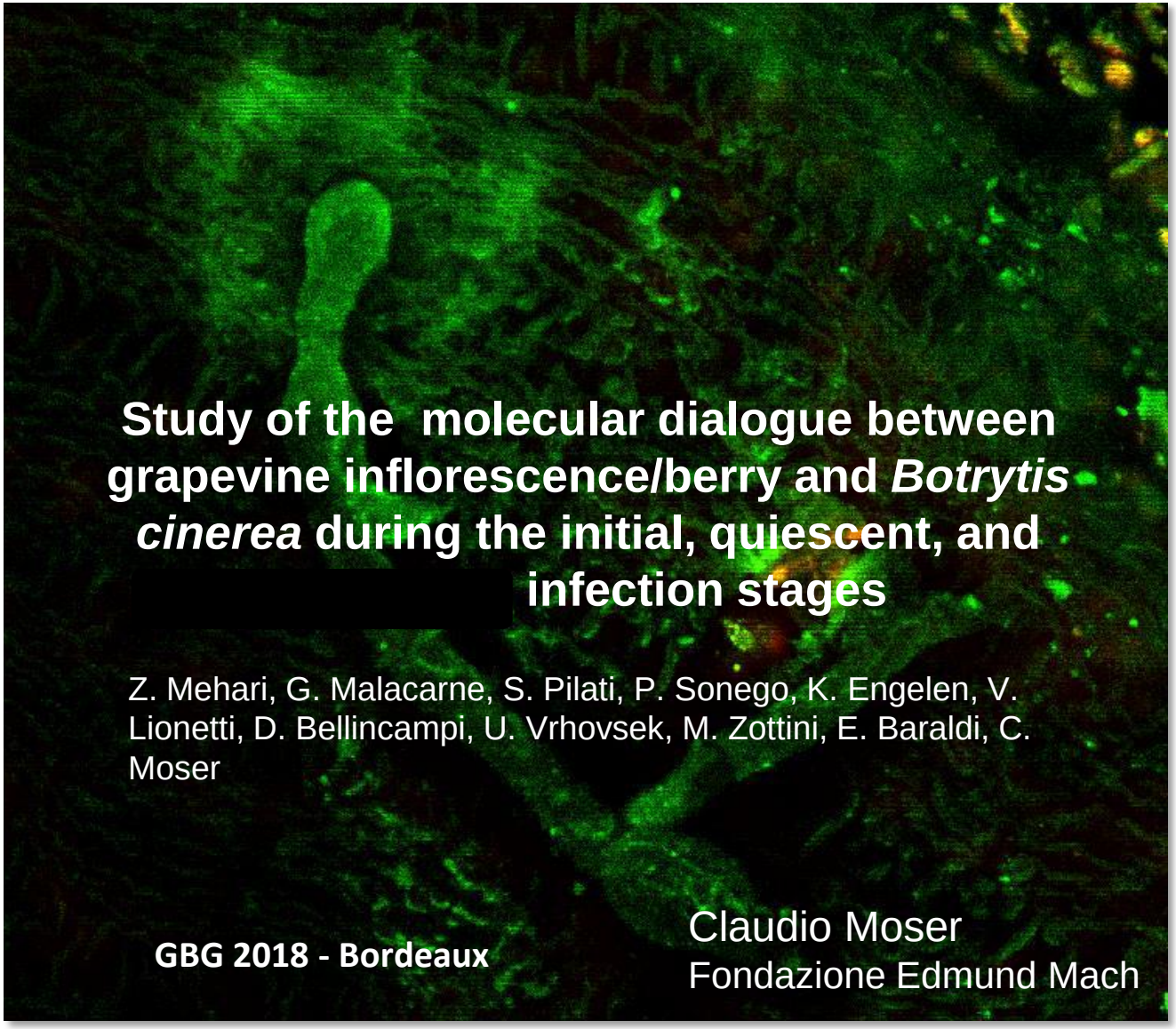
FONDAZIONE EDMUND MACH



Study of the molecular dialogue between grapevine inflorescence/berry and *Botrytis cinerea* during the initial, quiescent, and infection stages

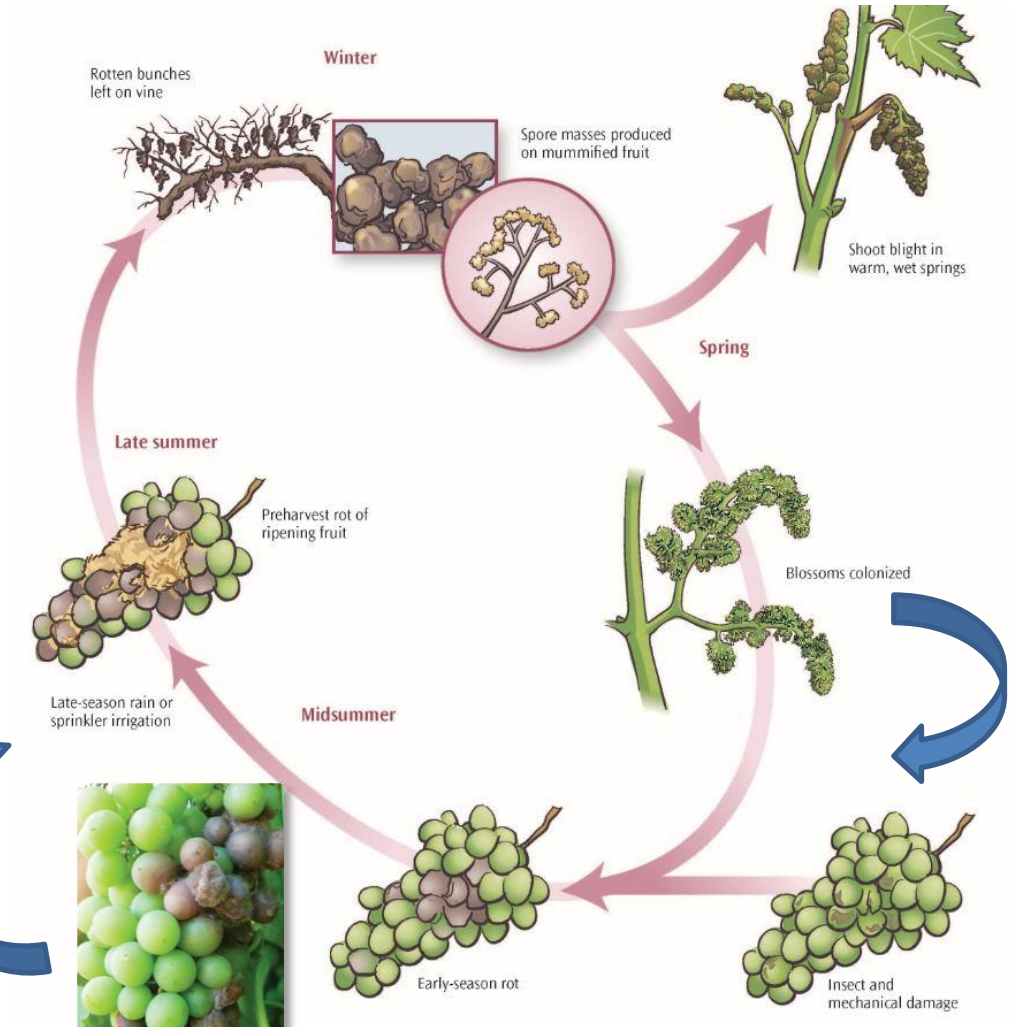
Z. Mehari, G. Malacarne, S. Pilati, P. Sonego, K. Engelen, V. Lionetti, D. Bellincampi, U. Vrhovsek, M. Zottini, E. Baraldi, C. Moser

GBG 2018 - Bordeaux

Claudio Moser
Fondazione Edmund Mach

Botrytis cinerea is one of the major grapevine pathogens: it causes bunch rot.

SYMPTOMS



LATENCY, no SYMPTOMS

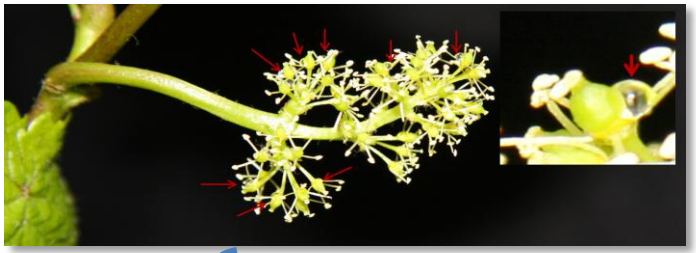
From: http://www.lodigrowers.com/botrytis-cinerea/

Control strategies

- Agronomical practices: leaf removal in the cluster zone, shoot topping to obtain looser clusters or better wind and sun exposure
- GA treatment: to obtain less compact bunches
- Fungicides: applied at 3-4 different phenological stages (mid-bloom, at bunch closure, vèraison, 2 w after vèraison)
- New interspecific varieties: (e.g. Carminoir, Galotta, IASMA Eco 1,2,3,4) or transgenic lines (e.g. chitinase overexp.)

