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

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10th ROSACEAE GENOMICS CONFERENCE

RGC10 VIRTUAL 2020

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

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

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GAFL INRA AVIGNON

PSH Plantes et Systèmes de culture Horticoles

Instituto Nacional de Investigación Agropecuaria URUGUAY

CONTEXT

Brown rot

- a very commun 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

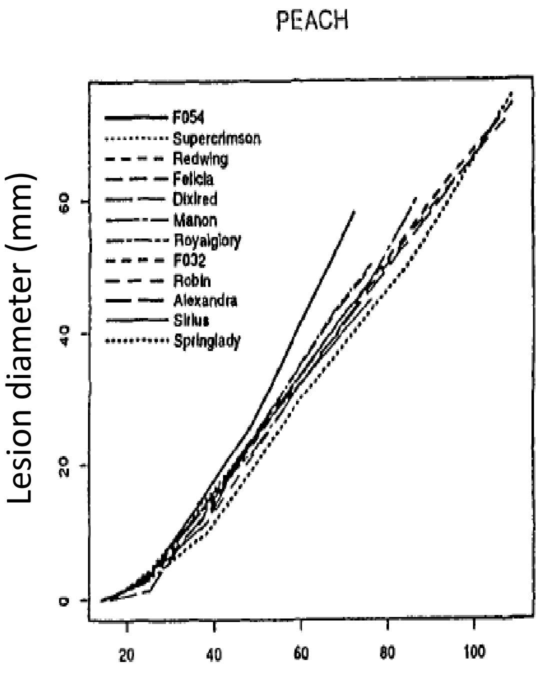
The use of chemicals is the rule both on flowers and fruit

- environmental impacts
- public health trouble

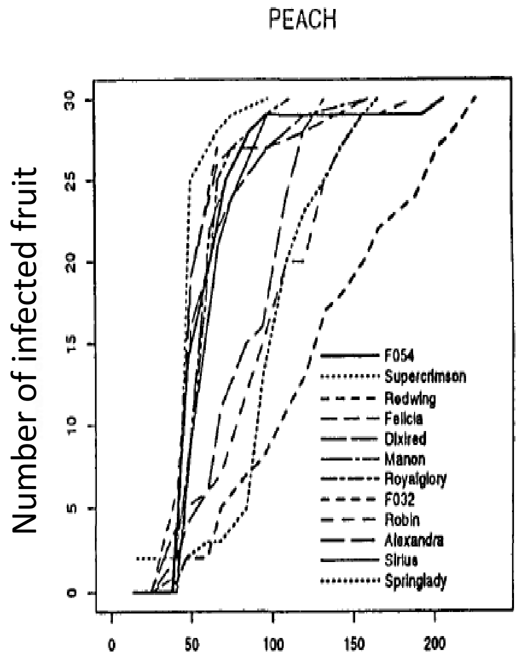
'The' solution ⤳ grow resistant cultivars

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)



