The effect of HLB on pre-harvest development of Diplodia stem-end rot

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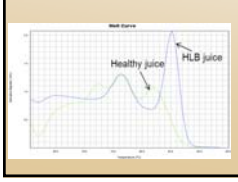
Stem-end rot of Florida Citrus

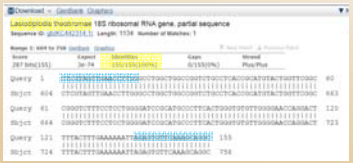
- Diplodia stem-end rot: by *Diplodia natalensis* (Syns. *Lasiodiplodia theobromae*)
- Phomopsis stem-end: by *Phomopsis citri*
- Alternaria stem-end rot: by *Alternaria citri*

- Diplodia infects citrus fruit under the calyx
  --- usually remains latent until after harvest
  --- invades from the calyx abscission zone into fruit
- The decay rate is greatly enhanced by exposure to ethylene

High incidence of Diplodia DNA was found in HLB-affected orange juice

- **DNA from a fungus consistently present in HLB-affected juice, but not in juice from healthy fruit.**
- **By DNA sequencing, the fungus was identified—it was Diplodia**
- **The finding was confirmed by Diplodia specific primers.**





Studies conducted on citrus fruit harvested from trees


Asymptomatic (AS)

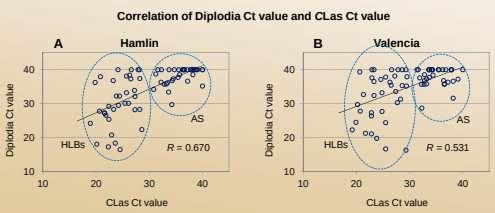

HLB-symptomatic (HLBs)

- PCR detection for Diplodia and *C*las
- Fruit Detachment Force (FDF) measurement
- Diplodia isolation
- Electron Microscopy
- Fruit ethylene production measurement
- Fruit decay assay

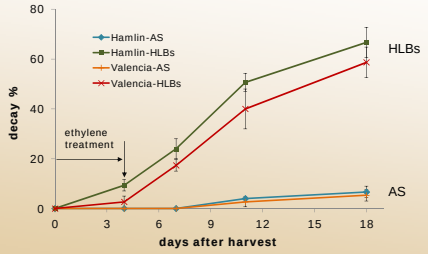
AS and HLBs fruit were compared in the assays

Higher incidence of Diplodia was found in HLB-symptomatic than non-symptomatic fruit

- Positive correlations between Diplodia and *C*Las titers for both cultivars
- Significantly higher Diplodia titers in HLBs than in AS fruit (P<0.001)



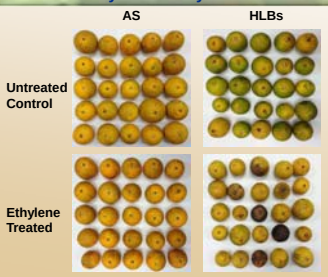
decay incidence of orange fruit during ethylene treatment and subsequent air storage



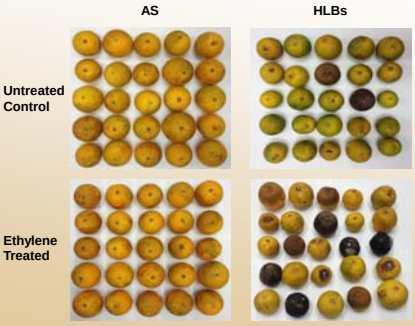
- After harvest, the fruits were treated in 27.8°C, and 90%–95% relative humidity, with 10 ppm ethylene for 4 d (ethylene degreening treatment).
- Then fruits were transferred to air storage for up to another two weeks

Before ethylene treatment

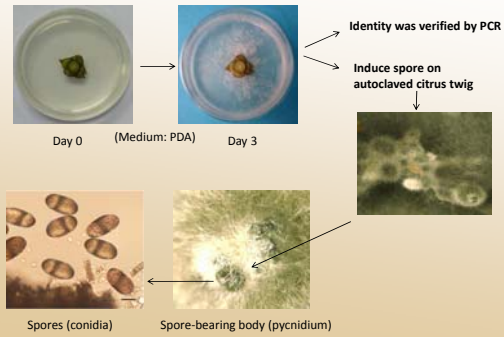
3 days after ethylene treatment



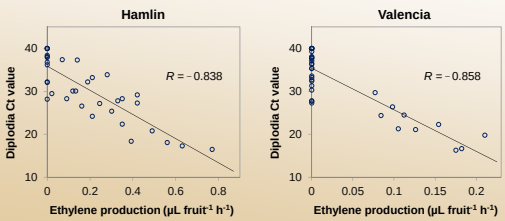
7 days after ethylene treatment



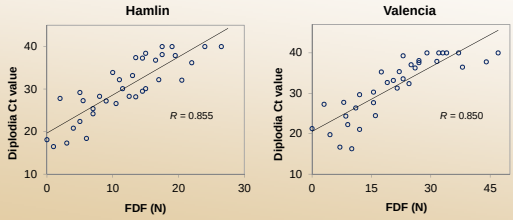
Isolation and identification of Diplodia from HLBs fruit