Objective

1. PRIMARY INFECTION AT FLOWERING STAGE



2. GREEN BERRIES: QUIESCENCE

3. RIPE BERRIES: EGRESSION

To understand the interactions between grapevine inflorescence and B. cinerea during initial infection, quiescence, and egression stages

This knowledge can improve control strategies

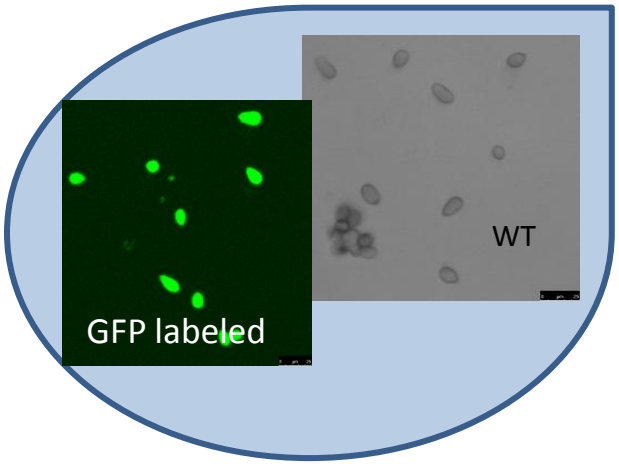
Material and Methods

- Flowers raised from fruiting cuttings (cv. Pinot Noir)

- *B. cinerea* B05.10 strain (WT & GFP labelled)

Anthesis

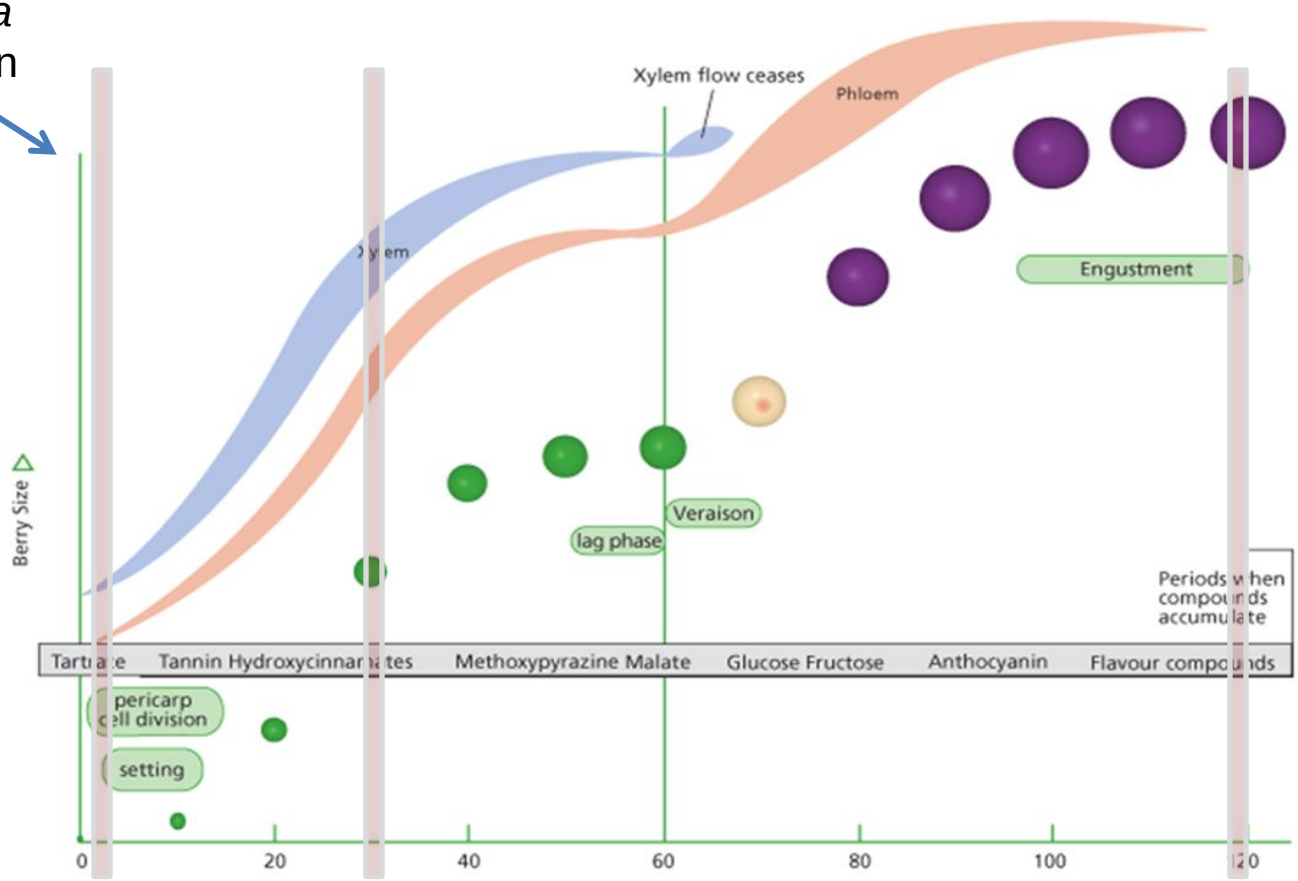
B. cinerea B05.10



300 conidia/flower

1. Macroscopic and microscopic analysis (B05.10-GFP)
2. Dual Transciptome analysis (RNA-seq)
3. Targeted secondary metabolite analysis (mainly phenols)

B. cinerea inoculation



12 - 96 hpi

5 time pts– sec. Met.
2 time pts– RNA-seq.

EARLY INFECTION

4 wpi

1 sample – sec. Met. & RNA-seq.

