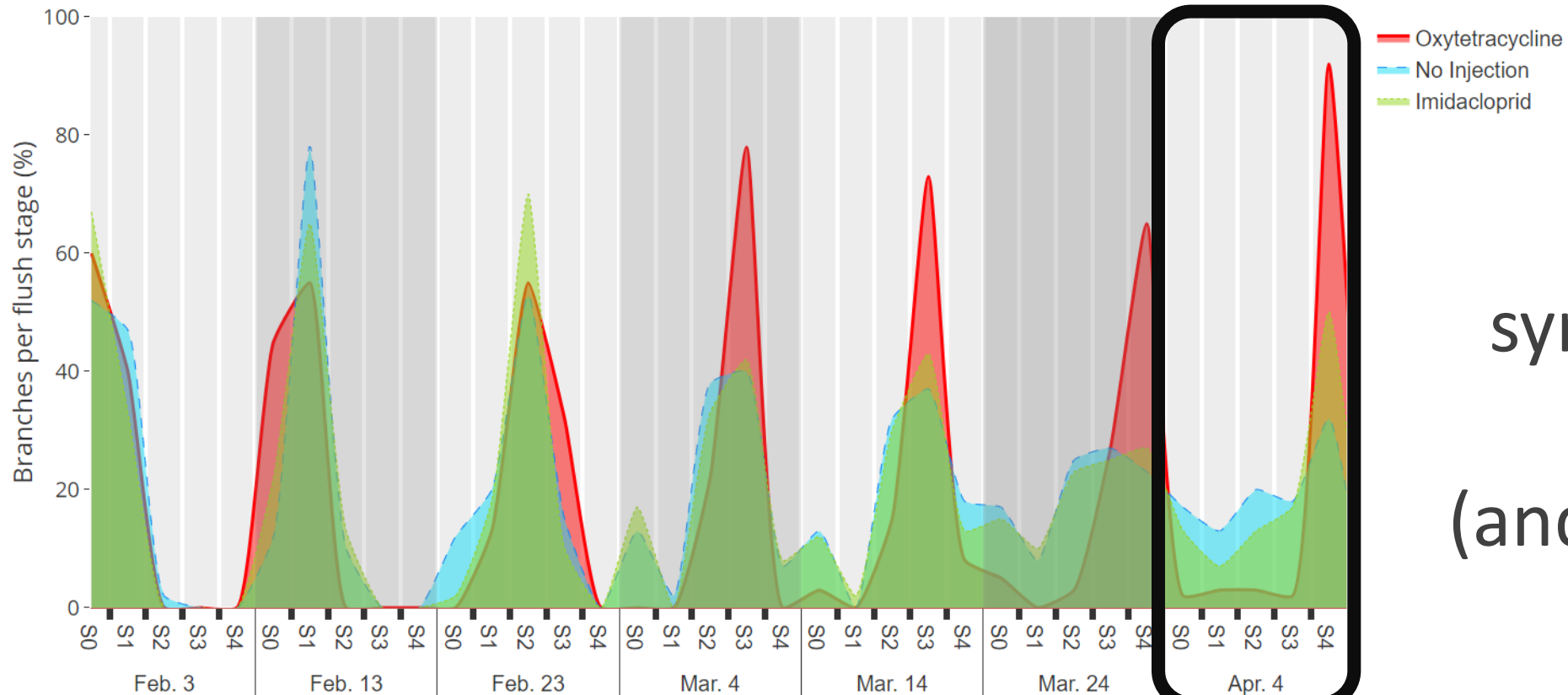
Stage 2: Flush Elongation



Stage 3: Leaf Expansion



Stage 4: Leaf Hardening



OTC
synchronizes
flushing
(and flowering)

Flushing phenology



Oxytetracycline

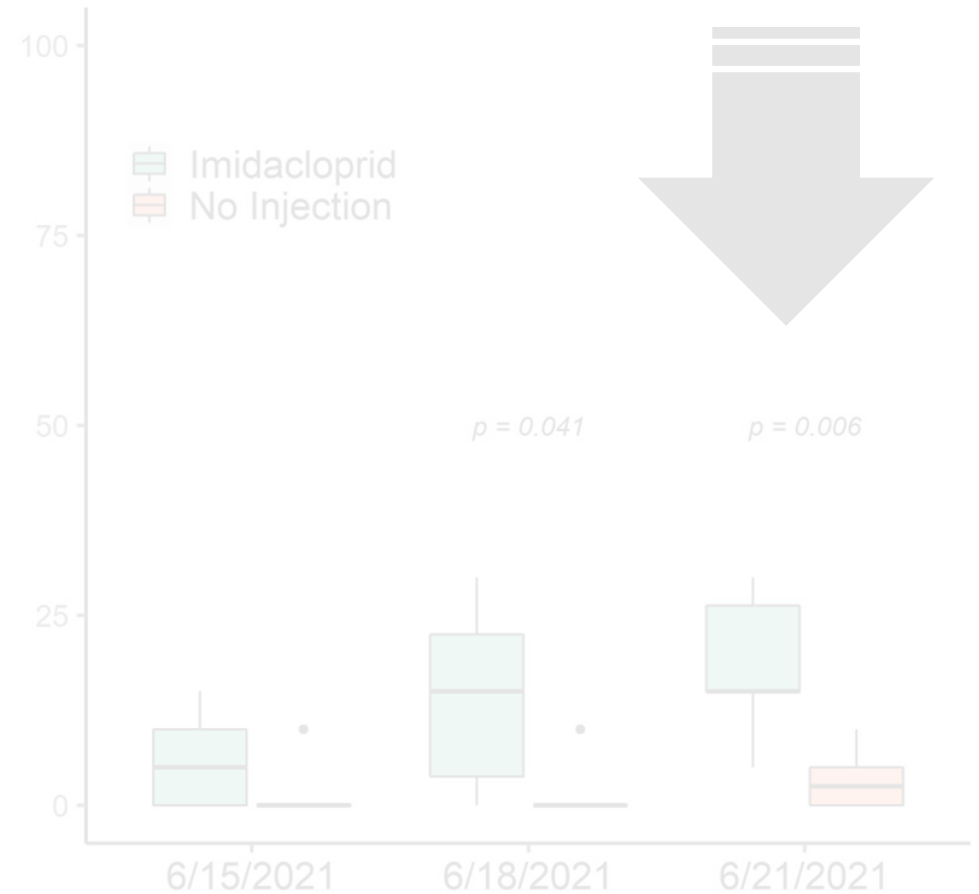
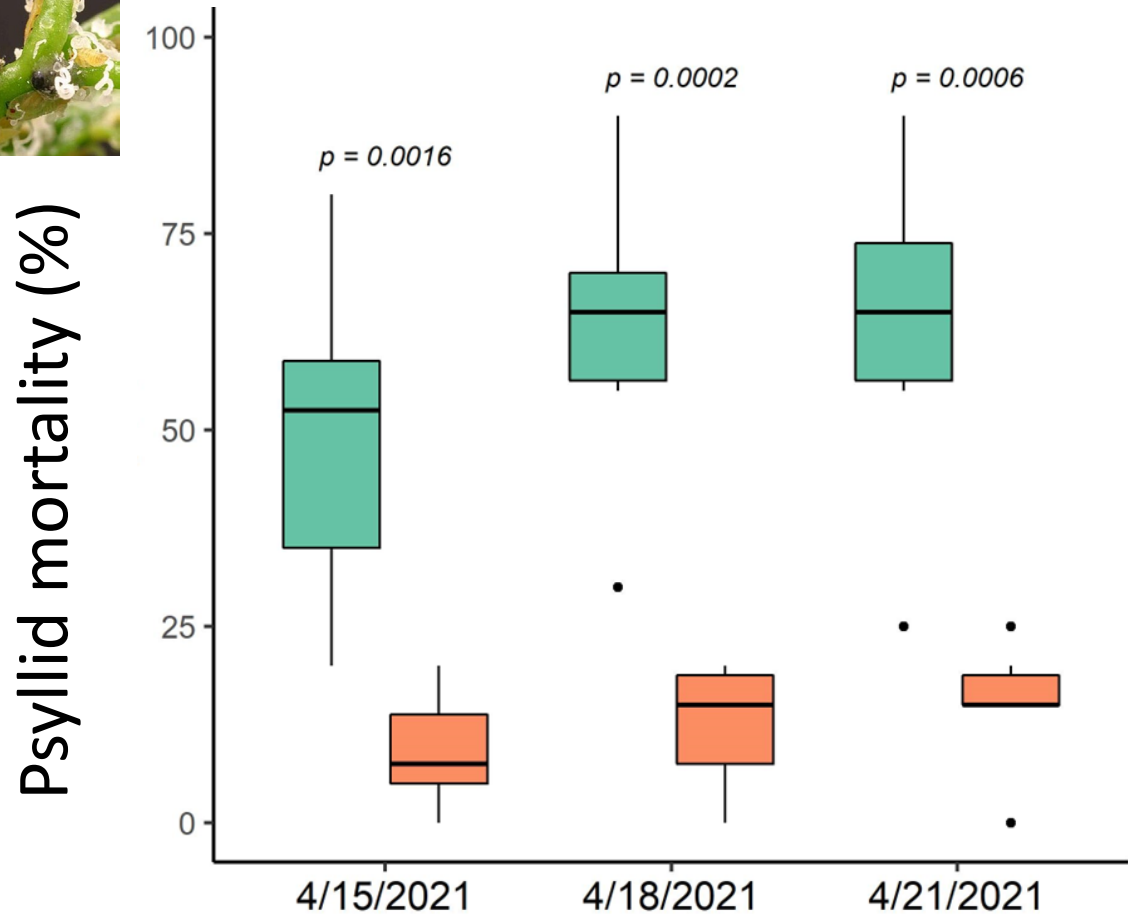