Time (h)
Infection of artificially injured fruit

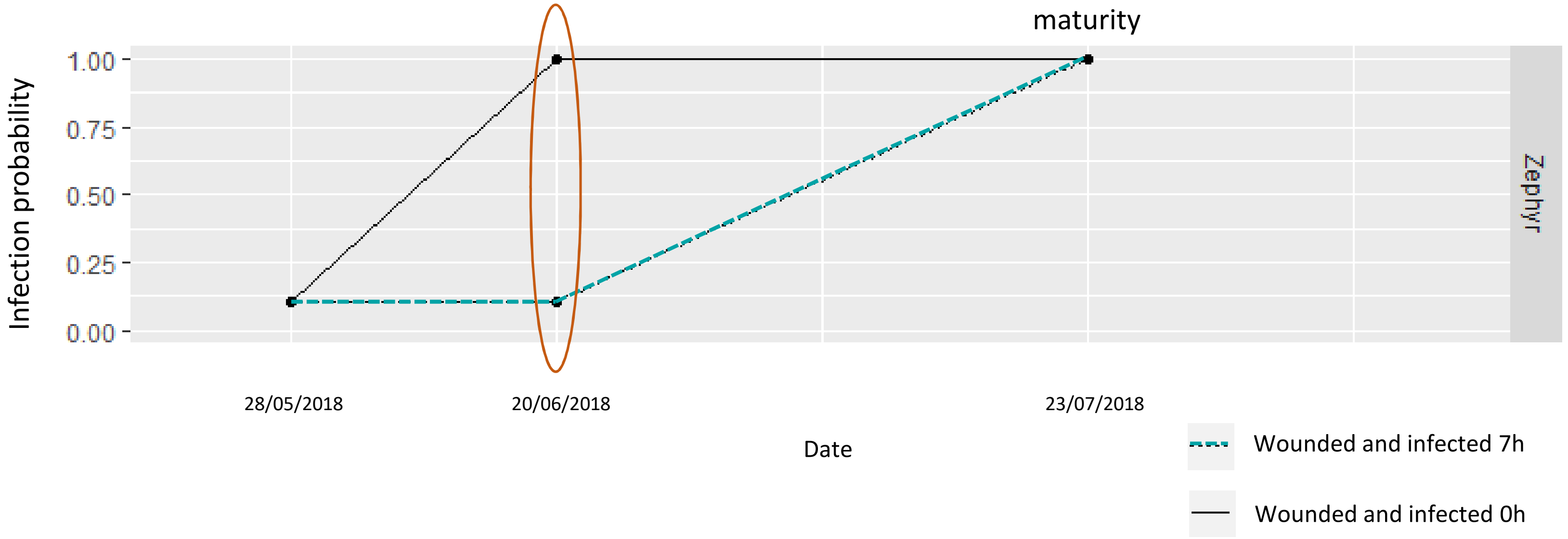


Time (h)
Infection of NOT injured fruits

Some resistance factors to brown rot relie on fruit skin

Pascal et al 1994 ; Lino et al 2016

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

Does wounding Nectarine Fruit Disrupt *Monilinia Laxa* Infection ?

Experimental protocol

Cultivars : Zephyr and C216
End of June: immature fruit

Fruit harvest

0h 1h 7h

For each modality

14 fruit	wounding / no wounding
5 replicates of 3 fruit	wounding / no wounding
4 replicates of 3 fruit	wounding / no wounding
10 fruit	wounding / no wounding

Experimental protocol

Cultivars : Zephyr and C216
End of June: immature fruit

Fruit harvest

0h — 1h — 7h

For each modality

Volatil organic compounds GC-MS

14 fruit	wounding / no wounding	
5 replicates of 3 fruit	wounding / no wounding	
4 replicates of 3 fruit	wounding / no wounding	
10 fruit	wounding / no wounding	

28 samples

2 genotypes

Experimental protocol

Cultivars : Zephyr and C216
End of June: immature fruit

Fruit harvest

0h — 1h — 7h

For each modality

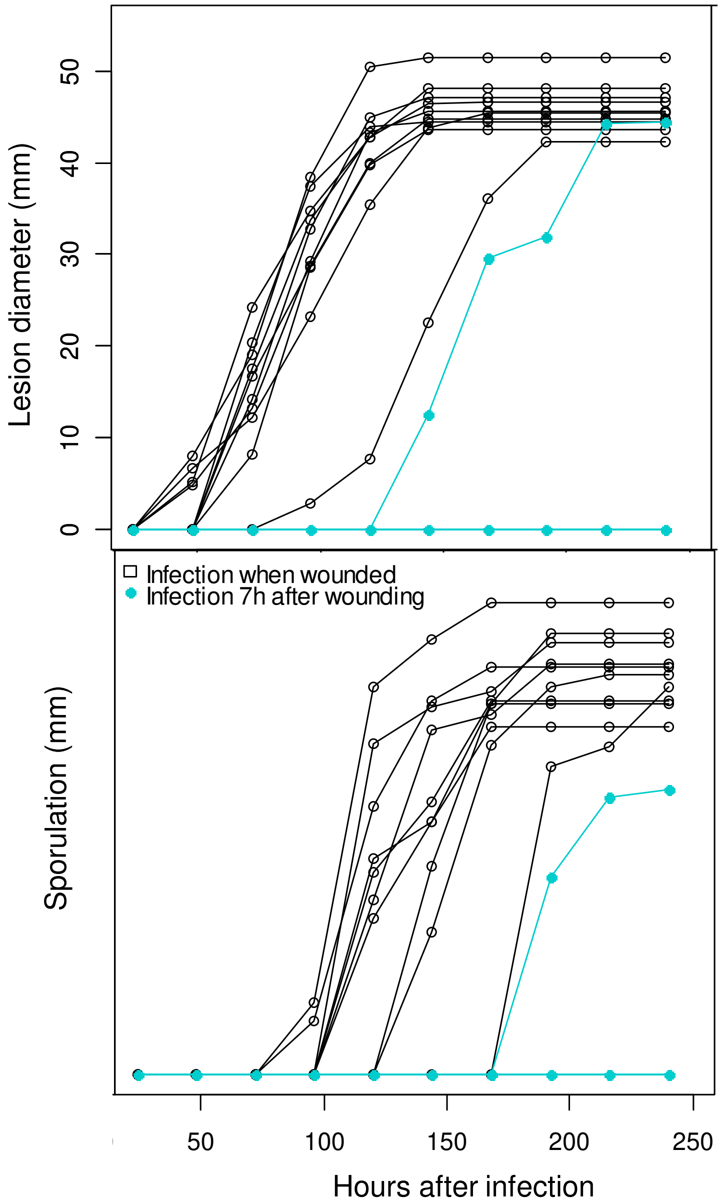
14 fruit	wounding / no wounding	Volatil organic compounds GC-MS	28 samples	2 genotypes
5 replicates of 3 fruit	wounding / no wounding	Phenolics and triterpenoids HPLC	20 samples	2 genotypes
4 replicates of 3 fruit	wounding / no wounding	Gene expression RNAseq	16 libraries	Zephyr
10 fruit	wounding / no wounding	infection		