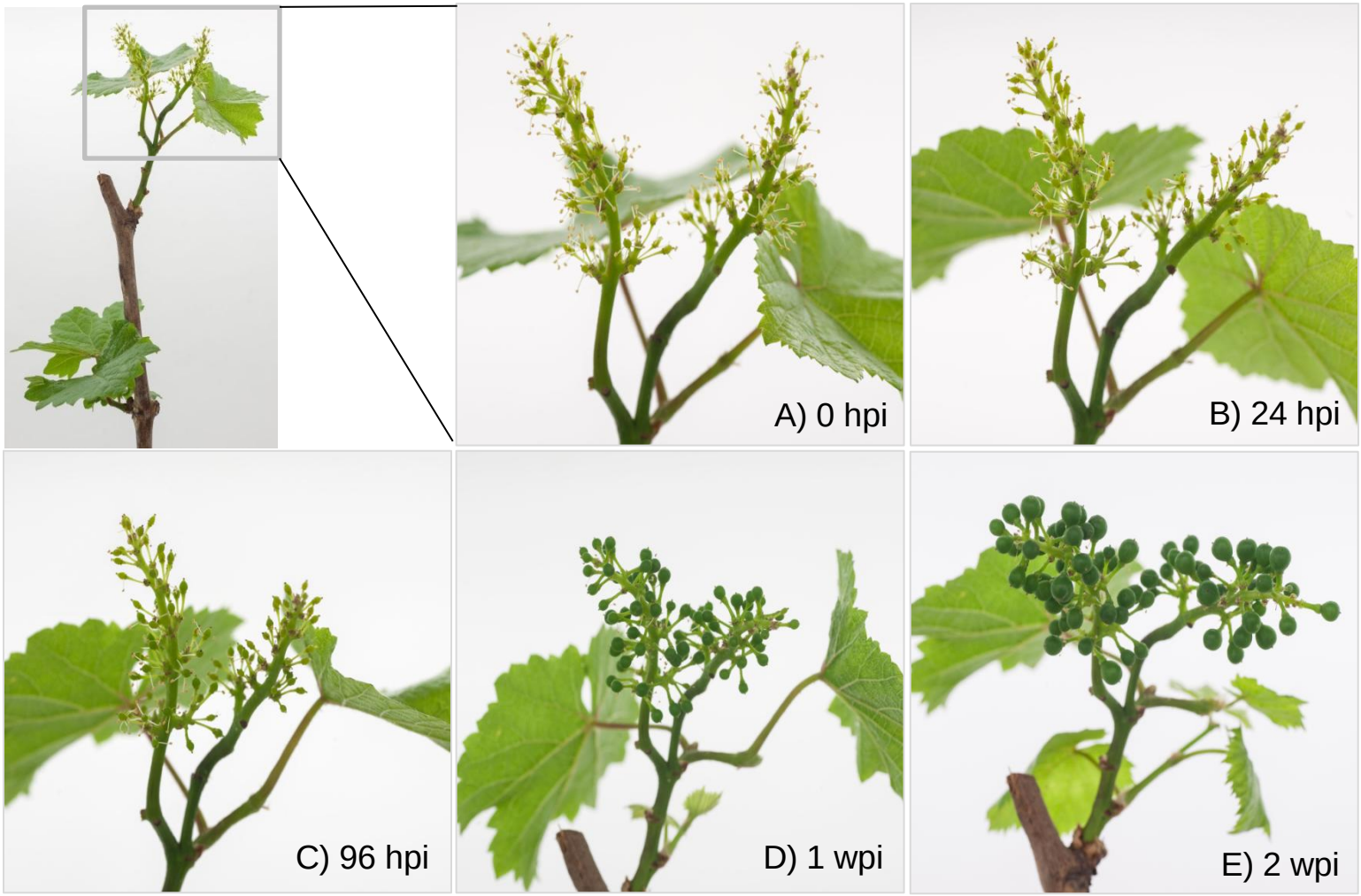
QUIESCENCE

12 wpi

2 samples – sec. Met. & RNA-seq.

EGRESSION

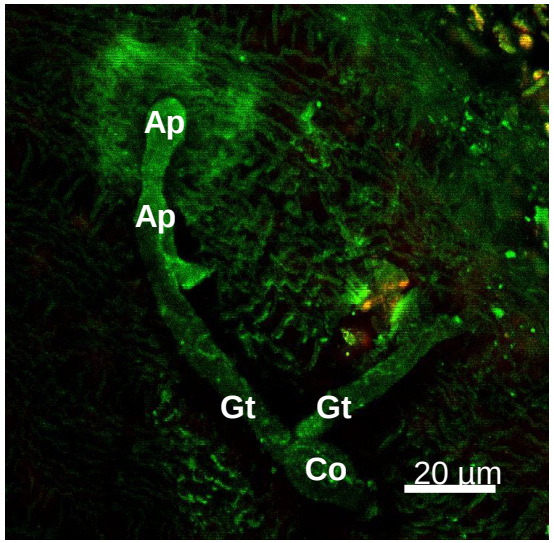
Macroscopic analysis

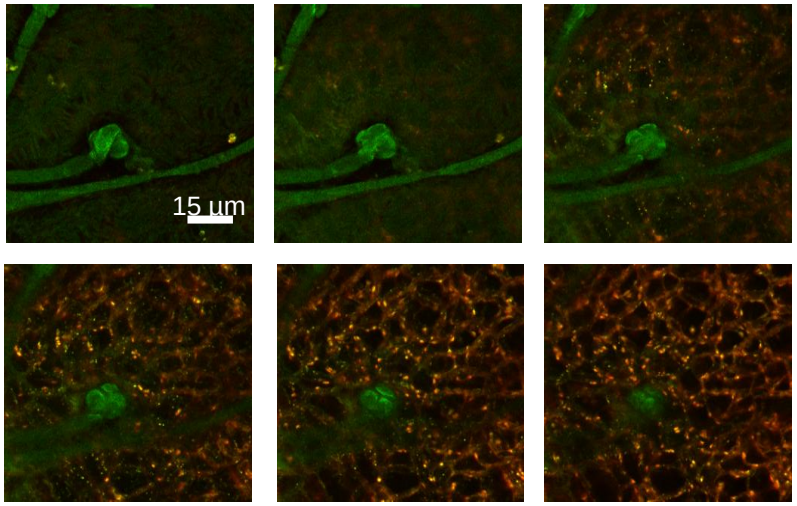


No visible symptoms of infection or fungal growth within the first 2 wpi

Microscopic confocal analysis

24 hpi

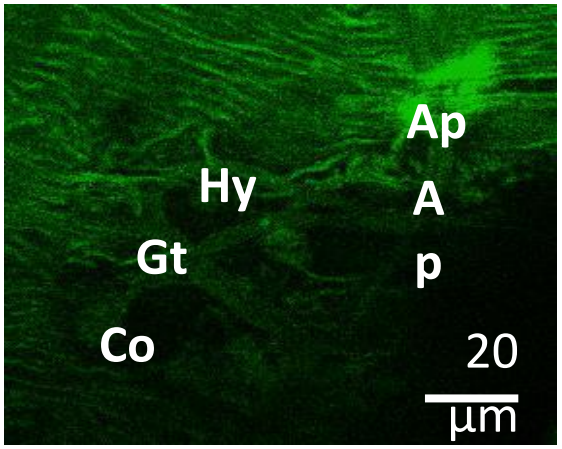


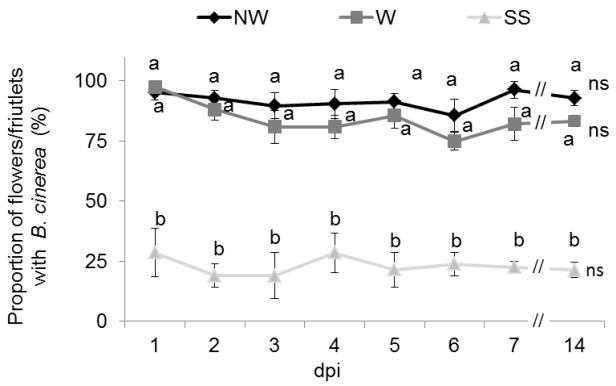


Z stack images with a pass of 1 µm each

Conidia germination and penetration of the first epidermal layers

96 hpi





Botrytis is still present but in a quiescent state: no growth progress

Transcriptome analysis

Summary of reads mapping of the 15 RNA-seq libraries

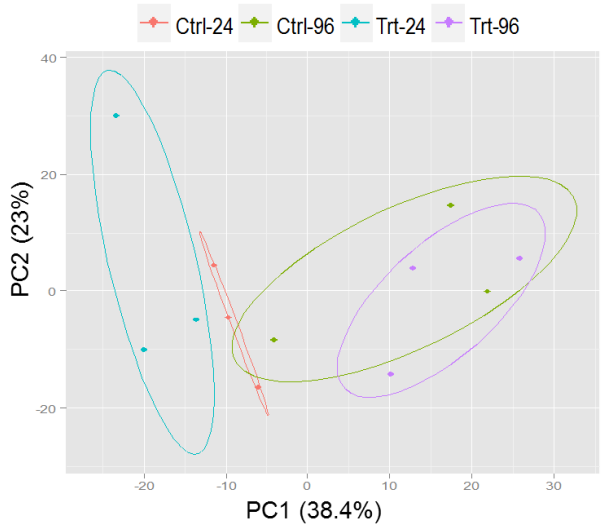
	Library	Total quality-trimmed reads	Reads mapped to *V. vinifera* reference	Reads uniquely mapped to *V. vinifera* reference	Reads mapped to *B. cinerea* reference	Reads uniquely mapped to *B. cinerea* reference
CTRL 24hpi	Ctrl 1-24	33,582,861	26,185,827 (77.97%)	24,938,558 (74.26%)	60,629 (0.18%)	9,736 (0.03%)
	Ctrl 2-24	30,634,590	24,156,916 (78.86%)	23,009,237 (75.11%)	63,570 (0.21%)	10,476 (0.03%)
	Ctrl 3-24	28,509,351	21,919,491 (76.89%)	20,541,510 (72.05%)	301,999 (1.06%)	26,834 (0.09%)
CTRL 96hpi	Ctrl 1-96	22,410,851	16,848,719 (75.18%)	15,496,486 (69.15%)	472,766 (2.11%)	7,195 (0.03%)
	Ctrl 2-96	37,394,962	24,336,710 (65.08%)	23,155,616 (61.92%)	60,179 (0.16%)	7,598 (0.02%)
	Ctrl 3-96	27,843,357	21,949,631 (78.83%)	20,838,437 (74.84%)	92,550 (0.33%)	11,458 (0.04%)
Bc-inf 24hpi	Trt 1-24	27,812,146	19,069,689 (68.57%)	16,282,036 (58.54%)	1,281,100 (4.6%)	35,213 (0.12%)
	Trt 2-24	31,644,086	25,622,237 (80.97%)	24,469,696 (77.33%)	134,816 (0.43%)	72,414 (0.23%)
	Trt 3-24	30,211,586	24,631,219 (81.53%)	23,391,950 (77.43%)	164,851 (0.55%)	77,040 (0.26%)
Bc-inf 96hpi	Trt 1-96	25,919,449	21,240,182 (81.95%)	20,270,161 (78.20%)	77,351 (0.34%)	16,453 (0.06%)
	Trt 2-96	26,077,701	20,388,725 (78.18%)	19,359,716 (74.24%)	116,774 (0.45%)	54,139 (0.21%)
	Trt 3-96	23,503,720	18,957,227 (80.66%)	18,077,115 (76.91%)	79,818 (0.34%)	28,891 (0.12%)
Bc-PDB	Bc 1	22,223,388	76,740 (0.35%)	21,423 (0.01%)	20,072,229 (90.32%)	14,108,503 (63.48%)
	Bc 2	21,289,297	47,738 (0.22%)	28,559 (0.13%)	19,256,732 (90.45%)	16,603,159 (77.99%)
	Bc 3	22,254,222	50,719 (0.23%)	17,798 (0.08%)	20,086,452 (90.25%)	16,522,732 (74.24%)

