Botrytis cinerea activates virulence-related metabolism in the interaction with tolerant green grapes

Flávio Soares[1], Diana Pimentel[1], Alexander Erban[2], Catarina Neves[1], Pedro Reis[3], Marcelo Pereira[1], Cecilia Rego[3], Margarida Gama-Carvalho[1], Joachim Kopka[2], and Fortes Ana Margarida[1]

[1]Universidade de Lisboa Instituto de Biossistemas e Ciencias Integrativas
[2]Max-Planck-Institut fur molekulare Pflanzenphysiologie
[3]Universidade de Lisboa Instituto Superior de Agronomia

May 17, 2022

Abstract

Botrytis cinerea is responsible for the gray mold disease, severely affecting *Vitis vinifera* grapevine and hundreds of other economically important crops. However, many mechanisms of this fruit-pathogen interaction remain unknown. The combined analysis of the transcriptome and metabolome of green fruits infected with *B. cinerea* from susceptible and tolerant genotypes was never performed in any fleshy fruit, mostly because green fruits are widely accepted to be resistant to this fungus. In this work, peppercorn-sized fruits were infected in the field or mock-treated, and infected berries were collected at green (EL32) stage from a susceptible (Trincadeira) and a tolerant (Syrah) variety. RNAseq and GC-MS data suggested that Syrah exhibited a pre-activated/basal defense relying on specific signaling pathways, hormonal regulation, specifically jasmonate and ethylene metabolism, and linked to phenylpropanoid metabolism. In addition, putative defensive metabolites such as shikimic, ursolic/ oleanolic, and *trans*-4-hydroxy cinnamic acids, and epigallocatechin were more abundant in Syrah than Trincadeira before infection. On the other hand, Trincadeira underwent relevant metabolic reprogramming upon infection but was unable to contain disease progression. RNA-seq analysis of the fungus *in planta* revealed an opposite scenario with higher gene expression activity within *B. cinerea* during infection of the tolerant cultivar and less activity in infected Trincadeira berries. The results suggested an activated virulence state during interaction with the tolerant cultivar without visible disease symptoms. Together, this study brings novel insights related to early infection strategies of *B. cinerea* and the green berry defense against necrotrophic fungi.

Botrytis cinerea activates virulence-related metabolism in the interaction with tolerant green grapes

Flávio Soares[1], Diana Pimentel[1], Alexander Erban[2], Catarina Neves[1], Pedro Reis[3], Marcelo Pereira[1], Cecilia Rego[3], Margarida Gama-Carvalho[1], Joachim Kopka[2], Ana Margarida Fortes[1*]

[1] BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal

[2] Max-Planck-Institut für Molekulare Pflanzenphysiologie, 14476 Potsdam-Golm, Germany

[3] Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal

* Author for correspondence:

Ana Margarida Fortes, BioISI, University of Lisbon, Portugal

Email: amfortes@fc.ul.pt

Significance statement

Virulence-related metabolism is highly activated in *Botrytis cinerea* in the interaction with tolerant green *Vitis vinifera*grapes that remain largely unaffected in their molecular and metabolic responses whereas susceptible grapes conduct a complex defensive reprogramming while the fungus shows low transcriptional activity.

Abstract

Botrytis cinerea is responsible for the gray mold disease, severely affecting *Vitis vinifera* grapevine and hundreds of other economically important crops. However, many mechanisms of this fruit-pathogen interaction remain unknown. The combined analysis of the transcriptome and metabolome of green fruits infected with *B. cinerea* from susceptible and tolerant genotypes was never performed in any fleshy fruit, mostly because green fruits are widely accepted to be resistant to this fungus.

