Tale of two diseases: HLB and secondary infection by the fungus *Lasiodiplodia theobromae* (Diplodia)

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&

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HLB and Diplodia Diseases

- HLB causes off-flavor in orange fruit and juice due to decreased sugars, sometimes increased acids and increased bitter limonoids and astringent flavonoids – effects are worse earlier in the harvest season especially for Hamlin compared to Valencia
- HLB causes pre-harvest fruit drop, sometimes up to 30 % of the crop
- When looking for *Candidatus* liberibacter asiaticus (*C*las) DNA in OJ found also fungal DNA in HLB fruit juice but not in healthy juice – was *Lasiodiplodia theobromae* (Diplodia)
- Subsequently found that the fungus, Diplodia, normally a postharvest pathogen causing SER, was causing more SER on HLB fruit than healthy postharvest and later was found to be infecting fruit pre-harvest on the tree and may be contributing to fruit abscission
- Question: Do fruit that are loose on the tree (partial abscission zone formation) have different chemical and flavor properties?

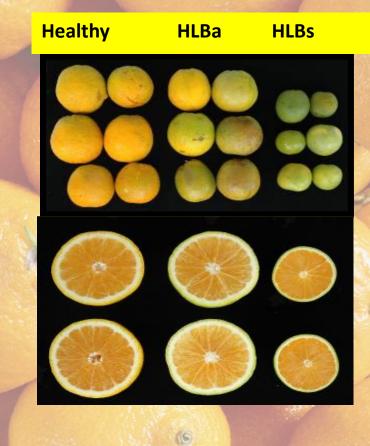




Diplodia PH SER







Diplodia Stem End Rot - 7 days after ethylene treatment

Healthy

HLBs

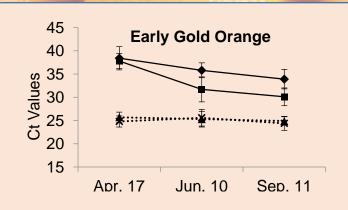


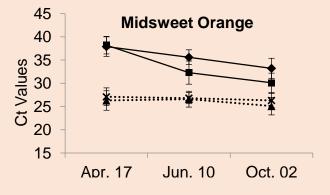
Untreated Control

Ethylene Treated

Diplodia titer – trees sprayed with Quadris Top

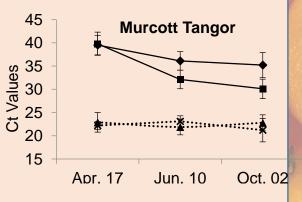
Tim Gottwald and Greg McCollum

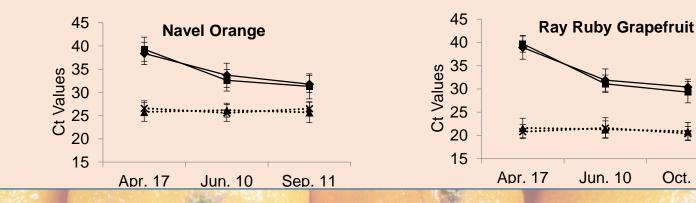




Jun. 10

Oct. 02



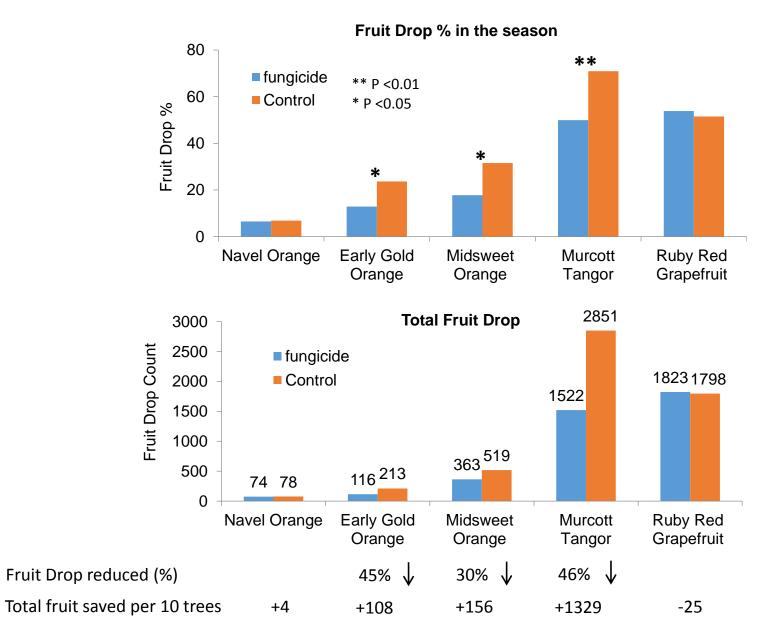




---- Diplodia-Quadris Top — Diplodia-Control CLas-Quadris Top CLas-Control

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Effects of Fungicide QUADRIS Spray



