

# Florida Bactericide Trials

1. AgroSource, Inc./USDA Trial (CRDF Grant Supported)
  - Initial 3 year grant to develop delivery methods and demonstrate field effectiveness
2. CRDF Grower Trial Results (One Year)
3. Cooperator 1 Trial (One year trial performed by Premier Citrus, Tom Jerkins)

# Three Independent Trials

- **3-Years AgroSource, Inc./USDA CRADA (CRDF Supported) Trial:**

- Individual tree treatments within blocks: initially 24 different treatments at 17 sites.
- In third year, 12 sites 560 trees.
- Randomized Complete Block design for treated and control trees.
- Resulted in recommended application method, rate and schedule.
- Led to emergency use and section 18 approval.

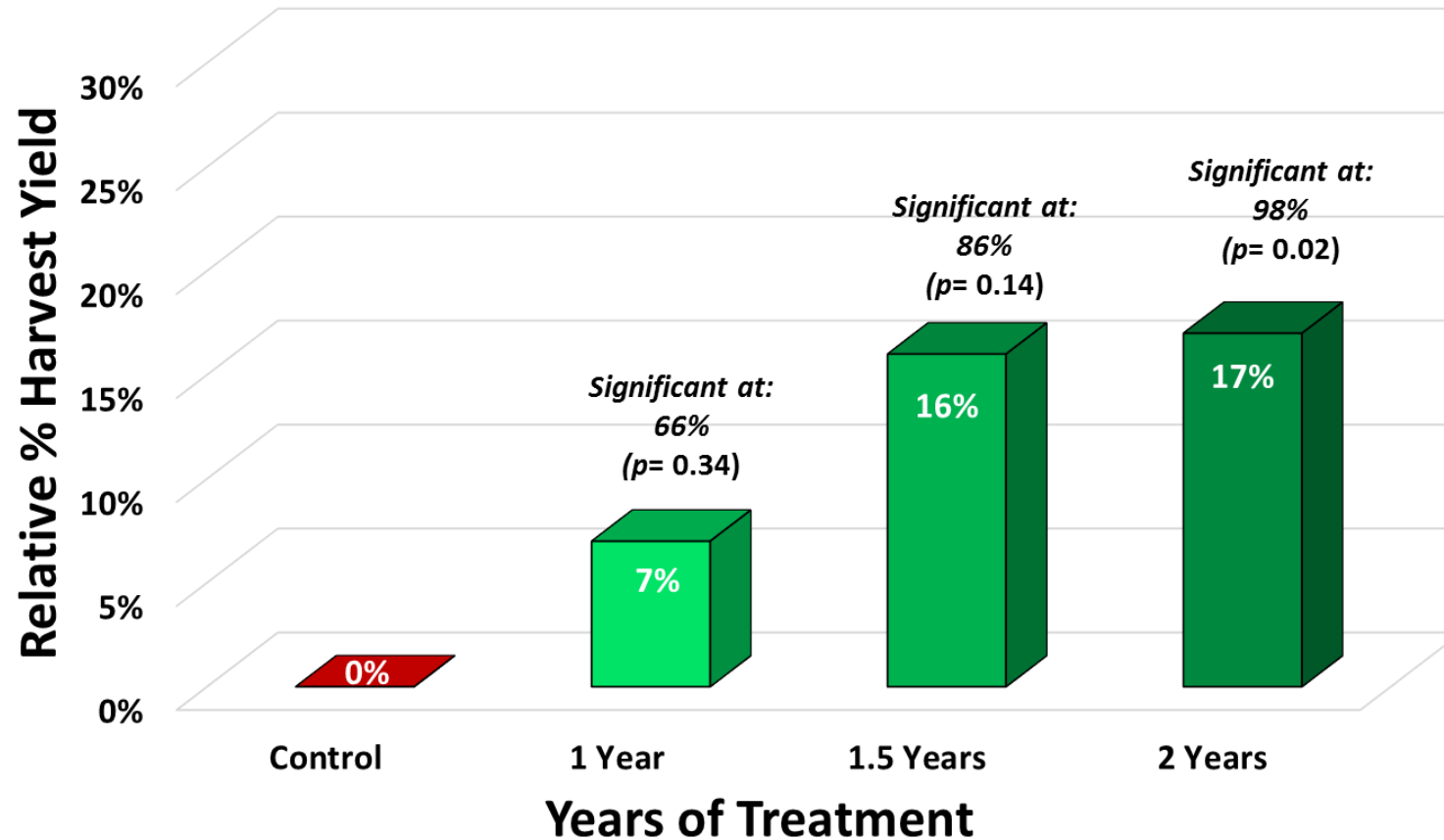
- **CRDF Grower Trial:**

- Statewide, grower applied whole block treatments with within block controls.
- 39 sites and 1850 trees.

- **First Year Cooperator 1 Trial: FireLine/FireWall + Adjuvant Program:**

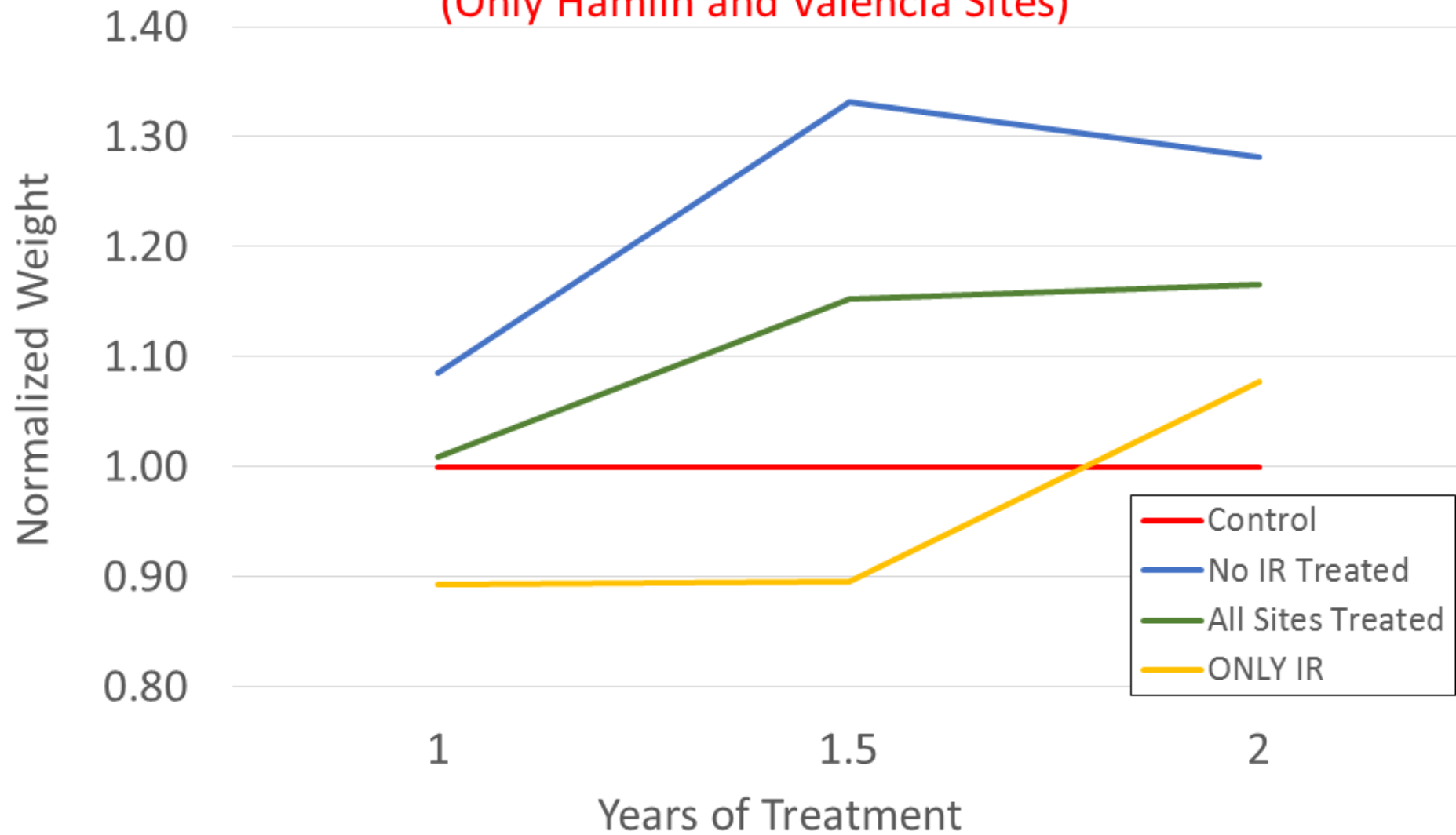
- Whole block treatments represents 1000s of acres and **over 1.6 million trees.**
- 27 sites 342 blocks across the state.
- Paired blocks for control and treated trees.
- Comparison made by comparing to previous years harvest.