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

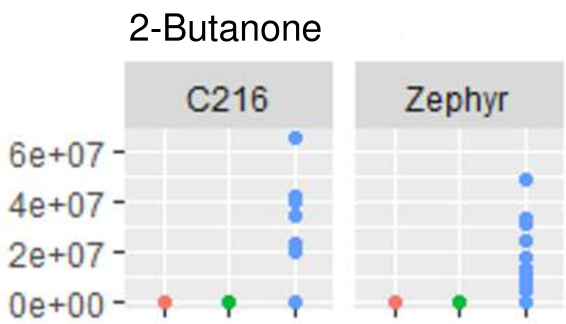
immediately after wounding

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

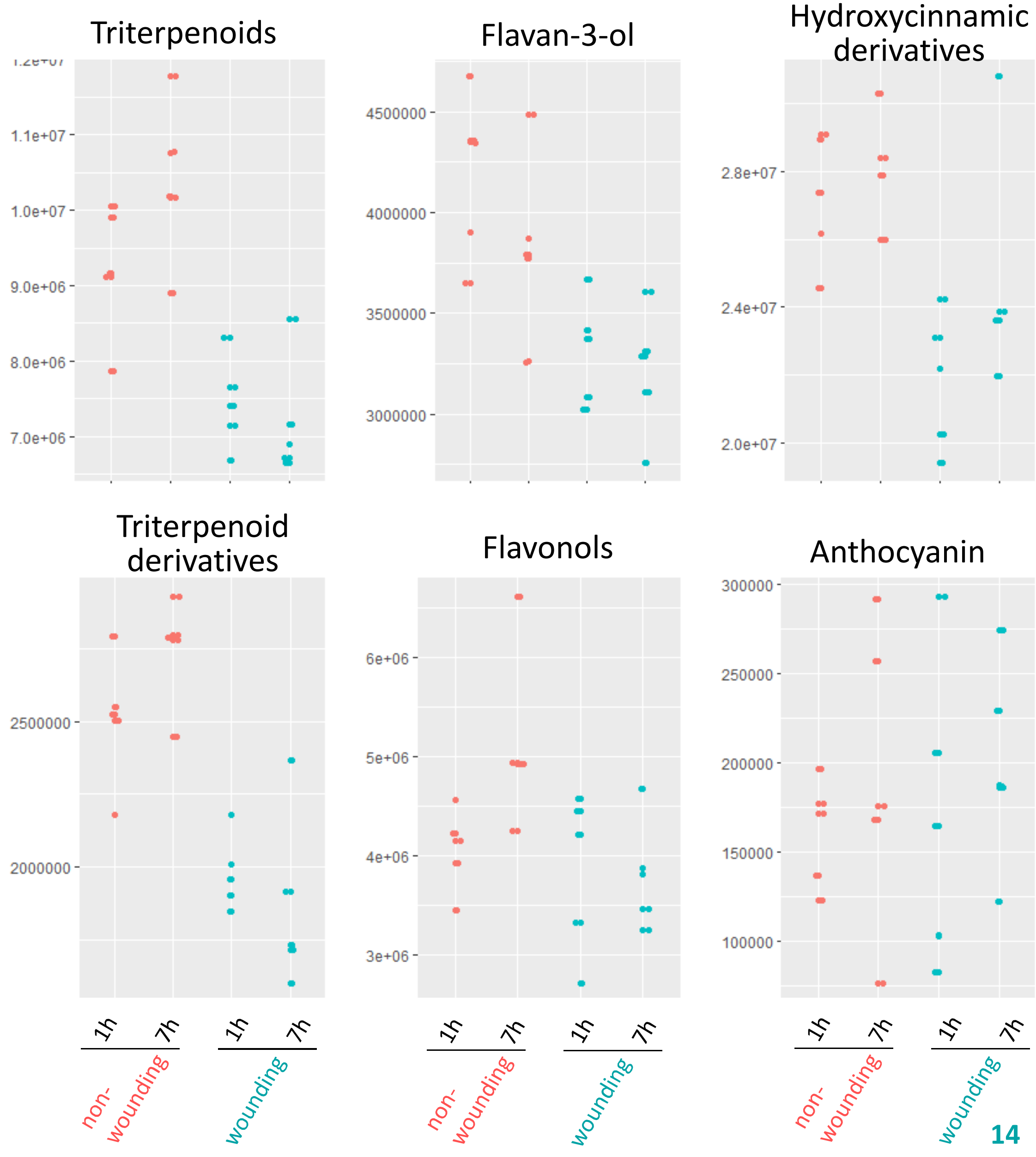
7h after wounded

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

HPLC analyses : phenolic and terpenoid compounds

30 phenolic and terpenoid compounds

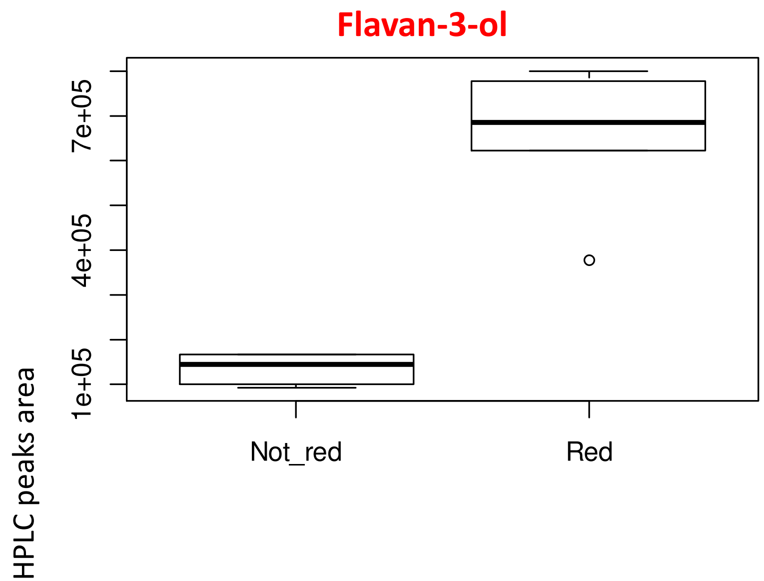
Samples from fruit with wounds presented lower contents



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^{-4}	***