Water Control

Imidacloprid efficacy

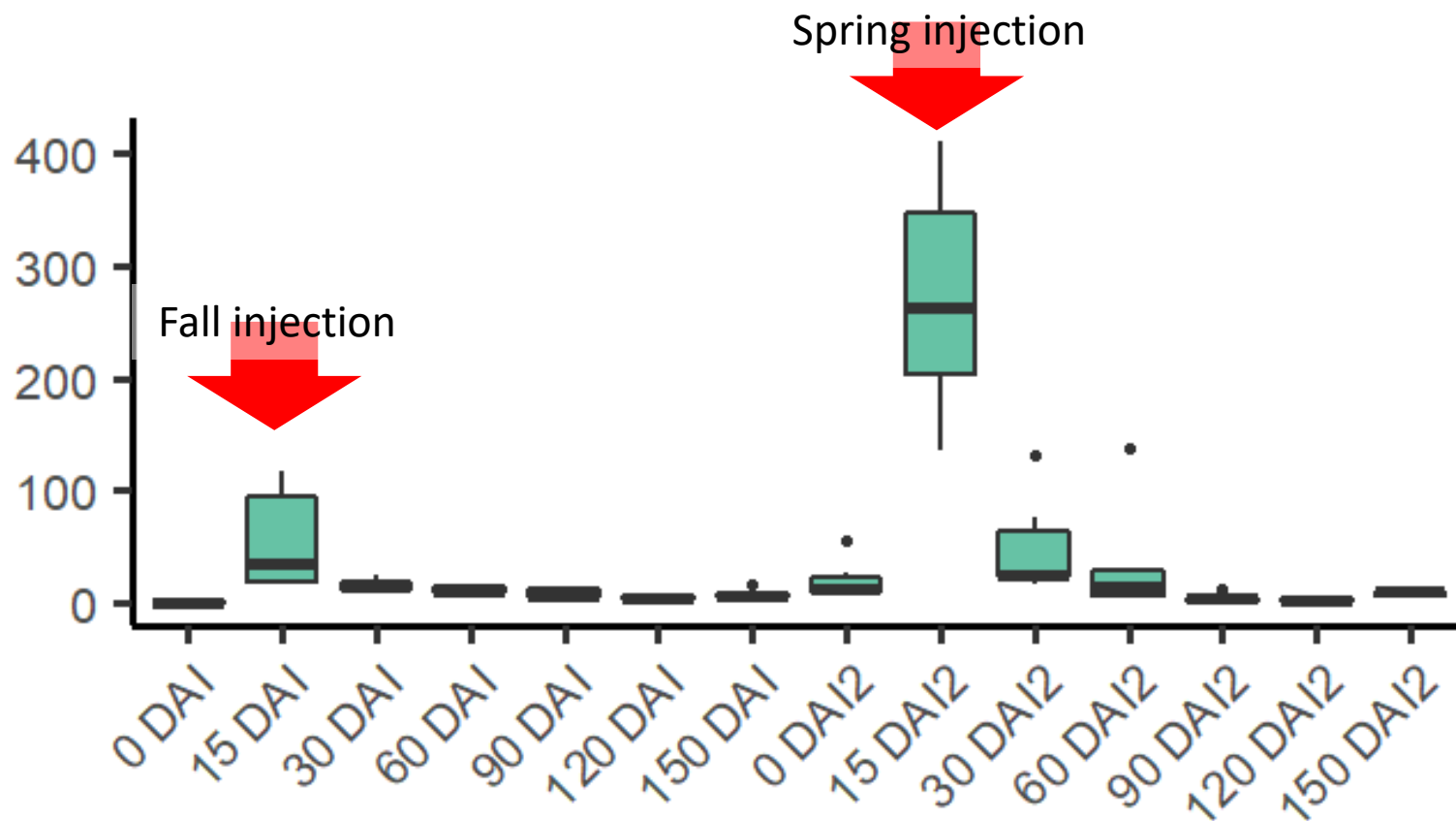


Imidacloprid efficacy



IMI significantly increased psyllid mortality 2 weeks after injection (4/5/2021),
but not after 2 months

Leaf residue levels



Higher leaf residues were found after spring injection. But residue levels were low within 1-2 months after injection

Other Trials

Trees

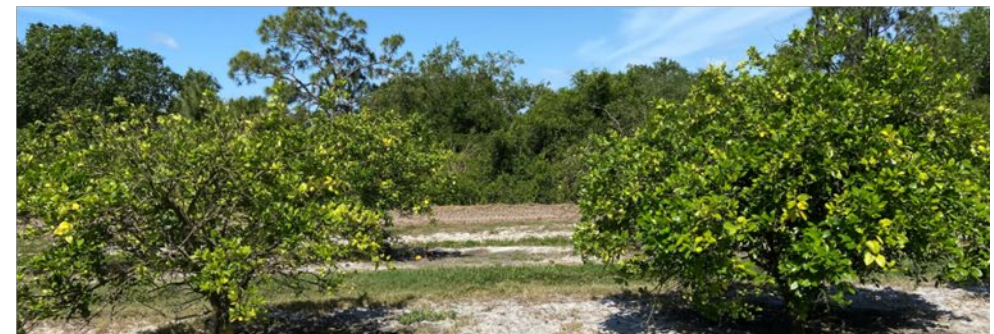
Mature Hamlin,
Valencia, and Duncan

Scion trunk diam. 4.0-5.5"

Treatments

OTC injected in April,
October, or both

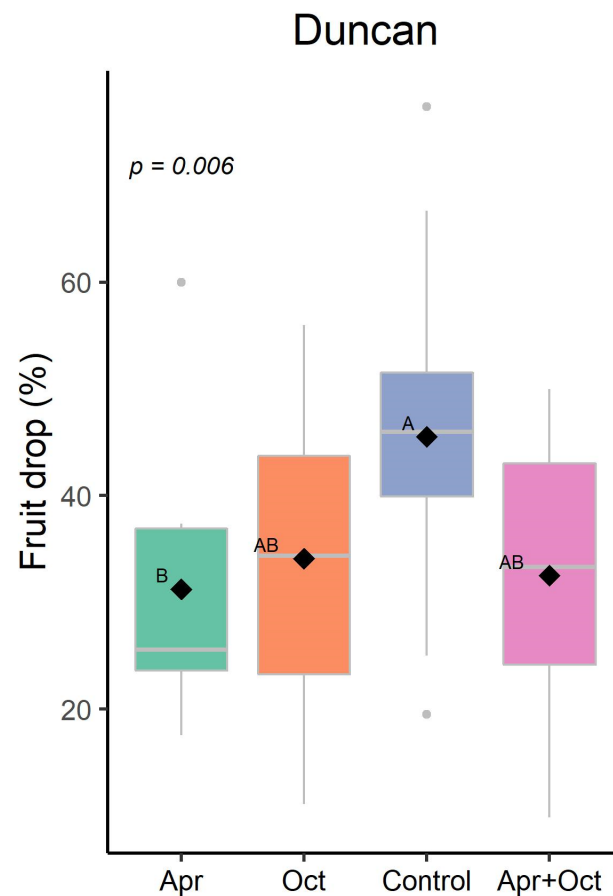
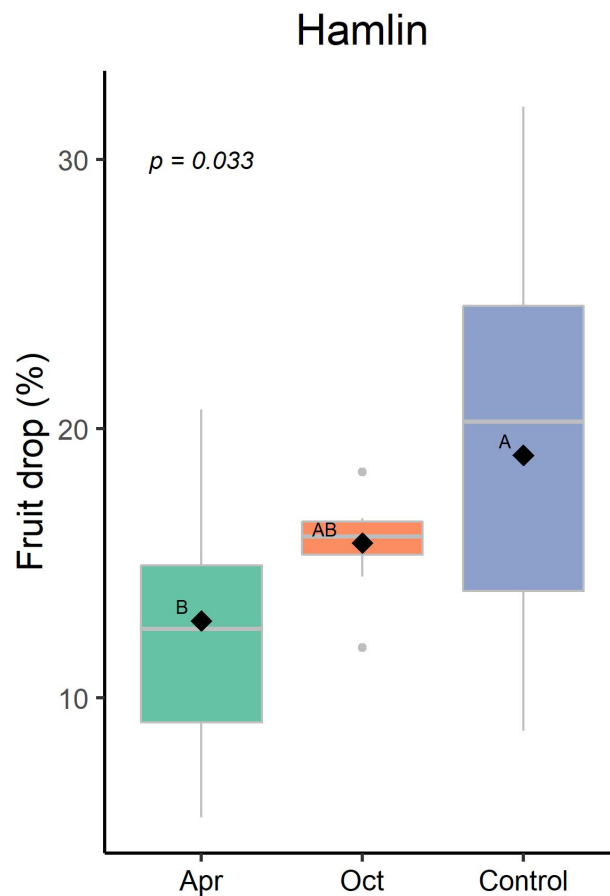
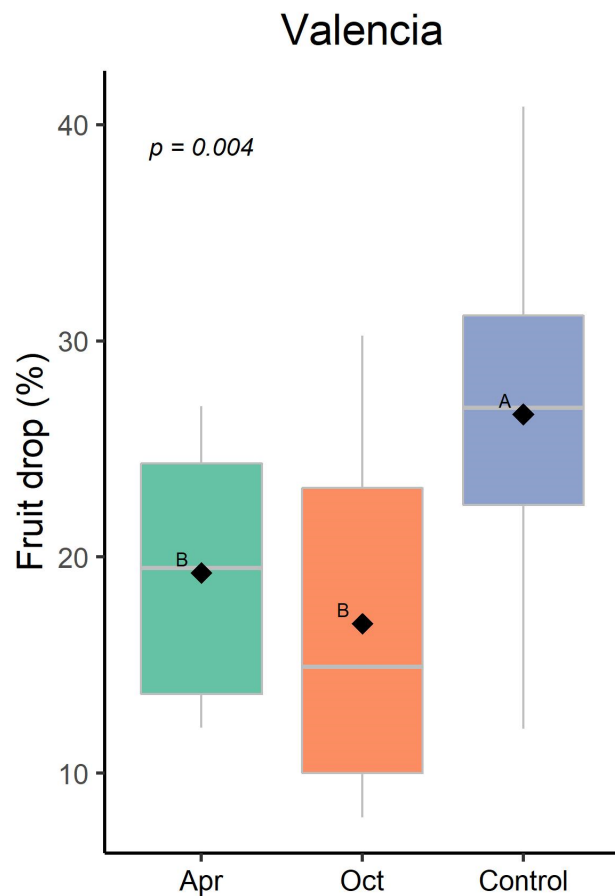
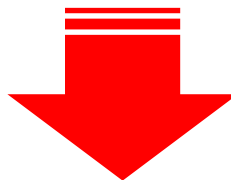
*The rate of OTC was the
same as for the smaller
trees (0.79g a.i. per tree)*





