

**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* (Clas) 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?

# HLB Disease



Diplodia PH SER



Healthy HLBa HLBs



# Diplodia Stem End Rot - 7 days after ethylene treatment

Healthy

HLBs

Untreated Control

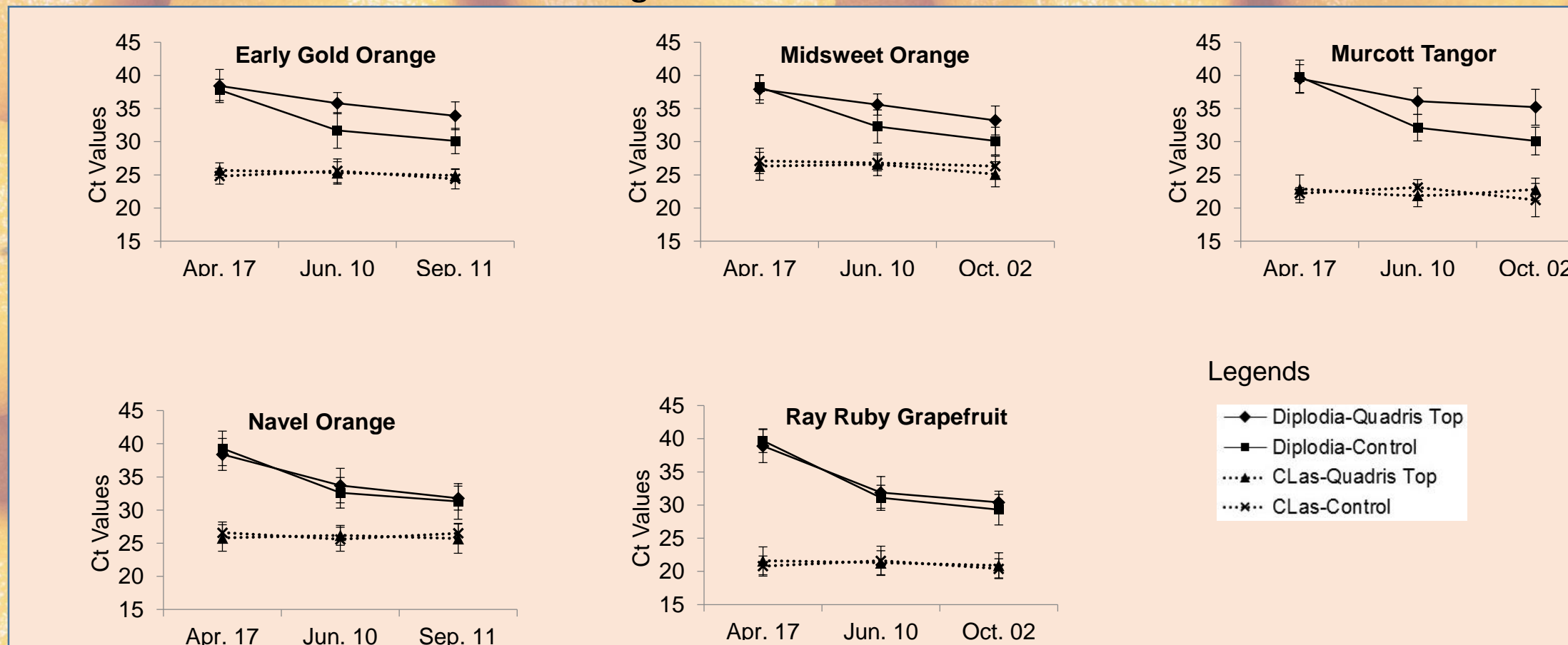


Ethylene Treated



# Diplodia titer – trees sprayed with Quadris Top

Tim Gottwald and Greg McCollum

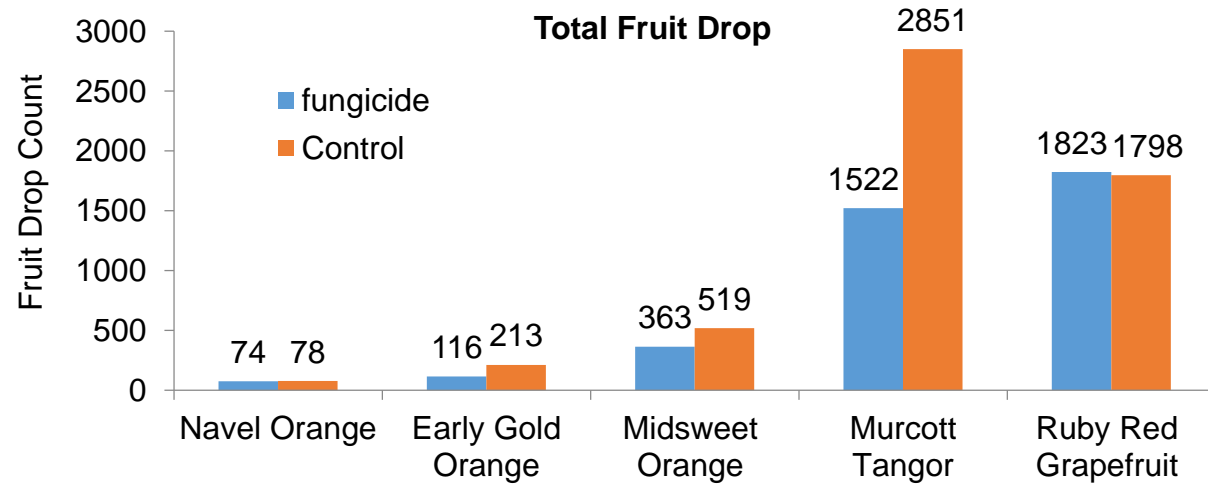
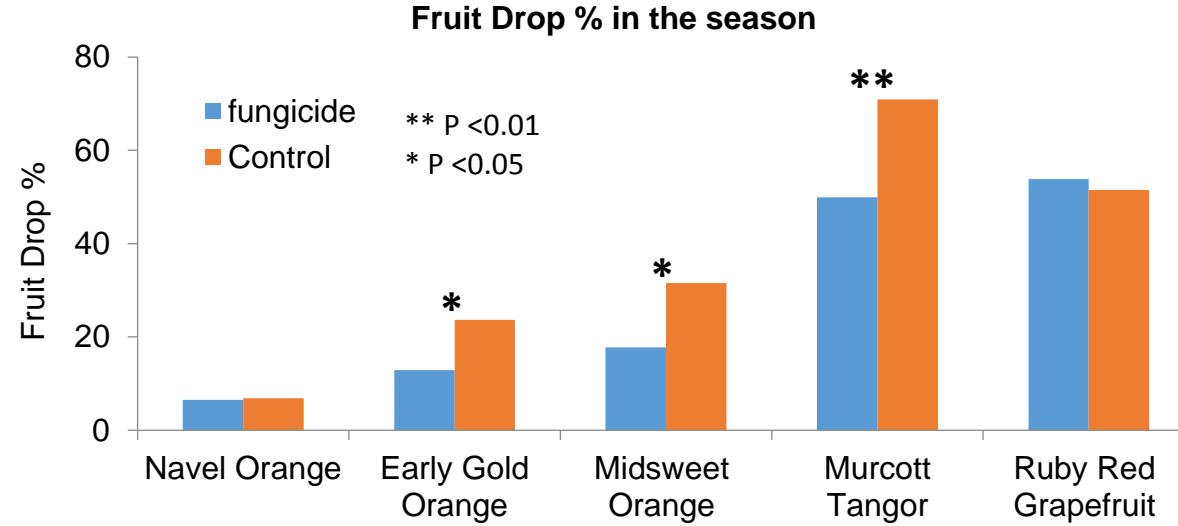