# USDA, ARS/AgroSource CRDF Funded Trial

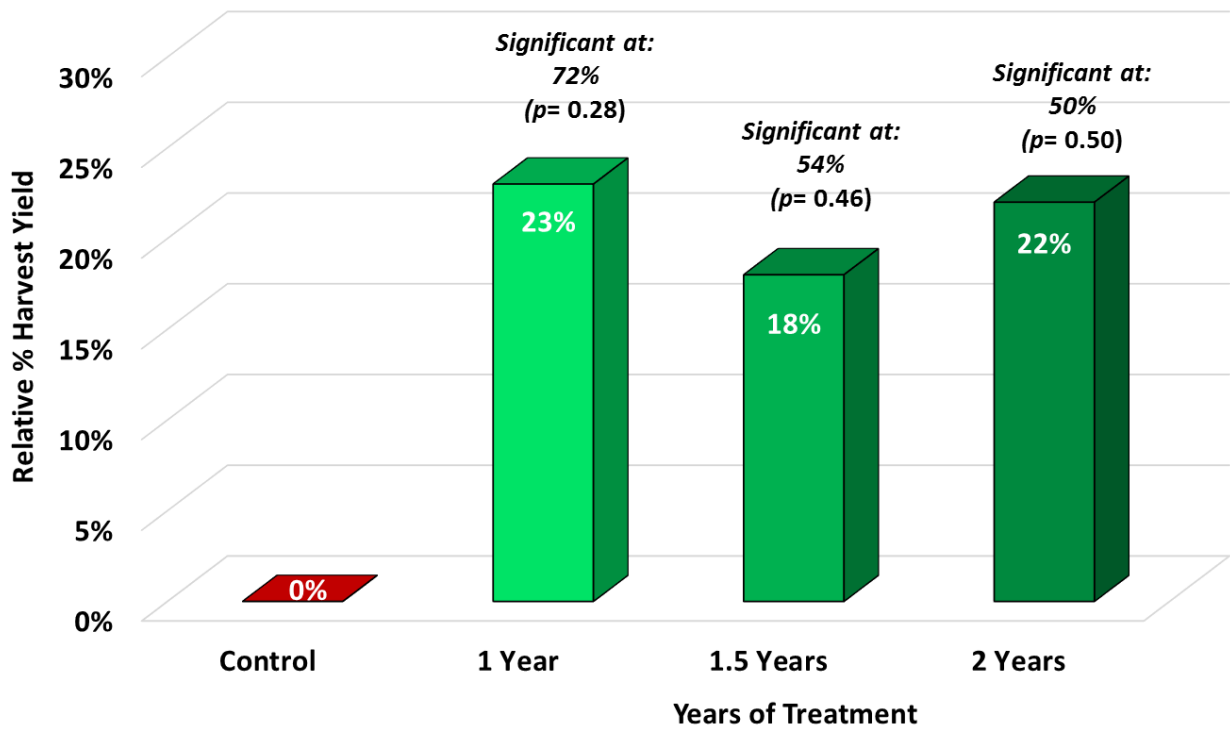


# Normalized Yield By Year of Treatment

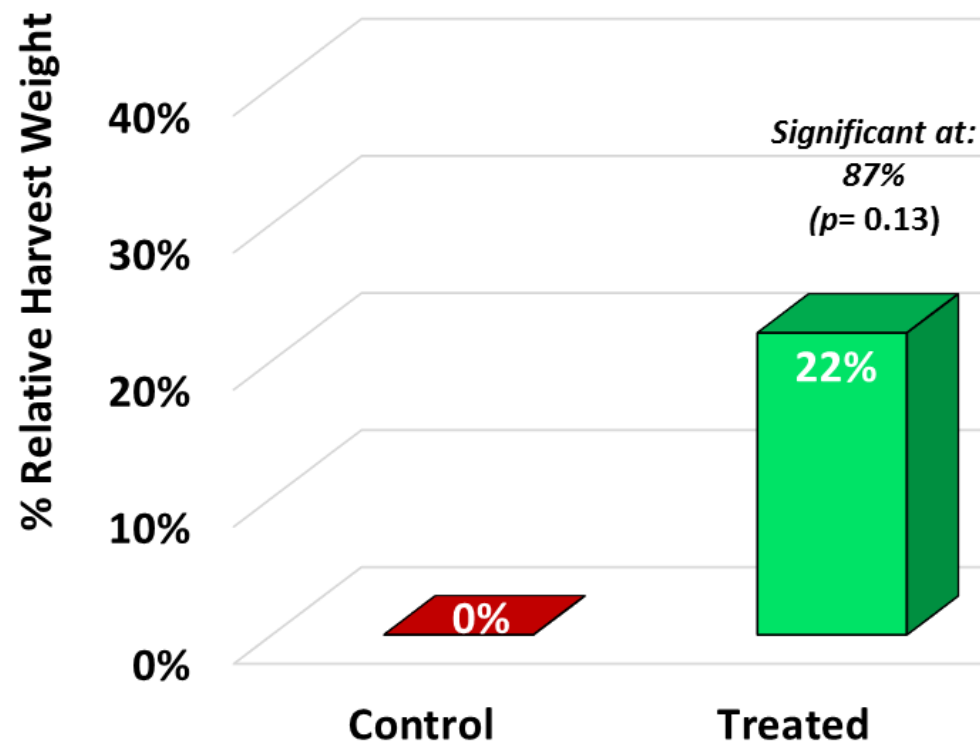
(Only Hamlin and Valencia Sites)