Correlation of Diplodia Ct value and ethylene production in HLB-symptomatic fruit

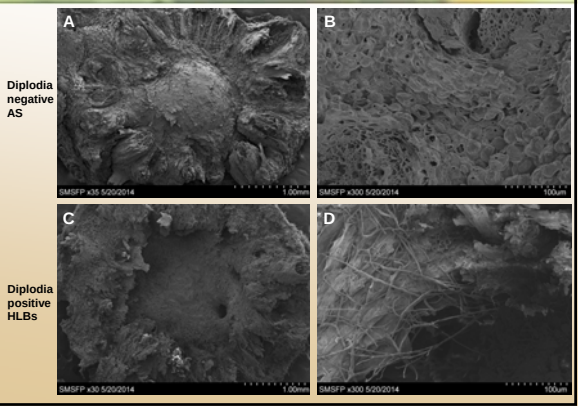


Correlation of Diplodia Ct value and FDF in HLB-symptomatic fruit



Fruit Detachment Force (FDF)
= the pulling force required for detaching fruit from the stem

Diplodia invasion of fruit abscission zone observed under an SEM



Increased on-tree decay of fruit on the HLB-affected trees

Many of the on-tree decay were caused by Diplodia

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Summary

HLB leads the infected citrus under a great biotic stress

Diplodia pre-harvest invasion of HLB-affected fruit

Consequences

- Pre-harvest Diplodia SER is not unusual
- Pre-harvest fruit drop is exacerbated
- Post-harvest fruit decay is increased
- ❑ Contribute to HLB-related off-flavor of orange juice ??
  ---Need study to know

❖ **Diplodia management should be enhanced and applied pre-harvest**

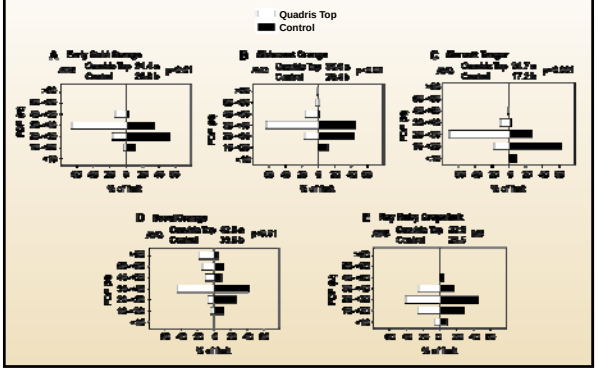
Fungicide spray trial

- **Five citrus cultivars**:
  --Navel Orange
  --Early Gold Orange
  --Midsweet Orange
  --Murcott Tangor
  --Ruby Red Grapefruit
- **20 trees used for each cultivar**
- **Quadris Top Fungicide: four times (15.4 oz/35 gal of water)**

The schedules:

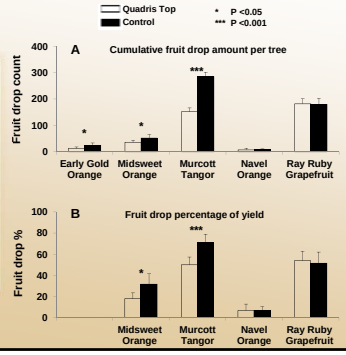
	Dates applied fungicide (2014)				Dates sampled for qPCR (2014)			Dates measured FDF (2014)		Dates evaluated fruit drop (2014-2015)			
	#1	#2	#3	#4	#1	#2	#3	#1	#2	#1 (2014)	#2 (2014)	#3 (2014)	#4 (2014-2015)
Early Gold Orange	4/4	5/30	8/29	10/31	4/17	6/10	9/11	9/11	10/30	9/11-10/2	10/2-10/30	10/30-11/12	--
Navel Orange	4/4	5/30	8/29	10/31	4/17	6/10	9/11	9/11	10/30	9/11-10/2	10/2-10/30	10/30-11/12	--
Midsweet Orange	4/4	5/30	9/19	12/12	4/17	6/10	10/2	10/2	11/24	10/2-10/30	10/30-11/24	11/24-12/22	12/22/14-1/16/15
Murcott Tangor	4/4	5/30	9/19	12/12	4/17	6/10	10/2	10/2	11/24	10/2-10/30	10/30-11/24	11/24-12/22	12/22/14-1/16/15
Ray Ruby Grapefruit	4/4	5/30	9/19	12/12	4/17	6/10	10/2	10/2	11/24	10/2-10/30	10/30-11/24	11/24-12/22	12/22/14-1/16/15

Average (AVG) and Distribution of Fruit Detachment Force (FDF)



Cumulative fruit drop count and drop percentage during the maturation season

- 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'



Conclusion from the field trial

- Fungicide foliar application may be an effective strategy in control of HLB-related fruit drop for some citrus types and cultivars
- More work needs to be done to confirm these results
- Fungicide resistance and cost-benefit ratios need to be considered also