	Procyanidin B1	0.254	ns
	Flavan-3-ol	5.16^{-3}	**
	Flavan-3-ol	9.08^{-3}	**
	Catechin	8.95^{-4}	***
Flavanone	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	
Hydroxycinnamic derivatives	cis-Neochlorogenic acid	9.19^{-3}	**
	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^{-3}	**
	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

HPLC analyses : phenolic and terpenoid compounds in red zones

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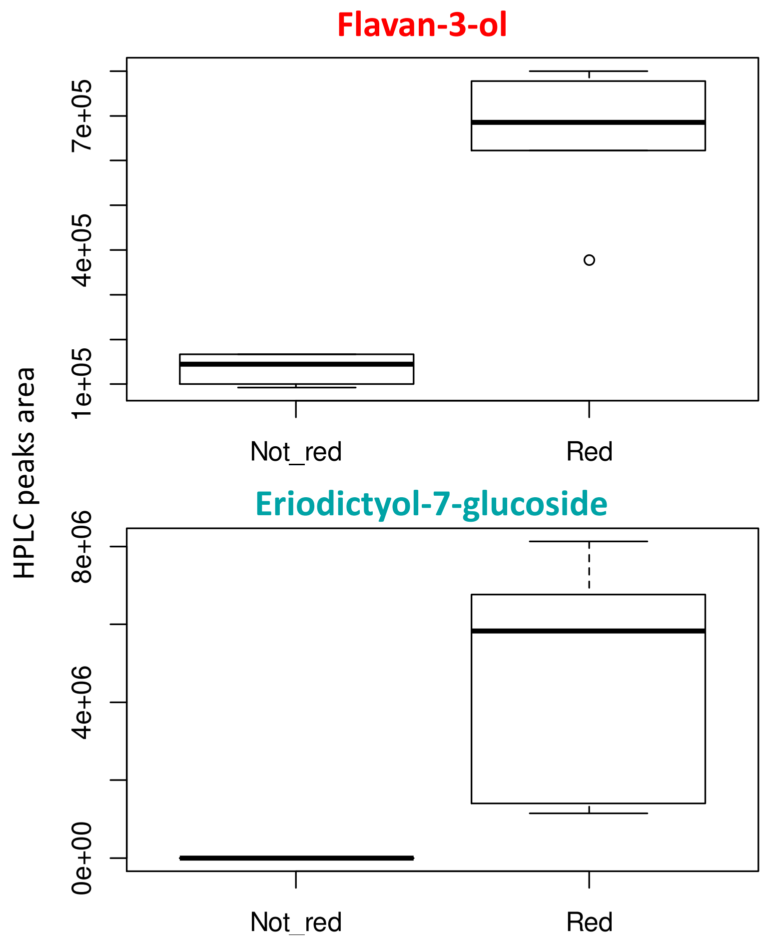
6 compounds present in greater proportion in red zones