Relative Percent Harvest Yield Over Time for Grapefruit

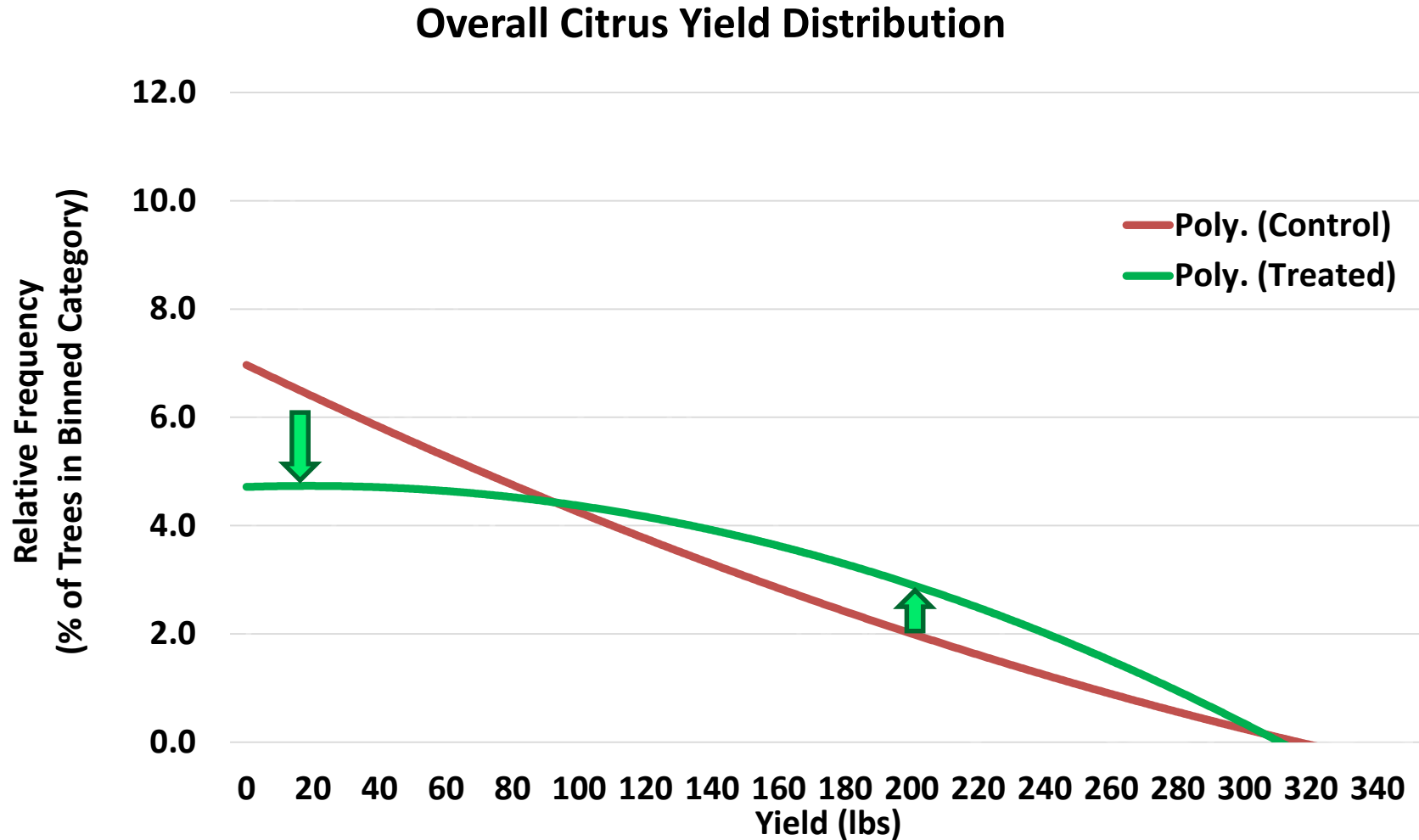


Percent Relative Harvest Weight for Control vs Treated Grapefruit, Y2+Y3



# FireLine™ & FireWall™ HLB Control Trials

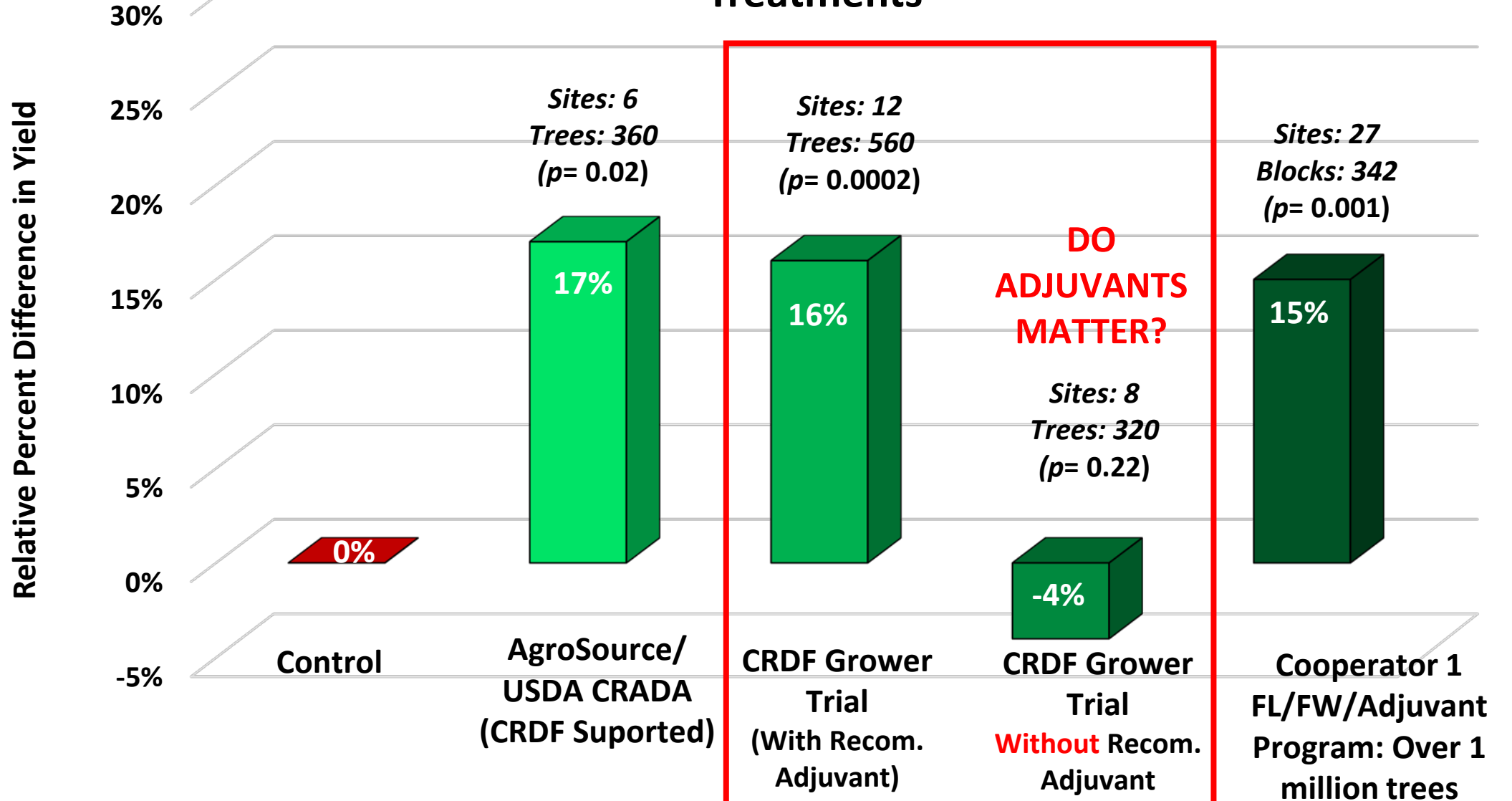
- Yield Results by Distribution -



# DO BACTREICIDES WORK?

## Relative Percent Yield Response to FireLine™ and FireWall™

### Treatments



How Did Each Treatment Site Respond Within Each  
Trial?

