



# **Diplodia infection exacerbates pre-harvest fruit drop of HLB-affected trees**

Wei Zhao, Elizabeth A. Baldwin, Jinhe Bai, Greg McCollum,  
Tim Gottwald, Anne Plotto

USDA-ARS Horticultural Research Laboratory, Ft. Pierce, FL

Mike Irey

Southern Gardens Citrus, US Sugar Corporation, Clewiston, FL



# Excessive pre-harvest fruit drop

- Excessive pre-harvest fruit drop is severe since HLB epidemic
  - Significant loss of yield
- The reason for the HLB-related fruit drop is uncertain
  - The proposed reasons:
    - a. Water shortage due to root loss
    - b. Nutritional deficits due to phloem plugging
- ❖ The involvement of *Diplodia* infection

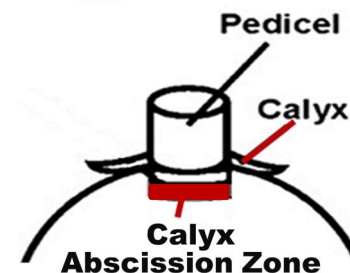


# About *Diplodia* latent infection and invasion

- Causes citrus fruit Stem-End-Rot (SER) decay
  - A postharvest disease
- Latent infection of citrus fruit occurs during the fruit development
  - But remains inactive until harvest



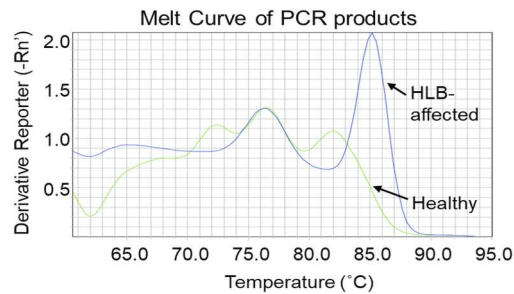
**Stem-End-Rot**



- ❖ **In HLB-affected fruit, *Diplodia* invasion can happen before harvest**
  - an accidental discovery in orange juice

# The finding of preharvest invasion of *Diplodia* in HLB-affected fruit

- DNA from a fungus frequently present in HLB-affected juice, but not in juice from healthy fruit



- A peak at 85 °C was frequently present in the HLB juice, but not in healthy juice.

- By DNA sequencing, the fungus was identified—it was *Diplodia*

Download ▾ GenBank Graphics ▾

*Lasiodiplodia theobromae* 18S ribosomal RNA gene, partial sequence  
Sequence ID: [gb|KC442314.1](#) Length: 1134 Number of Matches: 1

Range 1: 604 to 758 GenBank Graphics ▾ Next Match ▴ Previous Match

Score	Expect	Identities	Gaps	Strand
287 bits(155)	3e-74	155/155(100%)	0/155(0%)	Plus/Plus

Query	1	CTCGTAGTTGAACCTTGGGCGCTGGCTGGCCGGTCTGCCTCACCGCATGTACTGGTTCGGC	60
Sbjct	604	CTCGTAGTTGAACCTTGGGCGCTGGCTGGCCGGTCTGCCTCACCGCATGTACTGGTTCGGC	663
Query	61	CGGGTCTTTCTCCTCTGGGGATCCGCATGCCCTTCACTGGGTGTGTTGGGGAAACCAGGACT	120
Sbjct	664	CGGGTCTTTCTCCTCTGGGGATCCGCATGCCCTTCACTGGGTGTGTTGGGGAAACCAGGACT	723
Query	121	TTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC	155
Sbjct	724	TTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC	758

## Follow-up Studies of Citrus Fruit in Groves

❖ Three harvests of Hamlin and three harvests of Valencia fruit



❖ To confirm *Diplodia* invasion of on-tree fruit

- qPCR quantification of *Diplodia* and CLas in abscission zone tissue
- *Diplodia* isolation
- Electron Microscopy
- Fruit decay assay

❖ To study the impact on pre-harvest fruit drop

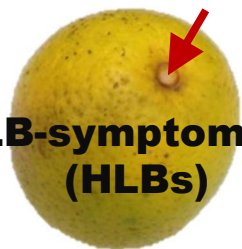
- Fruit Detachment Force (FDF) measurement
- Fruit ethylene production measurement