Other Trials

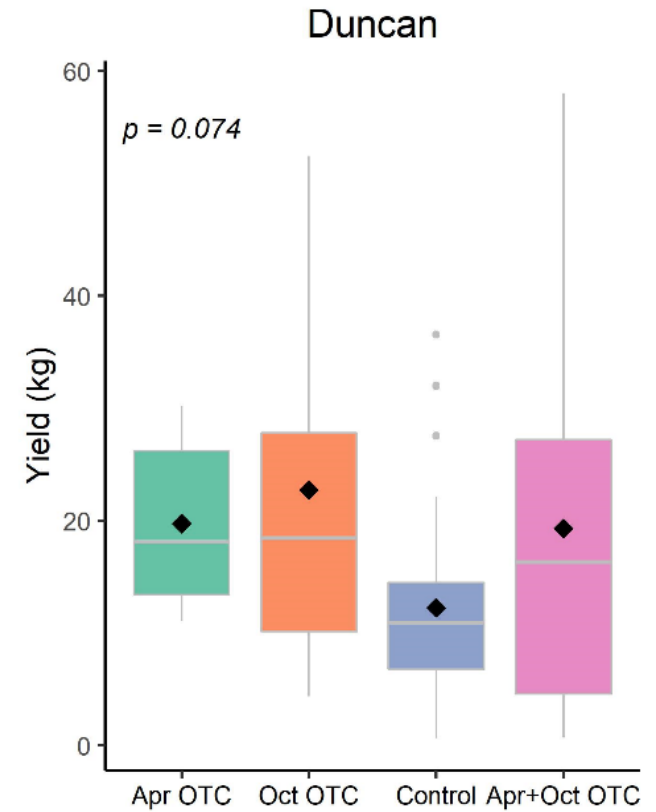
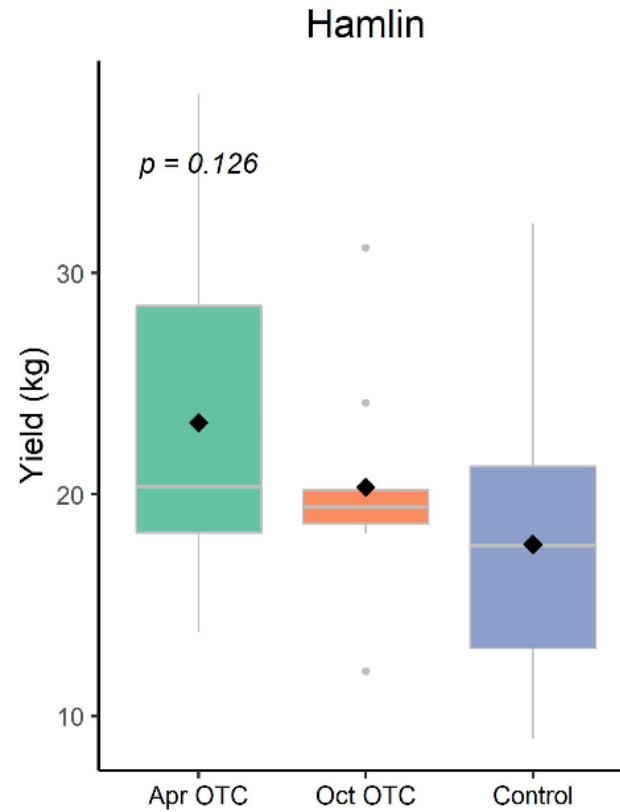
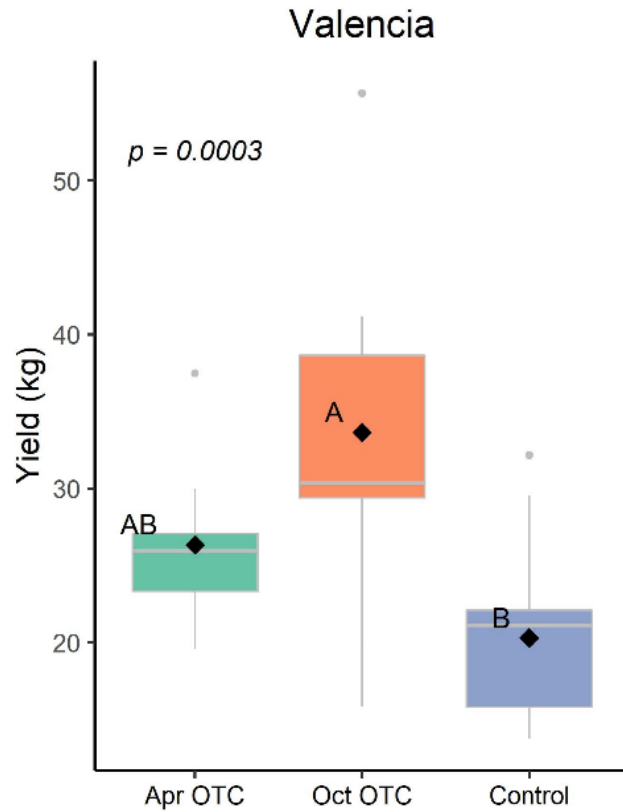
FRUIT DROP