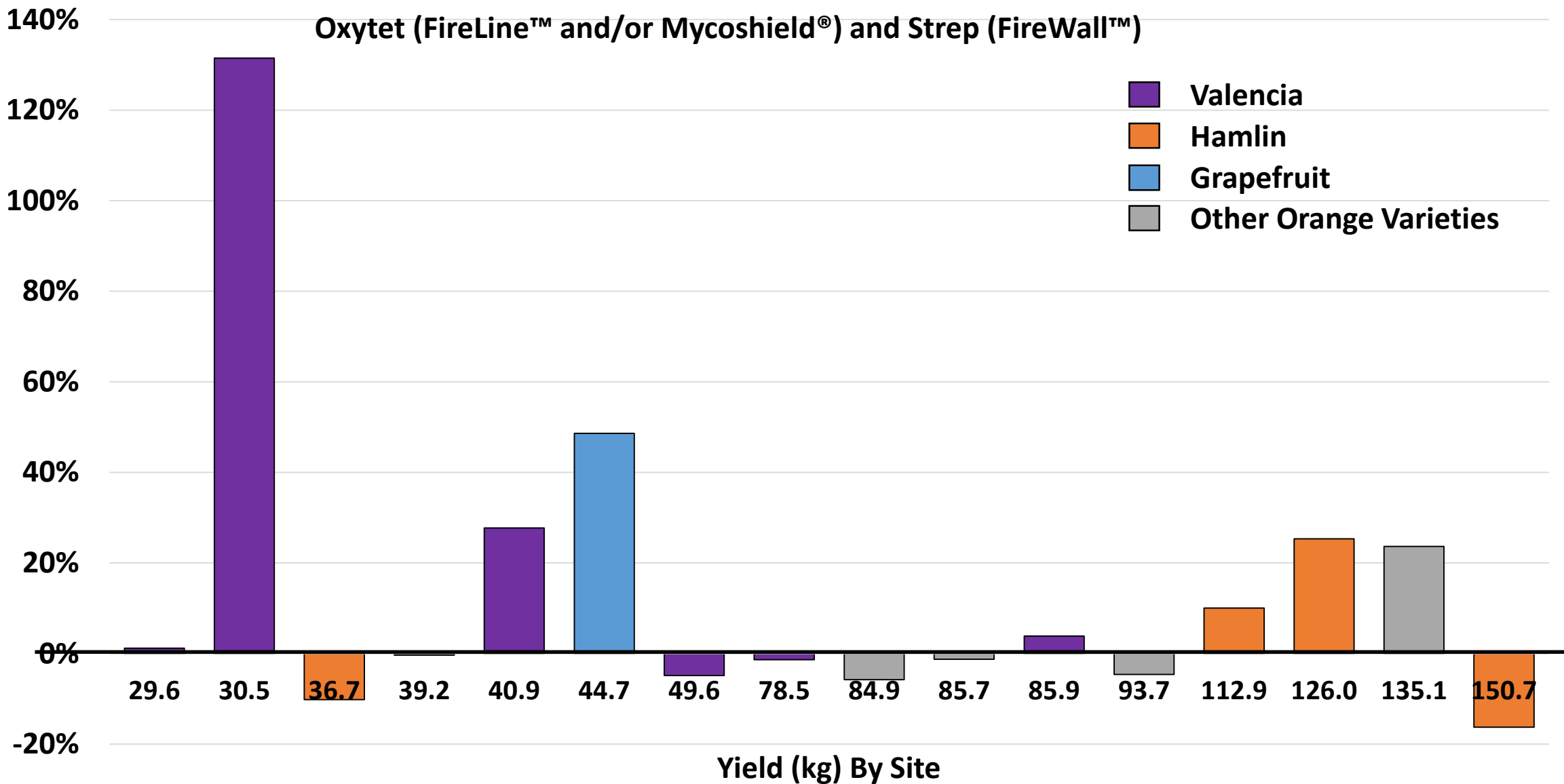


# Trial 1: CRDF Grower Trial: Yield Data Compared to Control

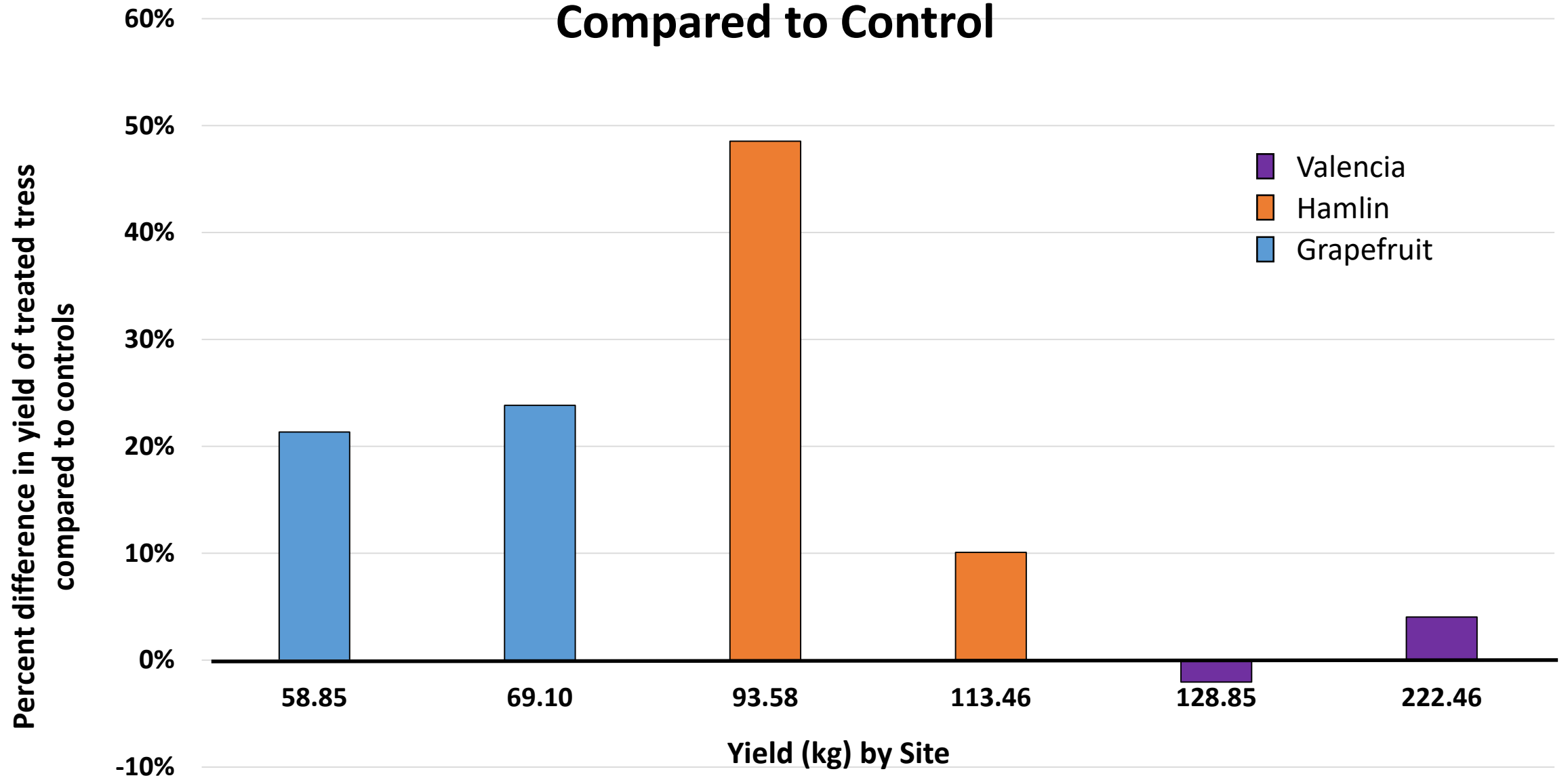
Oxytet (FireLine™ and/or Mycoshield®) and Strep (FireWall™)

% difference in yield of treated trees compared to control

- Valencia
- Hamlin
- Grapefruit
- Other Orange Varieties



# AgroSource, Inc./USDA CRADA (CRDF Supported) Trials Yield Compared to Control



# Single Grower Independent Trial

## Premiere Citrus (Tom Jerkins)

- **First Year Cooperator 1 Trial: FireLine/FireWall + Adjuvant Program:**

- Whole block treatments represents 1000s of acres and **> 1.6 million trees.**
- Total 23 Valencia (only) Groves involved
  - 330 Blocks: 127 Control, 203 Treated
    - 11,643 Acres
- Managers of treated blocks were instructed to use only FireLine and FireWall, with recommended adjuvants
- Control blocks were managed the same, but with no FL/FW treatment or adjuvant
- Treatment Started in Spring of 2016, after the 2015-2016 harvest.
  - Not all blocks had control/treatment pairs but each site did.
  - Comparison made by comparing to previous years harvest.