**Asymptomatic (AS)**

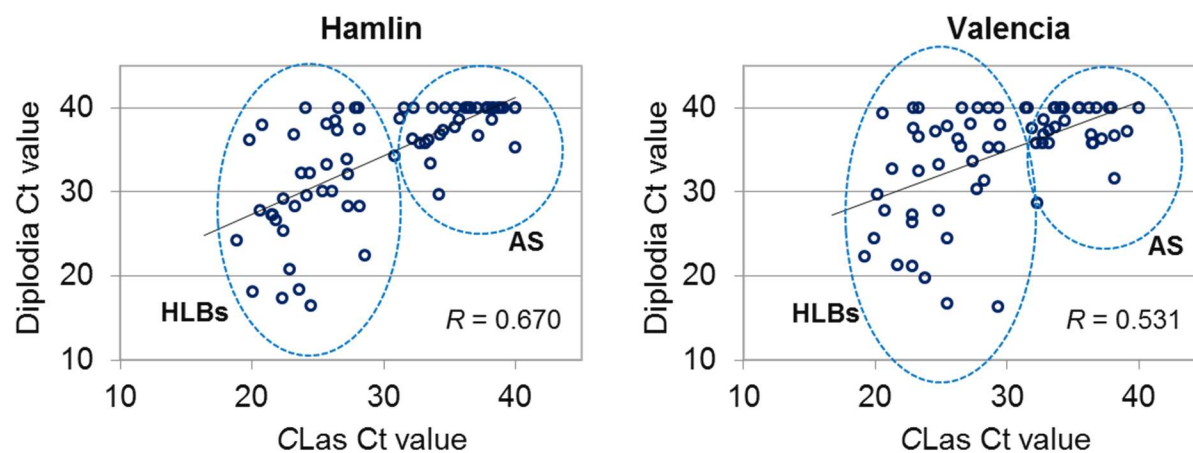


**abscission zone**

**HLB-symptomatic (HLBs)**



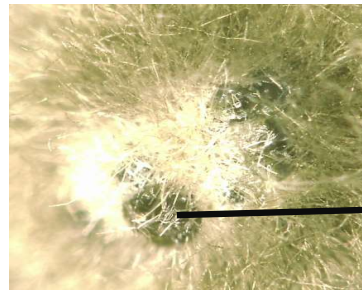
## qPCR results



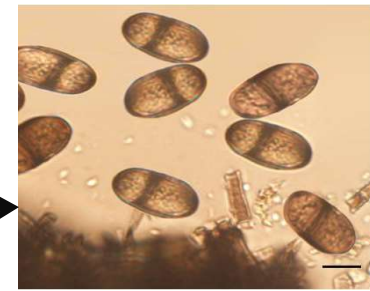
- ❖ Higher Diplodia titers in HLB-symptomatic (HLBs) than in asymptomatic (AS) fruit
- ❖ Positive correlation between Diplodia level and CLas level

❖ **Diplodia was isolated from abscission zone of HLB-symptomatic fruit**

Identity was validated  
-- by PCR  
-- morphology of spore

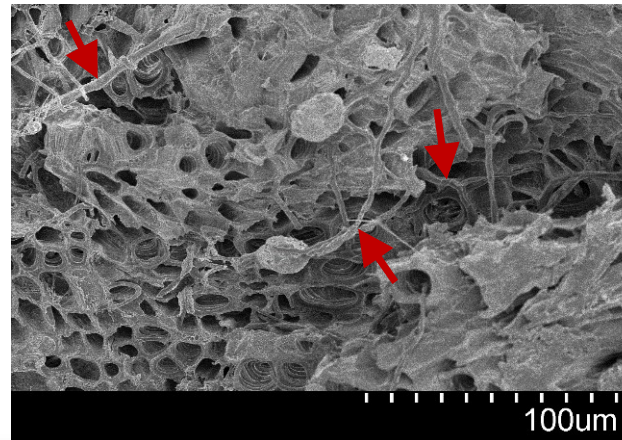


Spore-bearing body (pycnidium)