Other Trials

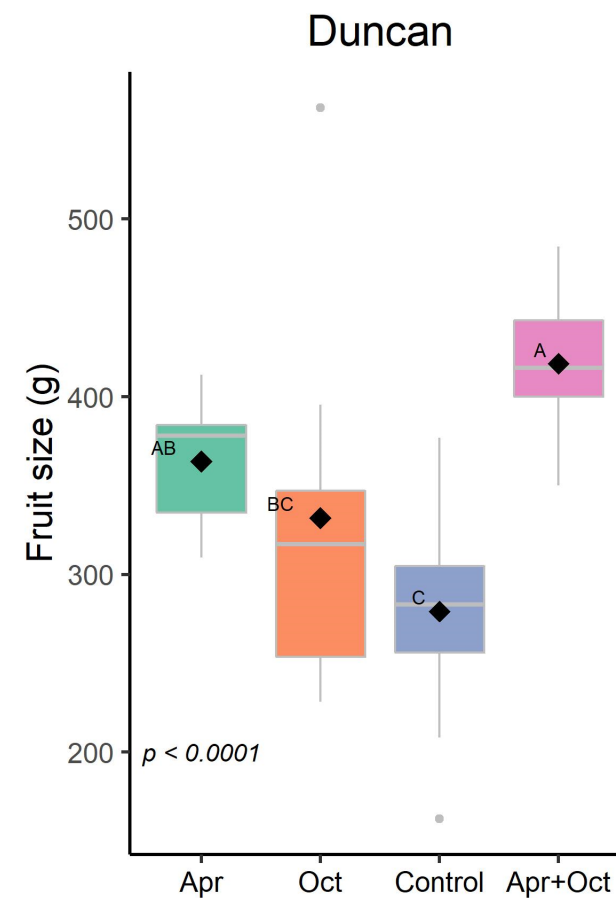
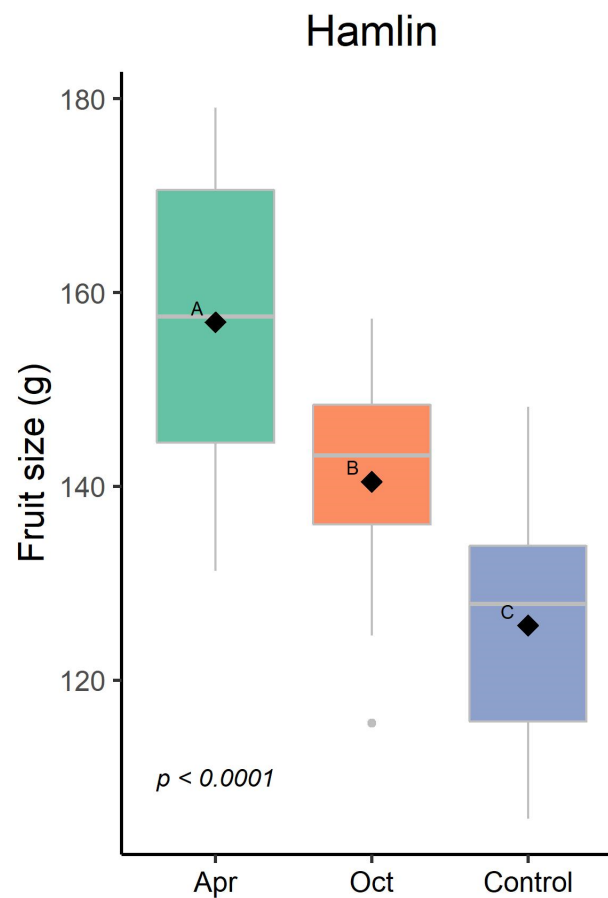
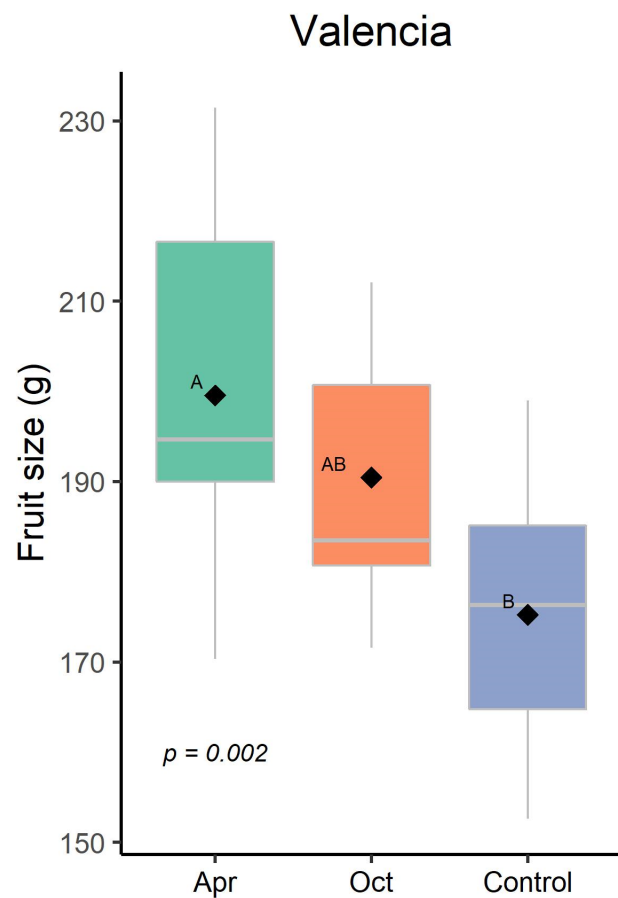
YIELD





Other Trials

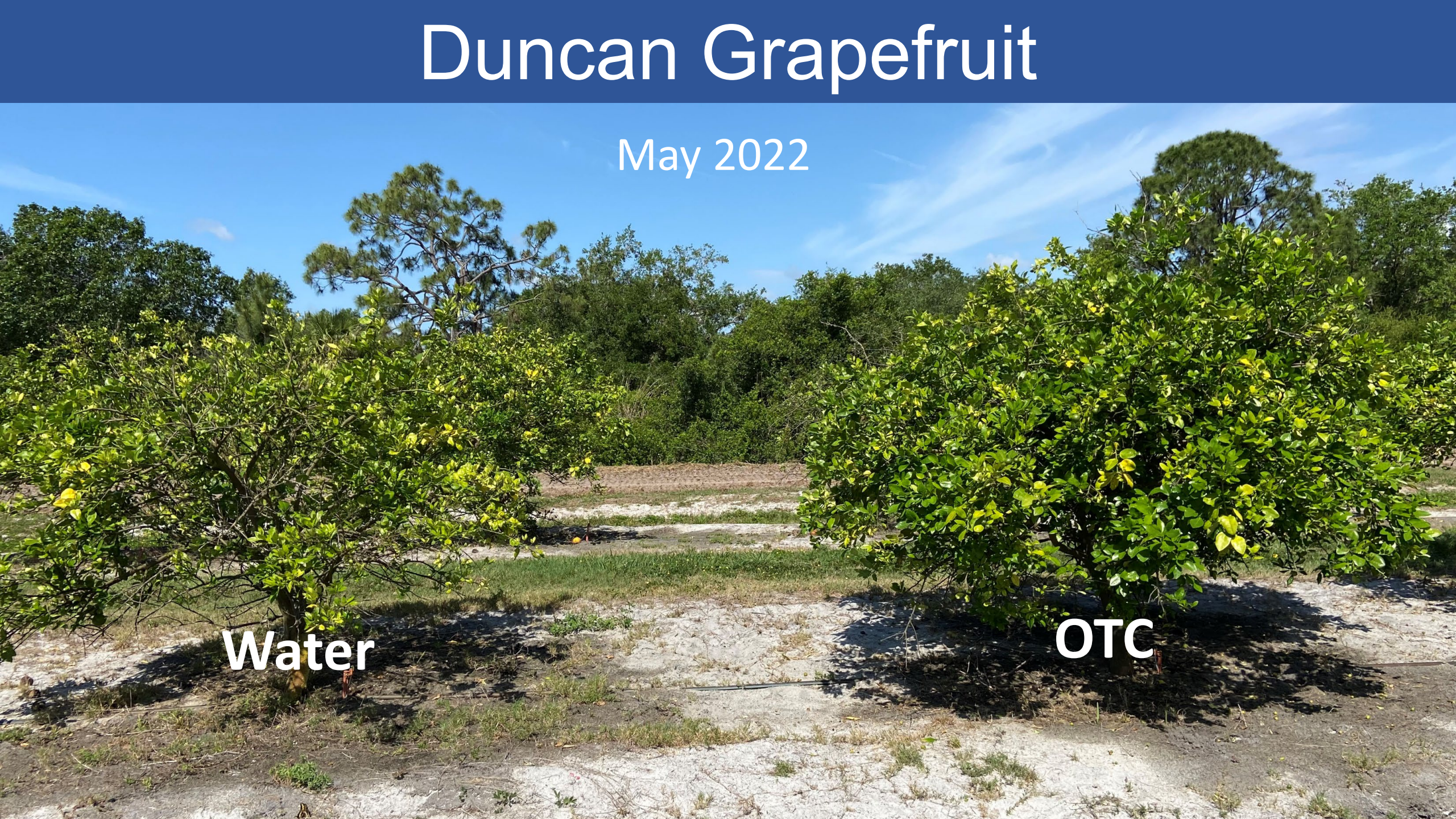
FRUIT SIZE



Duncan Grapefruit

May 2022

Water

A photograph of two Duncan Grapefruit trees in a field. The tree on the left is labeled 'Water' and the tree on the right is labeled 'OTC'. Both trees are green and have some yellow fruit. The ground is sandy and there are other trees in the background under a blue sky.

OTC

Ongoing Trials

1. Valencia/Carrizo – 8?-yrs-old – SW Florida

- Different OTC rates
- Timing of injections

5.5"

2. Valencia/Kuharske – 8-yrs-old – SW Florida

- Different OTC rates
- Different volumes

5"

3. Valencia/SO – 9-yrs-old – East Coast

- Different OTC rates
- Different OTC formulations
- Different technologies/methodologies

4.5"

4. Valencia/X639 – 4-yrs-old – East Coast

- Different OTC rates
- Timing of injection
- Different technologies/methodologies
- Rootstock vs. scion

2.5 – 3"