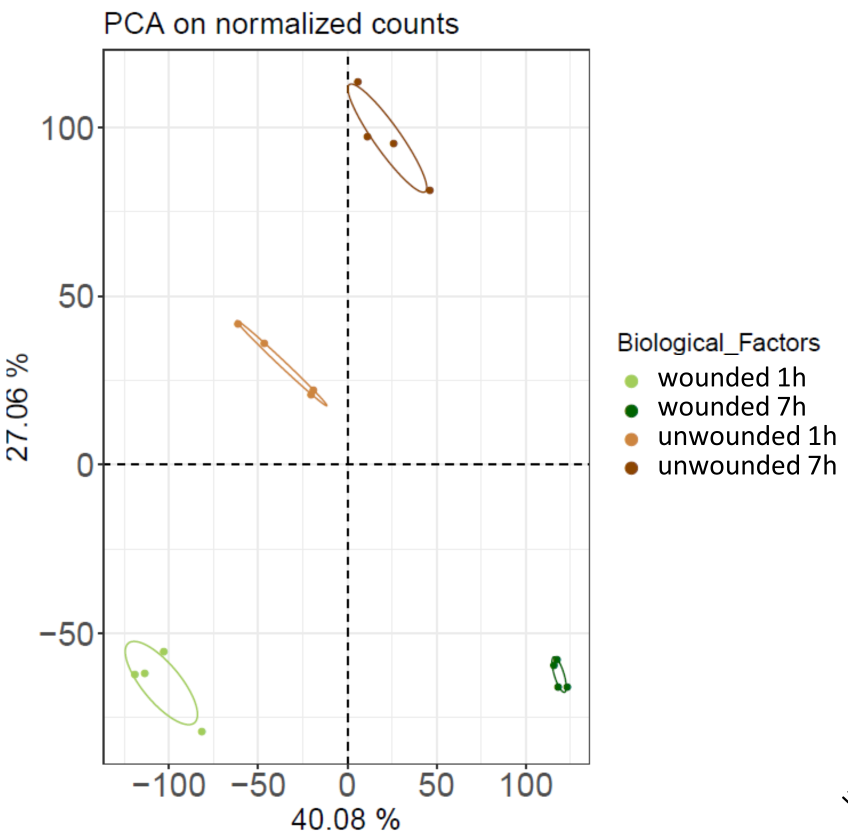
9 compounds **only** in red zones

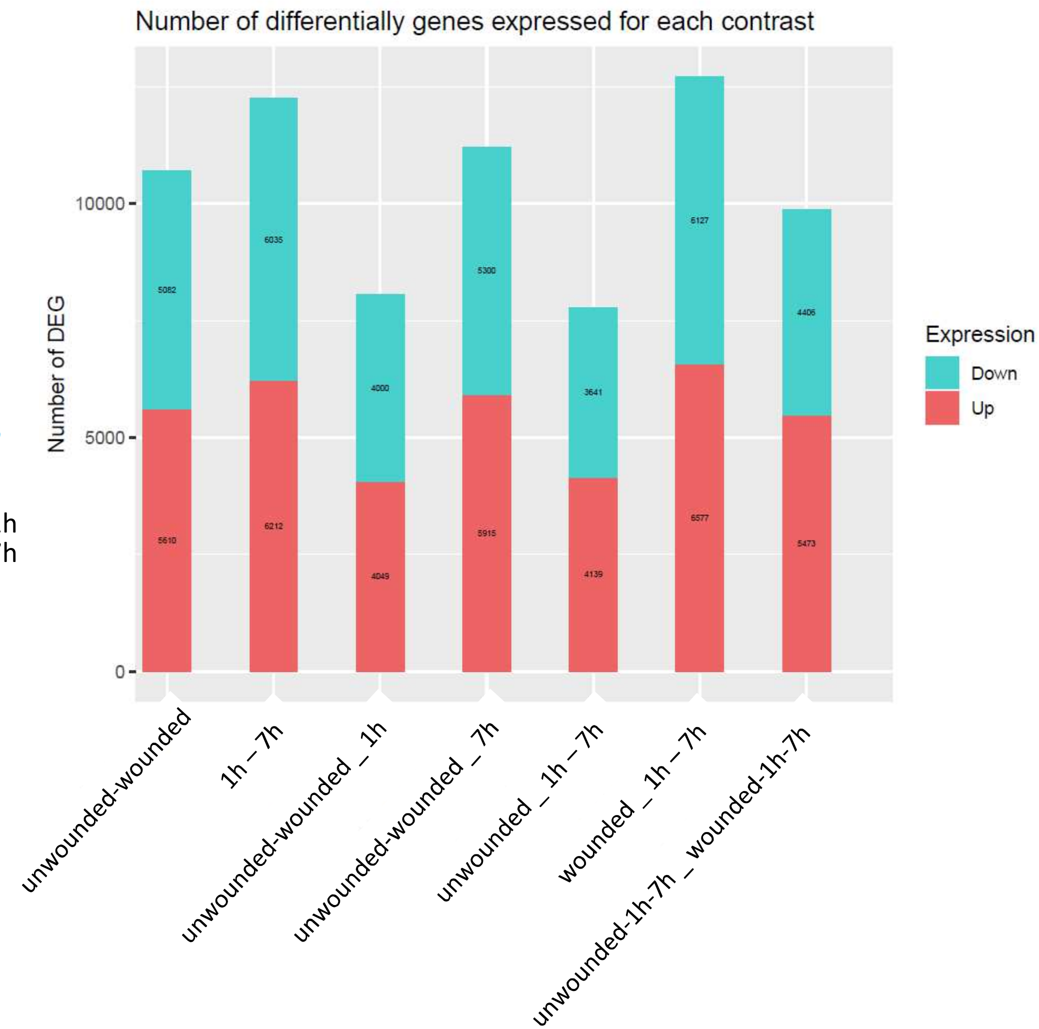


The two major phenolic compounds
- Eriodictyol-7-glucoside = prunine
- Naringenin-7-glucoside