In this work, peppercorn-sized fruits were infected in the field or mock-treated, and infected berries were collected at green (EL32) stage from a susceptible (Trincadeira) and a tolerant (Syrah) variety. RNAseq and GC-MS data suggested that Syrah exhibited a pre-activated/basal defense relying on specific signaling pathways, hormonal regulation, specifically jasmonate and ethylene metabolism, and linked to phenylpropanoid metabolism. In addition, putative defensive metabolites such as shikimic, ursolic/ oleanolic, and *trans* -4-hydroxy cinnamic acids, and epigallocatechin were more abundant in Syrah than Trincadeira before infection. On the other hand, Trincadeira underwent relevant metabolic reprogramming upon infection but was unable to contain disease progression. RNA-seq analysis of the fungus *in planta* revealed an opposite scenario with higher gene expression activity within *B. cinerea* during infection of the tolerant cultivar and less activity in infected Trincadeira berries. The results suggested an activated virulence state during interaction with the tolerant cultivar without visible disease symptoms. Together, this study brings novel insights related to early infection strategies of *B. cinerea* and the green berry defense against necrotrophic fungi.

Keywords: *Botrytis cinerea* , fruit-fungus interaction, transcriptome, metabolome,

Vitis vinifera.

Author Contributions: A.M.F. designed and supervised the study; F.S., D.P., A.M.F., extracted RNA; D.P., A.E., J.K., performed metabolomics; P.R., C.R., performed infections; F.S., M.P., M.G.C. performed bioinformatic analysis of RNAseq; C. N. Performed one figure and assisted in tables; F.S. wrote the initial draft, completed by A.M.F.; M.G.C., J.K., A.M.F. revised the manuscript.

1 INTRODUCTION

Grapevine is one of the most valuable and cultivated crops throughout the world. Most of the cultivars used for wine and table grape are derived from the species *Vitis vinifera* , selected due to its organoleptic characteristics, albeit being highly susceptible to biotic stresses, mostly caused by oomycetes or fungi like *Botrytis cinerea* (Armijo et al. 2016). *B. cinerea* is a widespread, filamentous, and necrotrophic fungus that infects more than 200 plant species, causing serious economic losses every year (AbuQamar, Moustafa, and Tran 2017). This pathogen is the causal agent of grey mold (bunch rot), one of the most severe diseases in grapevines, affecting the yield and quality of production worldwide. As a result, frequent applications of fungicides are needed to protect vineyards, with tremendous economic implications and compromising environmental sustainability (Petrasch, Silva, et al. 2019).

The plant innate immune system (PIIS) is a multi-layer and tightly regulated signal transduction component that triggers proteins and metabolites with a defensive role and is focused on battling the invasion of different pathogens (W. Zhang et al. 2019). The PIIS is composed of the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (X. Wang et al. 2014). PAMPs are recognized by the plant pattern recognition receptors (PPRs), a system of receptor-like kinases or receptor-like proteins, crucial for cell-to-cell communication and extracellular signal sensing (Dalio, Magalhães, and

Machado 2017). Different examples of PAMPs have been described, such as flg22 (bacterial flagellin), elf18 (bacterial elongation factor-Tu) and, regarding fungi, cell wall polysaccharides, chitin, β-glucans, and ergosterol (Ono, Mise, and Takano 2020). For example, the endopolygalacturonases from *Botrytis* are recognized by the receptor-like protein RBPG1 in Arabidopsis and induce the PTI response, a relatively weak but broad-spectrum immune response (L. Zhang et al. 2014). The ETI is the second level of pathogen recognition and requires the perception of pathogen-specific effectors, recognized by plant R proteins and leading to a rapid and robust response (Jones and Dangl 2006; Lo Presti et al. 2015). Pathogen recognition by the PTI/ ETI systems is followed by a complex signaling network that regulates gene expression and the activation of several downstream defense-related pathways, such as the induction of reactive oxygen species (ROS), cell wall modifications to limit fungal growth, or/and the activation of calcium signaling and MAPK cascades resulting in the expression of many defense-related genes and production of secondary metabolites such as phytoalexins (Monteiro et al. 2003; Torres, Jones, and Dangl 2006; Xiao and Kachroo 2019;).

