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## Wounding nectarine fruit disrupts *Monilinia laxa* infection: deciphering fruit gene pathway involved and the role of phenolic and volatile compounds

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Bénédicte Quilot-Turion, Marie-Noëlle Corre, Guy Costagliola, Laure Heurtevin, Véronique Signoret, et al.. Wounding nectarine fruit disrupts *Monilinia laxa* infection: deciphering fruit gene pathway involved and the role of phenolic and volatile compounds. 10th Rosaceae Genomics Conference, Centre for research in agricultural genomics, Dec 2020, Barcelone, Spain. hal-03267785

**HAL Id: hal-03267785**

**<https://hal.inrae.fr/hal-03267785v1>**

Submitted on 22 Jun 2021

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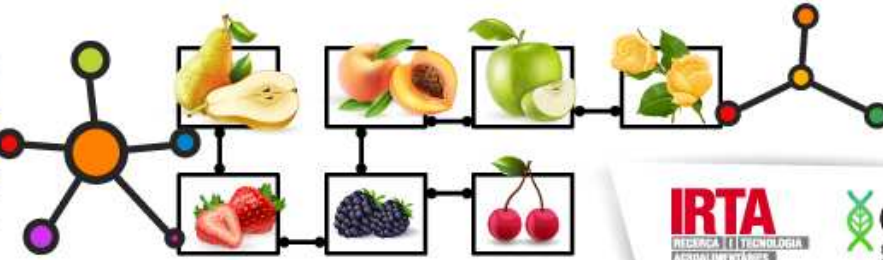
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10th ROSACEAE  
GENOMICS  
CONFERENCE

**RGC10**

**VIRTUAL 2020**

9-10-11 December  
16-17-18 December



**IRTA**  
RESEARCH | TECHNOLOGY  
AGRIALIMENTARY

**CRAG**<sup>FR</sup>  
CENTRE FOR RESEARCH  
IN AGRICULTURAL GENOMICS

**INRAE**

➤ **WOUNDING NECTARINE FRUIT DISRUPTS *MONILINIA LAXA*  
INFECTION: DECIPHERING FRUIT GENE PATHWAY INVOLVED AND  
THE ROLE OF PHENOLIC AND VOLATILE COMPOUNDS**

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HEURTEVIN Laure, SIGNORET Véronique, RASEIRA Maria Do Carmo,  
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Instituto Nacional de Investigación Agropecuaria  
URUGUAY

**Embrapa**

INRAE

➤ CONTEXT



## Brown rot

- a very common disease
- main species: *Monilinia laxa*, *M. fructicola* and *M. fructigena*
- flower and stem desiccation and fruit rot
- can provoke 30 to 40% of harvest losses



One of the most  
damaging diseases  
of peach and  
*Prunus* species

Prophylaxy is not effective enough

The use of chemicals is the rule both on flowers and fruit

- environmental impacts
- public health trouble



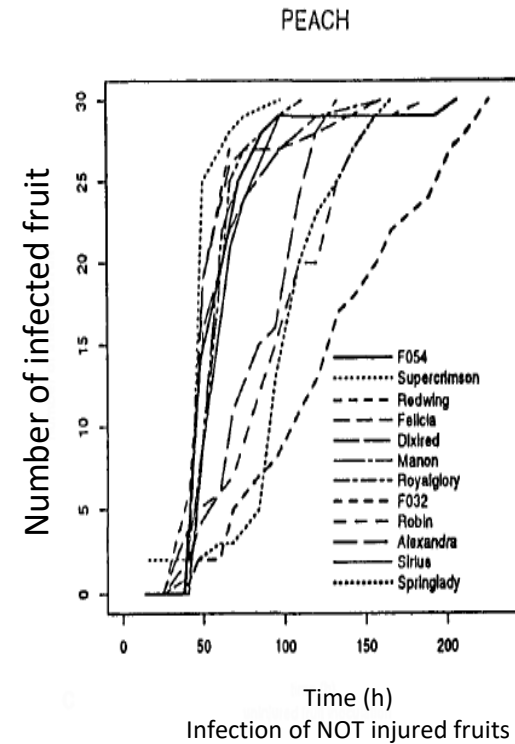
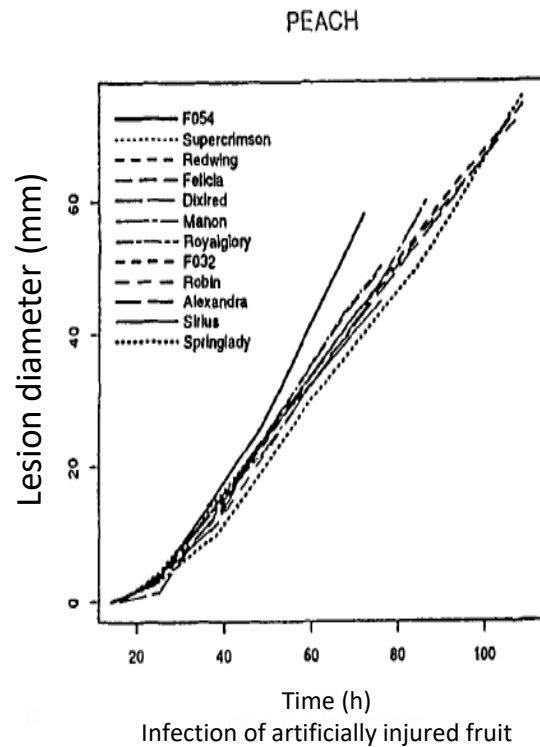
**‘The’ solution  $\leadsto$  grow resistant cultivars**