Legends

- ◆ Diplodia-Quadris Top
- Diplodia-Control
- ▲ CLas-Quadris Top
- × CLas-Control



# Effects of Fungicide QUADRIS Spray



Fruit Drop reduced (%)		45% ↓	30% ↓	46% ↓	
Total fruit saved per 10 trees	+4	+108	+156	+1329	-25

# 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

**HLB-fruit retained when shaking the tree**



**HLB-fruit dropped when shaking the tree**

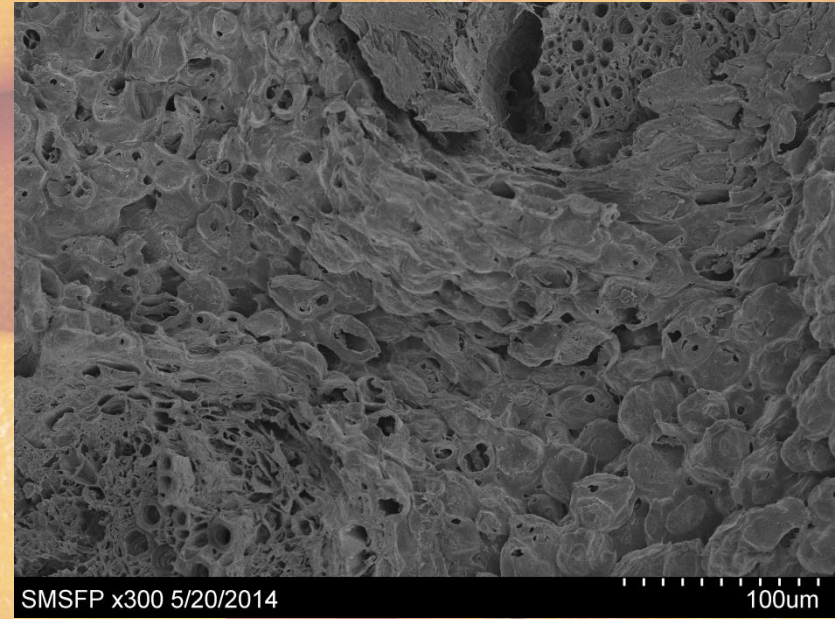
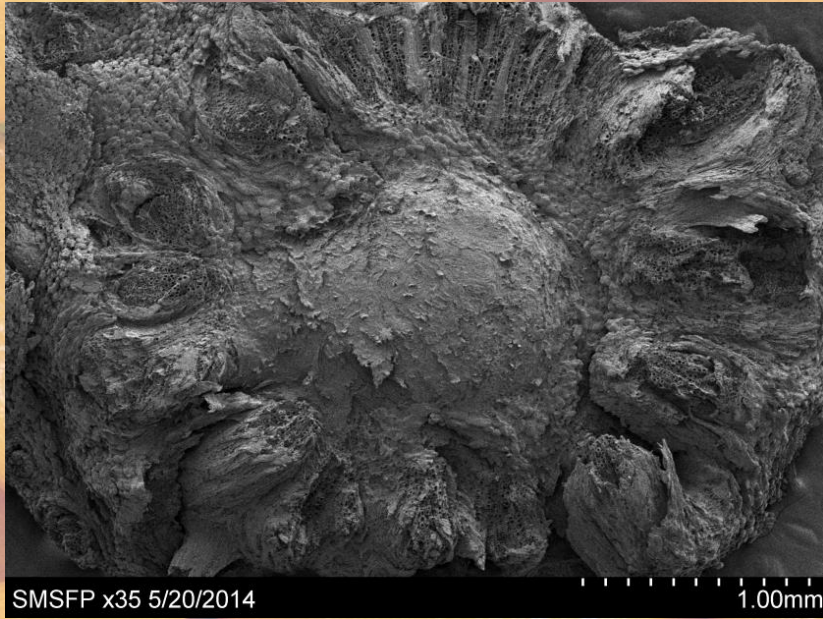
**HLB-fruit retained when shaking the tree**



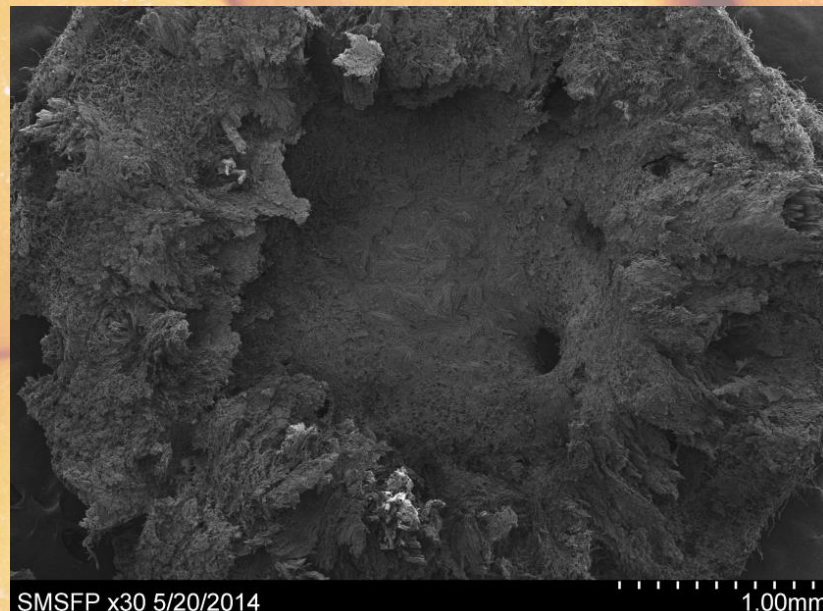
**HLB-fruit dropped when shaking the tree**