The aforementioned plant defenses are shaped by several phytohormones, including salicylic acid (SA), classically associated with resistance against biotrophic pathogens; and jasmonic acid (JA) and ethylene (ET) linked with resistance to necrotrophic fungi, including *B. cinerea* (Coelho et al. 2019; Xiao and Kachroo 2019).Nevertheless, there are several exceptions to this idea, and the participation of growth and stress-related hormones such as gibberellic and abscisic acid, auxin, and brassinosteroids in plant defense activity has also been described (S. AbuQamar, Moustafa, and Tran 2017; W. Zhang et al. 2019). On the other hand, necrotrophic pathogens have evolved complex strategies to subdue the host immune system. *B. cinerea*can infect grapevine by mycelium penetration through stomata and wounds or by conidia early invasion, infecting mainly the flower receptacle area and remaining quiescent until berry maturation (Viret et al. 2004; Haile et al. 2017). In favorable conditions, the conidium develops the appressorium, a specialized infective structure that secretes several phytotoxins and promotes an oxidative burst that facilitates host colonization (Gourgues et al. 2004). Nevertheless, in the early stages of infection and before the necrotrophic phase, the fungus can exhibit a short biotrophic behavior that allows the accumulation of biomass and establishment inside the host (Veloso and Kan 2018).

Recent studies have been trying to clarify the transcriptome landscape behind *B. cinerea* virulence in different species, such as cucumber and *A. thaliana* leaves (Kong et al. 2015; W. Zhang et al. 2019), kiwifruit (Zambounis et al. 2020), tomato fruit, and others (Srivastava et al. 2020). Moreover, the fungus transcriptome during infection of grapes (cv. Marselan) was accessed at harvesting stage by microarrays (Kelloniemi et al. 2015), and by RNAseq in cv. Pinot Noir at flowering (EL25/26) (Haile et al. 2017) and ripening stage (Haile et al. 2020). Haile and colleagues (2020) also reported the *in planta B. cinerea* transcriptome during fungus quiescent state in green hard berries. Nevertheless, all these studies addressed only one cultivar and the mechanisms behind the unusual infection of green berries remain undiscovered. Moreover, the complex host/*B. cinerea* pathosystem continues to stand poorly understood and more studies are needed, especially due to the extremely plastic transcriptomes of both organisms, which are influenced in a bidirectional manner (W. Zhang et al. 2019).

Grape clusters can be naturally infected by *B. cinerea* before bloom and after *veraison* with increasing susceptibility from*veraison* to ripening. Between flower and *veraison* , grape berries are known to be naturally resistant to *B. cinerea*infection (González-Domínguez et al. 2015). However, recent studies showed that certain varieties may be tolerant only at the early stage of grape development since they can become infected when artificial in-field infections are performed (Agudelo-Romero et al. 2015). Such is the case of Trincadeira, a very important Portuguese cultivar, which is extremely susceptible to *B. cinerea* . Additionally, recent studies focused on hormonal metabolism indicated that tolerance against this necrotrophic fungus is mostly based on basal defense, whereas susceptibility is due to delayed defensive responses (Coelho et al. 2019).

In the present work, we compared for the first time the transcriptome and metabolome associated with green hard berries infected with *B. cinerea* from susceptible (Trincadeira) and tolerant (Syrah) cultivars, bringing innovative insights regarding the early regulatory mechanisms involved in tolerance/susceptibility. Moreover, *in planta*associated pathogen transcriptomes were accessed in both grapevine cultivars, disclosing

the dynamics of early infection in opposite host scenarios.

2 Materials & Methods

2.1 Plant material and fruit inoculation

The *B. cinerea* isolate used was obtained from contaminated grapevine plants and kept in potato dextrose agar (Difco, Detroit, MI, USA) at 5 °C. The infections were performed by spraying berry clusters with a conidial suspension at the developmental stage of peppercorn size (EL29), following the procedure by Agudelo-Romero et al.(2015). Collection of Trincadeira and Syrah samples after visual inspection of symptoms was performed at the green stage, EL32 according to the modified E–L system (Coombe 1995). Five to six replicates were obtained for each variety, and each treatment (control sprayed with phosphate buffer). All berry clusters were briefly transported in ice to the laboratory, frozen in liquid nitrogen, and kept at -80 °C until further use. Preceding extraction for transcriptomic and metabolomic analyses, seeds were removed. Three-four biological replicates of Syrah and Trincadeira green berries (control and infected) were used for RNA-seq and metabolomics.