5. OLL-8/X639 – 4-yrs-old – Central Ridge

- Same as trial 4

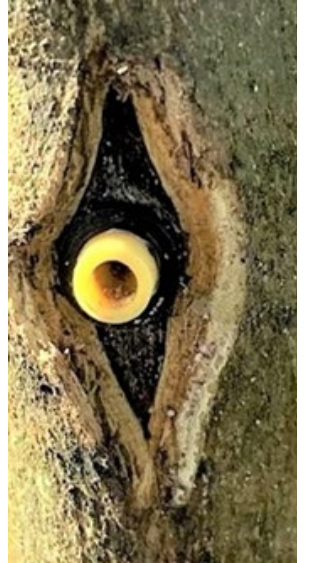


All crop will be destroyed

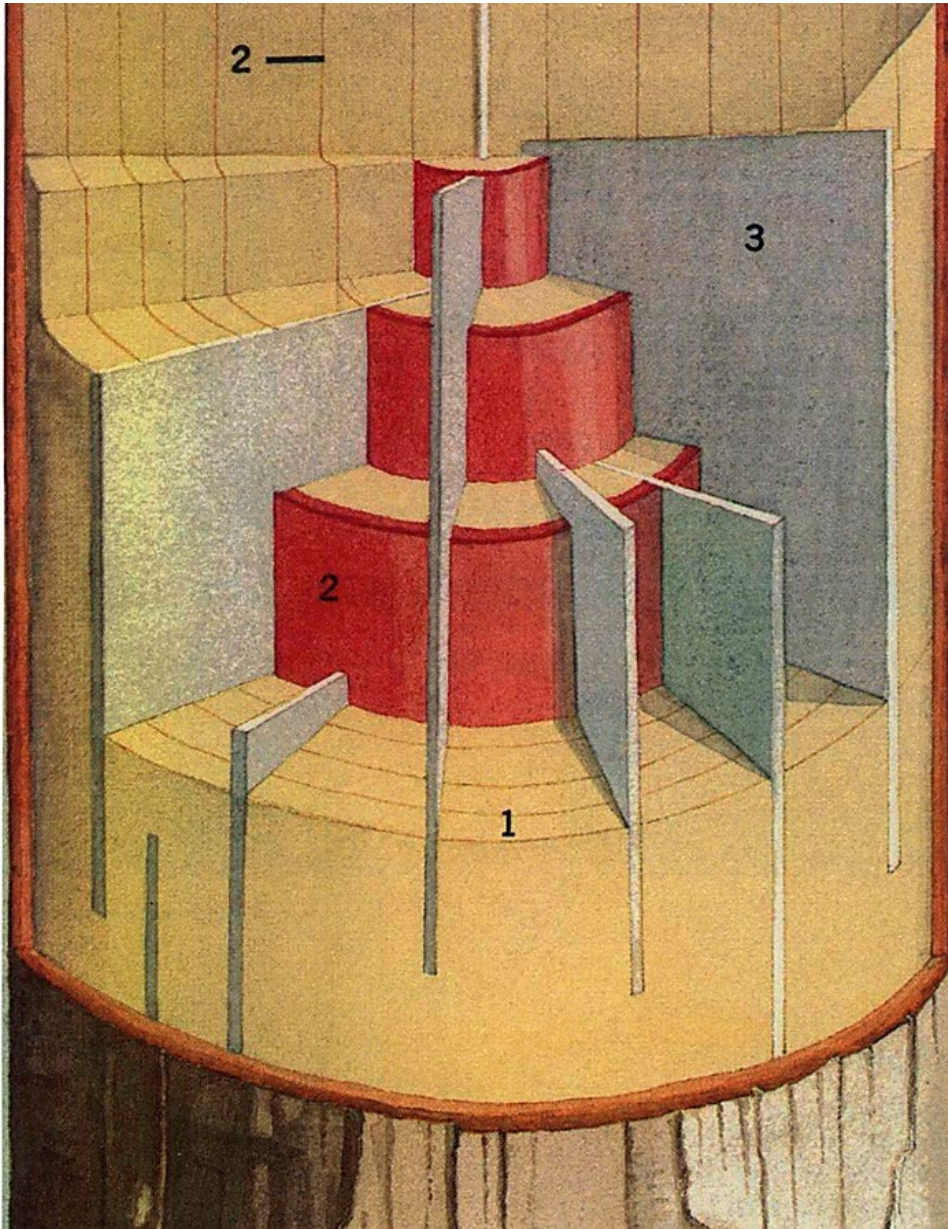
- ✓ Background Information
- ✓ Preliminary Research
- ✓ Field Trials
- ✓ **Tree Injury**



Tree injury



Wound compartmentalization



Compartmentalization of Decay in Trees (CODIT)

Shigo and Marx (1977)

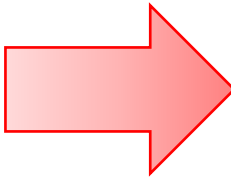
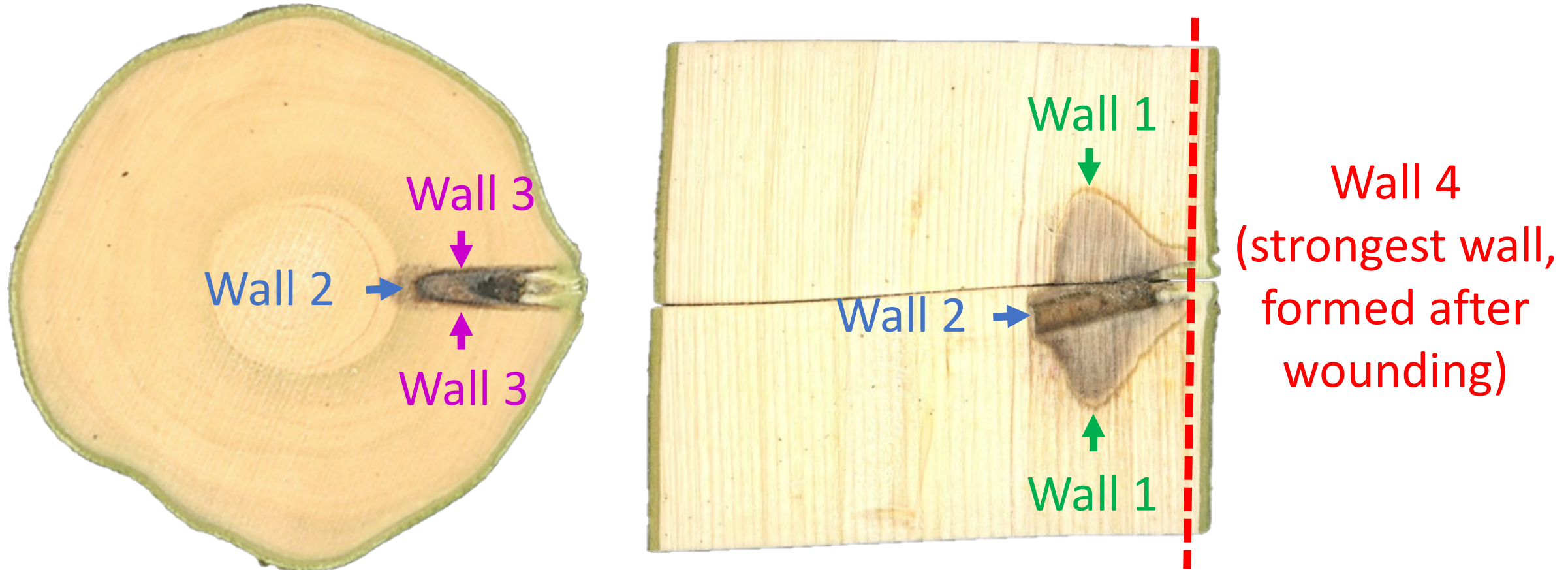
Wall 1 (weak)  Wall 3 (strong)

Fig. 64: The CODIT Model depicts the tree as a chambered organism in which there are structural walls that can react to decay by compartmentalization. Wall 1 occurs in the axial direction. Wall 2 provides barriers in the radial direction (toward the center of the trunk) and wall 3 in the tangential direction (to the sides). In the model, wall 1 represents the weakest compartment and wall 3 is the strongest (Shigo & Marx 1977).