Diplodia  
negative



Diplodia  
positive



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 HLB-affected trees (Wei Zhao)

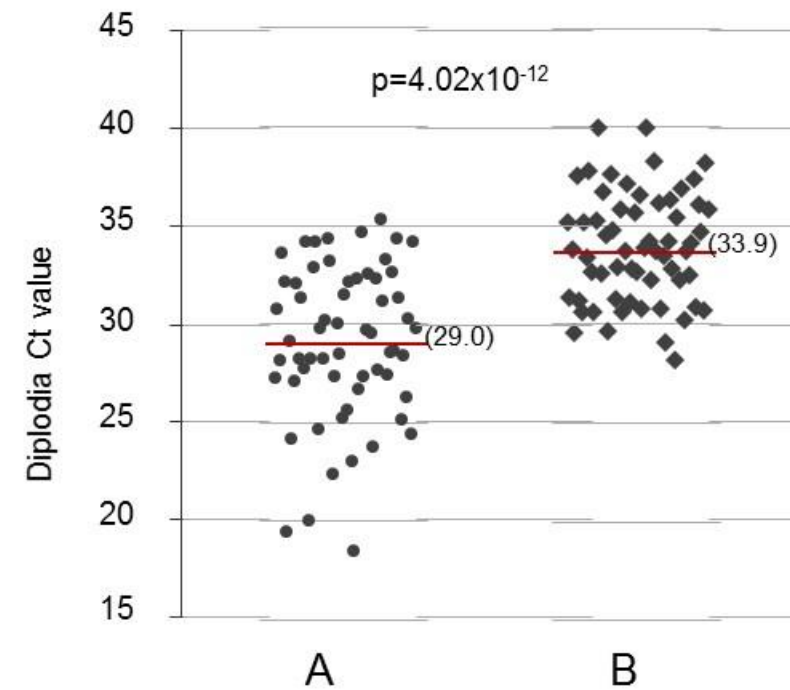
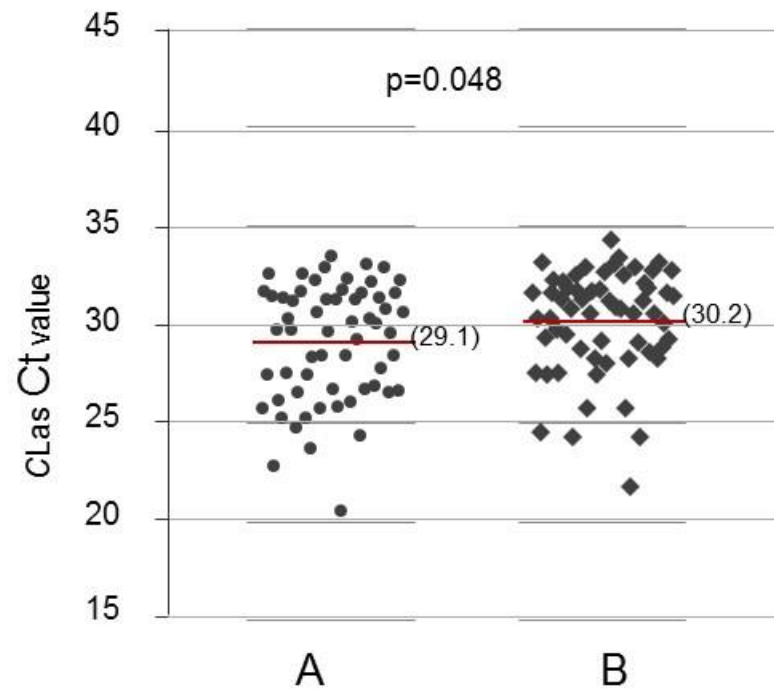


# qPCR analysis of CLas and Diplodia

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

A: Fruit dropped when shaking the trees

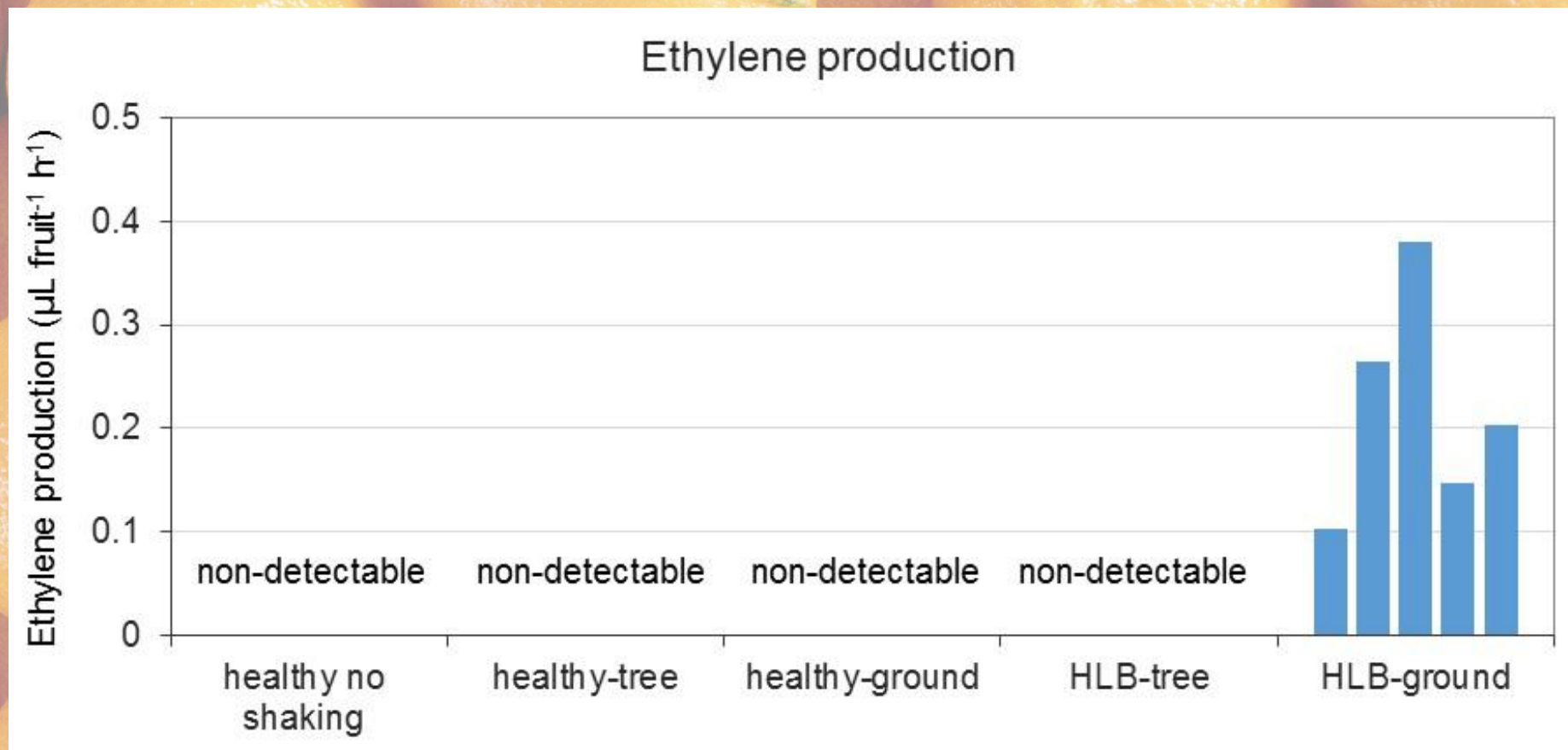
B: Fruit did not drop when shaking the trees