2.2 RNA extraction, sequencing, and gene expression analysis

Total RNA extraction was carried out as previously described by Fortes et al. (2011) with modifications (Coelho et al. 2019). RNA quantity and integrity were evaluated on Nanodrop 1000 (Thermo Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies).

Sequencing was performed at the Centre for Genomic Regulation (CRG; Barcelona, Spain). Libraries were prepared using the TruSeq Stranded mRNA Sample Prep Kit v2 (ref. RS-122-2101/2) according to the manufacturer's protocol. Libraries were subjected to 50 nt paired-end sequencing on the Illumina HiSeq 2500 using v4 chemistry. In total, 12 libraries were sequenced for Syrah and Trincadeira samples (6 control and 6 infected). Illumina raw read data were deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) and are accessible through PRJNA611792. Raw reads generated were checked for general quality and presence of adapters or contaminants via FastQC analysis (Andrews 2010). Quality trimming and filtering of raw reads were done with an in-house script with a threshold of 30 (quality score). Ten nucleotides at 5'-end were trimmed from each sequence of all libraries.

Genome assemblies of grapevine (12Xv1, http://genomes.cribi.unipd.it/) and B. cinerea (strain B05.10) (ASM83294v1, http://fungi.ensembl.org) were used as reference sequences. Both genomes were concatenated and used as a reference for mapping the quality-trimmed reads. HISAT2 (v. 2.1.0) was used to align the processed reads to the combined references with two consecutive mapping steps in order to find the splice sites independently of the annotations (Kim, Langmead, and Salzberg 2015). The mapping parameters were as follow: –rdg 2,2 –rfg2,2 – mp4,2 –rna-strandness RF. The software package SAMtools (v. 1.3.1) (http://samtools.sourceforge.net/) was used for intermediary processing of the mapped reads, such as removal of duplicates (rmdup). The HTSeq tool (version0.11.1) was used for strand-specific counting of read-pairs mapped to the exon regions annotated in the grapevine (12Xv1) and B. *cinerea* (ASM83294v1) genomes (Anders, Pyl, and Huber 2015). Differential gene expression analysis was performed using the Bioconductor package EdgeR (v. 3.24.2) (Robinson, McCarthy, and Smyth 2010). Total read counts were first normalized by library size using the trimmed means of median (TMM) method (Robinson and Oshlack 2010). Depth and gene length were normalized transforming pair-read to fragments per kb per million counts (RPKM). For differential expression analysis a RPKM > 10 threshold was used. After dispersion between samples was evaluated, an ANOVA-like test was run for any pairwise comparison with exactTest function.

For grapevine genes, differentially expressed genes (DEGs) were considered significant at the FDR [?] 0.05 and $|\log_2$ fold change ($\log_2$FC)$| > 1.0$. *B. cinerea*genes were handled as present or absent, comparing infected vs. control conditions, for both cultivars. Genes were only considered present if normalized gene count was equal to zero in all three control replicates and higher than twenty in all three infected replicates. Moreover, *B. cinerea* genes present in Syrah and absent in Trincadeira were described as Syrah-specific, and *vice versa.*

2.3 Functional enrichment analysis

Vitis vinifera DEGs lists were analyzed with FatiGO (Al-Shahrour, Diaz-Uriarte, and Dopazo 2004) to identify significant functional enrichment following a grapevine-specific functional classification of 12X V1 genome assembly predicted genes (Grimplet et al. 2012). Fisher's exact test was carried out in FatiGO to compare each study list with the list of total non-redundant genes in the grapevine genome. Significant enrichment was considered for P-value [?] 0.05 after Benjamini and Hochberg correction for multiple testing. Transcripts of *B. cinerea* were functional annotated according to (Petrasch, Silva, et al. 2019); this annotation was manually updated by literature review.