No resistant cultivar is available for farmers

No major resistance factor has been identified



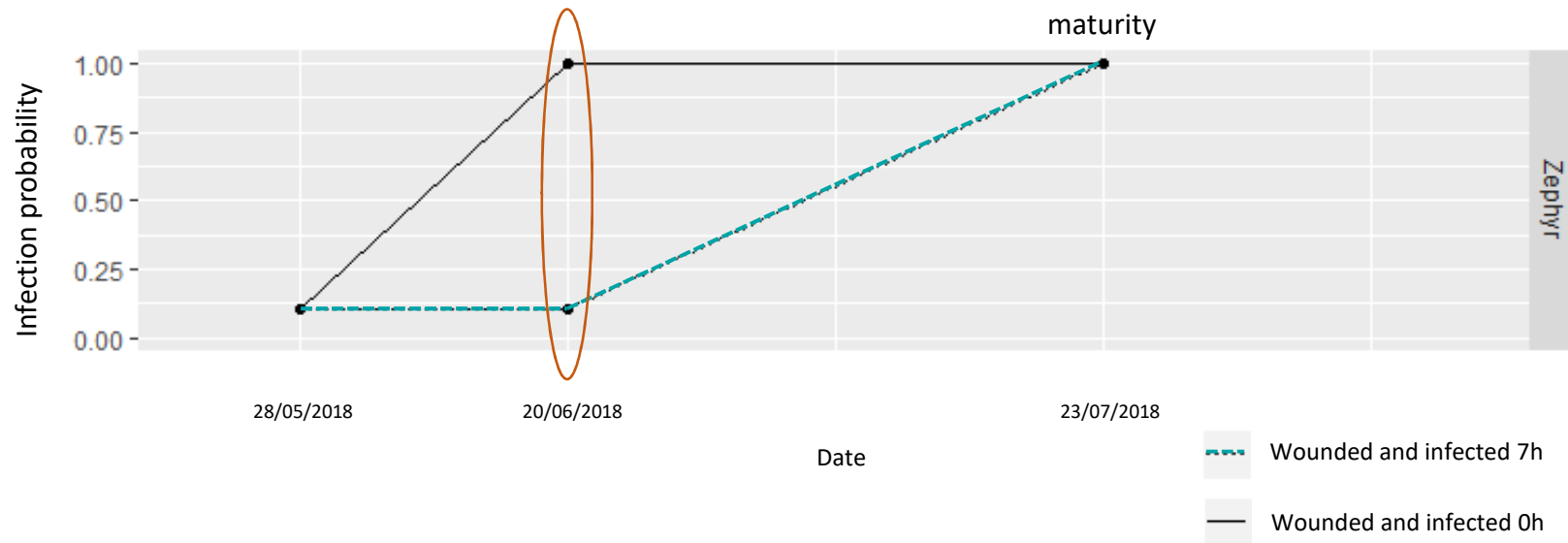
## The fungus penetrates using 'open doors' or directly through the skin (cutinases)



Some resistance factors to brown rot rely on fruit skin



## Infection immediately after wounding and 7 hours after wounding



The reaction depends on fruit stage

**Infection is limited by compounds synthesized by the fruit following the injury**

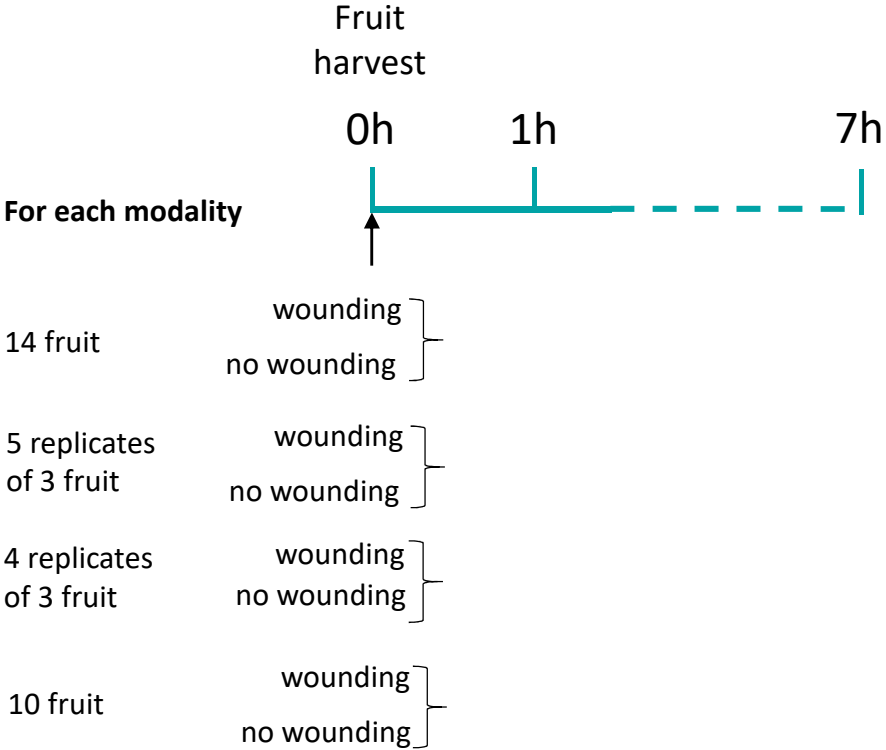


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➤ DOES WOUNDING NECTARINE FRUIT DISRUPT  
*MONILINIA LAXA* INFECTION ?