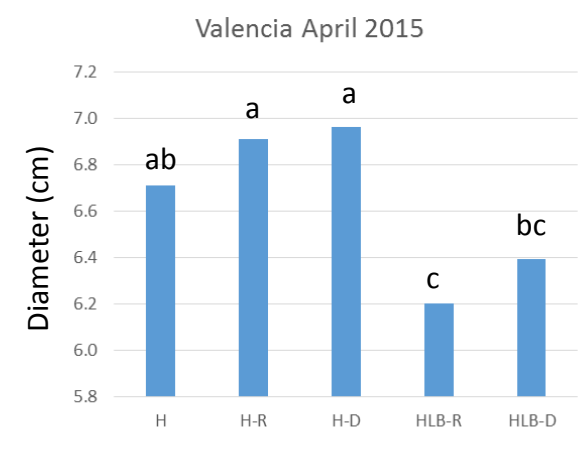
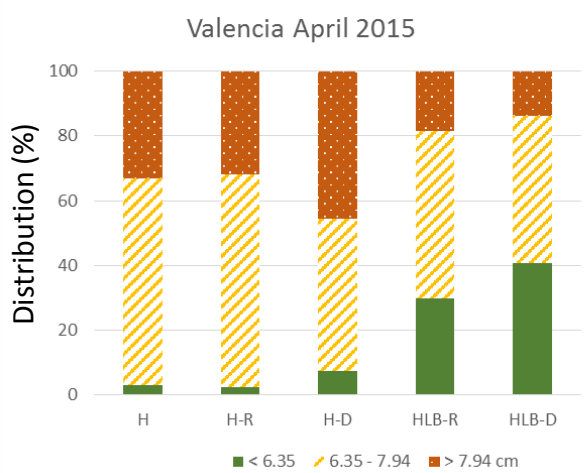
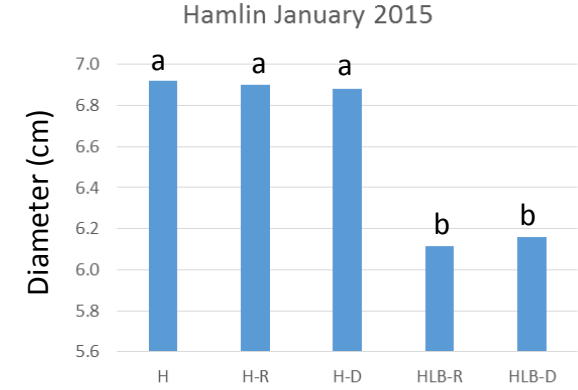
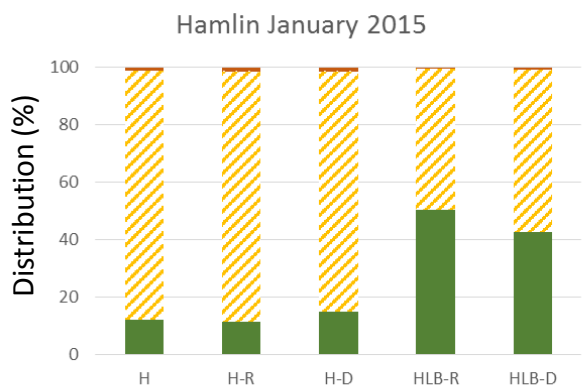
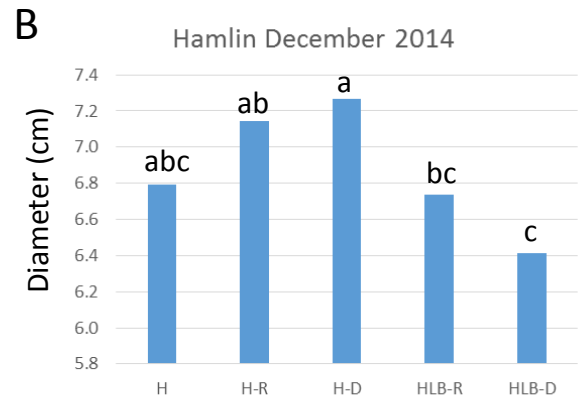
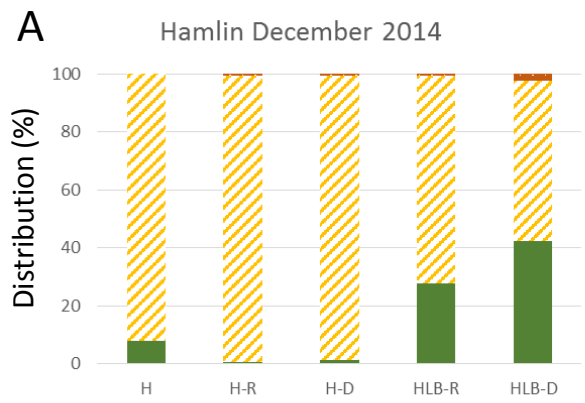
Wei Zhao