Wound compartmentalization



Effective wound compartmentalization in citrus trunks
after water injection

Wound compartmentalization

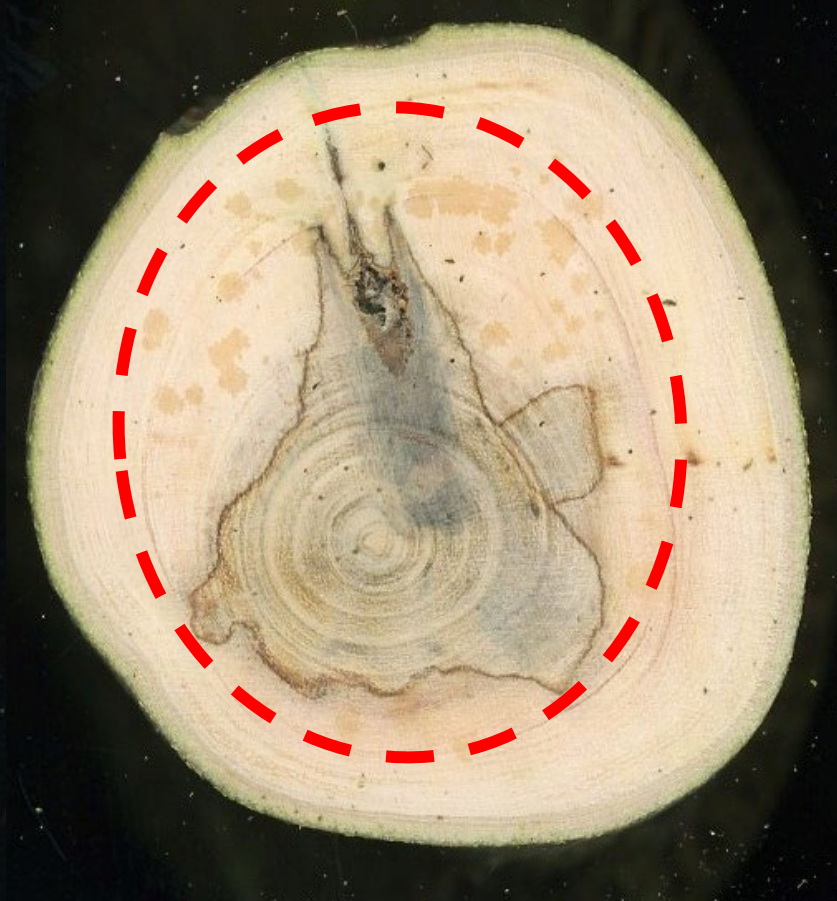
Water



Imidacloprid

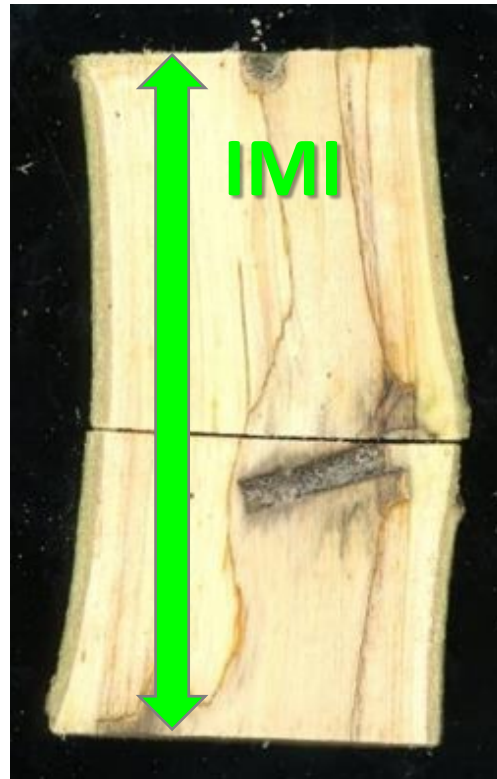
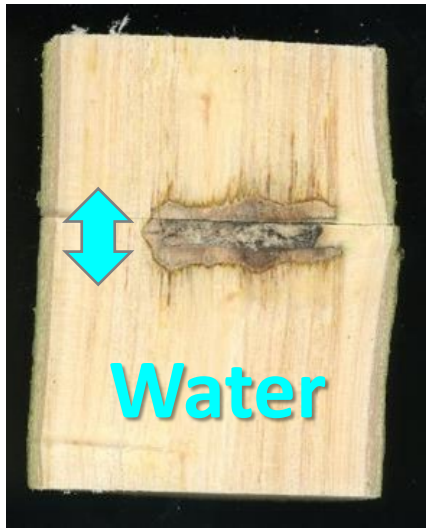


Oxytetracycline



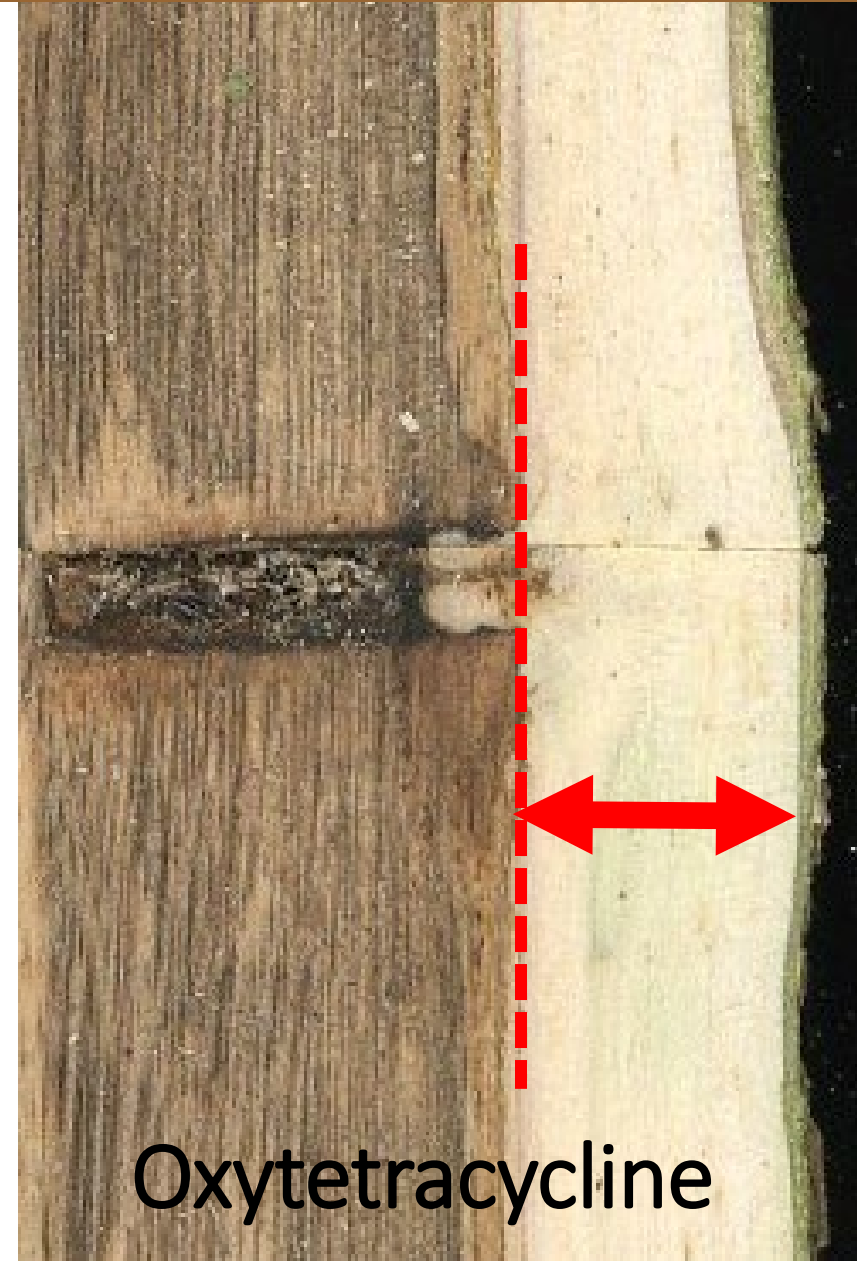
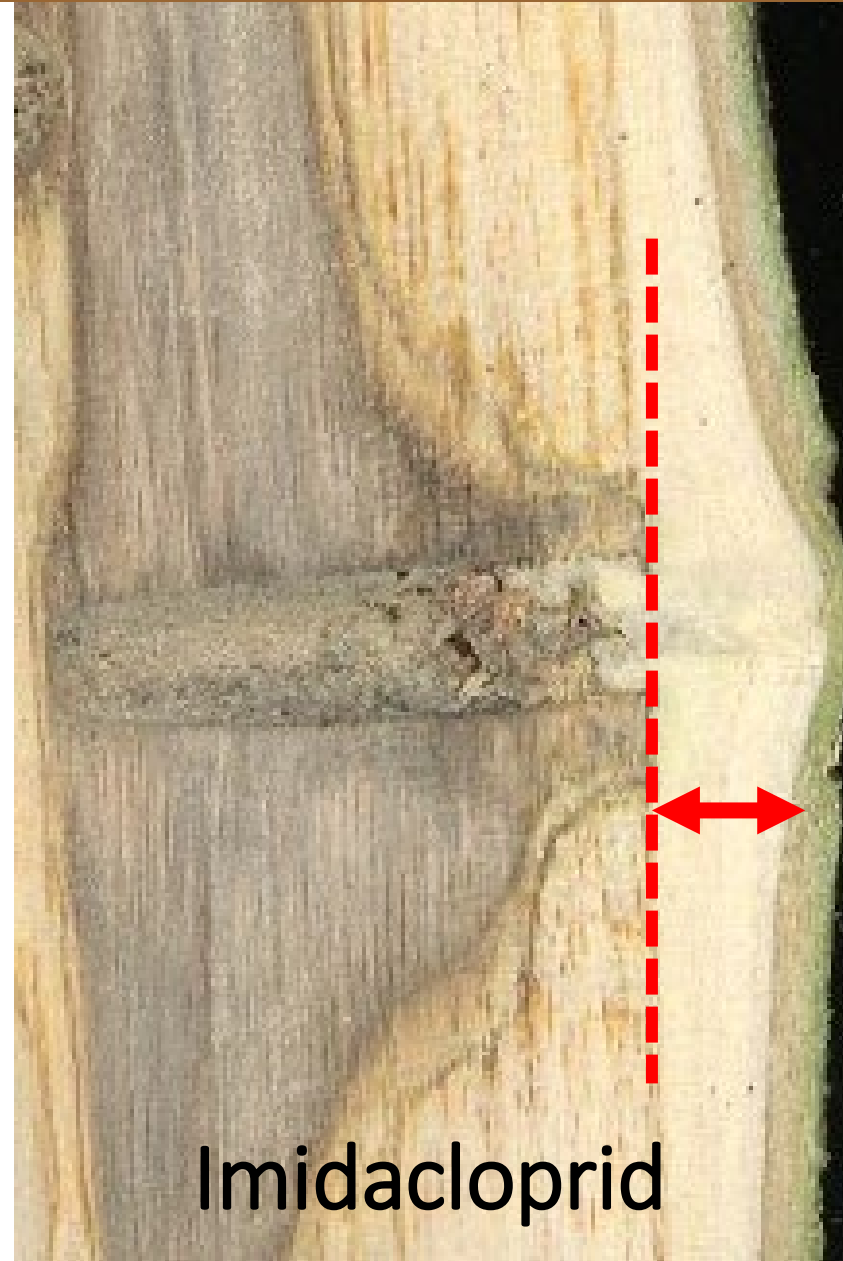
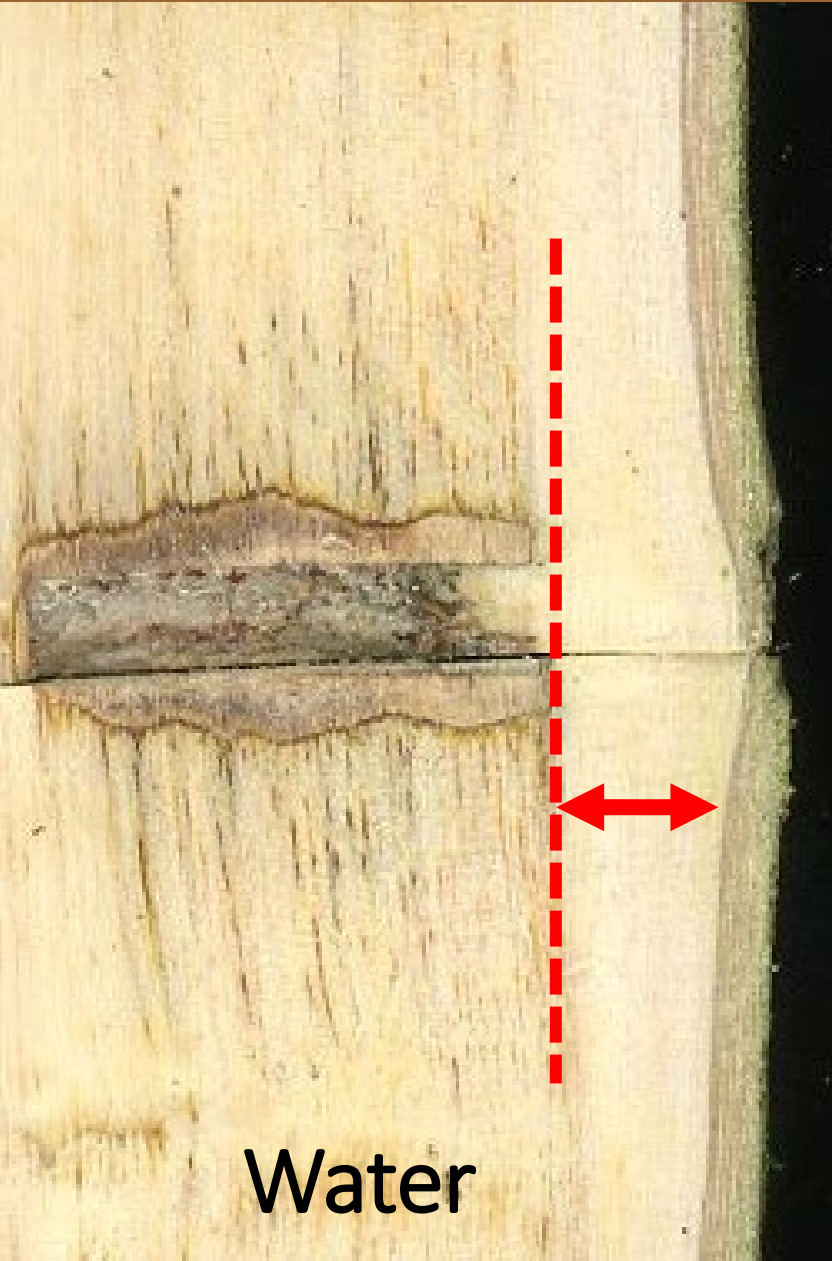
Compartmentalization is less effective after injection of chemicals