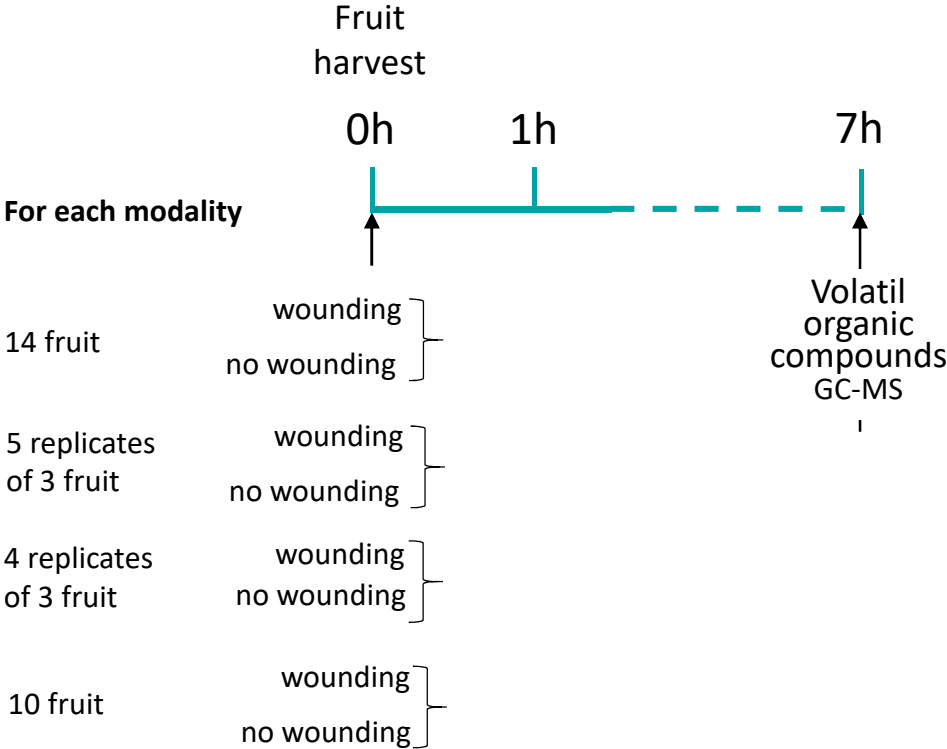
# Experimental protocol



Cultivars : Zephyr and C216  
End of June: immature fruit



# Experimental protocol

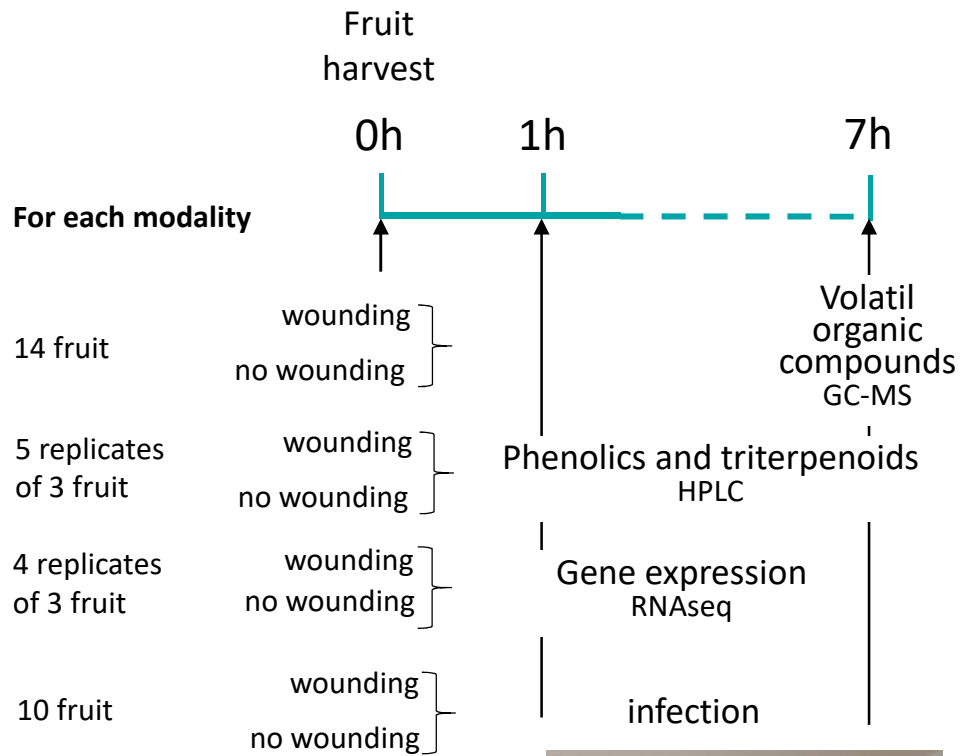


28 samples 2 genotypes

Cultivars : Zephyr and C216  
End of June: immature fruit



# Experimental protocol



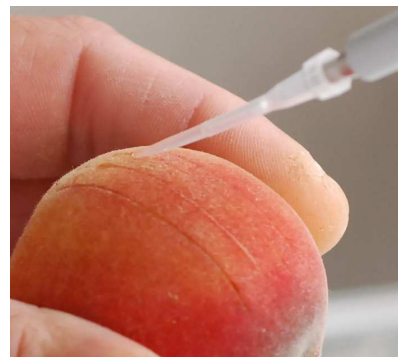
Cultivars : Zephyr and C216  
End of June: immature fruit



28 samples    2 genotypes

20 samples    2 genotypes

16 libraries    Zephyr

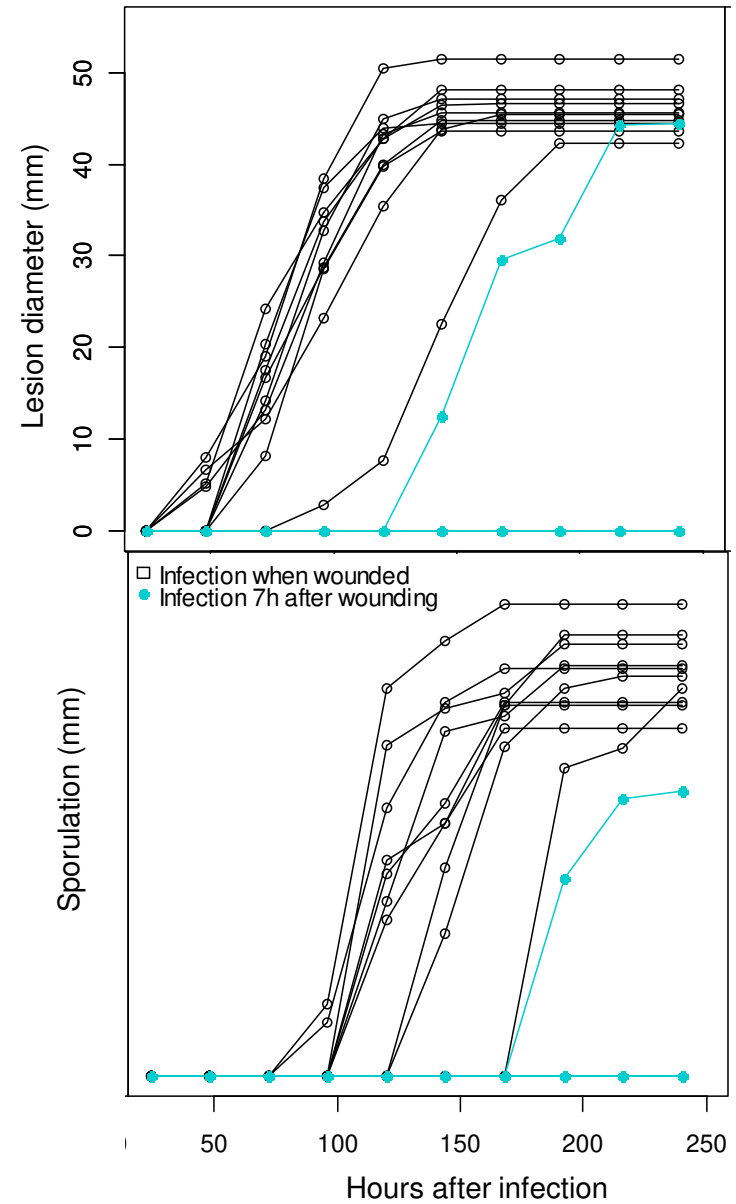
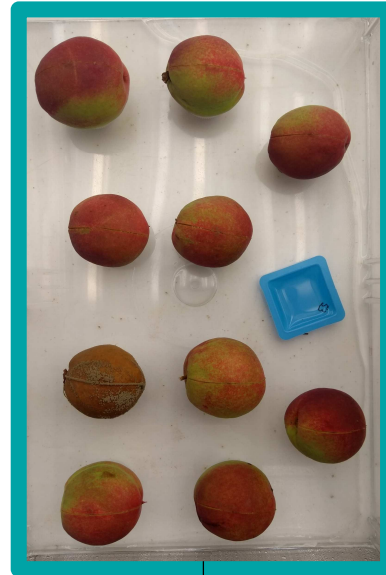


# Infections after wounding

240h after infection

Infection immediately after wounding

Infection 7h after wounding



The infection 7 hours after wounding resulted in slowed and reduced brown rot infection