# Ethylene production



Wei Zhao and  
Jinhe Bai

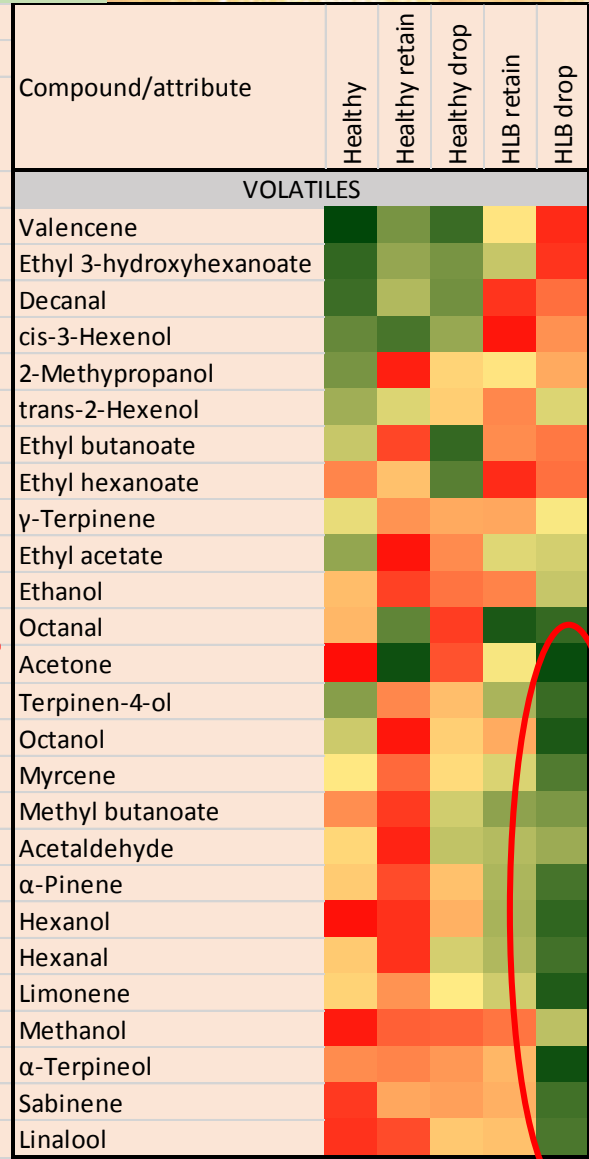