RNAseq analyses

26 873 genes

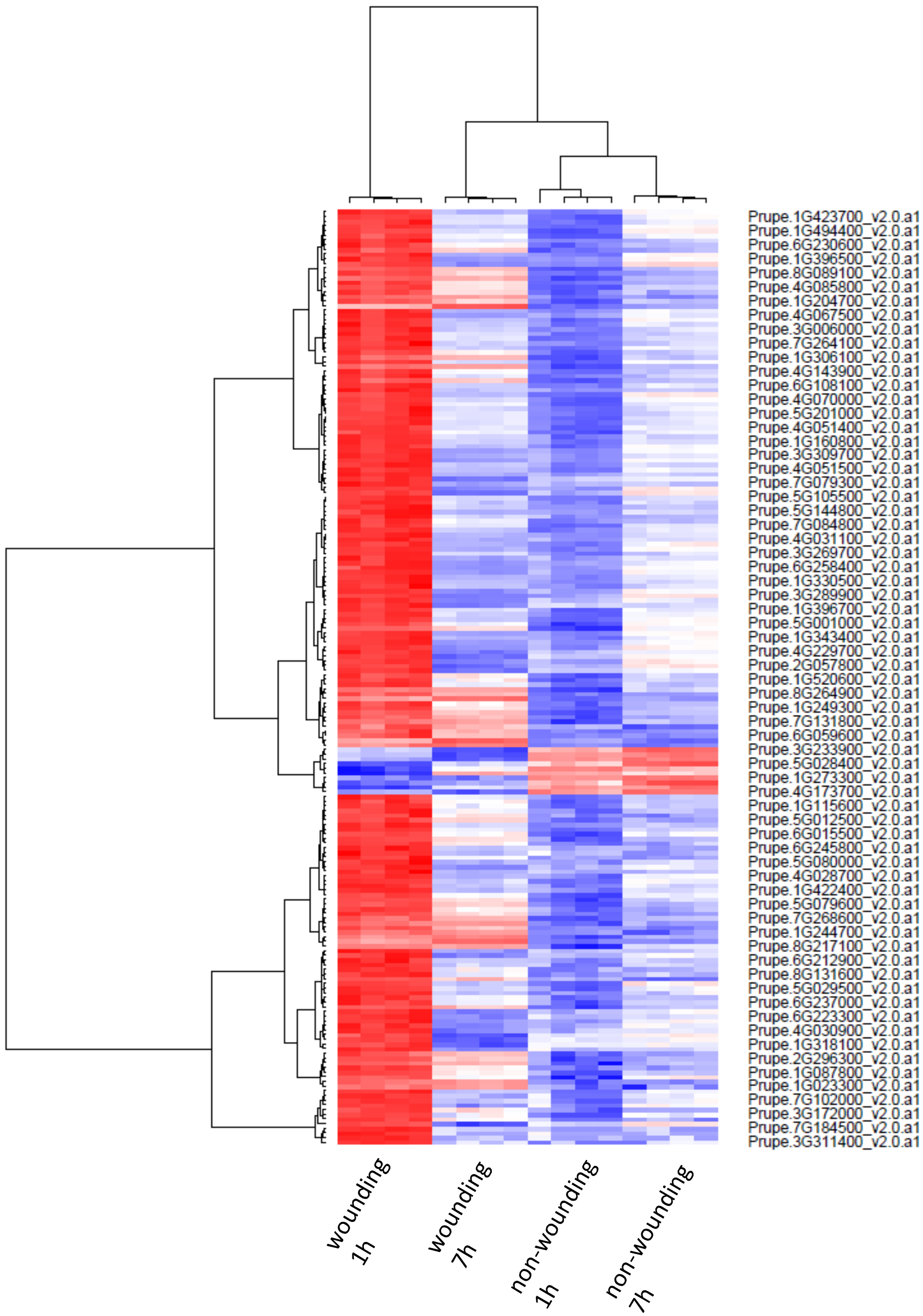




Lambert et al. 2020. DiCoExpress: a tool to process multifactorial RNAseq experiments from quality controls to co-expression analysis through differential analysis based on contrasts inside GLM models. *Plant Methods*, 16:68-68