Wound compartmentalization



..., especially in the axial direction

Wound compartmentalization



Summary

- Trunk injection can effectively and systemically deliver crop protection materials to target pests and diseases
- Injected OTC appears to move to the phloem, reduces CLas titers, and improves tree health, fruit quality, and yield
- Imidacloprid did not show long-term efficacy
- Trunk injections cause injury and long-term effects need to be evaluated
- USDA-NIFA-SCRI #2021-70029-36056 → new chemistries

Further information

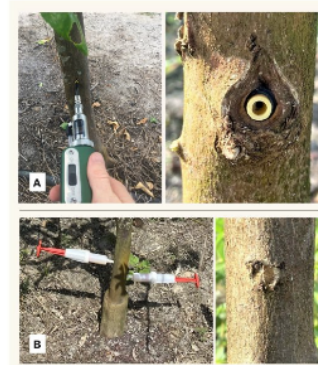
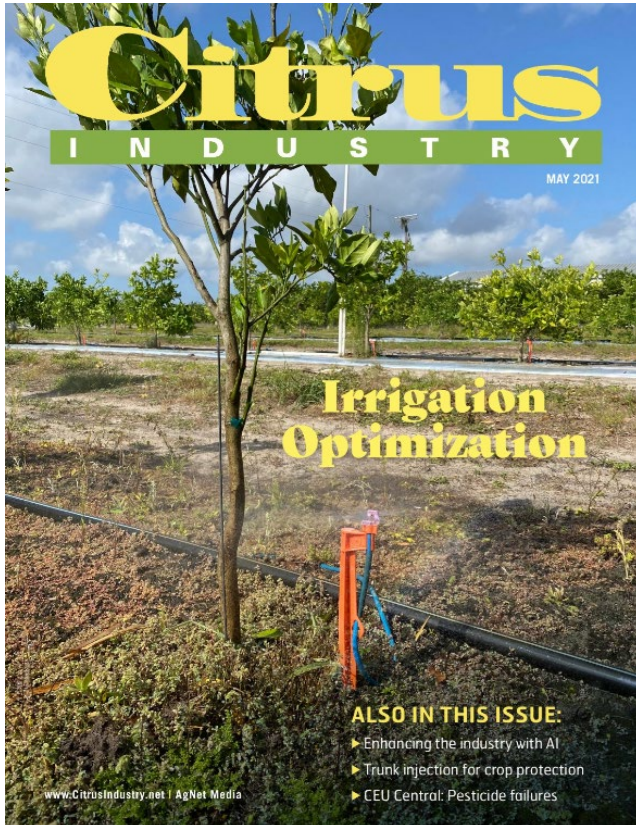


Figure 1. High-pressure injection (A) requires the use of a plastic plug, which causes more damage at the injection site than medium-pressure injection (B).

Principles and risks of trunk injection for delivery of crop protection materials

By Ute Albrecht and Leigh Archer

Trunk injection is a targeted delivery of materials into the stem or trunk of trees as an alternative to spraying or soil drenching. It is practical for disease and pest management in high-value forest trees and ornamental plants where aerial applications are problematic because of environmental and human health-related concerns. Interest in using the injection technique to protect agricultural crops has emerged more recently in areas where foliar applications and soil drenches have proven ineffective or pose environmental hazards.

"Injection" is defined as the act or process of forcing a liquid medicine or drug into someone or something,

usually by using a special needle. In botany, this term is used in a wider sense and applies to introducing any materials into a plant organ through cuts or holes with or without force.

The earliest evidence of plant injection is from the 12th century when Arabic horticulturists applied perfumes, spices, dyes and other substances through wounds to affect the smell, color or other qualities of flowers and fruits. Modern research on the use of trunk injection to deliver protection materials was incited by the devastating Dutch elm disease (a vascular fungal disease) wreaked in Europe and North America during the 1900s. This method is still used predominantly for forest trees and

ornamental plants, but also to treat diseases in some fruit tree crops.

TRUNK INJECTION METHODS

Different devices are available for delivering liquid materials into tree trunks. Many of them require drilling a relatively large hole, followed by injecting the desired material using pressures up to 100 pound-force per square inch or more. High-pressure injection usually requires inserting a plastic plug into the drill hole and is therefore only suitable for large-size trees.

Other devices require less pressure or no drilling and are less damaging and more suitable for smaller trees (Figure 1). University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) field experiments show that some pressure is necessary to effectively deliver the necessary volumes of material into a tree.

TREE PHYSIOLOGICAL PRINCIPLES

Trunk injection delivers materials into the xylem (wood) of trees. The xylem is the part of the vascular system that is responsible for transporting water and nutrients from the roots to the rest of the tree. It is mostly composed of non-living tissue that forms a pipe-like system. Transport in the xylem is passive and occurs with the plant transpiration stream. Because injected materials are easily distributed through the xylem and are spread relatively homogeneously throughout the canopy, trunk injection is primarily used to target xylem-related diseases such as wood-boring insects or xylem-inhabiting fungi and leaf chewing, piercing or sucking insects.

The urgent need for an HLB cure and the discovery of novel therapeutic compounds have sparked interest in using trunk injection for effective delivery of materials into citrus trees. In contrast to pests and diseases commonly targeted by trunk injection, HLB is associated with a phloem-limited pathogen.

While the xylem occupies most of the trunk, the phloem is a thin layer of tissue located in the inner bark. The phloem is a living tissue that transports sugars and other organic substances throughout the plant. Phloem transport occurs from source tissues with a high sugar content (usually

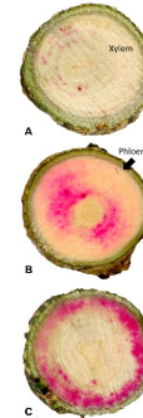


Figure 2. Trunk distribution of three dyes with different chemical properties: (A) dye with low mobility; (B) dye dispersing throughout much of the inner trunk and (C) dye moving predominantly in the outer wood beneath the bark. Dyes were injected 8 inches below the stem section shown.

photosynthetically active leaves) to sink tissues where sugars are needed, such as roots and developing fruits. It is not possible to inject large amounts of materials directly into the phloem.

OTHER CONSIDERATIONS

For trunk-injected crop protection materials to reach pathogens that reside in the phloem, such as the HLB-associated bacteria, the materials need to be able to move readily from the xylem to the phloem. The exchange of materials between xylem and phloem is not well understood but depends on the properties of the injected chemical.

Figure 2 demonstrates the different movements of three dyes with different chemical properties. For a crop protection material to be effective against phloem-inhabiting pathogens, it must be mobile enough to reach the phloem, but not so mobile that it moves back



Figure 3. Effect of oxytetracycline trunk injections on tree health (A) and fruit drop (B) of 4-year-old Valencia trees. The tree on the right received one injection of oxytetracycline in October 2020; the photo was taken in February 2021. Fruit drop was assessed from December 2020 to March 2021.

out and is transported primarily in the faster-moving xylem.