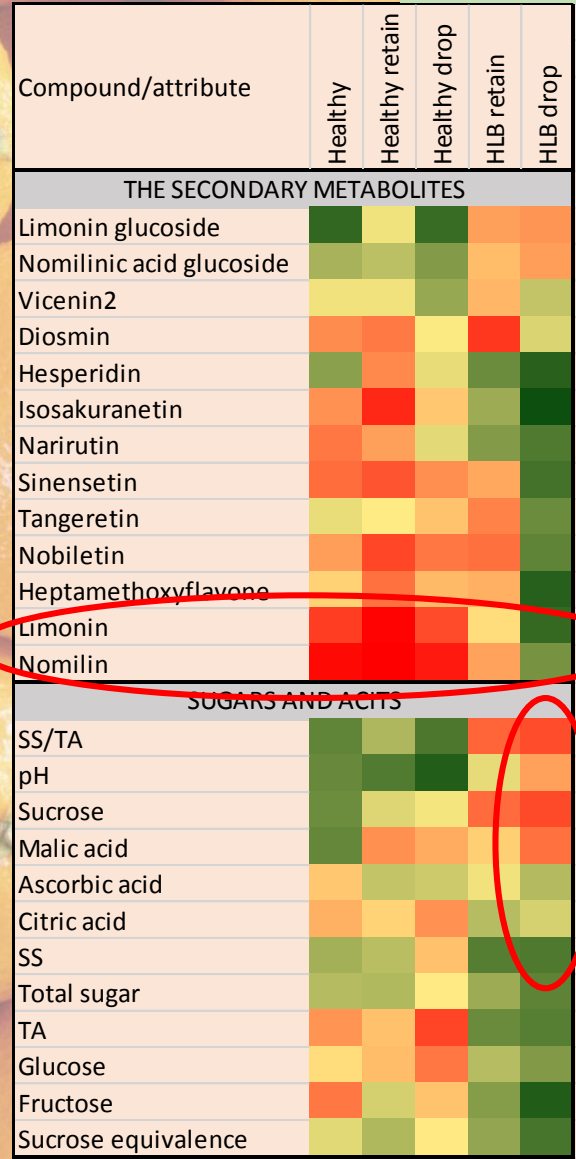
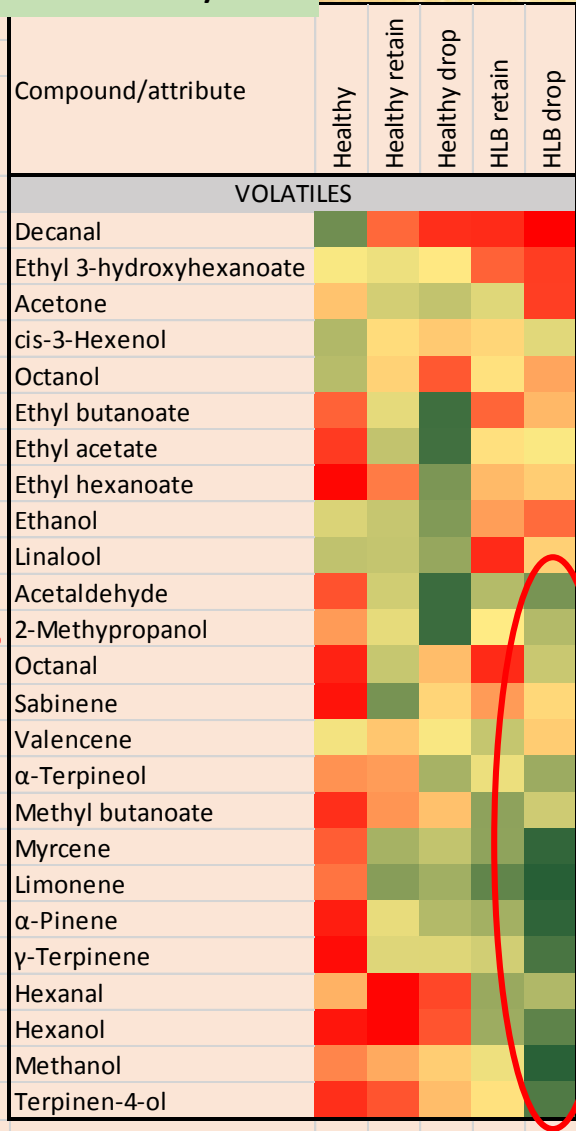
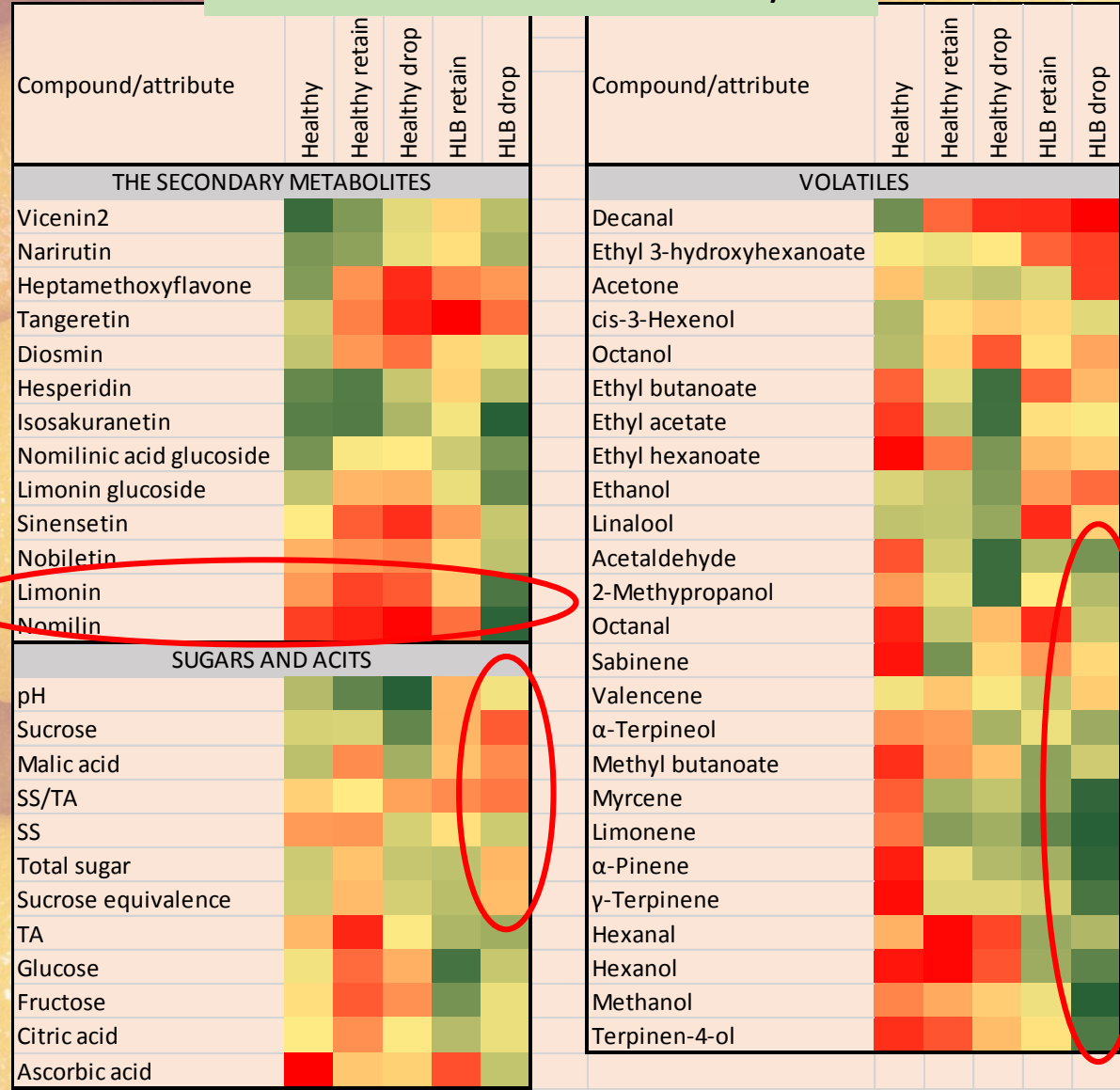