## Red reactions

immediately  
after wounding



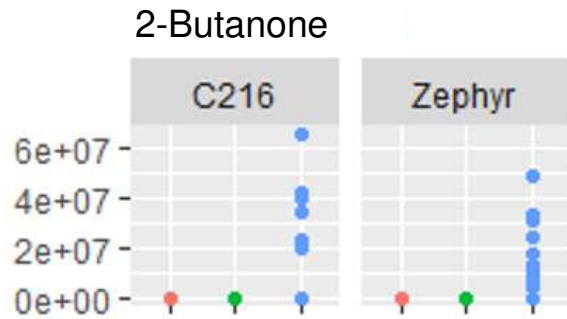
7h after wounding



## GC-MS analyses : volatile compounds

70 were detected

The treatment effect was significant for 32 volatile compounds



11 were present **only** in case of wounded samples, for both genotypes

Good candidates to explain wounding effect on infection

7h after wounded

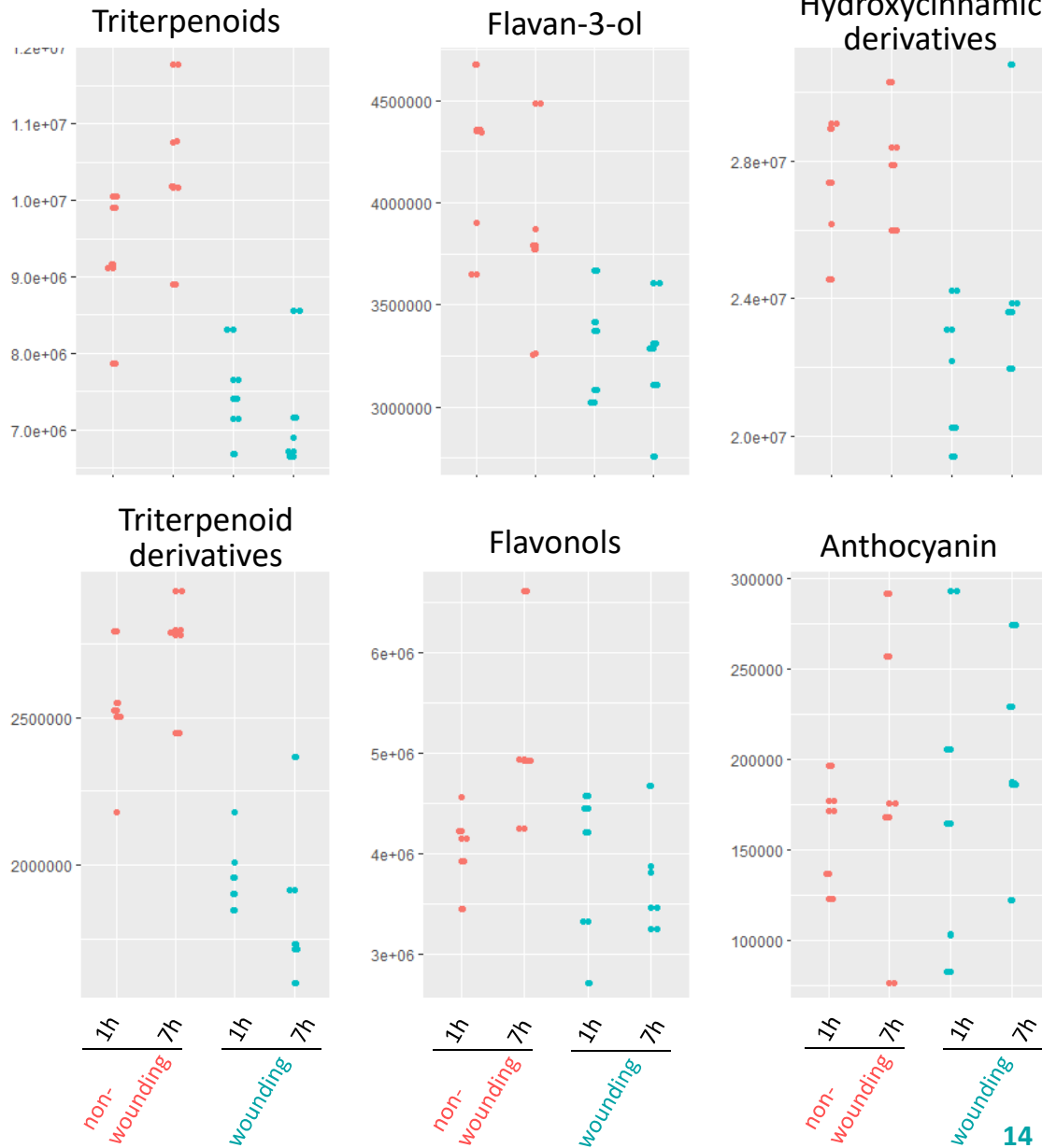
Volatile compound	Treatment	Volatile compound	Treatment
Aac,methyl_ester	4.06 <sup>-3</sup> **	1-Pentene	8.17 <sup>-8</sup> ***
2-Butanone	1.35 <sup>-6</sup> ***	Terpinolene_B	6.40 <sup>-9</sup> ***
Ethyl_Acetate	0.017 <sup>ns</sup>	P-Cymene	0.015 <sup>ns</sup>
Tropilidene_Toluene	0.617 <sup>ns</sup>	2-tert_Butyltoluene	0.457 <sup>ns</sup>
Hexanal	0.558 <sup>ns</sup>	Cyclohexane_A	4.35 <sup>-7</sup> ***
Unknown1	0.053 <sup>ns</sup>	Undecane	1.38 <sup>-8</sup> ***
Unknown2	3.57 <sup>-3</sup> **	β-Linalool	1.95 <sup>-3</sup> **
Ethylbenzene	0.899 <sup>ns</sup>	Nonanal	0.837 <sup>ns</sup>
1-Met_2prop_acet	0.333 <sup>ns</sup>	Cyclohexane_B	6.52 <sup>-9</sup> ***
P-Xylene_A	0.538 <sup>ns</sup>	neo_allo_Ocimene	5.78 <sup>-10</sup> ***
3-Met_3but1ol,acet	7.22 <sup>-3</sup> **	2,6-Dimethyl	0.012 <sup>ns</sup>
P-Xylene_B	0.211 <sup>ns</sup>	allo_Ocimene	2.11 <sup>-4</sup> ***
N-Amyl_acetate	0.049 <sup>ns</sup>	Unknown3	0.017 <sup>ns</sup>
α-Pinene_A	3.65 <sup>-3</sup> **	Diisopropyl_xanth	4.83 <sup>-3</sup> **
Benzaldehyde	0.513 <sup>ns</sup>	Dodecane	0.307 <sup>ns</sup>
5-Hep_2one,6met	0.491 <sup>ns</sup>	Decanal	0.759 <sup>ns</sup>
β-Myrcene	4.11 <sup>-8</sup> ***	Benzothiazole	0.018 <sup>ns</sup>
Furan,2pentyl	0.021 <sup>ns</sup>	cis_3Hexenyl_isoval	6.98 <sup>-12</sup> ***
Heptane	0.064 <sup>ns</sup>	n-Hexyl_iso_val	5.02 <sup>-12</sup> ***
Decane	7.83 <sup>-8</sup> ***	trans-2Hexenyl_val	2.60 <sup>-6</sup> ***
Octanal	8.31 <sup>-4</sup> ***	Unknown4	0.018 <sup>ns</sup>
3-Hexen_1ol,acet	9.58 <sup>-6</sup> ***	Tridecane	0.997 <sup>ns</sup>
3-Heptene	0.661 <sup>ns</sup>	Undecanal	0.674 <sup>ns</sup>
α-Phellandrene	1.57 <sup>-4</sup> ***	Unknown5	0.067 <sup>ns</sup>
α-Pinene_B	0.015 <sup>ns</sup>	Unknown6	0.543 <sup>ns</sup>
Aac,hexyl_es	3.63 <sup>-6</sup> ***	Unknown7	0.012 <sup>ns</sup>
2-Hexen_1ol,ac	3.98 <sup>-3</sup> **	cis-Jasmone	1.18 <sup>-8</sup> ***
Terpinolene_A	0.018 <sup>ns</sup>	Tetradecane	0.812 <sup>ns</sup>
omp-cymene	3.80 <sup>-4</sup> ***	Trans-α-Bergamo	7.38 <sup>-4</sup> ***
Limonene	4.27 <sup>-4</sup> ***	Trans-Geranylacet	0.818 <sup>ns</sup>
O-cymene	6.90 <sup>-11</sup> ***	Cis-β-Farnesene	6.50 <sup>-3</sup> **
β-Ocimene	1.27 <sup>-7</sup> ***	Pentadecane	0.683 <sup>ns</sup>
γ-Caprolactone	0.238 <sup>ns</sup>	Butylated_Hydroxy	0.021 <sup>ns</sup>
γ-Terpinene	3.38 <sup>-4</sup> ***	Heneicosane	0.195 <sup>ns</sup>
Acetophenone	0.201 <sup>ns</sup>	Di_n_octyl_ether	0.808 <sup>ns</sup>