Fruit drop experiment

Shake trees – collect dropped fruit

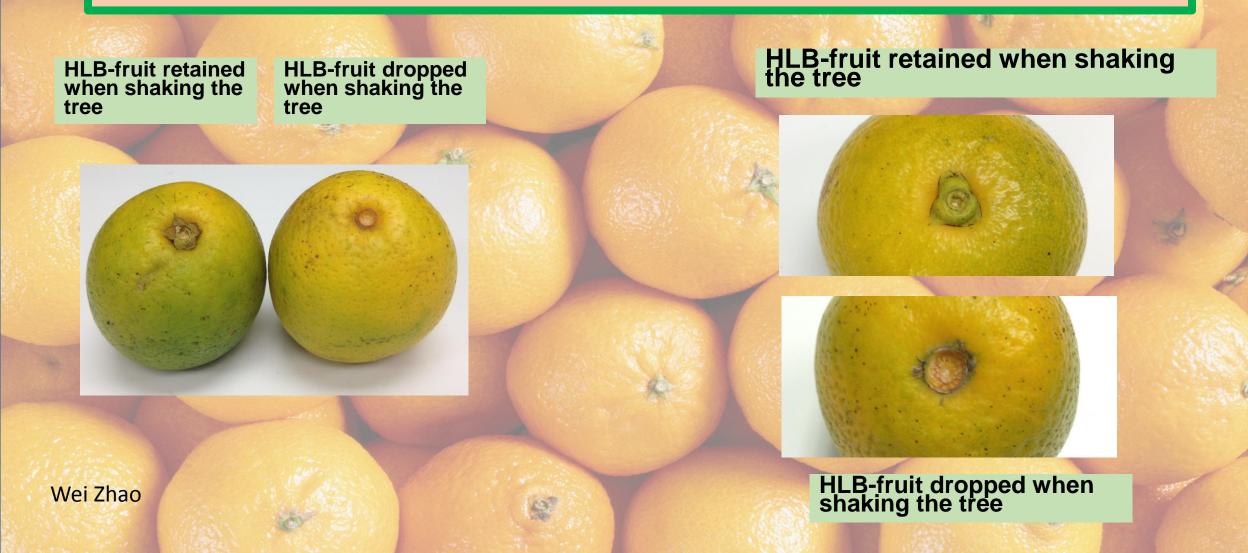
- Healthy trees not shaken
- Healthy trees shaken
 - Collected the fruit that fell off
 - Harvested fruit that remained on the tree

Harvest fruit that remain on the trees after shaking

- HLB trees shaken
 - Collected the fruit that fell off
 - Harvested the fruit that remained on tree

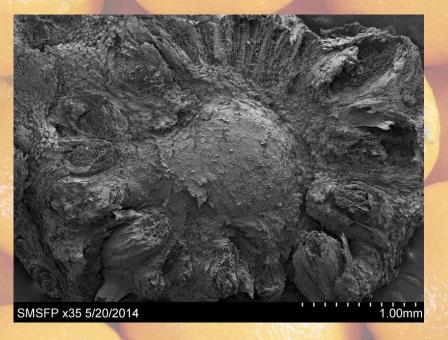
Hamlin, December (2014), January (2015); Valencia, April (2015)

Difference in calyx abscission zone of HLB-affected fruit between the dropped and retained fruit when shaking the tree