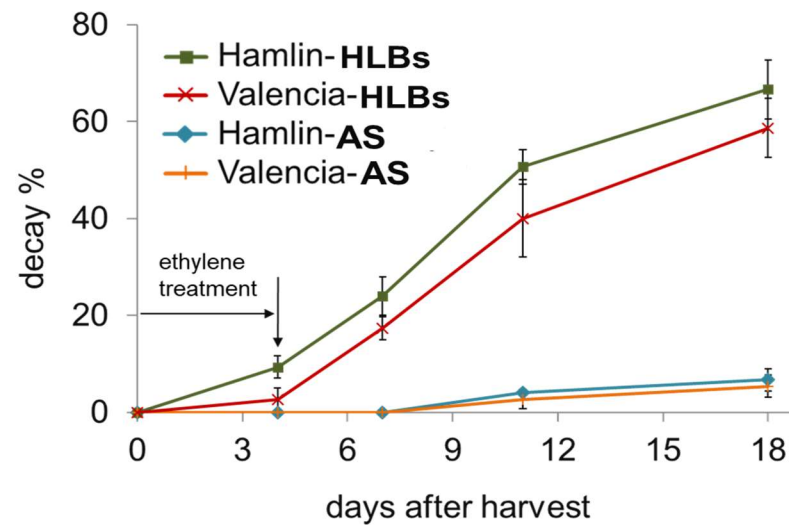


Spores (conidia)

❖ **Fungal hyphae were observed in abscission zone of HLB-symptomatic fruit**



## ❖ *Diplodia* SER decay incidence was higher in HLBs than in AS fruit

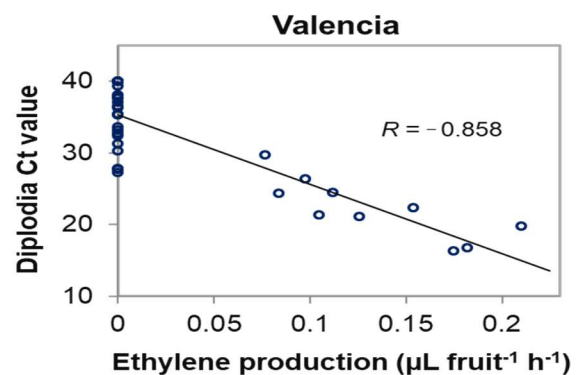
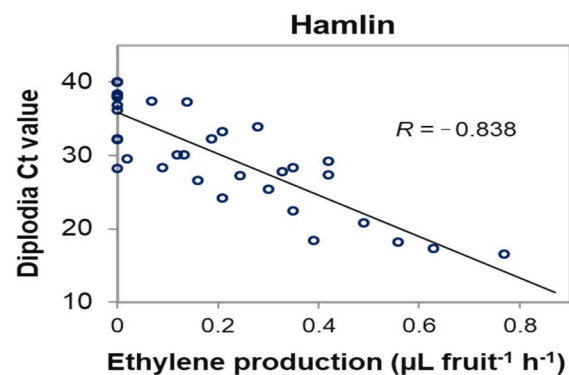


- After harvest, the fruit were treated in 27.8°C, and 90%–95% relative humidity, with 10 ppm ethylene for 4 d (ethylene degreening treatment).
- Then fruit were transferred to air storage for up to two weeks

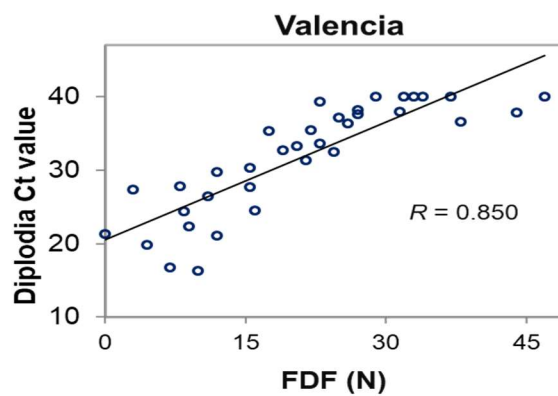
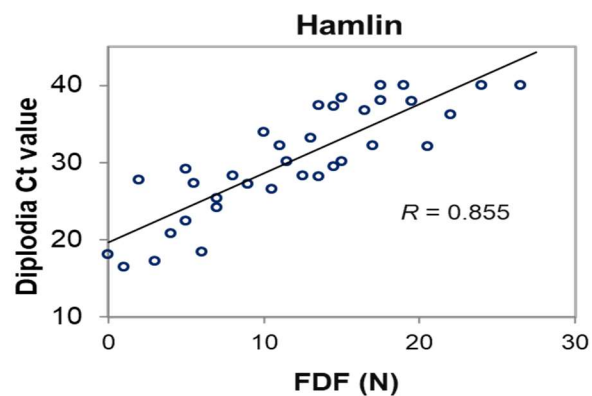
## Pre-harvest SER decay in HLB-affected groves