Premier Sites  
Grouped by Region  
for Data Analysis

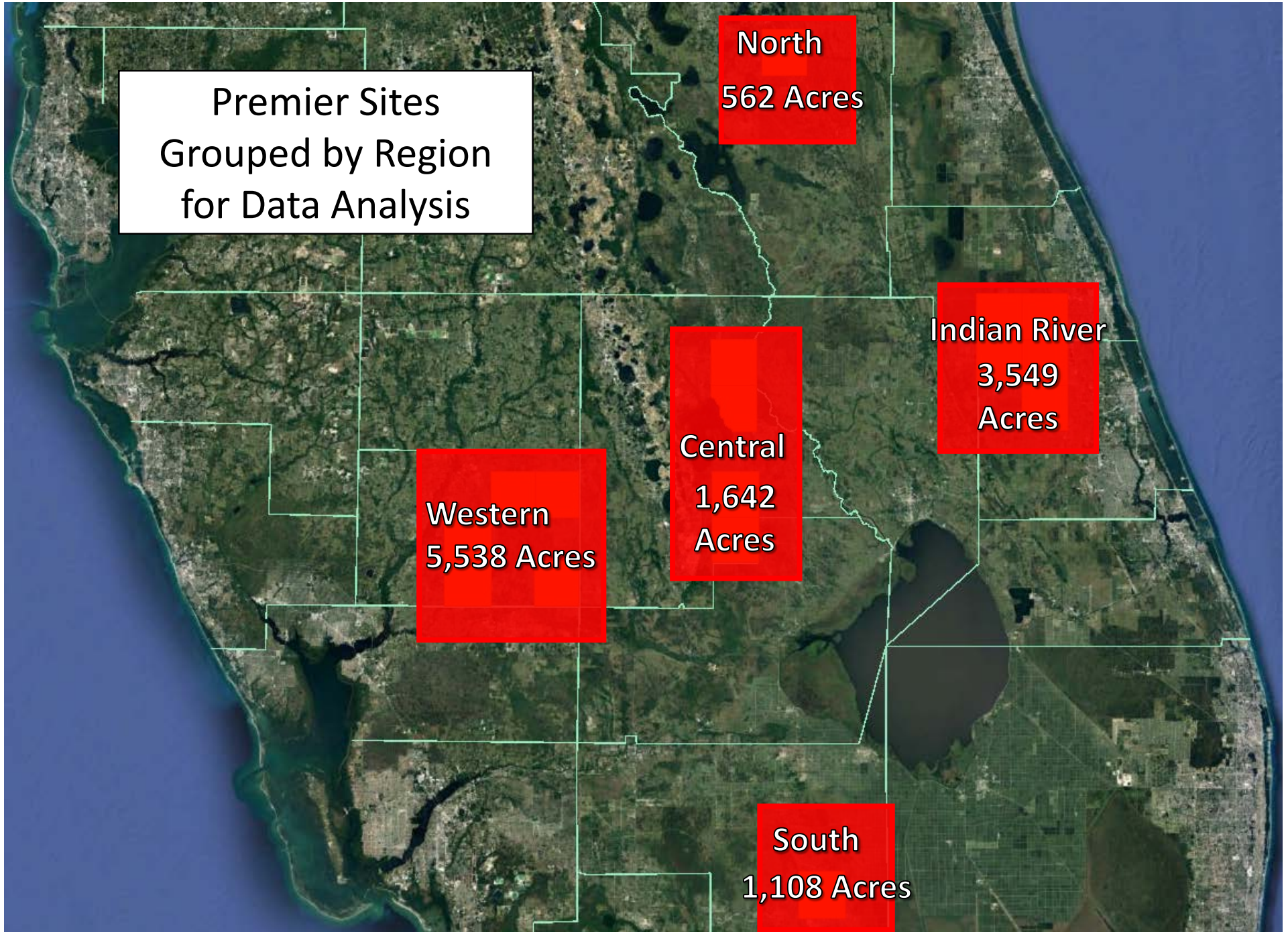
North  
562 Acres

Indian River  
3,549  
Acres

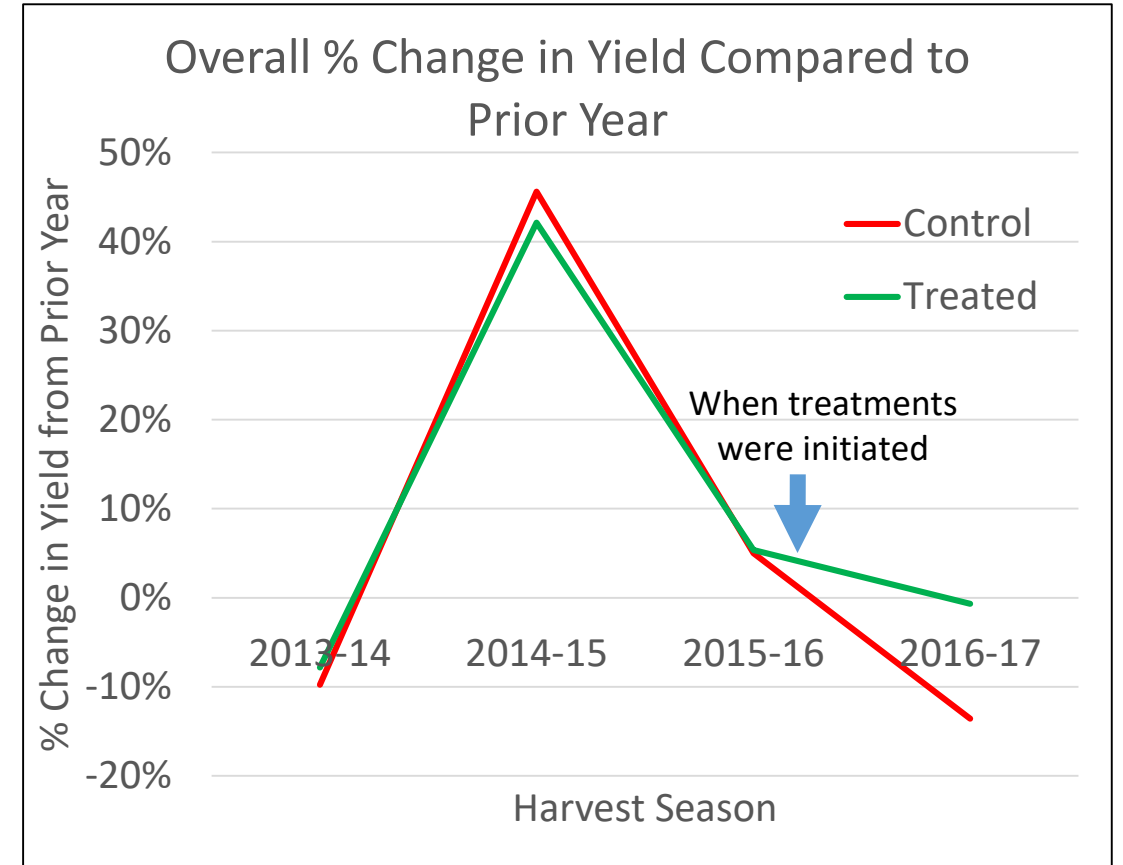
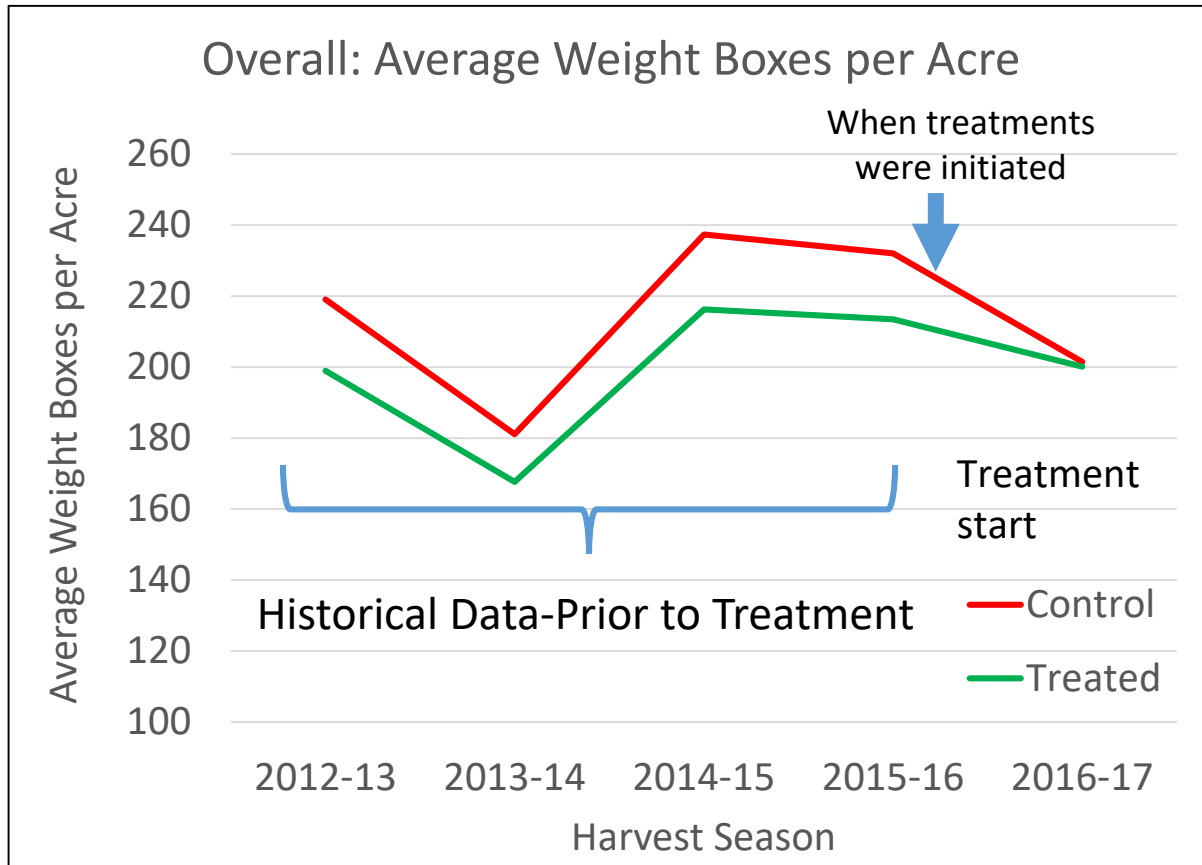
Central  
1,642  
Acres