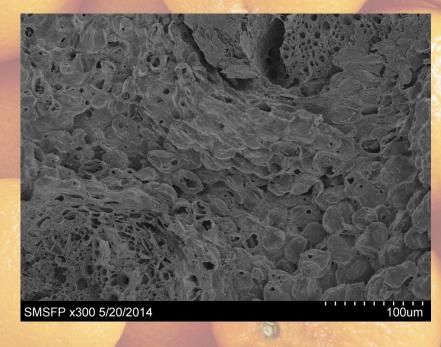
Diplodia negative





SMSEP x30 5/20/2014

Con the second





1.00mm

100um

Wei Zhao

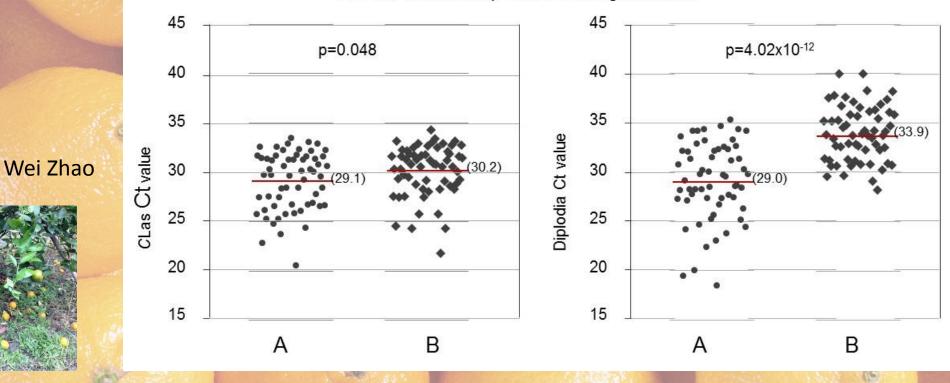
Three studies conducted

- Chemical, biochemical and physical analyses of juice from fruit firmly or loosely attached to the tree for healthy and HLB-affected trees (Liz Baldwin)
- Sensory analyses for juice from fruit firmly or loosely attached to the tree for healthy and HLB-affected trees (Anne Plotto)
- qPCR and RNA seq analysis of abscission zones from fruit firmly or loosely attached to the tree for healthy and HLBaffected trees (Wei Zhao)

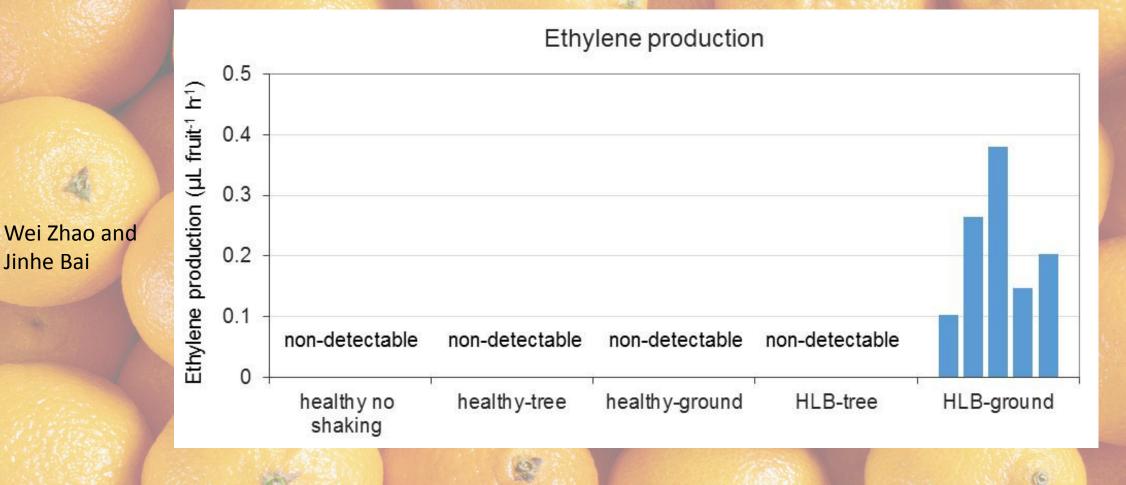
qPCR analysis of CLas and Diplodia

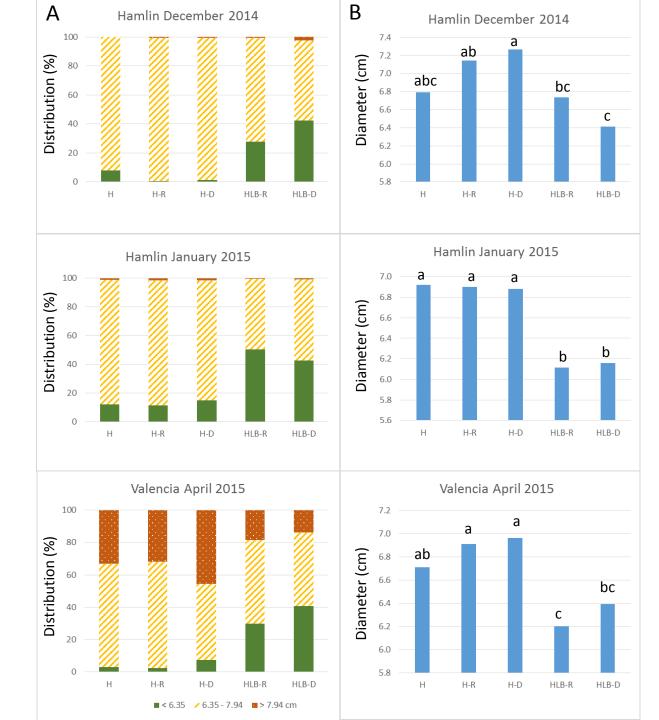
Comparison of CLas and Diplodia Ct values between dropped and retained fruit