Between 59 and 75 % map to the *V. vinifera* reference genome

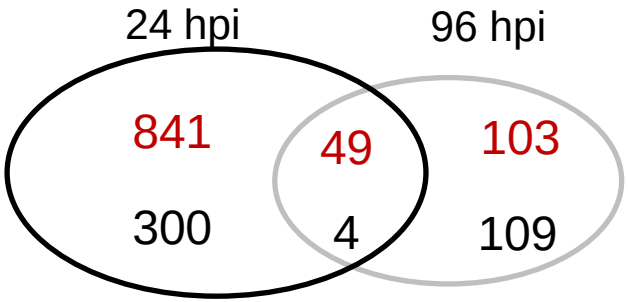
Only up to 0.26 % map to the *B. cinerea* genome

Transcriptome analysis

In Vitis



Grapevine DEGs (absolute fold-change > 1.5 and a *p*-value < 0.05)

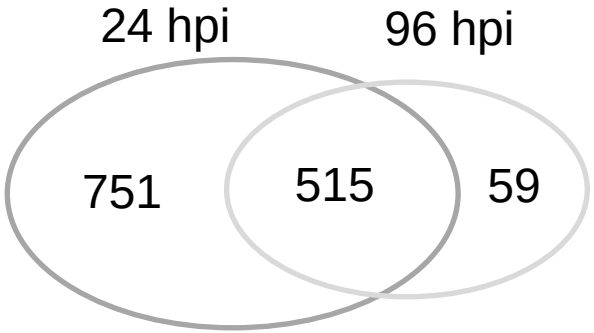


- At 24 hpi, *Botrytis* treated vs. untreated samples are separated
- At 96 hpi samples seem very similar at a whole transcriptome level

In Bc

- Fungal RNA in the samples is limited: i) 300 conidia per flower ii) arrest in fungal growth after penetration.

Bc genes expressed *in planta* => 1325 genes (at least 10 reads, on average)



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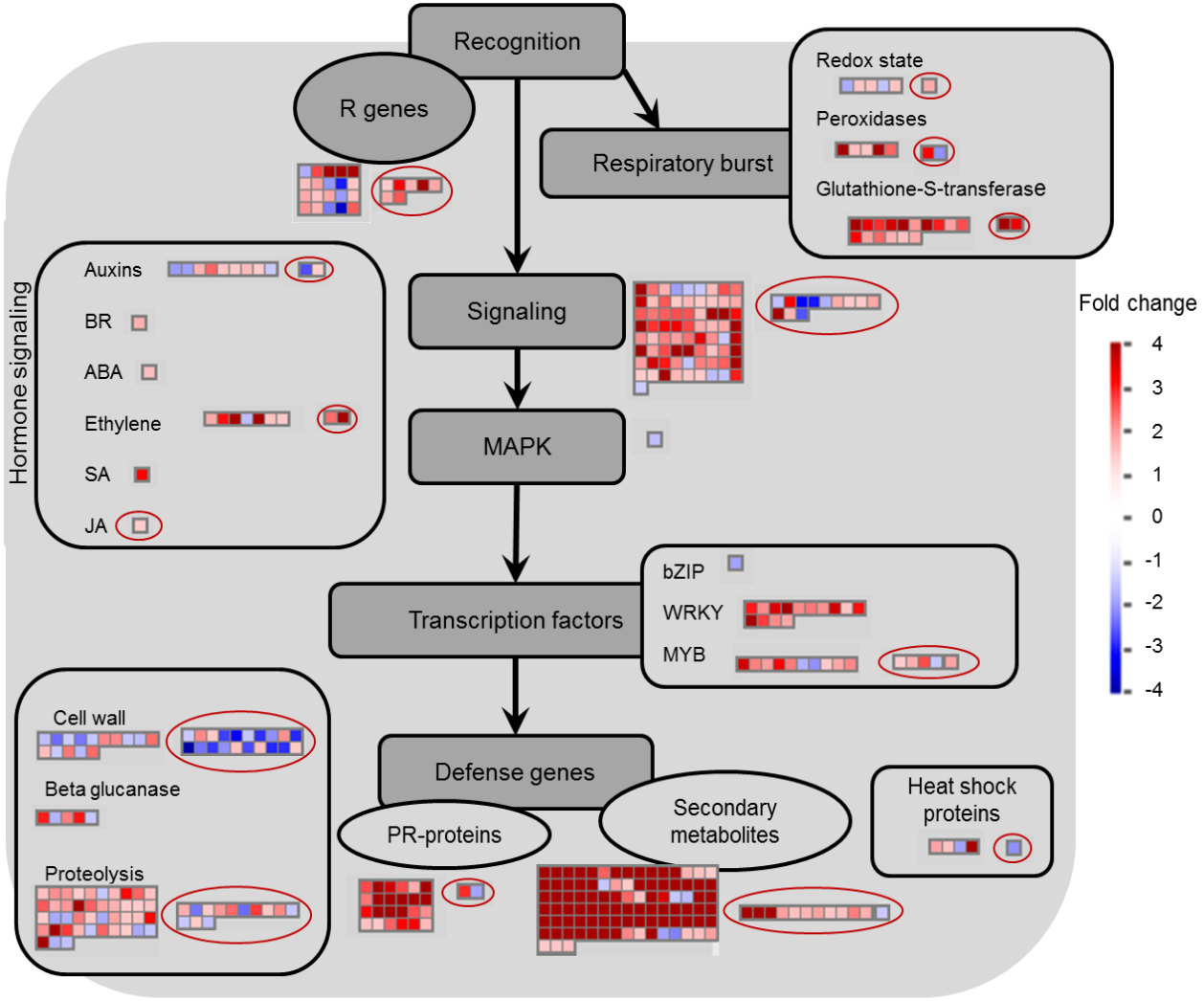
Botrytis transcripts expressed *in planta*

	Abbreviation	Transcript description	Gene ID	Average no. of raw reads from RNA-seq analysis: In planta expressed 24hpi	In planta expressed 96hpi	PDB culture
Redox	*BcSOD1*	Superoxide dismutase1	Bcin03g03390	76	26	9411[a]
	BcGOX1	Galactose oxidase	Bcin13g05710	21		609
	BcAOX	Alcohol oxidase	Bcin07g03040	24		490
	BcGST1	Glutathione S-transferase	Bcin10g00740			2655[a]
	BcPRD1	Dyp-type peroxidase	Bcin13g05720	19		312
	BcGPX3	Glutathione peroxidase	Bcin03g01480	23		2871[a]
	BcLCC2	Laccase2	Bcin14g02510	15		32[b]
CWDE	*BcCUTA*	Cutinase	Bcin15g03080	15		54[b]
	BcCUT-like1	Cutinase	Bcin01g09430	68	11	9[b]
	BcOAH	Oxaloacetate acetylhydrolase	Bcin12g01020	386		38[b]
	BcPG1	Polygalacturonase1	Bcin14g00850	209	175	147821[a]
	BcPG2	Polygalacturonase2	Bcin14g00610			362
	BcPG4	Polygalacturonase4	Bcin03g01680	44		47[b]
	BcPG6	Polygalacturonase6	Bcin02g05860	75		62
	BcPEL-like1	Pectate lyase	Bcin03g05820	87	24	40[b]
	BcGAR2	D-galacturonic acid reductase2	Bcin03g01500	37		104
	BcLGD1	D-galactonate dehydrogenase	Bcin01g09450	61	10	804
	BcGLA1	2-keto-3-deoxy-L-galactonate aldolas	Bcin03g01490	70	13	66
	BcXYN11A	Endo-beta-1,4-xylanase	Bcin03g00480	18		129
	BcβGLUC	Beta-glucosidase 1 precursor	Bcin10g05590	32		75
	BcAP8	Aspartic proteinase8	Bcin12g02040	30	12	1064[a]
	BcAP9	Aspartic proteinase9	Bcin12g00180	16		569
Phytotoxins	*BcBOT1*	Botrydial biosynthesis1	Bcin12g06380	58		86
	BcBOT2	Botrydial biosynthesis2	Bcin12g06390	41		55
	BcBOA6	Botcinic acid6	Bcin01g00060	13		1048[a]

