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


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Fondazione Edmund Mach

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

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

Introduction
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.)
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)

1. Macroscopic and microscopic analysis (B05.10-GFP)
2. Dual Transcriptome analysis (RNA-seq)
3. Targeted secondary metabolite analysis (mainly phenols)
**B. cinerea** inoculation

- **EARLY INFECTION**
  - 12 - 96 hpi
  - 5 time pts– sec. Met.
  - 2 time pts– RNA-seq.

- **QUIESCENCE**
  - 4 wpi
  - 1 sample – sec. Met.
  - & RNA-seq.

- **EGRESSION**
  - 12 wpi
  - 2 samples – sec. Met.
  - & RNA-seq.
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

96 hpi

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

Conidia germination and penetration of the first epidermal layers

Results
**Transcriptome analysis**

Summary of reads mapping of the 15 RNA-seq libraries

<table>
<thead>
<tr>
<th>Library</th>
<th>Total quality-trimmed reads</th>
<th>Reads mapped to V. vinifera reference</th>
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<tbody>
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<td><strong>CTRL 24hpi</strong></td>
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<tr>
<td>Ctrl 1-24</td>
<td>33,582,861</td>
<td>26,185,827 (77.97%)</td>
<td>24,938,558 (74.26%)</td>
<td>60,629 (0.18%)</td>
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<td>30,634,590</td>
<td>24,156,916 (78.86%)</td>
<td>23,009,237 (75.11%)</td>
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<tr>
<td>Ctrl 3-24</td>
<td>28,509,351</td>
<td>21,919,491 (76.89%)</td>
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<td>26,834 (0.09%)</td>
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<tr>
<td>Ctrl 1-96</td>
<td>22,410,851</td>
<td>16,848,719 (75.18%)</td>
<td>15,496,486 (69.15%)</td>
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<tr>
<td>Trt 1-24</td>
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<td>19,069,689 (68.57%)</td>
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<td>25,622,237 (80.97%)</td>
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<td>Trt 3-24</td>
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<td>24,631,219 (81.53%)</td>
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<td>164,851 (0.55%)</td>
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<td>116,774 (0.45%)</td>
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<td>Bc 1</td>
<td>22,223,388</td>
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<td>21,423 (0.01%)</td>
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<td>Bc 2</td>
<td>21,289,297</td>
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</table>

Between 59 and 75 % map to the V. vinifera reference genome
Only up to 0.26 % map to the B. cinerea genome
In Vitis

- 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.

Transcriptome analysis

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

24 hpi

- 841 genes
- 300 genes

96 hpi

- 49 genes
- 103 genes
- 4 genes
- 109 genes

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

24 hpi

- 751 genes

96 hpi

- 515 genes
- 59 genes

Results
# Botrytis transcripts expressed in planta

## Results

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Transcript description</th>
<th>Gene ID</th>
<th>Average no. of raw reads from RNA-seq analysis</th>
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<td>In planta expressed</td>
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<td>BcSOD1</td>
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<td>BcGOX1</td>
<td>Galactose oxidase</td>
<td>Bcin13g05710</td>
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<td>BcAOX</td>
<td>Alcohol oxidase</td>
<td>Bcin07g03040</td>
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<td>BcGST1</td>
<td>Glutathione S-transferase</td>
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<td>BcPRD1</td>
<td>Dyp-type peroxidase</td>
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<td>Laccase2</td>
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<td>BcCUT-like1</td>
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<td>BcOAH</td>
<td>Oxaloacetate acetylhydrolase</td>
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<td>Polygalacturonase2</td>
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<td>D-galactonate dehydrogenase</td>
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<td>BcBOA6</td>
<td>Botcinic acid6</td>
<td>Bcin01g00060</td>
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</table>

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**Redox**

**CWDE**

**Phytotoxins**

* 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
**Results**

**Flower**

**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
Several polyphenols involved in grapevine defense are induced together with some key biosynthetic genes.
Very low signal of \textit{B. cinerea} in hard green 4 wpi berries, still some specific genes are expressed.

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

In summary:
Acknowledgements

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University of Padova
Michela Zottini

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Paul Tudzynski
Ulrike Siegmund

Thank you!
B. cinerea inoculation

EARLY INFECTION
12 - 96 hpi
5 samples – sec. Met.
2 samples – RNA-seq.

QUIESCENCE
4 wpi
1 sample – sec. Met.
& RNA-seq.

EGRESSION
12 wpi
2 samples – sec. Met.
& RNA-seq.
# Transcriptome analysis

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

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<th>Reads uniquely mapped to B. cinerea reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG Ctrl1</td>
<td>18,764,162</td>
<td>17,226,166 (91.80 %)</td>
<td>16,552,848 (88.22 %)</td>
<td>46,268 (0.25 %)</td>
<td>520 (0.01 %)</td>
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<tr>
<td>HG Ctrl2</td>
<td>18,245,810</td>
<td>13,462,443 (73.78 %)</td>
<td>10,041,596 (55.04 %)</td>
<td>1,675,246 (9.18 %)</td>
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<td>HG Ctrl3</td>
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<td>17,125,573 (84.24 %)</td>
<td>16,376,294 (80.55 %)</td>
<td>520 (0.01 %)</td>
<td>545 (0.00 %)</td>
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<td>HG Trt1</td>
<td>23,828,415</td>
<td>21,594,466 (90.62 %)</td>
<td>20,837,836 (87.45 %)</td>
<td>45,065 (0.19 %)</td>
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<td>HG Trt2</td>
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<td>22,578,478</td>
<td>5,287,093 (23.42 %)</td>
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<td>Ripe Peg1</td>
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<td>23,815 (0.05 %)</td>
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Very low signal of *B. cinerea* in hard green and pre-egressed samples
Transcriptome analysis

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Comparisons</th>
<th>DEGs</th>
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<tr>
<td>4 wpi</td>
<td>Trt Vs Ctrl</td>
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<td></td>
<td>Eg. Vs Ctrl</td>
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<td>12 wpi</td>
<td>Peg. Vs Ctrl</td>
<td>310</td>
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<td>Bc Eg. Vs Ctrl</td>
<td>1425</td>
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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.

*Results (berry)*
**Vitis defense response**

Hormone signaling:
- Auxin
- ABA
- ET
- SA
- JA

Recognition:
- R genes

Respiratory burst:
- Redox state
- Peroxidases
- Glutathione-S-transferase

Signaling:
- MAPK

Transcription factors:
- bZIP
- WRK
- MYB

Defense genes:
- PR-proteins
- Secondary metabolite
- Heat shock protein

**Results (berry)**
Polyphenol secondary metabolism

Results (berry)
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