Western  
5,538 Acres

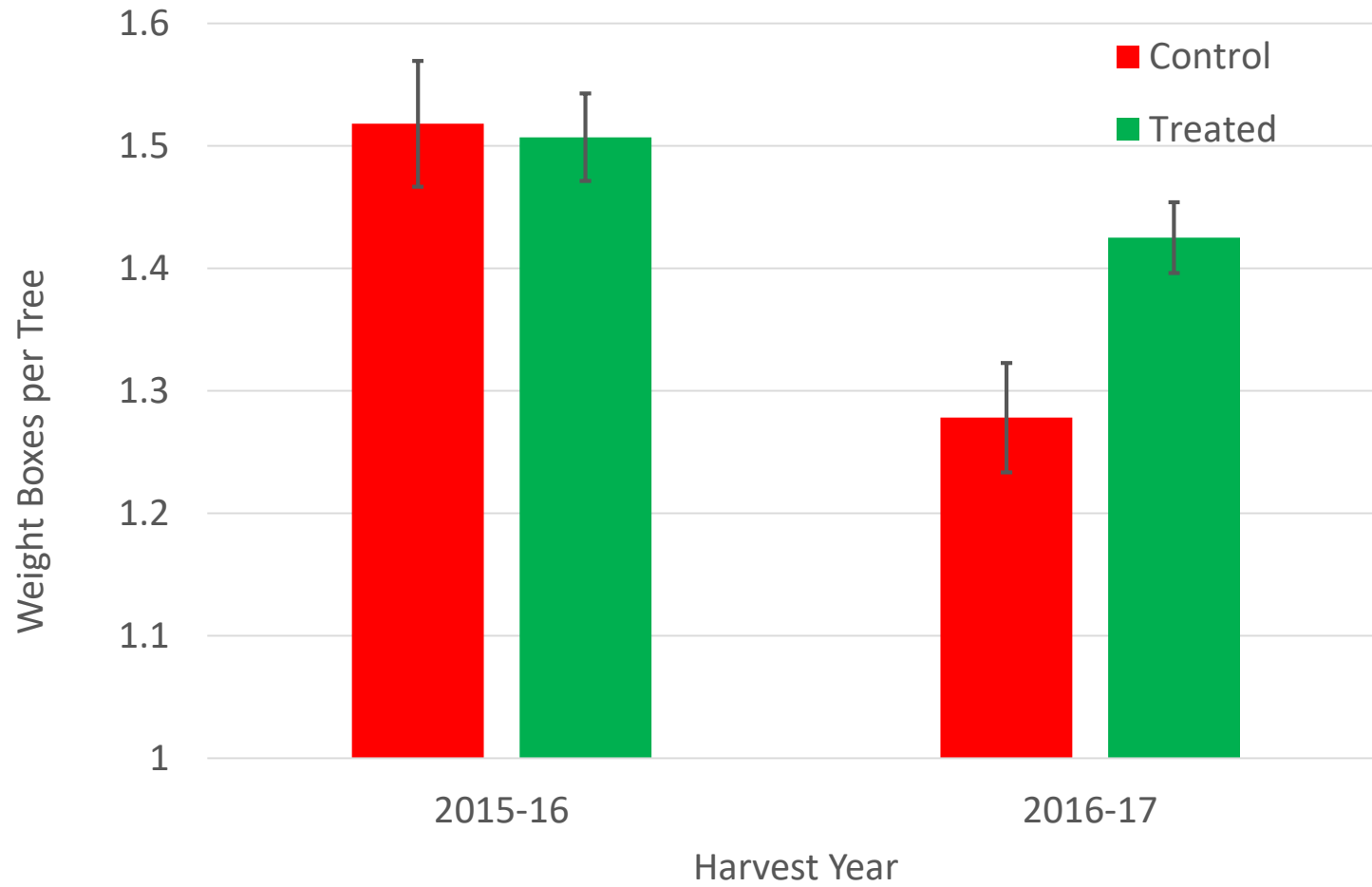
South  
1,108 Acres



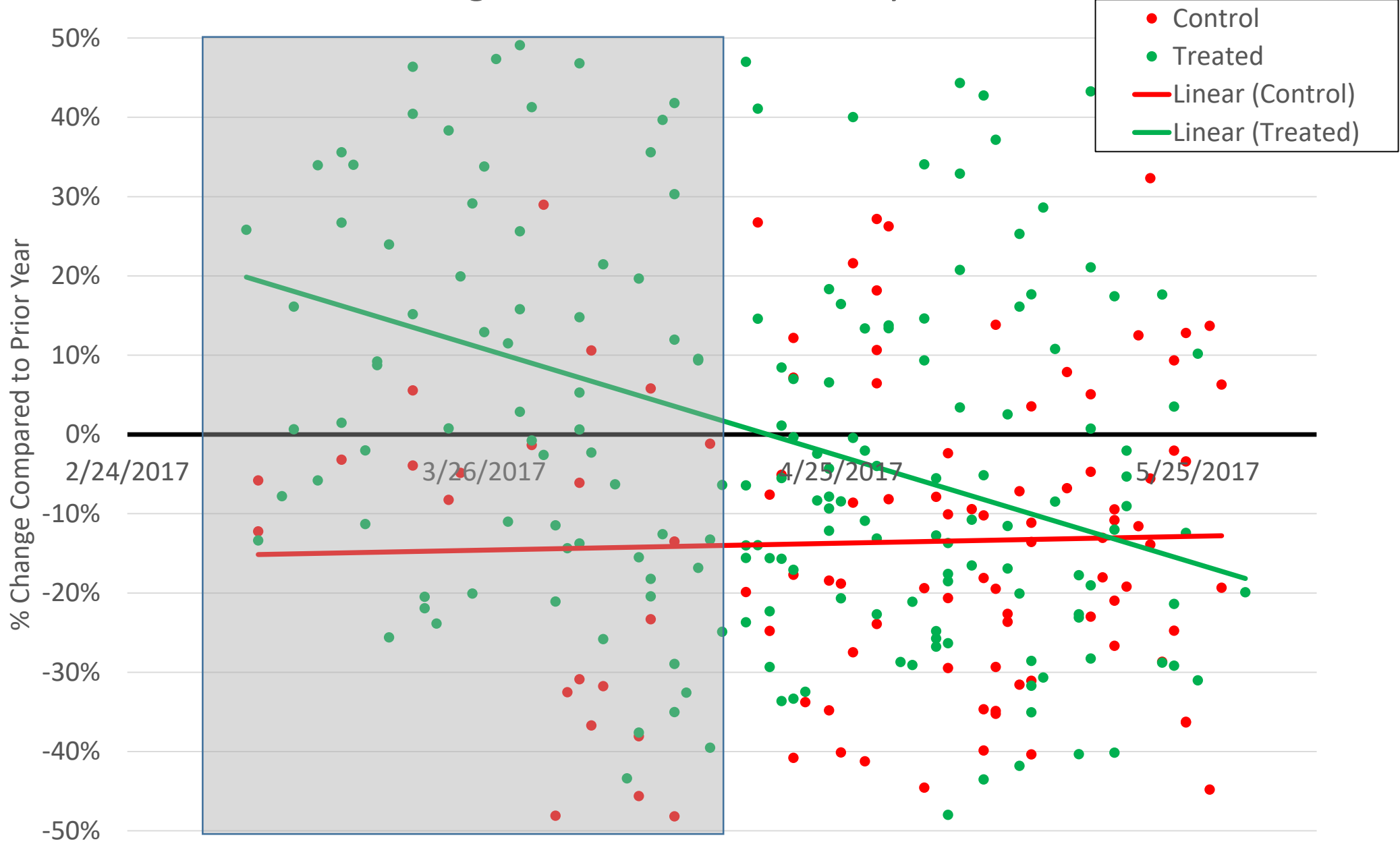
# Overall Comparison of Control vs Treated



Weight Boxes per Tree

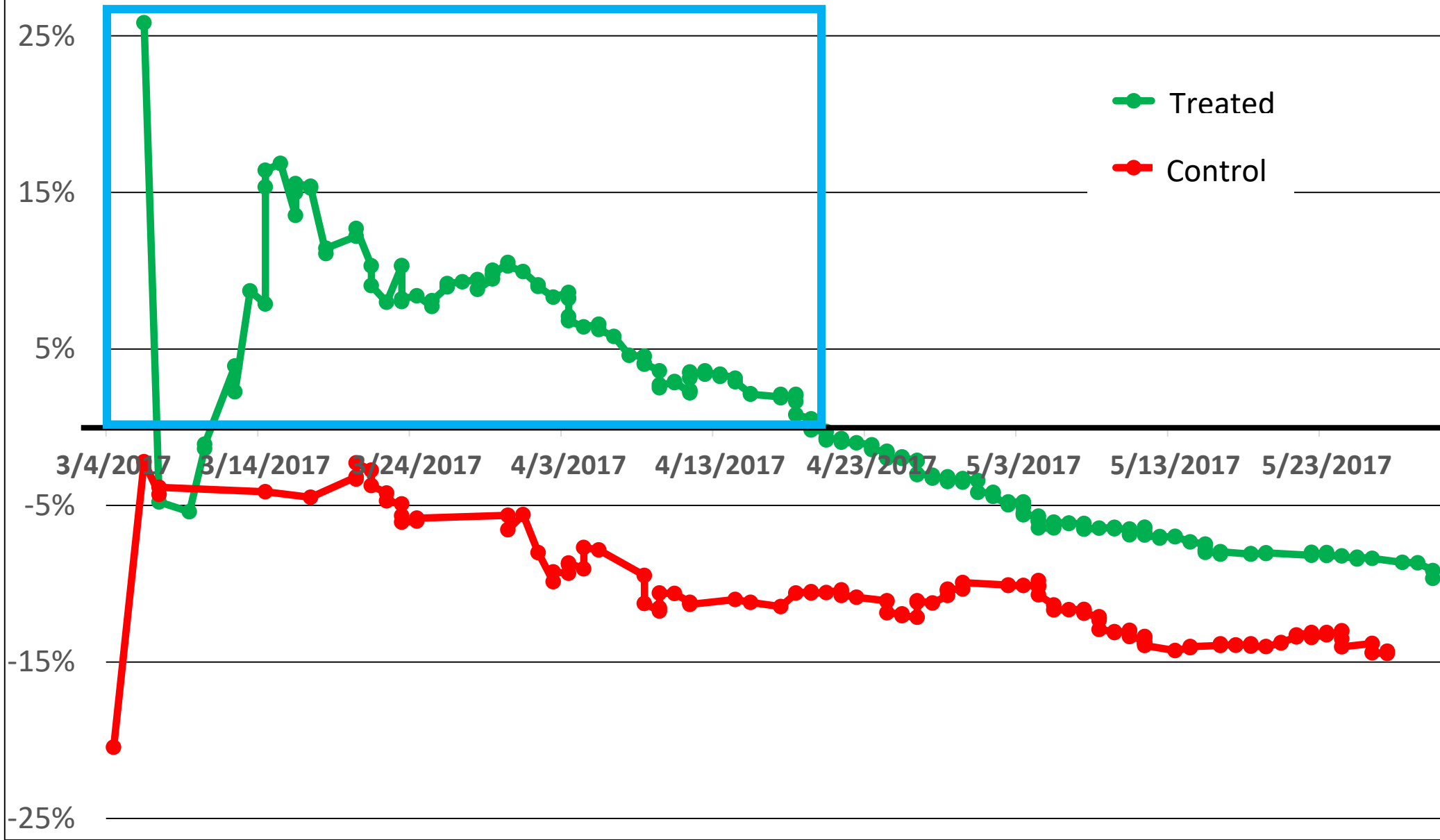


# All Sites: Change from Previous Year, by Harvest Date



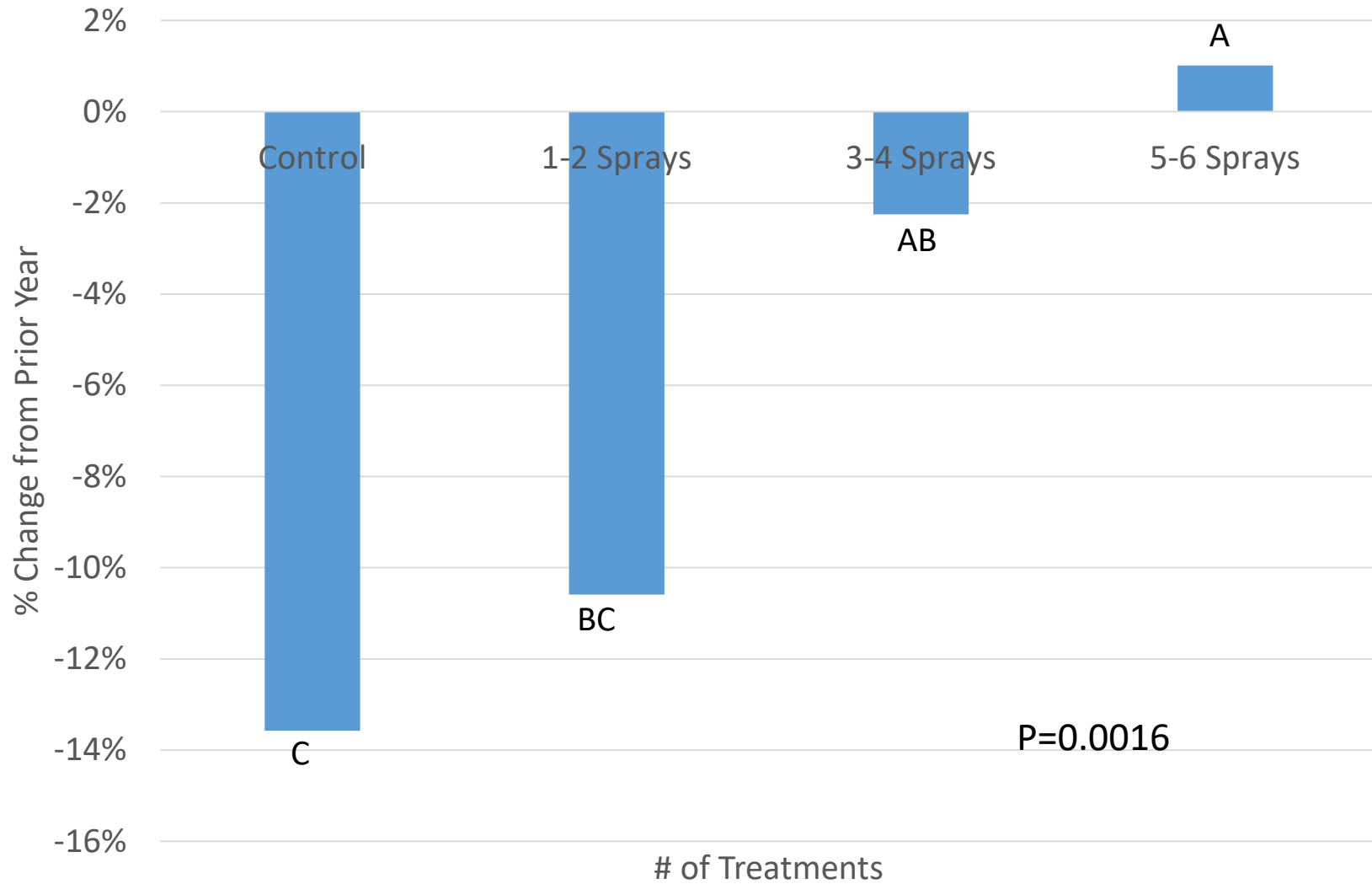
# Cooperator 1 Data:

Treated vs Untreated Cumulative % Change in Yield Compared to Previous Year and Sorted by Date Harvested



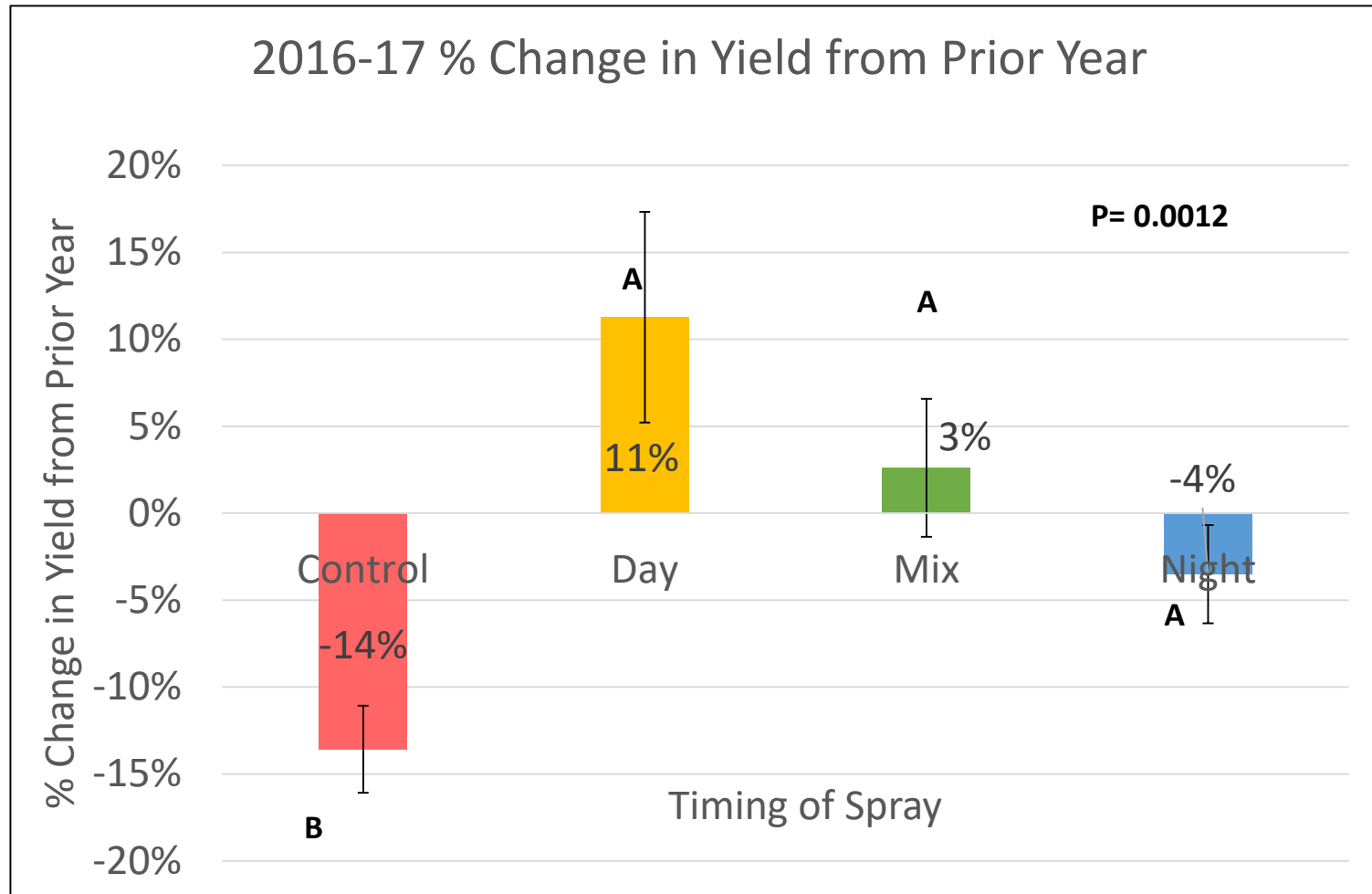


# 2016-17 % Change in Yield from Prior Year by # of Treatments



# Night vs. Day Applications Timing

- Night: 128 Blocks, Day: 35 Blocks, Mix: 56 Blocks



# Results from these Trials answer Three Questions:

## 1. Do Bactericides Work?

- a. Yes, Initial result indicates an on average yield improvement of ~15% after 1 year with FireLine and FireWall + Adjuvant.
- b. There are still variable results across groves throughout the state and we need to understand where this variability is coming from.

## 2. Do All Bactericides Work Equally?

- a. Current observations suggest both oxytetracycline and streptomycin are needed.
- b. Best results observed with combination of FireLine and FireWall; however, more trials are needed to confirm.

## 3. Do Adjuvants Matter?

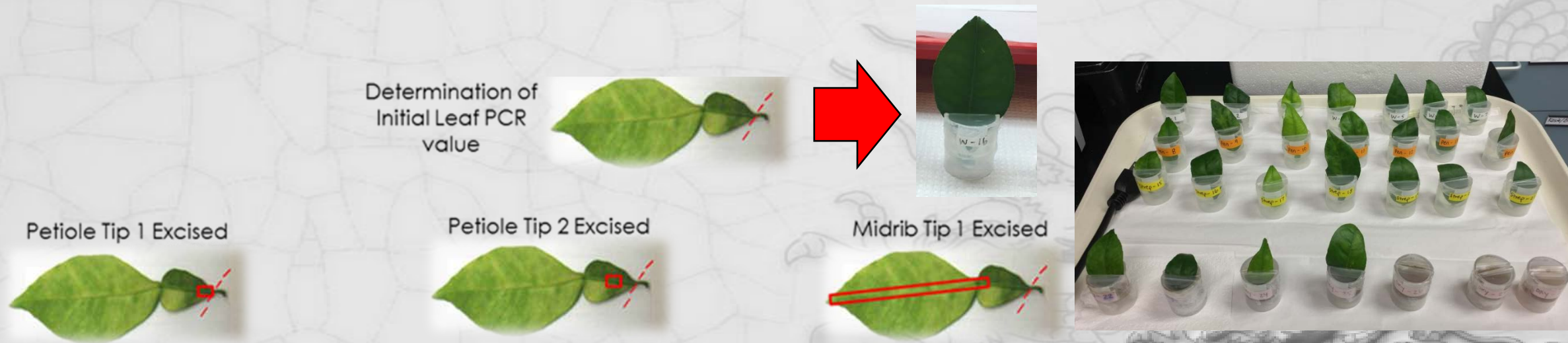
- a. Yes, positive results were obtained with FireLine and FireWall **ONLY** when recommended adjuvants were used.

## **Will Bactericide Effects Continue to Improve Tree Health and Crop Yield?**

- a. Need longer term studies (second year is in progress)
- b. Although the CRDF sponsored AgroSource/USDA CRADA trial was 3 years, the first two years were used to determine dose rate, penetration, and application schedule. The current recommended schedule was only applied for 1 year.
- c. There is significant variation in treatment effects. If this source of variation can be identified, more consistent positive results may be realized.