B.c. is ready to infect and cause disease

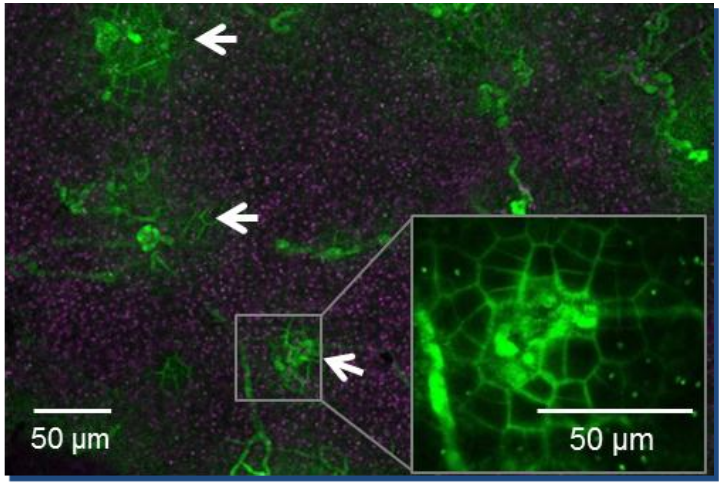
Vitis defense response



- Redox:
  - GST & ROS
- Membrane-localized receptor like kinases:
  - CLV1, WAK1, & BAK1
- Phytohormones
- Transcription factors:
  - WRKY & MYB
- Secondary metabolism
- PRs and Proteases
  - Chitinases
  - Glucanases
  - Thaumatin
  - Lipases
  - PR10, PR1

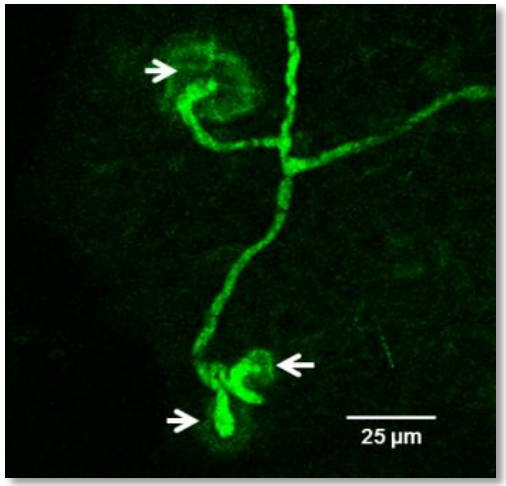
Large transcriptional reprogramming toward defense at 24hpi > at 96hpi

ROS accumulated at the site of penetration

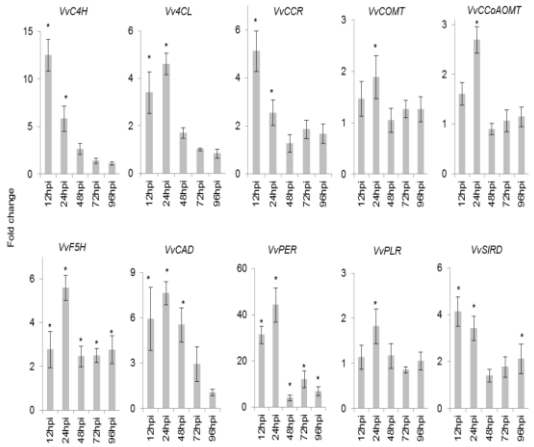


Cytoplasmic HyPer grapevine transgenic line

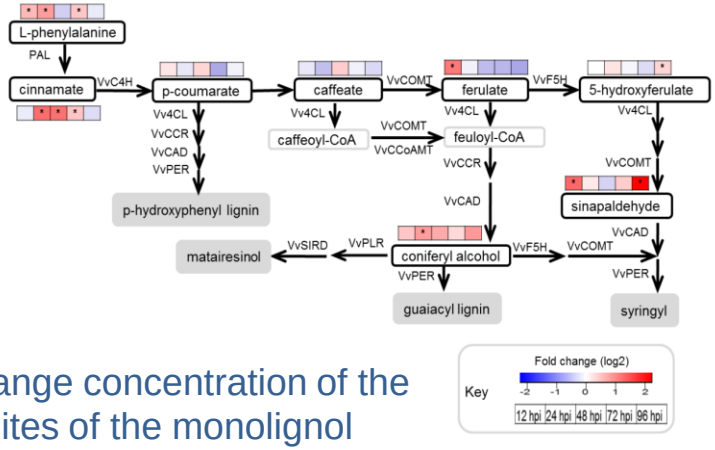
Infection triggers cell wall reinforcement



Autofluorescence (cell wall apposition)



Gene expression of the monolignol biosynthesis pathway



Fold change concentration of the metabolites of the monolignol biosynthesis pathway

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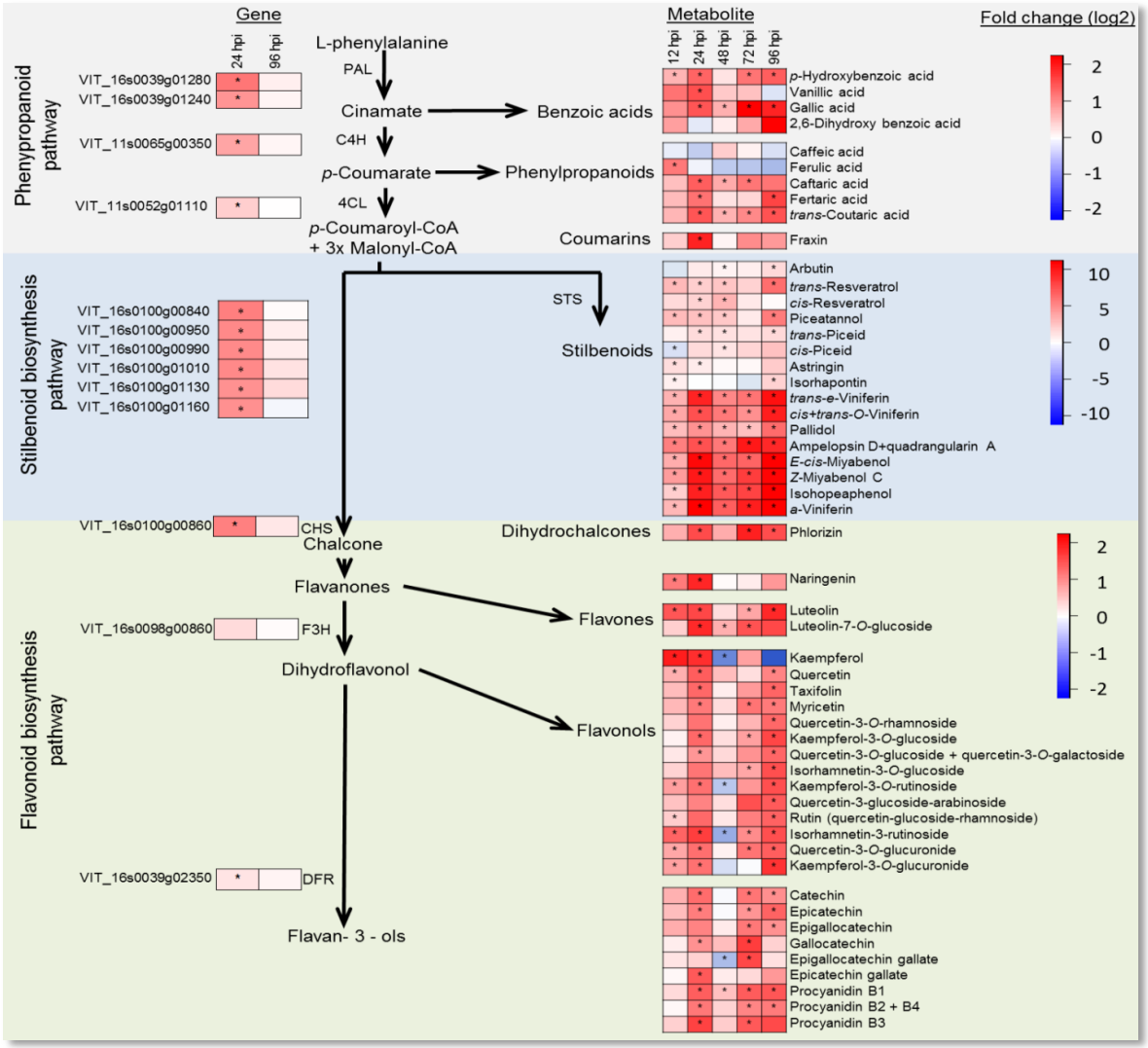
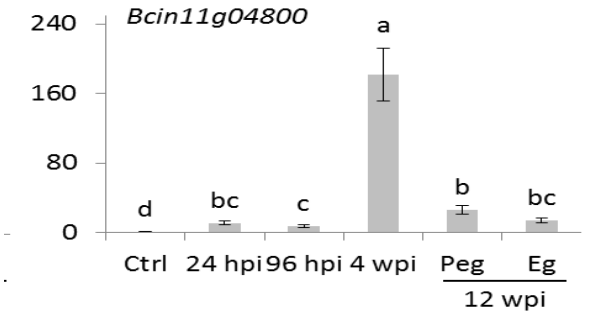
Polyphenol secondary metabolism