# HPLC analyses : phenolic and terpenoid compounds

30 phenolic and terpenoid compounds

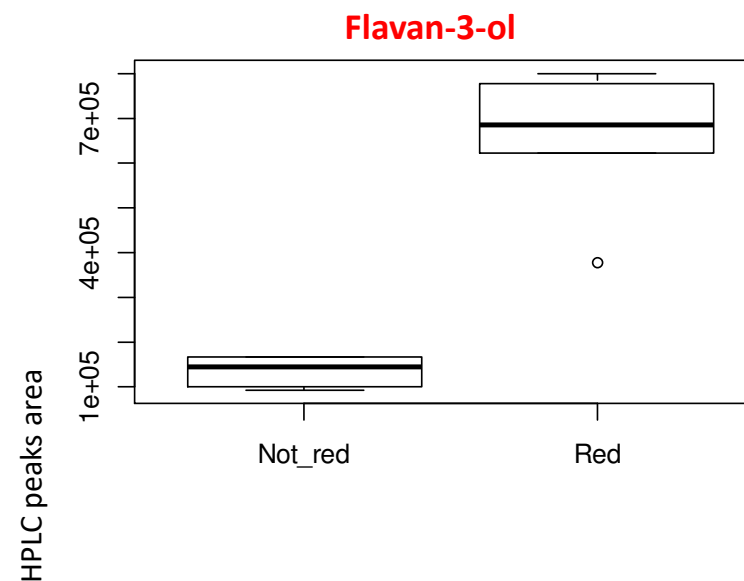
Samples from fruit with wounds presented lower contents



## HPLC analyses : phenolic and terpenoid compounds in red zones

Compound Family	Compound	p-value	
Triterpenoid	trihydroxy-urs-12-en-28-oic acid	0.030	ns
	trihydroxy-urs-12-en-28-oic acid	0.049	ns
	Oleanolic acid	0.496	ns
	Ursolic acid	0.676	ns
Flavan-3-ol	Flavan-3-ol	1.54 <sup>-4</sup>	***
	Procyanidin B1	0.254	ns
	Flavan-3-ol	5.16 <sup>-3</sup>	**
	Flavan-3-ol	9.08 <sup>-3</sup>	**
Flavanone	Catechin	8.95 <sup>-4</sup>	***
	Flavanone	only in red reaction	
	Flavanone	only in red reaction	
	Eriodictyol-7-glucoside	only in red reaction	
	Flavanone	only in red reaction	
	Naringenine-7-glucoside	only in red reaction	
	Flavanone	only in red reaction	
	cis-Neochlorogenic acid	9.19 <sup>-3</sup>	**
Hydroxycinnamic derivatives	Neochlorogenic acid	0.012	ns
	Hydroxycinnamic derivative	only in red reaction	
	Chlorogenic acid	0.739	ns
	cis-Chlorogenic acid	0.733	ns
	Hydroxycinnamic derivative	0.437	ns
	5-p-Coumaroylquinic acid	9.90 <sup>-3</sup>	**
	3,5-Dicaffeoylquinic acid	0.334	ns
	Hydroxycinnamic derivative	only in red reaction	
Hydroxycinnamic derivative	only in red reaction		

6 compounds present in greater proportion in red zones



### The two major phenolic compounds

- Eriodictyol-7-glucoside = prunine
- Naringenin-7-glucoside

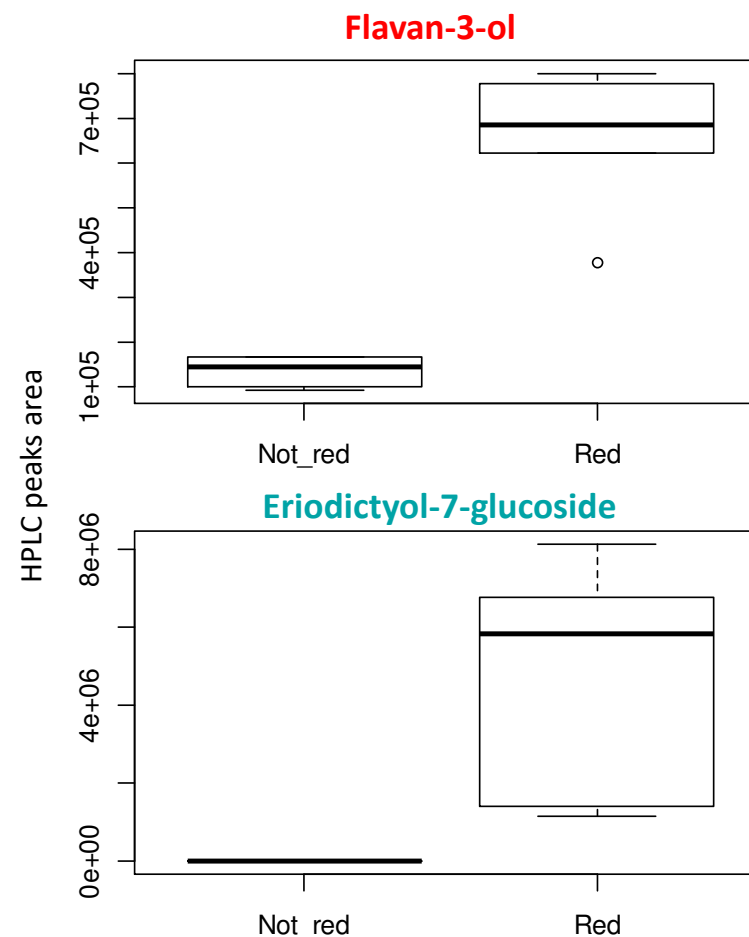


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6 compounds present in greater proportion in red zones