➤ **Diplodia positively correlated with fruit ethylene production**



➤ **Negatively correlated with fruit detachment force (FDF)**

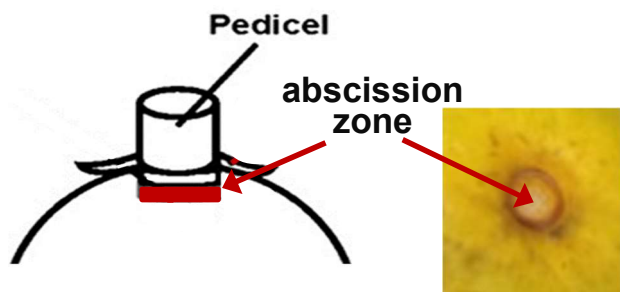
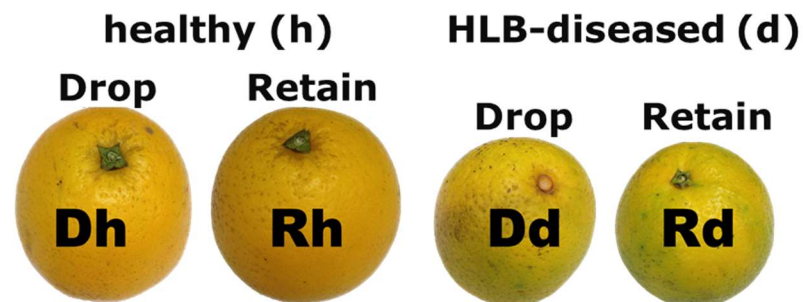


# Fruit Drop Experiment



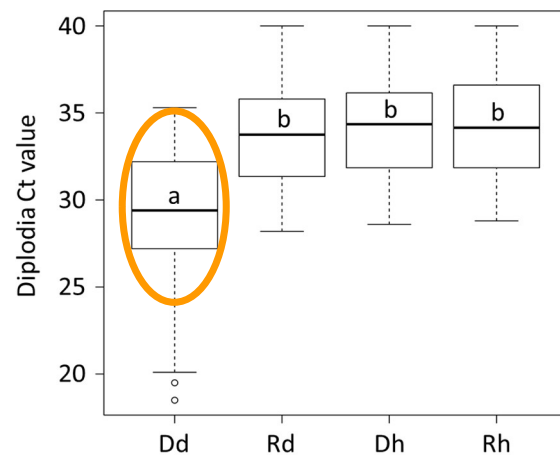
1) Identify Healthy and HLB-diseased trees (qPCR)