A: Fruit dropped when shaking the trees B: Fruit did not drop when shaking the trees



Ethylene production





Chemical heat maps Content Low

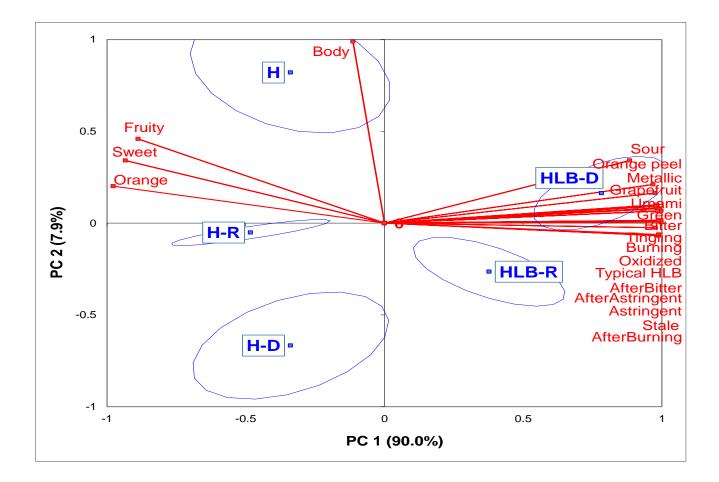
High

Hamlin December and January											Valencia Ap											ril 🥢 🔗					
Compo	ound/attribute	Healthy	Healthy retain	Healthy drop	HLB retain	HLB drop		Compound/attribute	Healthy	Healthy retain	Healthy drop	HLB retain	HLB drop			Compound/attribute	Healthy	Healthy retain	Healthy drop	HLB retain HLB drop		Compound/attribute	Healthy	Healthy retain	Healthy drop	HLB retain	HLB drop
THE SECONDARY METABOLITES								VOLATI	VOLATILES							THE SECONDARY METABOLITES				VOLATILES							
Vicenir	n2							Decanal								Limonin glucoside						Valencene					
Narirut	tin							Ethyl 3-hydroxyhexanoate						/		Nomilinic acid glucoside						Ethyl 3-hydroxyhexanoate					
Heptar	nethoxyflavone							Acetone							1	Vicenin2						Decanal					
Tanger	etin							cis-3-Hexenol								Diosmin						cis-3-Hexenol		_			
Diosmi	in							Octanol								Hesperidin						2-Methypropanol					
Hesper								Ethyl butanoate							5 A 1	Isosakuranetin				_		trans-2-Hexenol					
	uranetin							Ethyl acetate							100.00	Narirutin						Ethyl butanoate					
	nic acid glucoside							Ethyl hexanoate	_				_		325	Sinensetin						Ethyl hexanoate					
(C)-1	n glucoside							Ethanol								Tangeretin		_				γ-Terpinene					
Sinens								Linalool								Nobiletin						Ethyl acetate		_			
Nobile				_	_			Acetaldehyde							10 A 10	Heptamethoxyflavone		_				Ethanol					
Limoni							$\mathbf{>}$	2-Methypropanol	_					10	1000	Limonin						Octanal				-/	
Nomilin						Octanal								Nomilin						Acetone							
						Sabinene							SUGARS AND ACITS					Terpinen-4-ol									
pН					/_			Valencene					_		100	SS/TA		_			<u> </u>	Octanol		_			
Sucrose								α-Terpineol							202	pH			_			Myrcene	_		_		
Malic a	icid							Methyl butanoate					_		137	Sucrose						Methyl butanoate					
SS/TA			_					Myrcene							2010	Malic acid						Acetaldehyde	_	_			
SS								Limonene							6. S	Ascorbic acid						α-Pinene					
Total s	-							α-Pinene					_			Citric acid						Hexanol	_				
	e equivalence							γ-Terpinene	_							SS						Hexanal					
TA								Hexanal								Total sugar						Limonene					
Glucos								Hexanol						1	15	TA						Methanol					
Fructos								Methanol							23.57	Glucose						α-Terpineol					
Citric a								Terpinen-4-ol								Fructose						Sabinene					
Ascorb	ic acid													R	Ŀ	Sucrose equivalence						Linalool					