2.4 Soluble metabolites

Gas chromatography coupled to electron impact ionization time-of-flight mass spectrometry (GC-EI/TOF-MS) was used for soluble metabolite profiling, as described by Dethloff et al. (2014). Soluble metabolites were extracted according to Agudelo-Romero et al. (2013) from 300 +- 30 mg (fresh weight) of deep-frozen powder by 1 mL ethyl acetate for 2 h agitation at 30 degC. Extracts were centrifuged for 5 min at 14000 rpm, and two aliquots of 300 µL from the ethyl acetate fraction were dried by vacuum concentration and saved at -20 °C.

Chemical derivatization and retention index calibration were achieved before injection (Dethloff et al. 2014). GC-EI/TOF-MS analysis was performed using an Agilent 6890N24 gas chromatograph (Agilent Technologies, Germany) connected to a Pegasus III time-of-flight mass spectrometer (LECO Instrument GmbH, Germany), with splitless injection onto a Varian FactorFour capillary column (VF-5 ms) of 30 m length, 0.25 mm inner diameter, and 0.25 mm film thickness (Varian-Agilent Technologies, Germany). Chromatograms were acquired, visually controlled, baseline corrected, and exported in NetCDF file format using ChromaTOF software (Version 4.22; LECO, St. Joseph, USA).

Compounds were identified by mass spectra and retention time index matching to the reference collection of the Golm Metabolome Database (Kopka et al. 2005; Hummel et al. 2010) with manual supervision using TagFinder software (Luedemann et al. 2008). Guidelines for manually supervised metabolite identification were the presence of at least three specific mass fragments per compound and a retention index deviation of less than 1.0 % (Strehmel et al. 2008). Metabolite intensities were normalized by sample fresh weight and internal standard (C22), maximum scaled, and $\log_2$-transformed to approximate normal distribution for statistical analysis. A subset of metabolites was identified only by mass spectral match as indicated by square brackets, e.g. [ursolic acid]. This compound differs in in retention index from expected (Szakiel et al. 2012) and identified abundant oleanolic acid (Supplemental Table S1) and matches best to ursolic acid. Due a deviation from the expected retention index and the presence of other pentacyclic triterpenoids (Burdziej et al. 2019) in *Vitis vinifera* , we annotated [ursolic acid] as a triterpenoid of the ursolic/ oleanoic acid family.

2.5 Volatile metabolites

Volatile profiling used 500 ± 50 mg (fresh weight) of deep-frozen grape berry powder and was performed by solid-phase micro-extraction (SPME) and GC coupled to an electron impact ionization/quadrupole MS (GC-EI/QUAD-MS) using an Agilent 5975B VL GC-MSD system and a StableFlex^{TM} SPME-fiber with 65 µm polydimethylsiloxane/divinylbenzene (PDMS-DVB) coating (Supelco, USA), as described by *Vallarino* et al. (2018). SPME samples were taken from the headspace with 10 min incubation at 45 °C, 5 min adsorption at 45 °C, and 1 min desorption at 250 °C onto a DB-624 capillary column with 60 m length, 0.25 mm inner diameter, and 1.40 µm film thickness (Agilent Technologies, Germany). GC temperature programming was 2 min isothermal at 40°C followed by a 10 °C/min ramp to 260 °C final temperature, which was held constant for 10 min. The Agilent 5975B VL GC-MSD system was operated with a continuous flow of helium at 1.0 mL/min. Desorption from the SPME fiber was at 16.6 psi with an initial 0.1 min pulsed-pressure at 25 psi. The subsequent purge was 1 min at a purge flow of 12.4 mL/min. System stability was controlled, and the sample sequence randomized. GC-EI/QUAD-MS chromatograms were acquired with the mass range set to 30–300 m/z and a 20 Hz scan rate. Chromatograms were obtained, visually controlled, and exported in NetCDF file format using Agilent ChemStation-Software (Agilent) and baseline-corrected with MetAlign software (Lommen 2009).