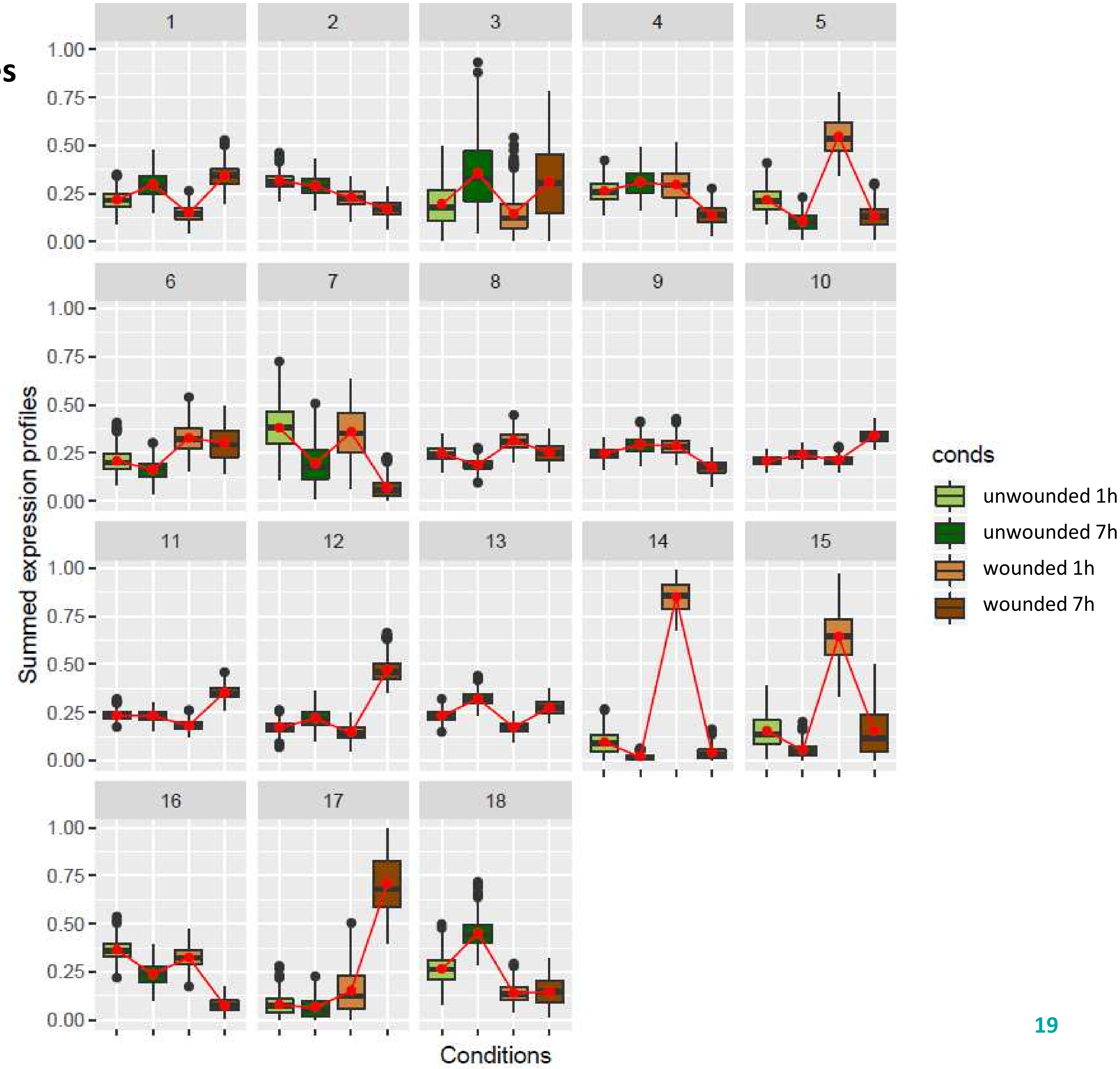
RNAseq analyses

Pathways activated after injury



RNAseq analyses

Co-expression analysis



CONCLUSIONS

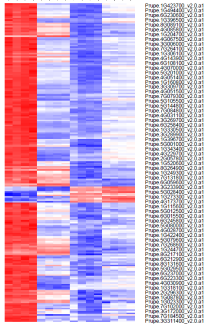
Take home messages

- 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*

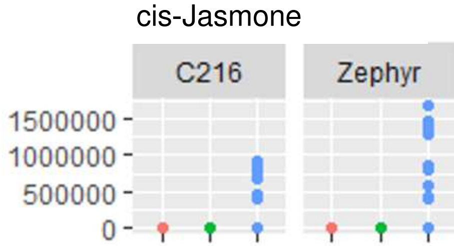
Wounding

1h

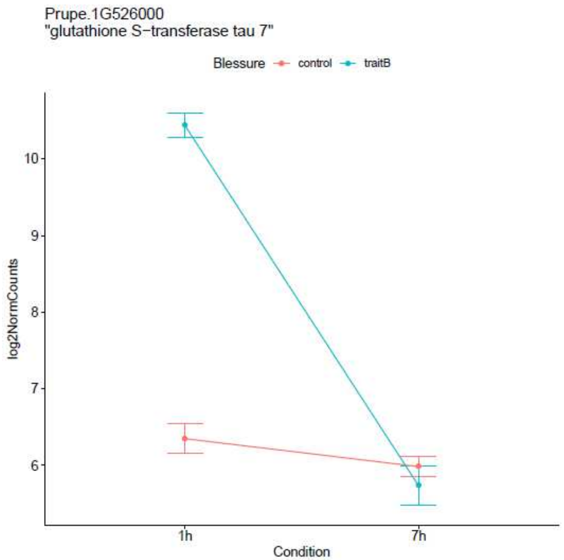


gene pathways
activated after injury

7h



volatile compounds
gave off