9 compounds only in red zones

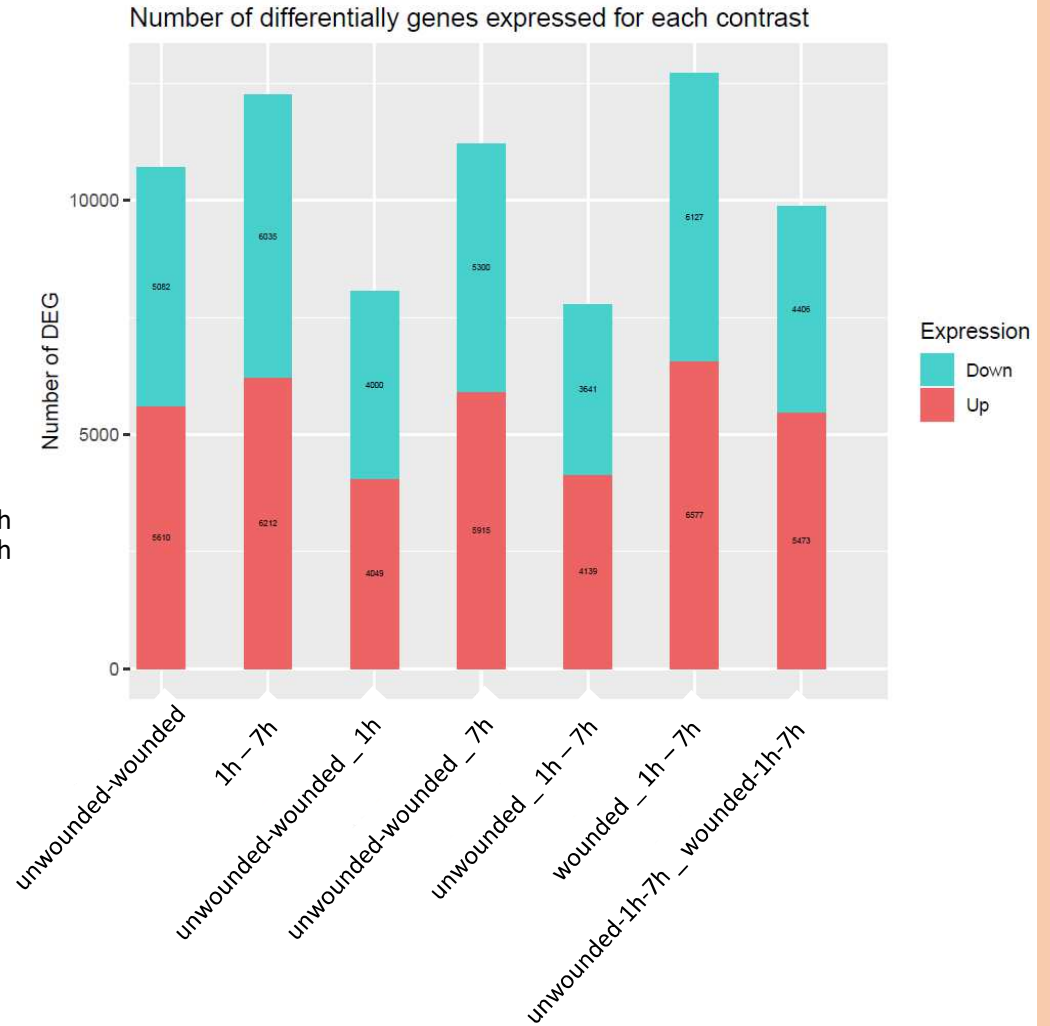
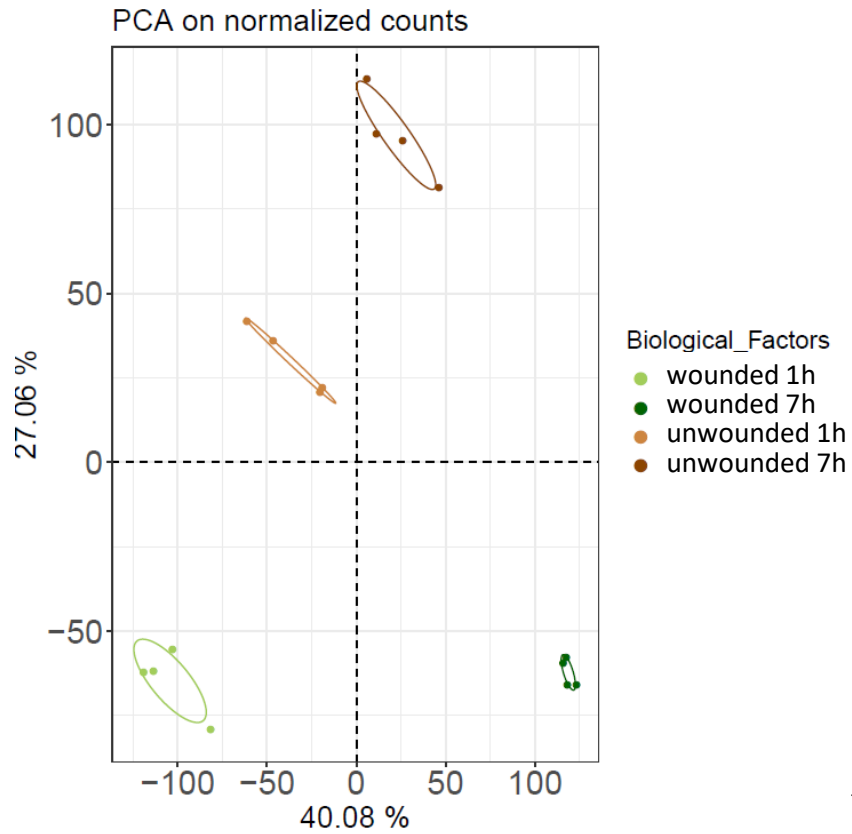