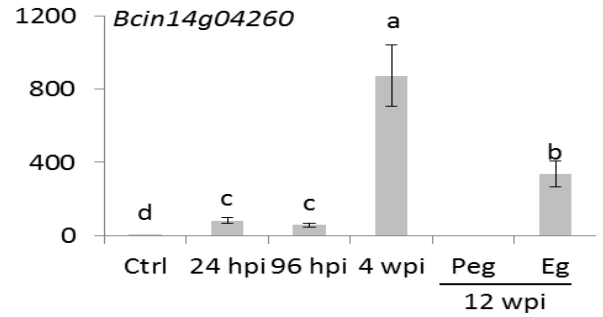


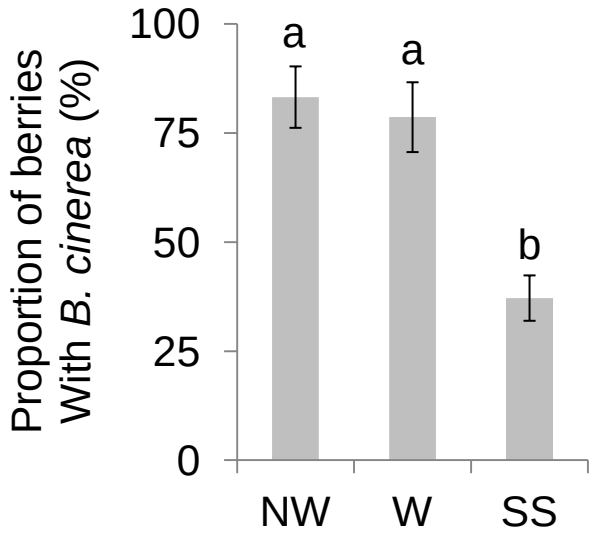
Several polyphenols involved in grapevine defense are induced together with some key biosynthetic genes.

Macroscopic analysis

4 wpi: QUIESCENCE







Very low signal of *B. cinerea* in hard green 4 wpi berries, still some specific genes are expressed.

Few hundred (ca. 600 genes) Vitis genes are modulated (most up-regulated), several in common with 12hpi

In summary:

Upon recognizing *Botrytis*, the flower within 24 hpi upregulates PR-proteins, monolignol precursors, stilbenoids, and reactive oxygen species, together with cell wall reinforcement.

=> forces *B. cinerea* into quiescence (still basal specific activity)

Plant, Cell & Environment

Plant, Cell and Environment (2017) doi: 10.1111/pce.12937

Original Article

Molecular analysis of the early interaction between the grapevine flower and *Botrytis cinerea* reveals that prompt activation of specific host pathways leads to fungus quiescence

Zeraye Mehari Haile[1,6], Stefania Pilati[1], Paolo Sonego[2], Giulia Malacarne[1], Urska Vrhovsek[3], Kristof Engelen[2], Paul Tudzynski[4], Michela Zottini[5], Elena Baraldi[6] & Claudio Moser[1]

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University of Bologna

Elena Baraldi

University of Padova

Michela Zottini

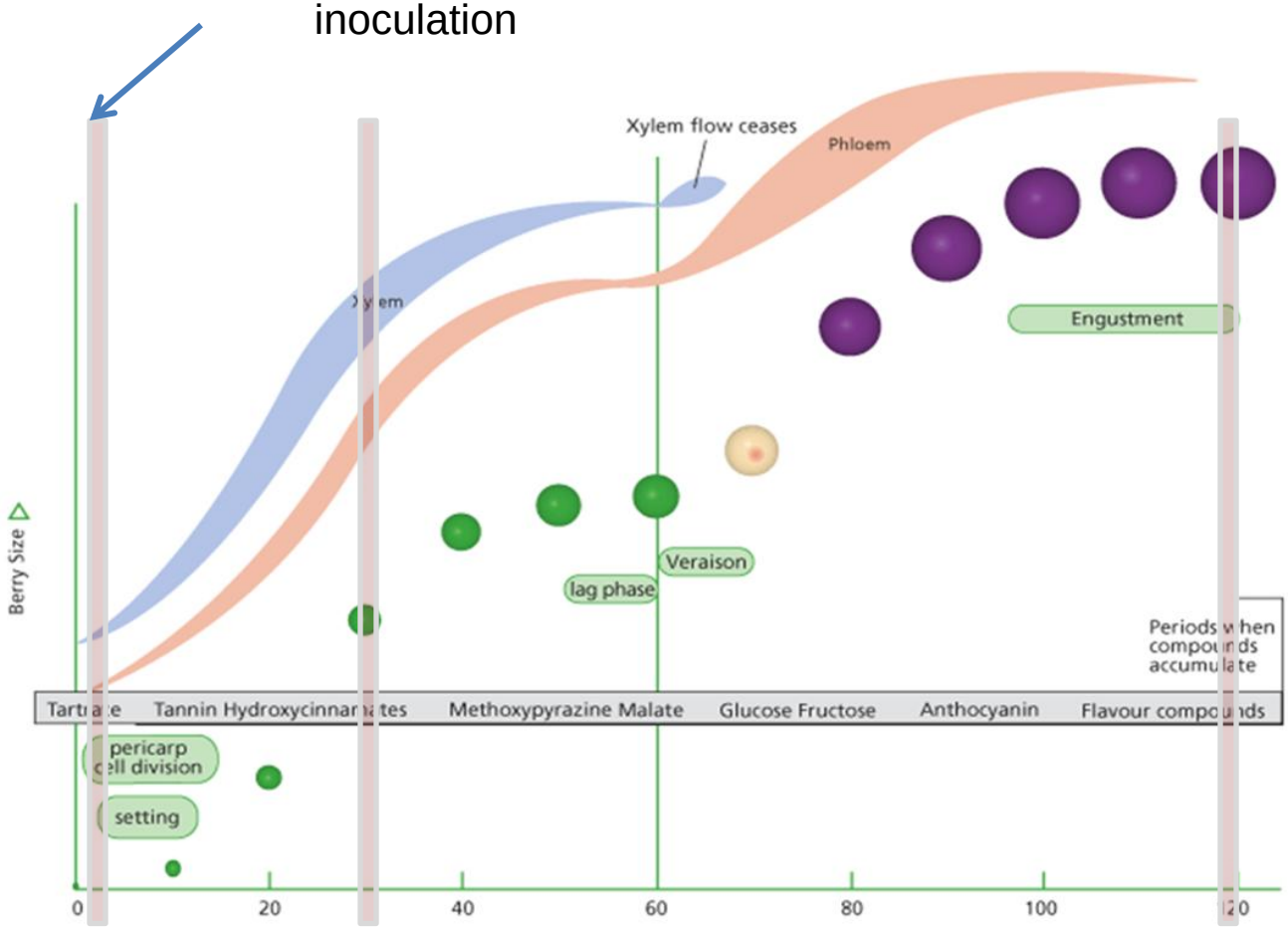
Westfälische Wilhelms-Universität Münster

Paul Tudzynski

Ulrike Siegmund

Thank you!

B. cinerea inoculation



12 - 96 hpi

5 samples – sec. Met.
2 samples – RNA-seq.

EARLY INFECTION

4 wpi

1 sample – sec. Met. & RNA-seq.

QUIESCENCE

12 wpi

2 samples – sec. Met. & RNA-seq.