2) Shake tree  Fruit retained on tree  
Fruit dropped on ground

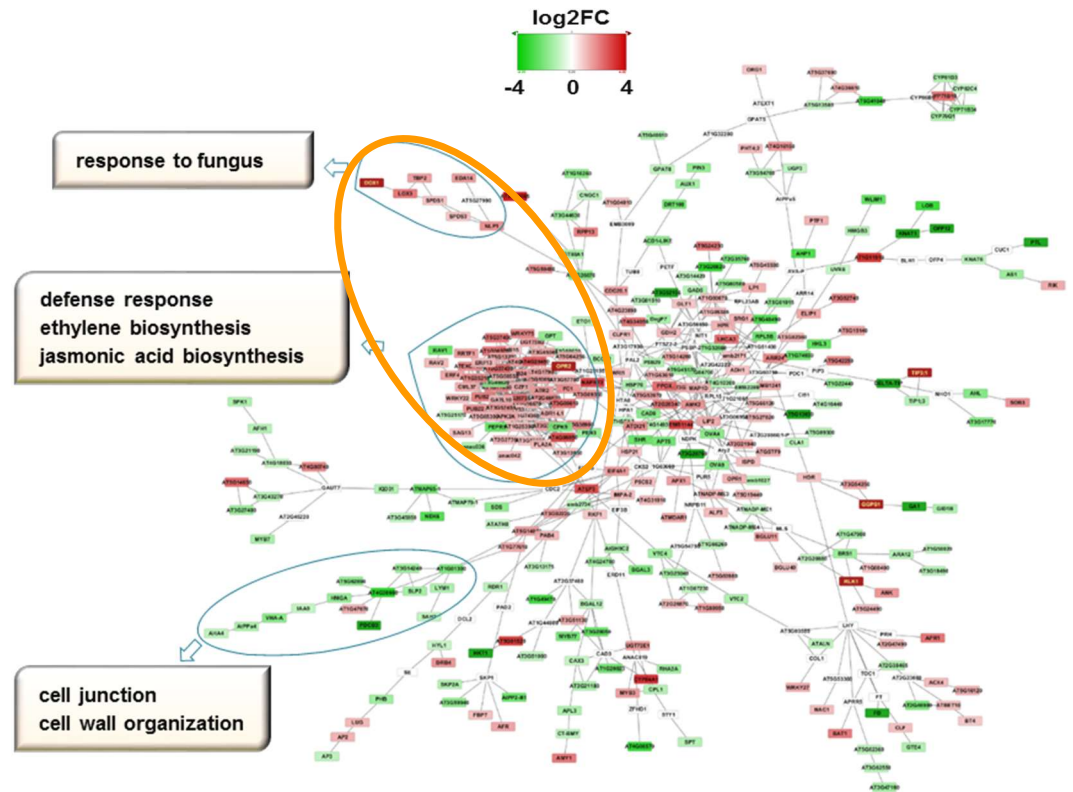


- Diplodia qPCR quantification
- RNA-sequencing analysis

- ❖ Diplodia titer in fruit dropped from HLB-diseased trees was significantly higher



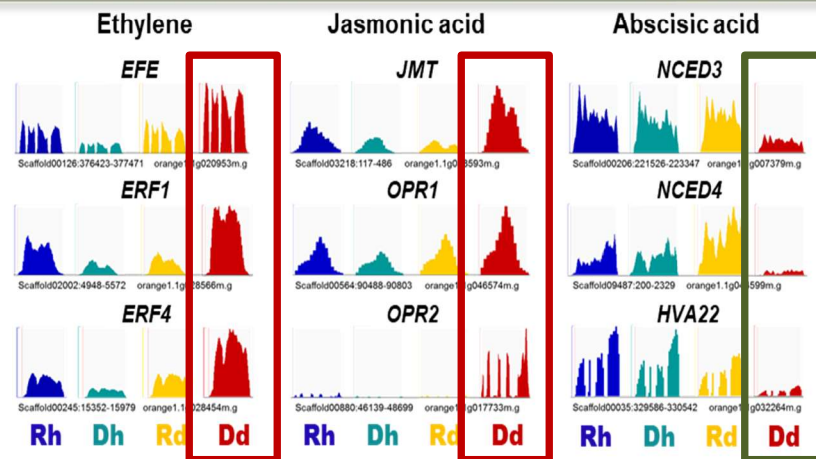
- ❖ A large number of fungus-induced defense response genes were up-regulated



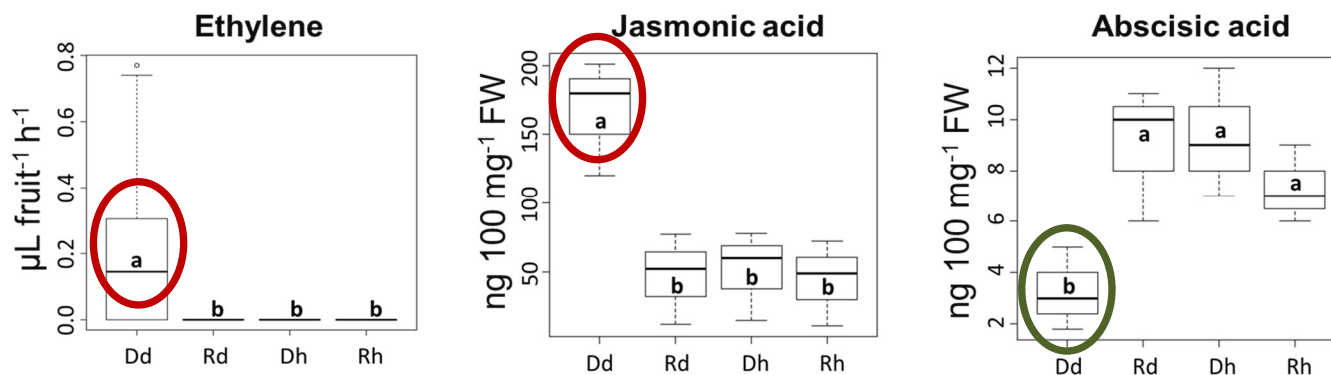
RNA-sequencing data viewed in Protein-protein interaction network