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

Wounding

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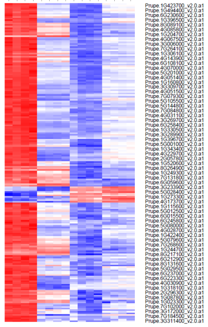
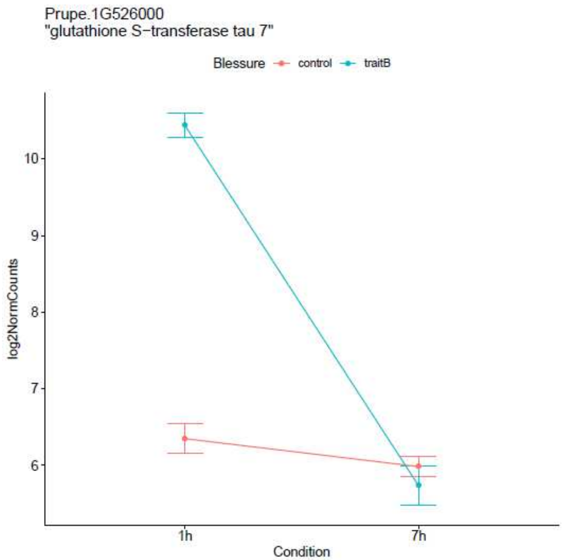


gene pathways
activated after injury

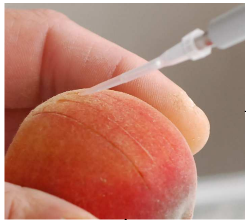
7h

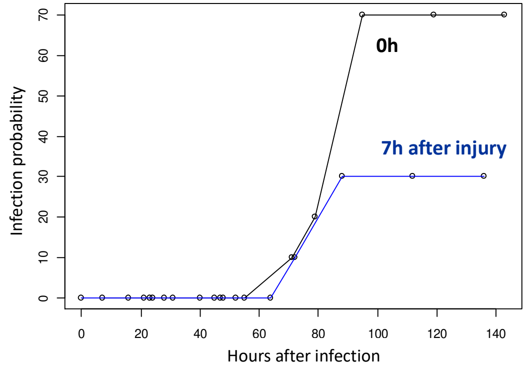
cis-Jasmone

volatile compounds
gave off



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

M Dini Viñoly

M Do C Raseira