### The two major phenolic compounds

- Eriodictyol-7-glucoside = prunine
- Naringenin-7-glucoside

# RNAseq analyses

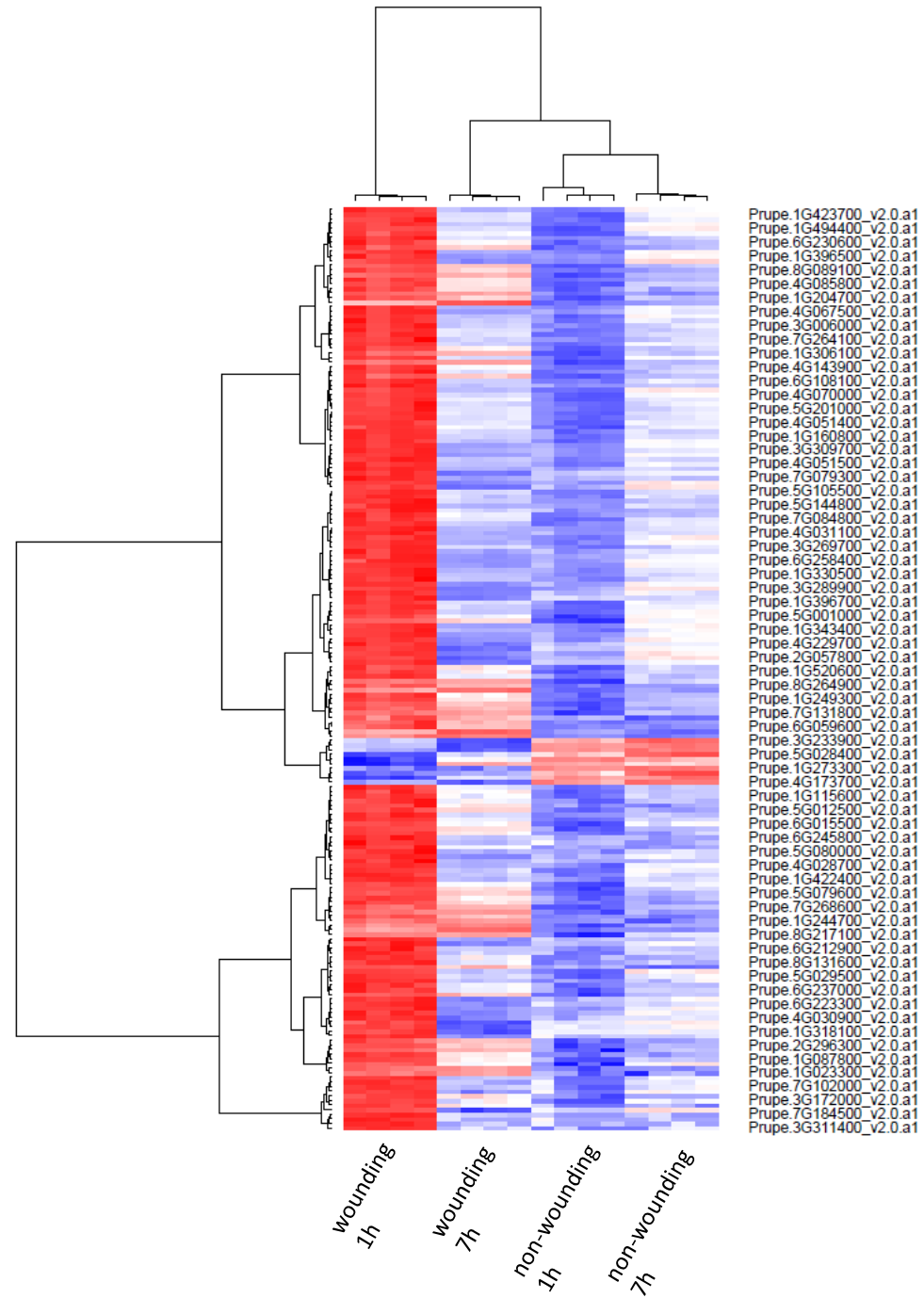
26 873 genes



Lambert et al. 2020. DiCoExpress: a tool to process multifactorial RNAseq experiments from quality controls to co-expression analysis through differential analysis based on contrasts inside GLM models. *Plant Methods*, 16:68-68