Using antimicrobial compounds to cure HLB has been a discussion for many decades. So far, these materials do not have the desired levels of activity when delivered in a foliar spray. In contrast, experiments with tetracyclines conducted in the 1970s in South Africa and other countries, and more recently Florida, demonstrated that it is possible to reduce bacterial titers and HLB severity through trunk injection.

Preliminary results from UF/IFAS ongoing field experiments support these findings and demonstrate that injecting oxytetracycline can improve tree health and dramatically reduce fruit drop in citrus trees that are severely affected by HLB (Figure 3). It is important to note that any materials injected into the trunk move readily into the fruits and that oxytetracycline is not labeled for trunk injection in bearing citrus trees. Nevertheless, these experiments show that trunk injection is effective for systemically delivering therapeutic materials and restoring health and productivity to HLB-affected trees.

Figure 2 demonstrates the different movements of three dyes with different chemical properties. For a crop protection material to be effective against phloem-inhabiting pathogens, it must be mobile enough to reach the phloem, but not so mobile that it moves back

WOUNDING

Another concern regarding the use of trunk injection is its effect on tree

health. Drilling or otherwise injecting materials into the trunk wounds the tree and provides entry points for opportunistic pathogens. Furthermore, upon injury, xylem vessels embolize and become dysfunctional, affecting the water- and nutrient-transport capability of a tree. Similarly, the phloem will be destroyed, affecting sugar transport.

Trees are generally very effective in compartmentalizing wounds. Figure 4A (page 17) shows the effective compartmentalization of a wound created after injecting water. During the next growing season, new xylem and phloem form, rendering the injured area functional again. As xylem transport is usually most active in the outer (newest) wood, the tree may fully regain its transport capacity in the season following injury.

It is imperative to determine any potential phytotoxic effects of the crop protection material before its use. For example, oxytetracycline prevents the closure of wounds after injection and causes considerable structural damage inside the tree (Figure 4B, page 17). The long-term effects of this are yet to be determined. Figure 4B also shows that a tree's ability to compartmentalize wounds is less effective in the up-and-down direction than in the left-to-right direction.

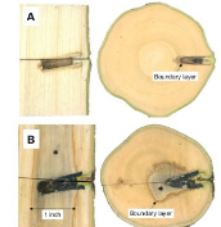


Figure 4. The wound is effectively compartmentalized after water injection (A), and new wood is visible above the injection site. In contrast, necrosis and ineffective compartmentalization is observed after oxytetracycline injection (B) as indicated by a broad zone of discoloration (C). The boundary layer marks the border of effective encapsulation of the wound area that prevents entry of opportunistic pathogens.

CONCLUSIONS

Trunk injection is an effective method for delivering crop protection materials systemically and with minimal impact on human health and the environment. However, trunk injection comes with risks ranging from residual chemicals in the fruits to the impact of wounding on long-term tree health. Trunk injection of most registered crop protection materials is not labeled for bearing citrus trees.

Currently, the cost associated with trunk injection impedes its widespread use in commercial citrus production. It is expected that automated delivery methods will be available soon that reduce cost and render trunk injection more practical for delivering novel therapeutic compounds currently being developed.

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Further information

Trunk Injection to Deliver Crop Protection Materials: An Overview of Basic Principles and Practical Considerations¹

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Introduction

Trunk injection is a targeted delivery of crop protection materials into the stem or trunk of woody plants as an alternative to spraying or soil drenching. It is sometimes referred to as "endotherapy." Trunk injection occurs into the xylem of trees from where the injected material is distributed through the plant with the transpiration stream. There are several advantages that trunk injection provides over conventional spray or soil drenching of crop protection materials: (1) products are applied more precisely and used more efficiently; (2) spray drift is eliminated; (3) if properly applied there is a lower risk for worker exposure; and (4) nontarget organisms are less affected (Wise et al. 2014). Because there is less concern for human health and the environment, the method can be used in urban environments and residential areas where aerial sprays are not an option. Trunk injection is predominantly used in forested areas, landscapes, and nonagricultural areas. However, there is a long history of using plant injection to deliver crop protection to commercial avocado trees, e.g., phosphonate injection of avocado trees in Australia and South Africa (Dann et al. 2013). In the United States,

most use in agricultural areas is in nonbearing crops, with Florida and California avocado trees being an exception. In California, injection of phosphonates to prevent phytophthora root rot has been implemented for decades. In Florida, approximately 20% of the commercial acreage has been injected prophylactically to prevent (suppress) the laurel wilt pathogen (*Raffaella lauricola*) on a 12-to-24-month basis since 2014 (Crane et al. 2020).

The earliest evidence for plant injection is from the 12th century, when Arab horticulturists applied perfumes, spices, dyes, and other substances through wounds in plants to affect the smell, color, or other attributes of flowers and fruits (Roach 1939). The first documented experimentation on trunk injections is from the 15th century by Leonardo da Vinci (Roach 1939), who injected arsenic and other poisonous solutions in apple trees to poison the fruit, possibly to prevent thieves from stealing his fruits. Other experimentation until the early 1900s included injection of different nutrient solutions to overcome nutrient deficiencies and different organic and inorganic substances to control insect, fungal, and other diseases. An excellent

Review

Trunk Injection as a Tool to Deliver Plant Protection Materials—An Overview of Basic Principles and Practical Considerations

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Abstract: Trunk injection is a targeted delivery of pesticides, insecticides, nutrients, or other plant protection materials into the stem or trunk of woody plants as an alternative to spraying or soil drenching. Trunk injection has historically been used for disease and pest management of high-value forest tree species or ornamental plants when aerial applications are problematic due to spatial problems and health-related concerns. An interest in using the injection technique for protection of agricultural crops in commercial production systems has emerged more recently, where foliar applications and soil drenches have proven ineffective or pose environmental hazards. This review provides an overview of the basic principles of trunk injection and the plant physiological implications, its current use in commercial agriculture and other plant systems, and associated risks.

Keywords: horticultural crops; crop protection; endotherapy; chemotherapy; systemic delivery



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High-Pressure Injection	Low-Pressure Injection	No-Pressure Injection
Drill-based Quik-Jet Micro-Injection System; Quik-Jet Air Injection System (Arborjet Inc., Woburn, MA, USA) arborjet.com ENDOplint (ENDOTerapia Vegetal, Girona, Spain) endoterapiavegetal.com Q-Connect (Rainbow Ecoscience, Minnetonka, MN, USA) rainbowecoscience.com Sidewinder Tree Injector (The Australian Made, Loganholme, Australia) australianmade.com.au	Drill-based Chemjet Tree Injectors (Chemjet, Queensland, Australia) chemjet.com.au Mauget Smart Shot (Mauget, Arcadia, CA, USA) mauget.com Manual Macro-Infusion (Rainbow Treecare, Minnetonka, MN, USA) rainbowtreecare.com EcoJet system (Bioforest Technologies, Ontario, Canada) bioforest.ca Tree IV Micro-Injection System; Viper Micro-Injection System (Arborjet Inc., Woburn, MA, USA) arborjet.com Microinjections (Tree Tech, Morrison, FL, USA) treetech.net Drill-free Wedge Direct-Injection System (ArborSystems, Omaha, NE, USA) arbor-systems.com	Drill-based Aacrop (Creative Sales, Fremont, NE, USA) aacrop-mediacap.com Mauget Capsules (Mauget, Arcadia, CA, USA) mauget.com Drill-free EZ Jet Lance (ArborSystems, Omaha, NE, USA) arbor-systems.com BITE Tree Infusion (Sorbus, Somerset, UK) sorbus-inf.co.uk/tree-care-bite

Figure 1. Some of the currently available trunk injection devices (links accessed 14 June 2022).

2.1. High-Pressure Injection

The Arborjet Quik-Jet Air (Arborjet Inc., Woburn, MA, USA) is one example of a high-pressure application device. Arborjet and similar high-pressure systems, such as ENDOplint (ENDOTerapia Vegetal, Girona, Spain), use 7.15 mm or larger diameter plastic plugs as injection ports, which are inserted into the tree after drilling of a hole. This creates a tight seal for injection, prevents leaking, and protects the wound from pathogens and insects. Injection of the compounds occurs through specialized metal injection tips at pressures of 60–100 psi (413–689 kPa) created by using compressed gas. Although the plastic plugs enable the rapid injection of large volumes of material, they can cause more damage to trees than no-plug methods as they increase the size of the injection hole, increase the probability of injury from bark cracking, and may interfere with full wound closure [23]. Dendrology research has shown that plugging tree core wounds does not provide any benefit and can even interfere with the natural healing capabilities of the tree [33]. Other high-pressure systems include the Q-Connect (Rainbow Ecoscience, Minnetonka, MN, USA) and the Sidewinder Tree Injector (The Australian Made, Loganholme, Australia).

2.2. Low-Pressure Injection

An alternative to high-pressure injection are syringe or needle-based methods sold by Chemjet (Kerrville, TX, USA), Mauget (Arcadia, CA, USA), Rainbow Ecoscience (Minnetonka, MN, USA), and other manufacturers, which allow the plug-free injection of materials. Injection using these devices occurs at relatively low pressures (<60 psi) by manual squeezing or use of a spring-loaded syringe system. Most of these systems rely on drilled holes prior to application; however, ArborSystems (Omaha, NE, USA) has developed a low-pressure drill-free system, the Wedge® Direct-Inject. With this device, a shallow stem core is removed, and liquid is manually pushed into the trunk. The rate of liquid uptake associated with lower-pressure devices is slower than that of higher-pressure

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Questions

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