# Development of a Single Leaf Assay to Test Antimicrobial Compounds with:

- 1) Activity Against Candidatus Liberibacter Asiaticus
- 2) No Toxicity to Citrus
- 3) Ability to Move Systemically in the Citrus Vascular System



# Antimicrobial Peptide Reduces CLas in Citrus Leaves

Determination of Initial Leaf PCR value



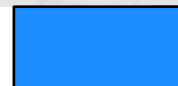
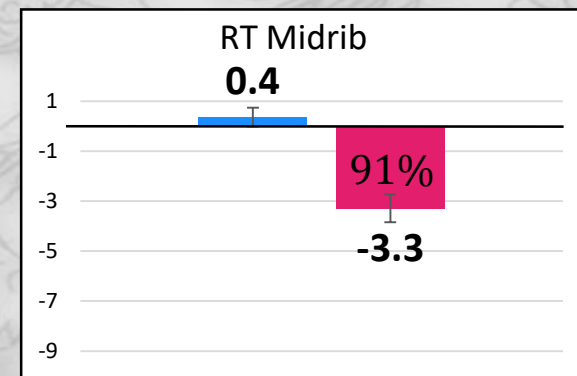
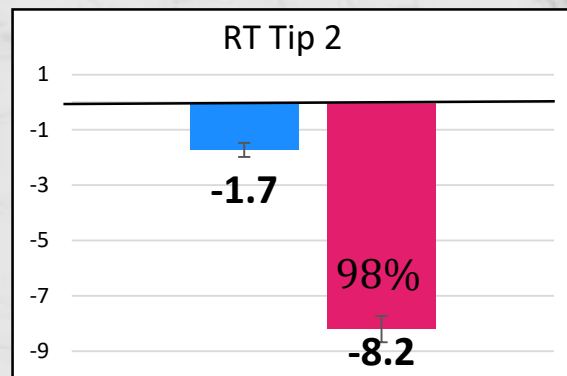
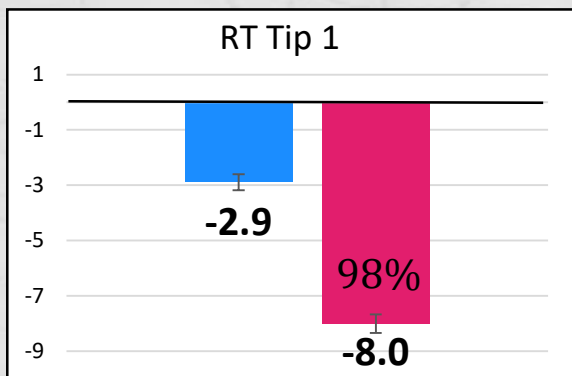
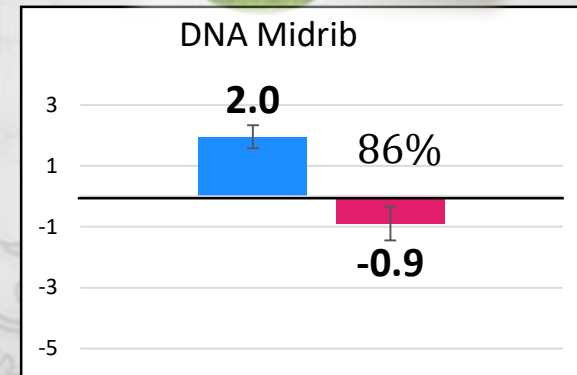
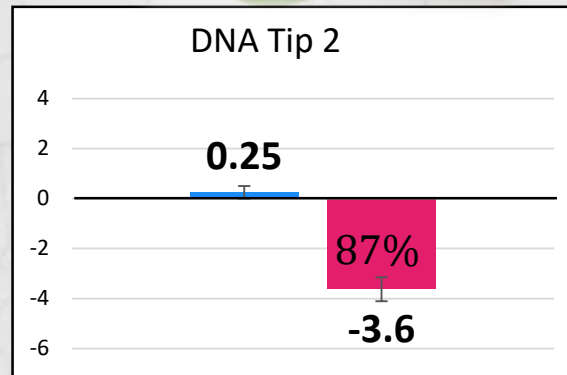
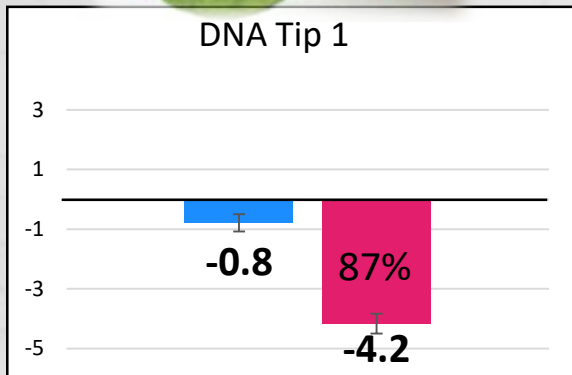
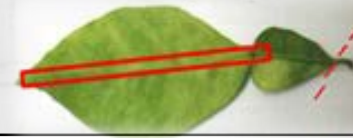
Petiole Tip 1 Excised



Petiole Tip 2 Excised



Midrib Tip 1 Excised



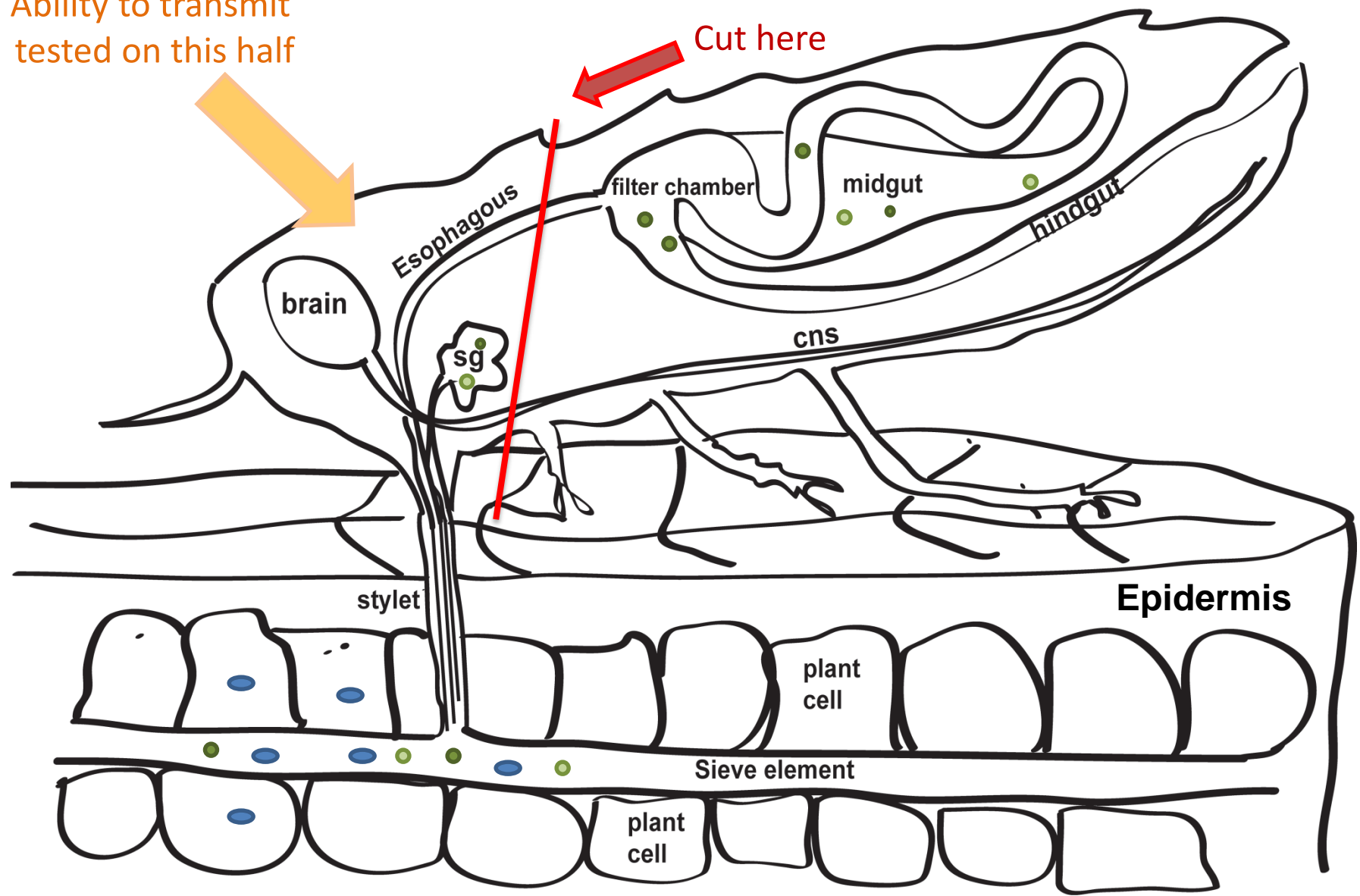
Water Control



AMP-2

Ability to transmit  
tested on this half

Cut here



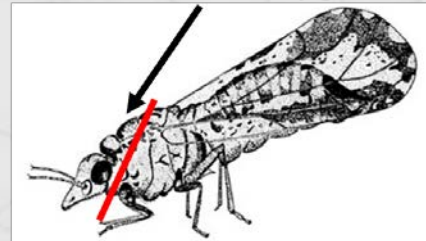
# Effect of AMPs on Acquisition of the Bacterium (CLas) During Feeding

Four Replicates with a total of 600 psyllids for all replications

Treatment	% Mortality	Psyllids that acquired (Detected in body)	Avg Ct
H2O-Control	79%	100%	29.9
AMP	95%	<1%	35.6



Location of the salivary glands



Three Replicates with a total of 260 psyllids for all replications

Treatment	% Mortality	Able to Transmit (Detected in salivary/head)	Avg Ct
H2O-Control	79%	42%	36.8
AMP	95%	0%	-





# DELIVERY



- **Direct Application**
  - **Spray**
  - **Infuse**
- **Transgenic**
  - **Nuclear Transformation of Citrus**
  - **Use of Citrus Tristeza Virus for production in the phloem (Collaboration with Dr. Bill Dawson at Univ. of FL)**

# Antimicrobial Peptide Uptake by Whole Plants