EGRESSION

Transcriptome analysis

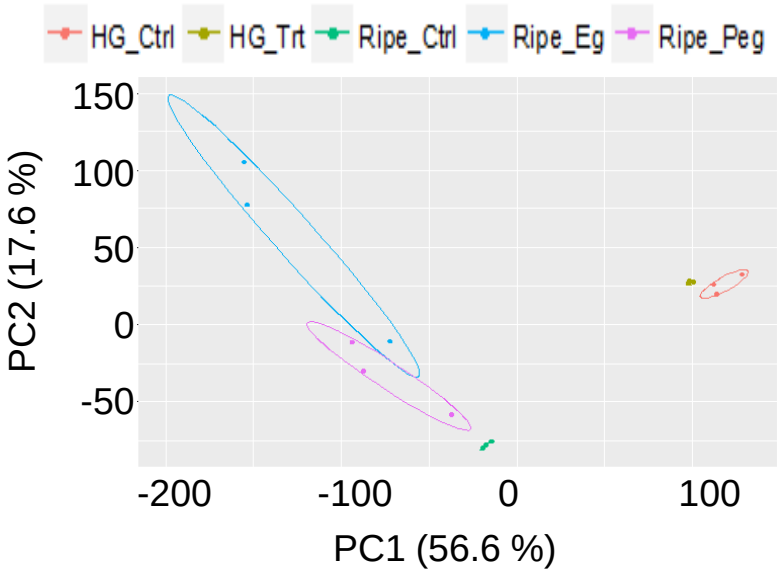
Summary of reads mapping of the 15 (+3) RNA-seq libraries

Library	Total quality-trimmed reads	Reads mapped to V. vinifera reference	Reads uniquely mapped to V. vinifera reference	Reads mapped to B. cinerea reference	Reads uniquely mapped to B. cinerea reference
HG_Ctrl1	18,764,162	17,226,186 (91.80 %)	16,552,848 (88.22 %)	46,268 (0.25 %)	520 (0.01 %)
HG_Ctrl2	18,245,810	13,462,443 (73.78 %)	10,041,596 (55.04 %)	1,675,246 (9.18 %)	1,786 (0.00 %)
HG_Ctrl3	20,330,170	17,125,573 (84.24 %)	16,376,294 (80.55 %)	68,337 (0.34 %)	545 (0.00 %)
HG_Trt1	23,828,415	21,594,466 (90.62 %)	20,837,836 (87.45 %)	45,065 (0.19 %)	6,958 (0.03 %)
HG_Trt2	21,976,001	19,853,734 (90.34 %)	19,120,028 (87.00 %)	56,664 (0.26 %)	16,928 (0.08 %)
HG_Trt3	21,146,332	18,828,955 (89.04 %)	18,070,467 (85.45 %)	75,234 (0.35 %)	24,680 (0.12 %)
Ripe_Ctrl1	24,635,902	22,048,745 (89.50 %)	21,007,364 (85.27 %)	37,989 (0.15 %)	818 (0.00 %)
Ripe_Ctrl2	26,843,805	24,151,620 (89.97 %)	23,069,555 (85.94 %)	27,883 (0.10 %)	836 (0.00 %)
Ripe_Ctrl3	29,860,937	26,527,044 (88.84 %)	25,136,297 (84.18 %)	169,478 (0.57 %)	885 (0.00 %)
Ripe_Eg1	21,296,699	3,254,793 (15.28 %)	2,756,774 (12.94 %)	16,664,468 (78.25 %)	14,235,574 (66.84 %)
Ripe_Eg2	22,578,478	5,287,093 (23.42 %)	4,550,569 (20.15 %)	14,815,131 (65.62 %)	12,490,580 (55.32 %)
Ripe_Eg3	28,787,397	12,703,484 (44.13 %)	11,577,654 (40.22 %)	12,750,536 (44.29 %)	10,718,882 (37.23 %)
Ripe_Peg1	45,369,750	32,808,151 (72.31 %)	27,381,446 (60.35 %)	2,547,394 (5.61 %)	22,842 (0.05 %)
Ripe_Peg2	55,880,939	45,269,916 (81.01 %)	42,976,225 (76.91 %)	2,315,554 (4.14 %)	1,698,079 (3.04 %)
Ripe_Peg3	47,239,407	40,062,424 (84.81 %)	38,243,440 (80.96 %)	118,269 (0.25 %)	23,815 (0.05 %)
Bc1	22,223,388	76,740 (0.35 %)	21,423 (0.01 %)	20,072,229 (90.32 %)	14,108,503 (63.48 %)
Bc2	21,289,297	47,738 (0.22 %)	28,559 (0.13 %)	19,256,732 (90.45 %)	16,603,159 (77.99 %)
Bc3	22,254,222	50,719 (0.23 %)	17,798 (0.08 %)	20,086,452 (90.25 %)	16,522,732 (74.24 %)

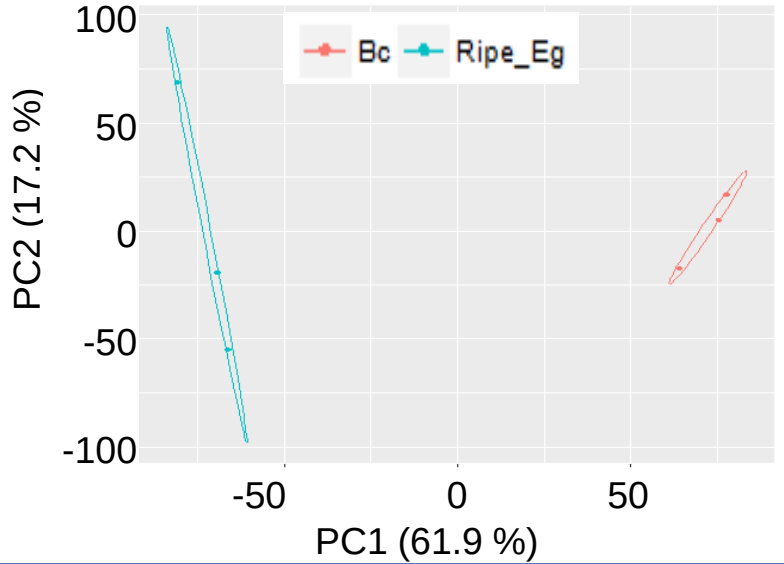
Very low signal of *B. cinerea* in hard green and pre-egressed samples

Transcriptome analysis

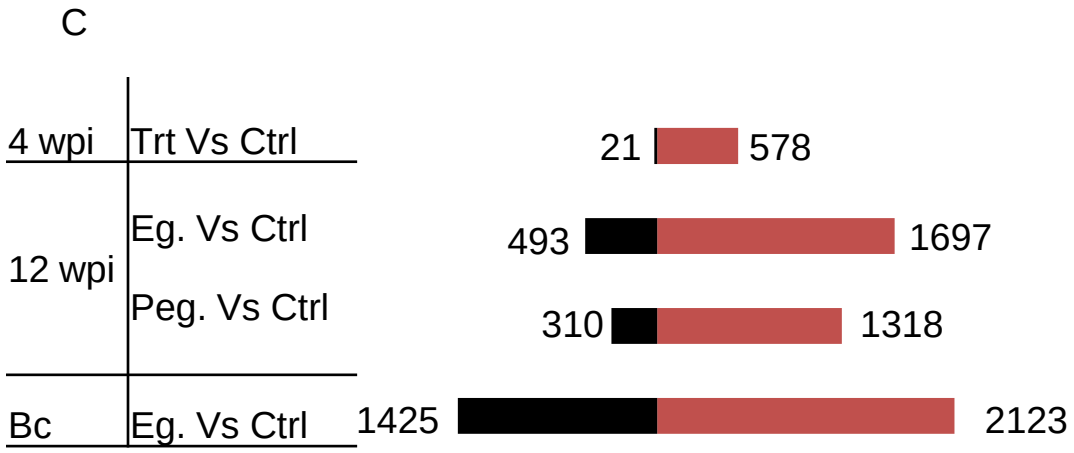
PCA Vitis transcriptome



PCA *B.c.* transcriptome



Vitis DEGs
(|FC |> 2 and P-value < 0.01)



Vitis:

- Few hundred genes are modulated in the quiescent stage
- A larger transcriptome re-arrangement is observed at Peg and Egression stages.

B. cinerea:

- in Eg. samples very different from PDB culture.