Compounds were identified as described above using TagFinder software (Luedemann et al. 2008) by mass spectra and retention time matching to the reference collection of the GMD for volatile compounds (Kopka et al. 2005; Hummel et al. 2010). Guidelines for manually supervised identification were the presence of at least three specific mass fragments per compound and a retention time deviation of less than 3 %. Metabolite intensities were normalized by sample fresh weight, maximum scaled, and $\log_2$-transformed to approximate normal distribution for statistical analysis.

2.6 Statistical analysis

Statistical analysis of metabolomic results was performed using log2-transformed response ratios and included Student's t-test, one- and two-way ANOVA, Kruskal–Wallis, and Wilcoxon rank-sum tests. Benjamini & Hochberg correction was used for multiple comparisons, and principal component analysis was performed applying the MetaGeneAlyse web application (v.1.7.1; http://metagenealyse.mpimp-golm.mpg.de) and the R function prcomp to the $\log_2$-transformed response ratios with missing value substitution, $\log_2 = 0$. Heatmaps were designed using the R package ComplexHeatmap (Gu et al., 2016). Venny 2.1 web tool (https://bioinfogp.cnb.csic.es/tools/venny/) was used to create all Venn diagrams.

3 RESULTS

3.1 Metabolic changes induced by B. cinerea infection in both Syrah and Trincadeira grape berries

Green berries are widely recognized as resistant to *Botrytis cinerea* infection. Notwithstanding, our previous work has shown that green berries of certain cultivars may exhibit heavy symptoms of infection under proper humidity and temperature conditions (Agudelo-Romero et al. 2015). In the present work, healthy and infected berries from Trincadeira and Syrah were sampled at green stage (EL32) according to the modified E–L system (Coombe 1995). The visual analysis showed that green Trincadeira berries already presented a high level of *B. cinerea* infection and, in contrast, Syrah showed only mild symptoms (Fig. 1). This was previously confirmed by Coelho et al. (2019) for the same samples by qPCR using primers specific to the fungal genomic DNA (*BcPG1*), this data indicated that the percentage of infection was ˜16X fold higher in Trincadeira at green stage than in Syrah (Coelho et al. 2019).

To gather insights on how grapes' metabolism was affected by the infection, a GC-EI-TOF/MS platform was used for the relative quantifications of sugars, organic acids, phenylpropanoids, and other soluble metabolites. Profiling of volatile metabolites was achieved using a GC-EI/QUAD-MS platform (Supplementary Table S1). Principal component analysis (PCA) was carried out with normalized responses (Supplementary Table S1 and Fig. 3). The two major PCs (PC1 and PC2) accounted for 47.70% of the total variability (Supplementary Fig. S1) and separated the samples based on cultivar and infection status, respectively. Within each cultivar, PC2 only established a clear separation between Trincadeira samples, as all Syrah samples were plotted together, revealing a similar metabolic content among control and infected berries. Twenty-five metabolites (23.4% of all detected species) displayed statistically significant differences in abundance between cultivars or between control and infected samples of the same cultivar (Fig. 2 and Supplementary Table S1).

Several differences were observed at basal levels when comparing tolerant and susceptible cultivars. Specifically, the phenylpropanoids epigallocatechin and *trans* -4-hydroxycinnamic acid were constitutively present in a larger amount in Syrah, together with shikimic acid, a precursor of phenylpropanoids; those compounds accumulated at higher levels in Syrah than in Trincadeira upon infection. Moreover, the volatile organic compounds (VOCs) 2-ethylfuran, hexanal, (E, E)-2,4-Hexadien-1-al, and phenylacetaldehyde were detected in higher amounts in control Syrah than in control Trincadeira.