- ❖ Ethylene and jasmonic acid were up-regulated
- ❖ Absciscic acid was down-regulated

➤ Gene Expression data



➤ GC-MS data



# Fungicide (Quadris TOP) Spray Trial

- **Five citrus cultivars:** Navel, Early Gold, and Midsweet orange, Murcott tangerine, and Ruby Red Grapefruit
  - 20 trees for each cultivar. 10 treated, 10 as control.
- **Quadris TOP Fungicide** spray: 4 times (15.4 oz/35 gal of water)
  - 4/4/14, 5/30/14, 8/29/14, and 10/31/14 (for Navel and Early Gold)
  - 4/4/14, 5/30/14, 9/19/14, and 12/12/14 (for Midsweet, Murcott and Ruby Red)
- Fruit sampled 10-14 days after each spray for Diplodia titer analysis
- Fruit drop count
  - once about every three weeks
  - started from 9/11/14 (for Navel and Early Gold) and 10/2/14 (for the other three)

Cleared ground before each count cycle



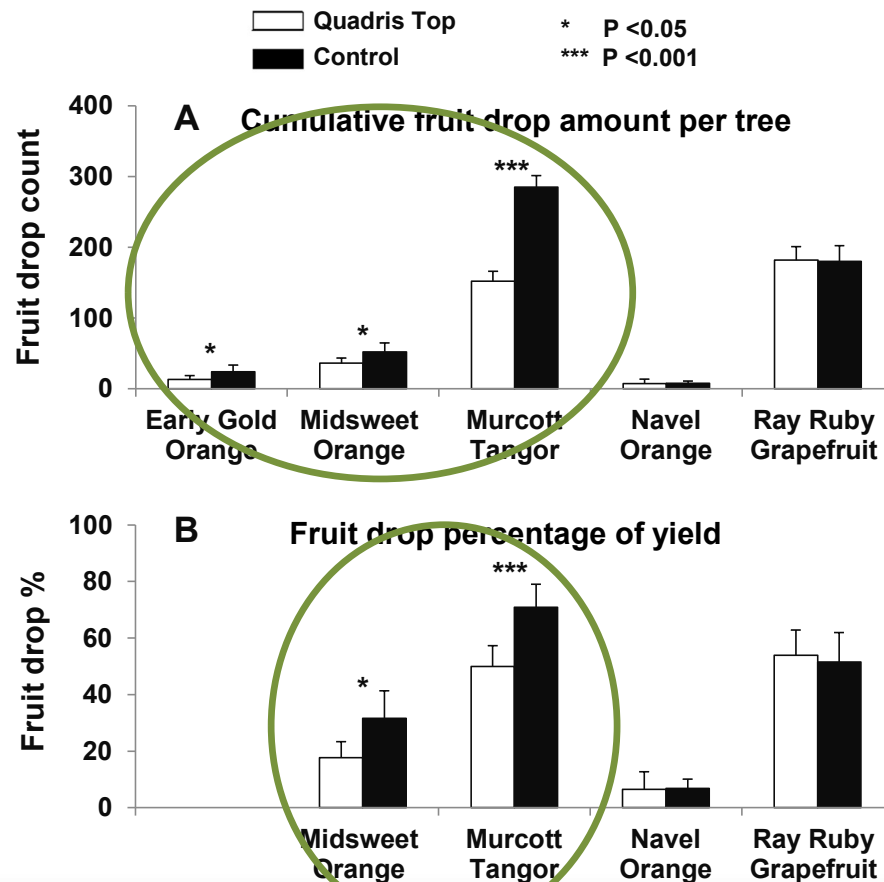
Count dropped fruit



# Cumulative fruit drop count and drop percentage during the maturation season

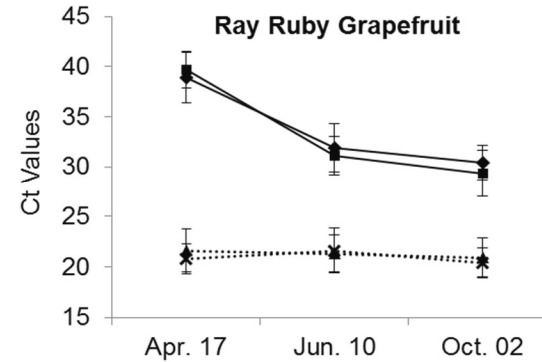
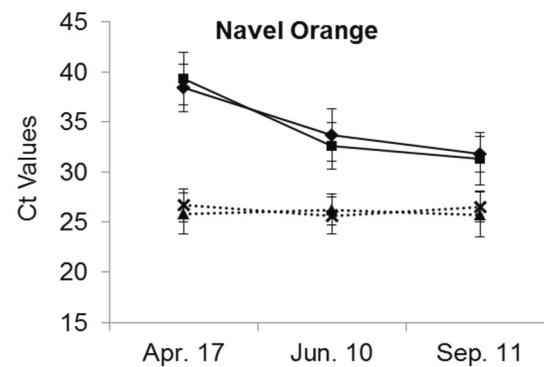
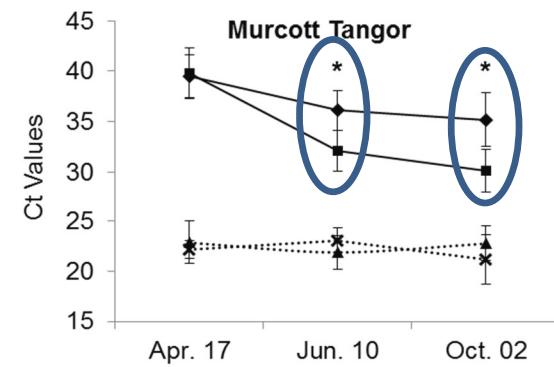
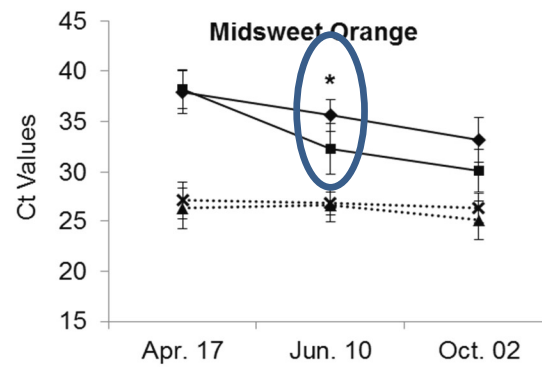
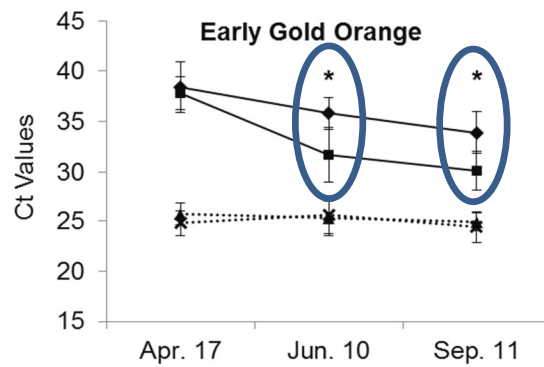
## Quadris TOP treatment

- reduced fruit drop significantly for
  - 'Early Gold' (45%)
  - 'Midsweet' (30%)