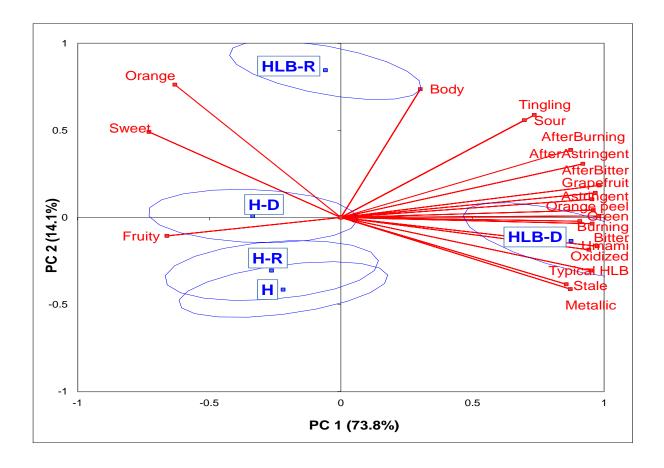
Sensory (Anne Plotto)

- Chemical differences suggest flavor differences
- In fact difference tests showed that panelists could tell the difference between HLB fruit that dropped off the shaken tree and those that remained on the tree for the most part
- Trained panels showed that HLB fruit that dropped were lowest in perception of desirable descriptors and highest in undesirable descriptors - to be described by Anne Plotto in the next talk.

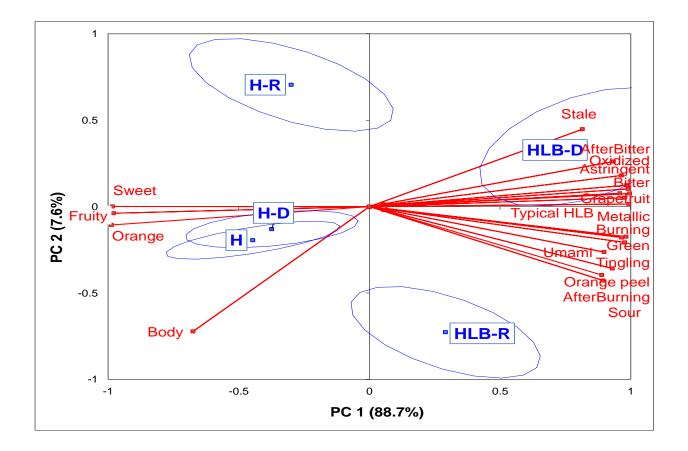
Early Hamlin



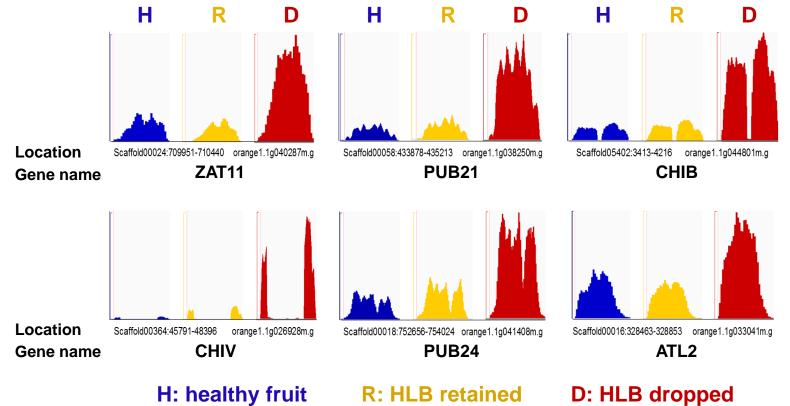
Late Hamlin



Valencia



RNA-seq by Integrative Genomics Viewer showing regulation of genes induced in response to chitin



H: healthy fruit

R: HLB retained

Conclusions

- The HLB fruit that dropped off the tree when the tree was shaken were loose due to a partially formed abscission zone
- The dropped HLB fruit AZs had more CLas and especially Diplodia titer, produced ethylene (fruit ripening and abscission hormone)
- The HLB fruit juice and especially the dropped HLB fruit juice had more bitter limonoids and astringent flavonoids, sometimes lower sugar/acid ratio
- The HLB fruit and especially the dropped HLB fruit juice had less fruity volatiles and more terpenoid volatiles
- So, secondary Diplodia infection is exacerbating fruit drop, SER and off-flavor on fruit loose on HLB-affected trees