# Chemical heat maps



Hamlin December and January

Valencia April

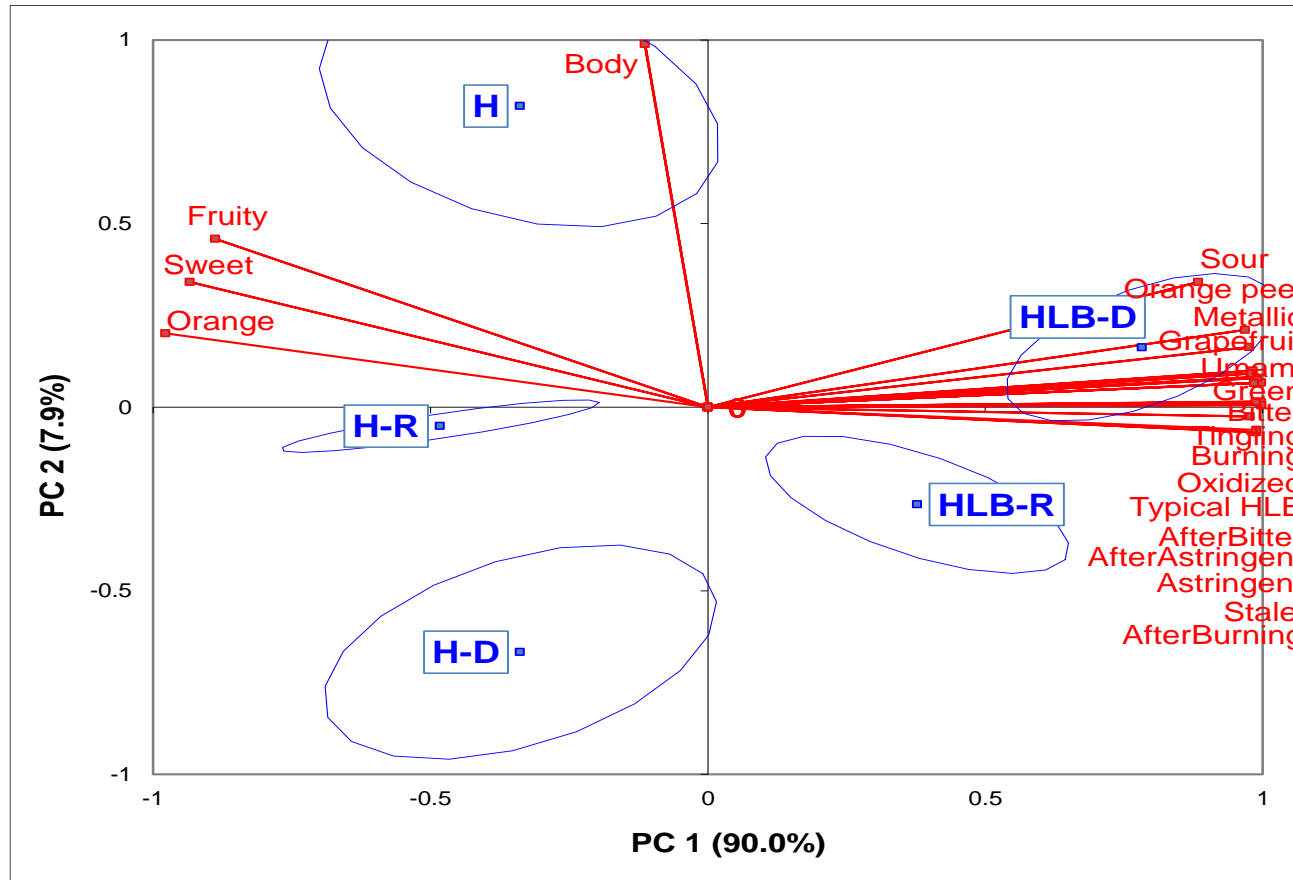


# Sensory (Anne Plotto)

- 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.