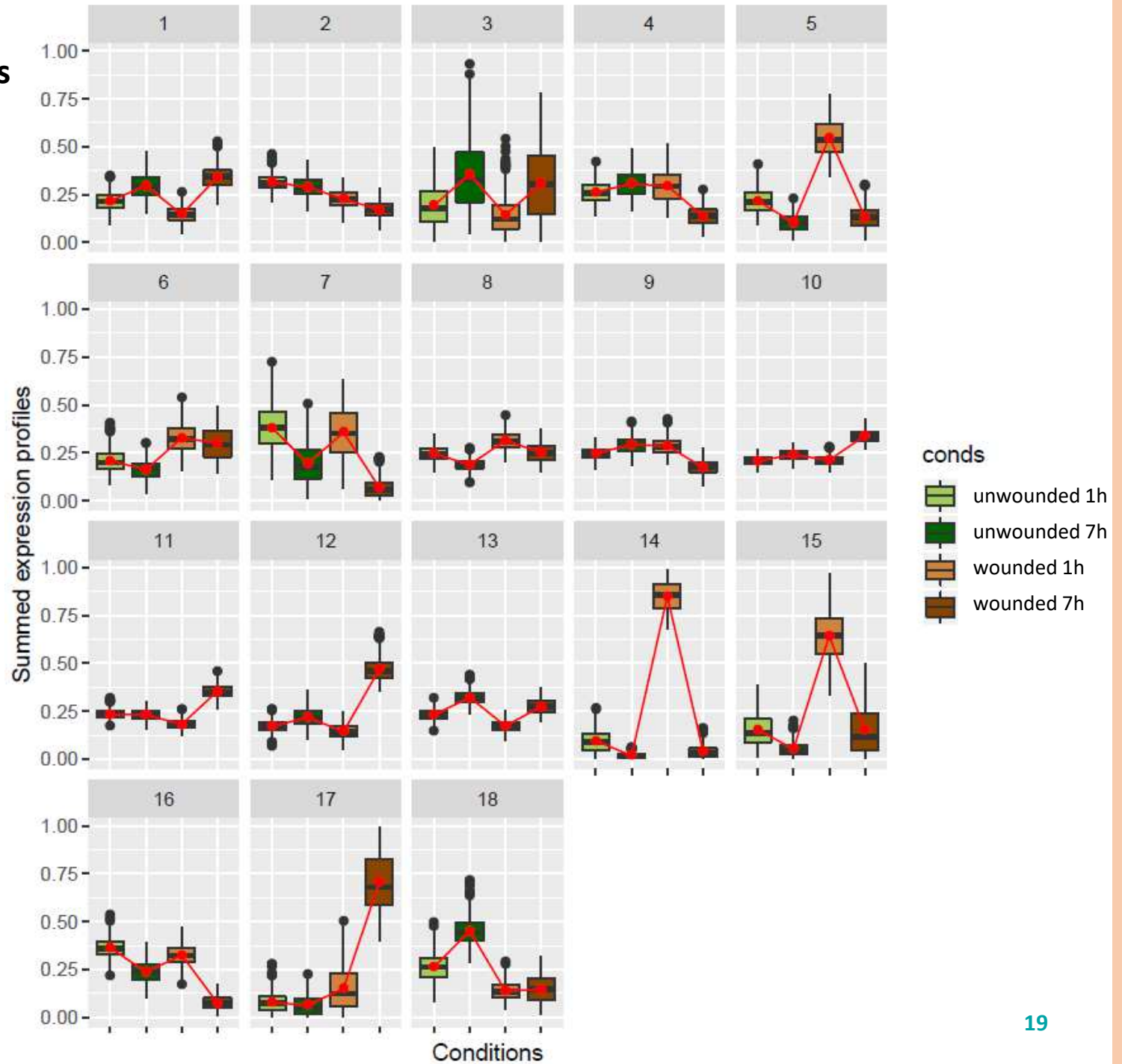
# RNAseq analyses

Pathways activated after injury



# RNAseq analyses

## Co-expression analysis



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➤ CONCLUSIONS

## Take home messages

- The inoculation done 7 hours after wounding resulted in **slowed and reduced brown rot** infection compared to inoculation done immediately after wounding
- **11 volatile compounds** were associated with wounded fruits only
- **phenolic and terpenoid compounds** were less present in fruit with wounds
- We observed **red reactions**
- **9 phenolic compounds** were present only in red zones  
2 major phenolic compounds : Eriodictyol-7-glucoside and Naringenin-7-glucoside
- RNAseq analyses highlighted **pathways activated after injury**

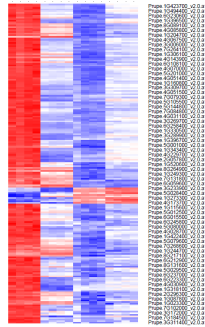


# Injury induces an activation of metabolic pathways involved in the susceptibility/resistance of peach to *M. laxa*

Wounding

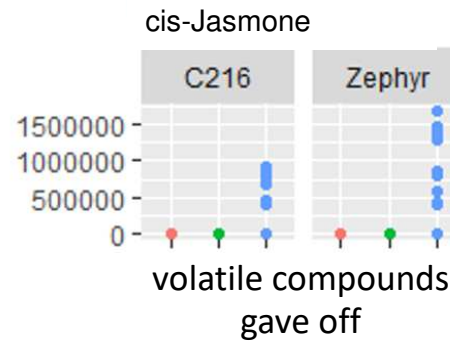


1h

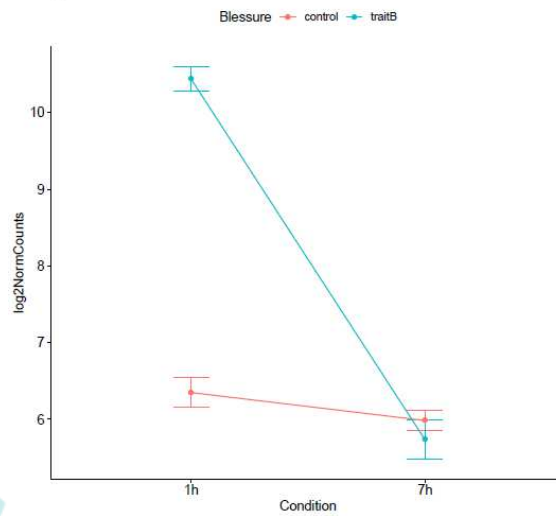


gene pathways activated after injury

7h



Prupe.1G526000  
"glutathione S-transferase tau 7"

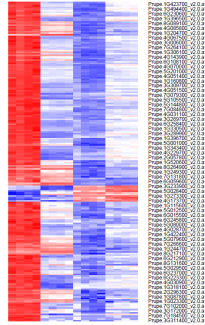


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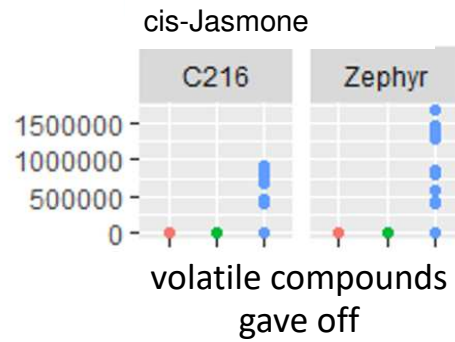


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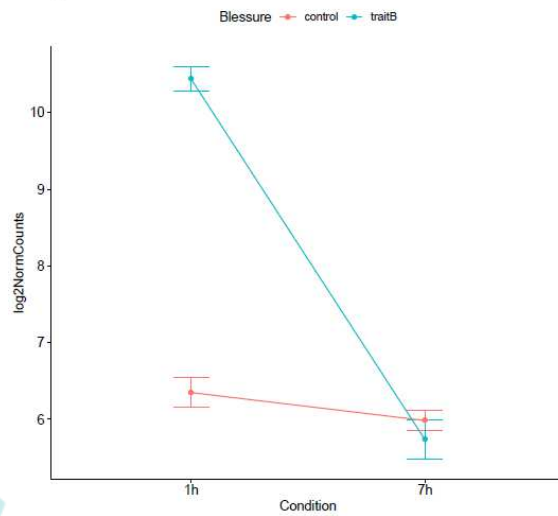


gene pathways activated after injury

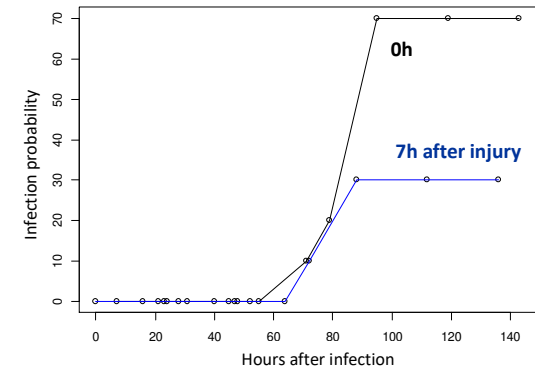
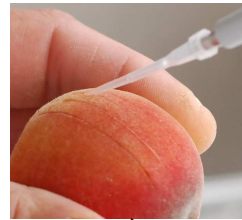
7h



Prupe.1G526000  
"glutathione S-transferase tau 7"



Infection



phenolic compounds



Eriodictyol-7-glucoside  
Naringenin-7-glucoside



Thanks to



MN Corre  
L Heurtevin  
V Signoret

**All colleagues  
from my team and unit**

**PhD student**  
M Dini Viñoly



G Costaglia



Instituto Nacional de Investigación Agropecuaria  
URUGUAY

M Dini Viñoly



M Do C Raseira