  - 'Murcott' (46%)
 during the maturation season
- No effect on fruit drop for 'Navel' or 'Ray Ruby'



## qPCR data

- Reduced Diplodia infection in 'Early Gold', 'Midsweet' and 'Murcott'
- Correlation of reduction in fruit drop with the reduction in Diplodia infection



### Legends

- ◆ Diplodia-Quadris Top
- Diplodia-Control
- ...▲ CLas-Quadris Top
- ...× CLas-Control
- \*  $p < 0.05$

## **The possible reasons why Quadris TOP worked better on some of the cultivars than others**

- Related to developmental stages of the fruit or fruitlet
- The scheduling of fungicide sprays might work better for some cultivars than for others
- Density of the tree canopy may influence the easiness of the fungicide to reach the target tissue

# Summary

- Diplodia pre-harvest invasion of HLB-affected citrus fruit is common.
- Diplodia invasion adds biotic stress and exacerbates fruit drop by causing fruit to produce abscission hormones (ethylene and jasmonic acid).
- Fungicide application may facilitate the control of excessive pre-harvest fruit drop  
--- needs optimization
- Removing dead branches timely to reduce the Diplodia population in the environment would also help

# *Thank You !*



**[wei.zhao@usda.gov](mailto:wei.zhao@usda.gov)**