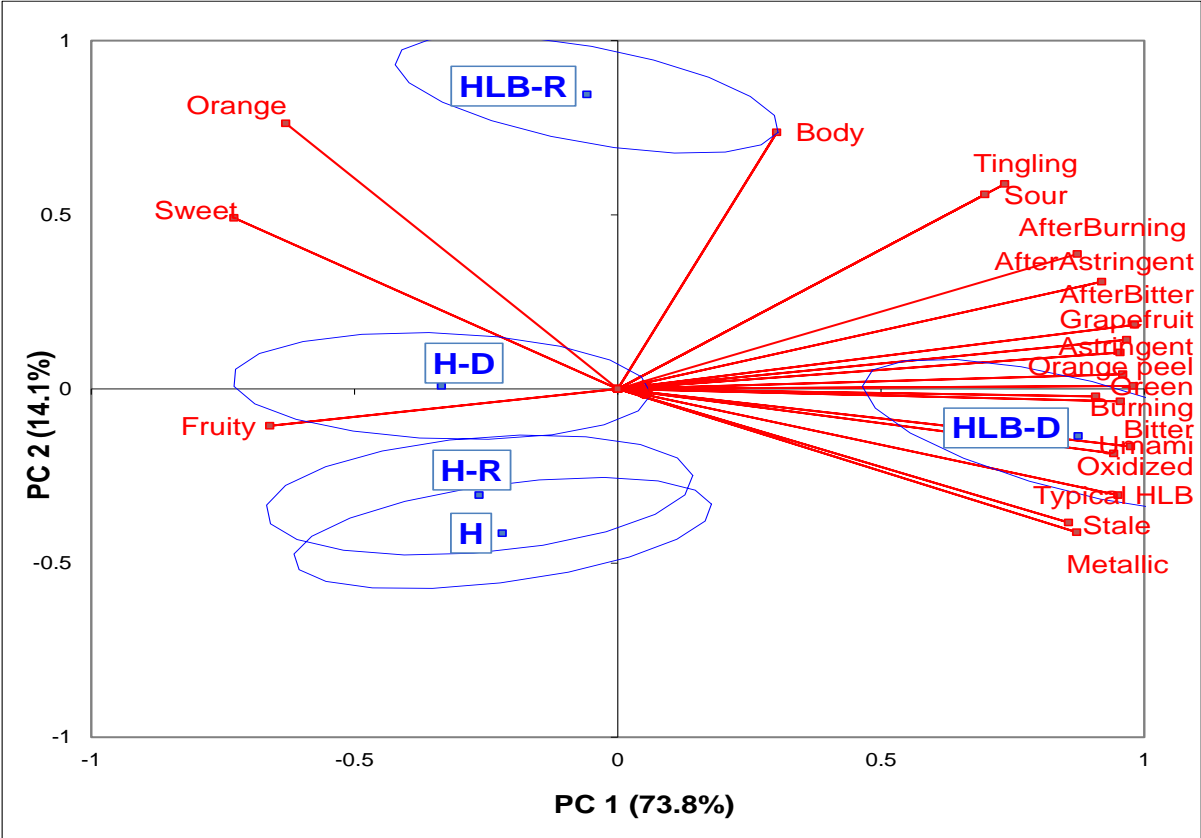


# Early Hamlin

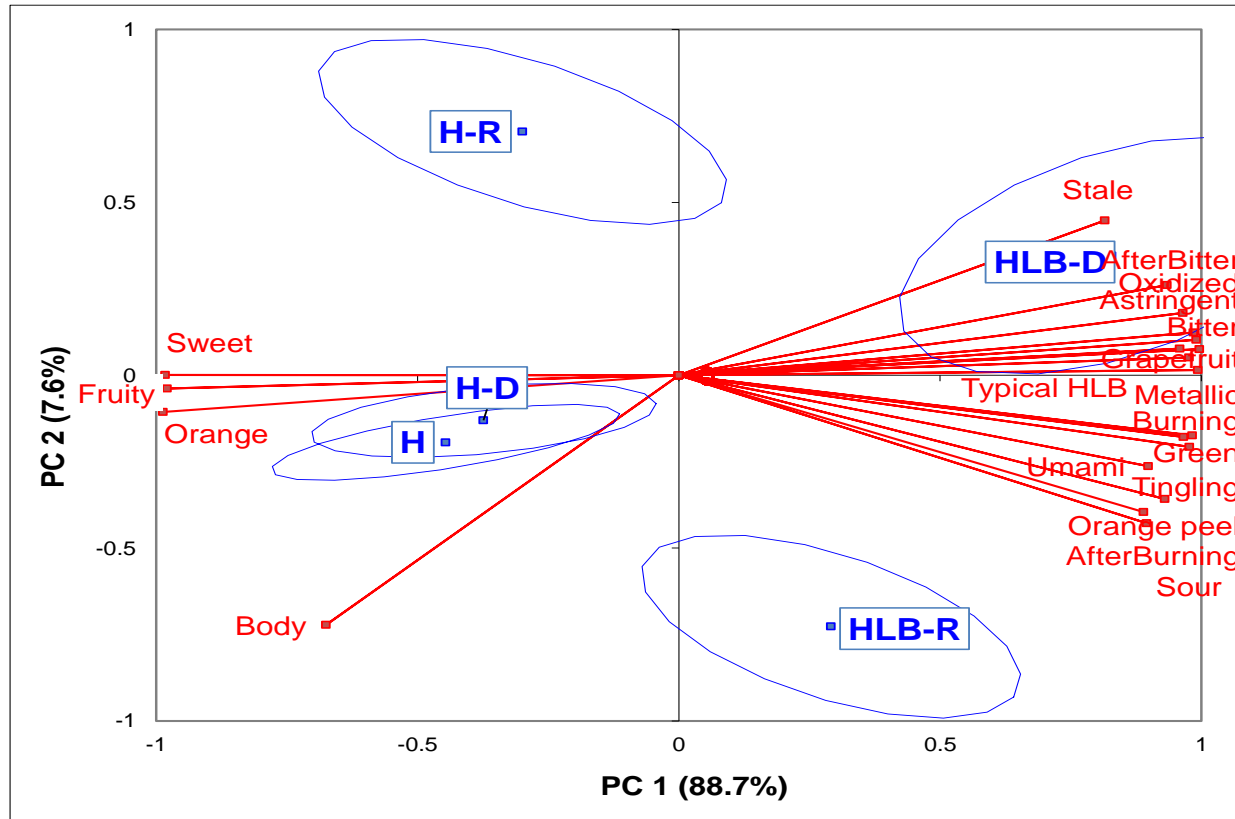




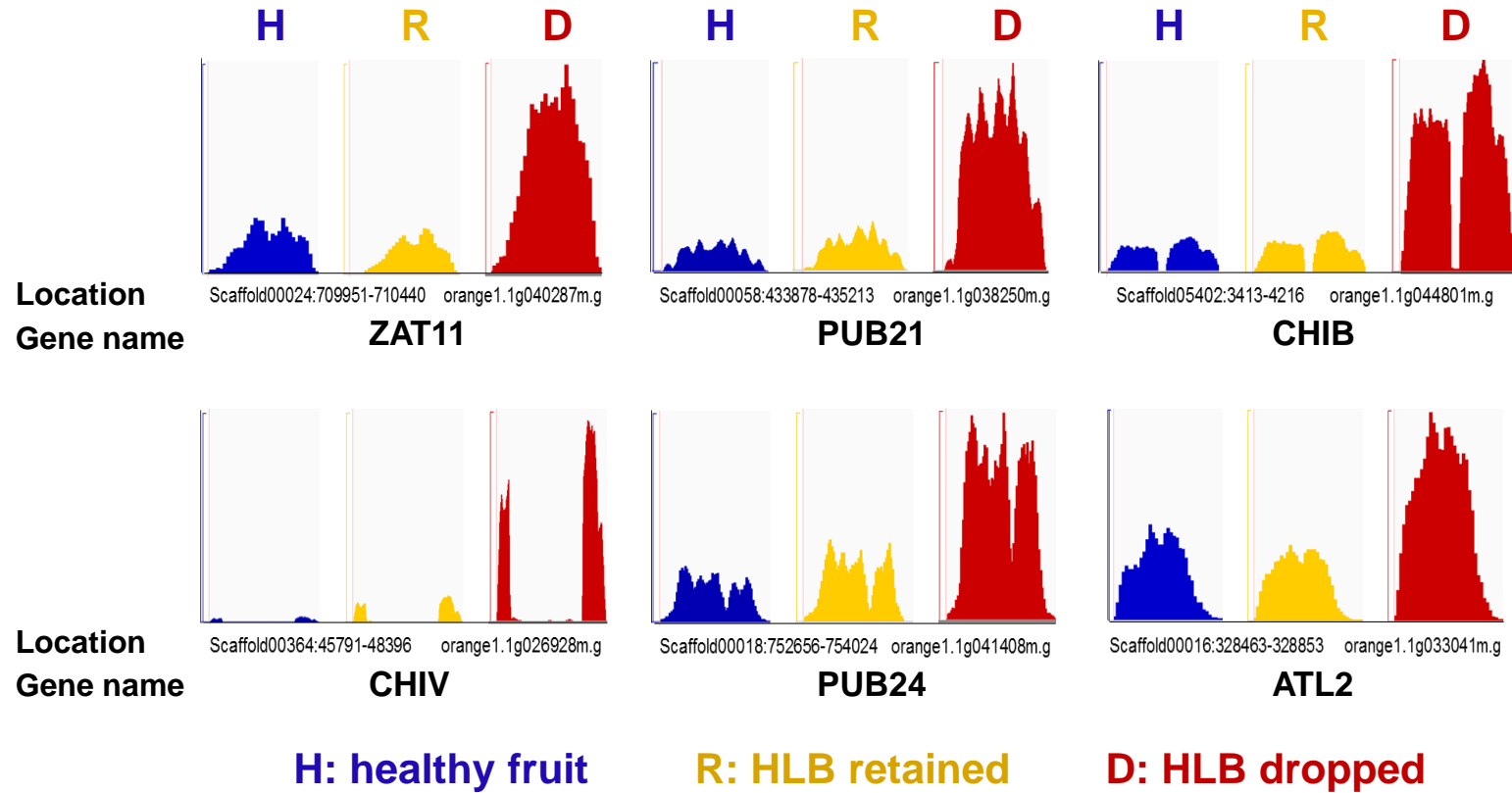
# Late Hamlin



# Valencia



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



# 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**