B. c. transcriptome at egression stage

Egression

86 % of total *B. cinerea* transcriptome is expressed

3,548 genes are differentially regulated

(compared to PDB cultured *B. cinerea*)

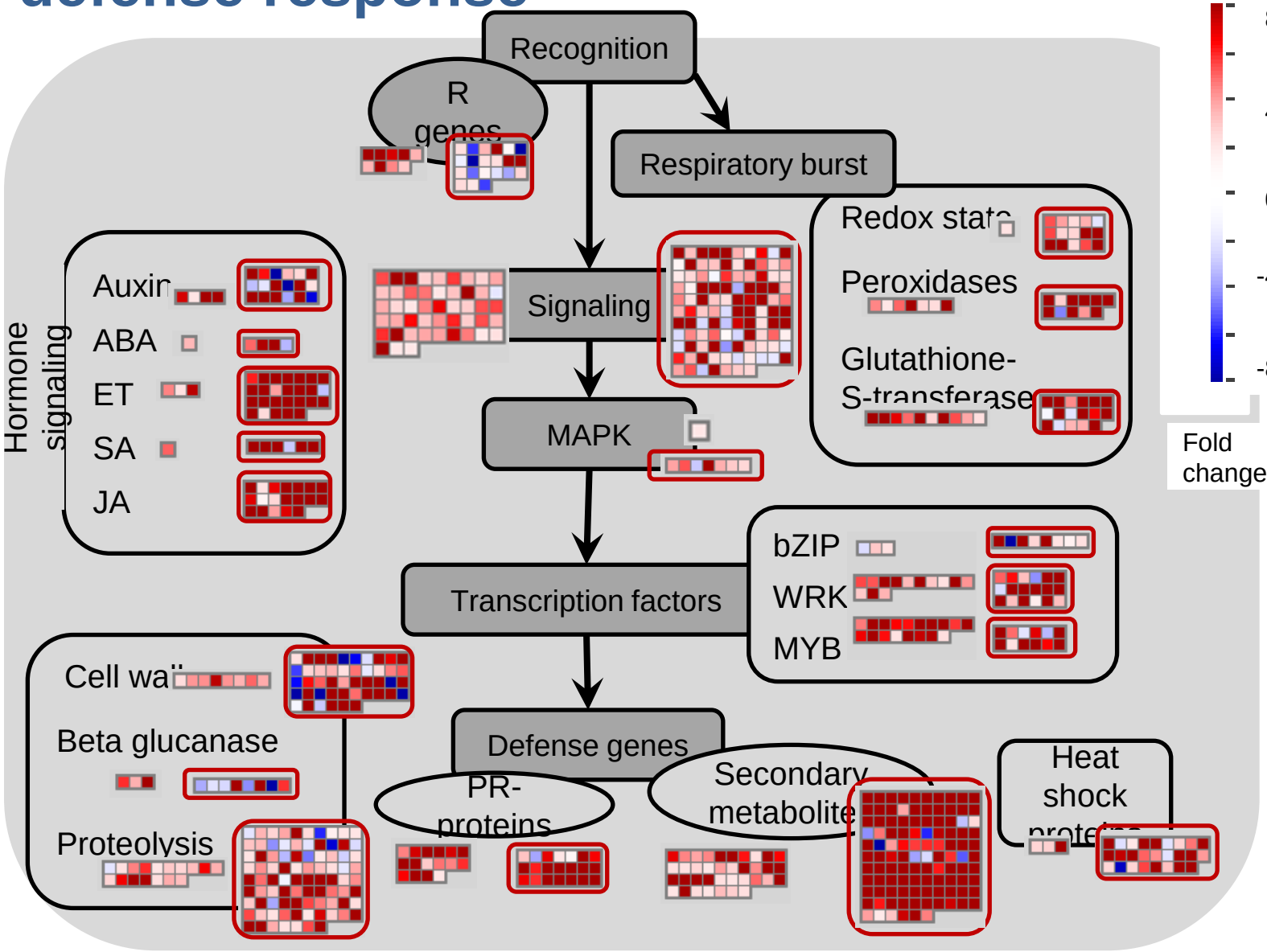
Biological functions:

- Carbohydrate-active enzymes and others involved in plant cell wall degradation

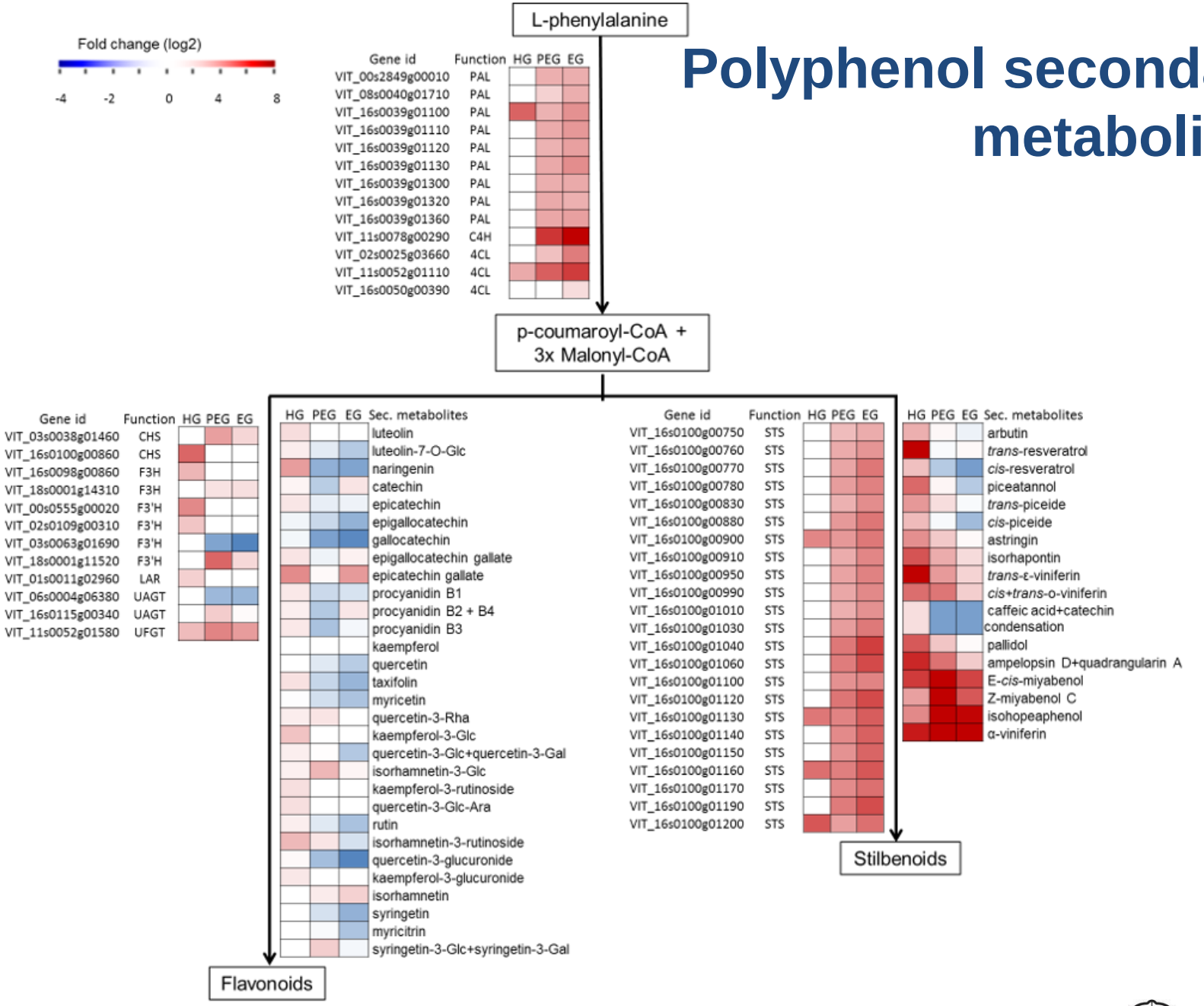
- Virulence and/or growth related genes

Utmost metabolic activity in the egressed *B. cinerea,* already starting at Peg stage.

Vitis defense response



Polyphenol secondary metabolism



Conclusions

The conjugated actions of induced defense responses in flowers and hard-green berries seem to be responsible for *B. cinerea* quiescence.

At ripening, the fungus exploits the ripening associated physico-chemical changes and recovers an active metabolism and pathogenic activity to cause bunch rot.

SUMMARY PAPER 2: During the quiescent state, the expressed fungal transcriptome highlighted that the fungus was undergoing basal metabolic activities besides remodeling its cell wall to evade plant chitinases. Berries responded by differentially regulating genes encoding for different PR proteins and genes involved in monolignol, flavonoid and stilbenoid biosynthesis pathways. At 12 wpi, the transcripts of B. cinerea in the pre-egressed samples showed that virulence-related genes were expressed, suggesting infection process was initiated. The egressed B. cinerea expressed almost all virulence and growth related genes that enabled the pathogen to colonize the berries. In response to egression, ripe berries reprogrammed different defense responses, though futile. Our results indicated that hard-green berries defense program was capable to contain B. cinerea; however, ripening associated fruit's cell wall self-disassembly together with high humidity created the opportunity for the fungus to egress and cause bunch rots