Regarding fatty acids, Syrah showed superior basal levels of hexacosanoic acid (C26) than Trincadeira (Fig. 2 and Supplementary Table S1). Very-long-chain fatty acids are required for the biosynthesis of the plant cuticle, generation of sphingolipids and have been associated with plant defense (Raffaele, Leger, and Roby 2009). Syrah also presented higher levels of a compound from the ursolic/ oleanolic acid family than Trincadeira in both basal and under infection conditions, and, in Trincadeira, this lipid increased in response to *B. cinerea* infection (Supplemental Table S1).

On the other hand, Trincadeira showed higher basal levels of glucose, succinic acid, and 2-oxoglutaric acid than Syrah; additionally, 2-oxoglutaric acid increased in Trincadeira upon infection together with fructose, benzaldehyde, and the sterols stigmasterol and campesterol (Fig. 2 and Supplementary Table S1). These compounds reached higher content in this susceptible cultivar than in Syrah, infected samples. Campesterol is a crucial precursor of brassinolide, which plays important role in several signaling pathways to reduce biotic stress damage (Anwar et al. 2018). Phenylacetaldehyde and decanal, putative markers of ripening in grape berries, were increased in Trincadeira upon infection, indicating an acceleration of ripening in susceptible Trincadeira promoted by the fungus *B. cinerea* as previously reported (Agudelo-Romero et al. 2015).

Finally, when comparing infected berries, triacontanoic acid, fumaric acid, and the fatty alcohol n-docosan1-ol were detected in higher amounts in Trincadeira, whereas the antioxidant α-tocopherol was increased in Syrah (Fig. 2 and Supplementary Table S1).

In general, *B. cinerea* infection had little influence on the Syrah metabolome at the green stage. On the other hand, ten metabolites were identified as potential markers of infection in Trincadeira, revealing an early metabolic reprogramming upon infection in this susceptible cultivar (Fig. 3 and Supplementary Table S1). Moreover, eight metabolites were more accumulated in Syrah at basal level and might thus be putative markers of tolerance (Fig. 2 and Supplementary Table S1).

3.2 RNAseq and functional enrichment analysis indicate a strong transcriptional reprogramming in Trincadeira under infection that was not observed in Syrah

Transcriptional profiling was performed using three biological replicates of control and infected berries of each cultivar. Supplementary Table S2 provides a summary of parsed reads and the reads mapped to the predicted transcriptomes, both for *B. cinerea* and *V. vinifera* . In our study, the average number of reads uniquely mapped to the grapevine genome was higher in Trincadeira infected samples than in Syrah (19 272 633 and 10 553 075 reads, respectively). On the other hand, *B. cinerea* average reads *in planta* were higher in the tolerant infected cultivar (6021 reads, representing 0.057% of the total) than in the susceptible cultivar (2570, representing 0.013% of total reads) (Supplementary Table S2).

The expression of 26110 different grape genes (87.11% of the total predicted grape genes (Jaillon et al. 2007)) and 5478 different *B. cinerea* genes (44.83% of the total predicted *B. cinerea* genes (Kan et al. 2017)) was detected across all samples. Multi-dimensional scaling (MDS) plot of all normalized grape gene counts separated the data into three groups (Supplementary Fig. S2). Similar to the metabolomic results, Syrah samples clustered together independently of the infection status whereas for Trincadeira the MDS plot discriminated infected from control samples.

Differences in gene expression between Trincadeira and Syrah were analyzed comparing the constitutive and under infection transcriptome of both cultivars, and individually for each variety (Infected vs Control) (Supplementary Table S3). The number of differentially expressed genes (DEGs) is reported in Supplementary Table S4. A total of 10555 grape genes were found to be differentially expressed (FDR [?] 0.05 and log2 $|FC| > 1.0$) due to the cultivar (7576 genes) or/and infection status (2979 genes). The Venn diagram illustrates that from the DEGs detected when comparing both infected cultivars, most of them were already detected when comparing the cultivars before infection, implying that the majority of differences are unrelated to specific responses to infection (Supplementary Fig. 3A). The remaining DEGs are likely explained by changes in the Trincadeira transcriptome since there were only 22 DEGs detected in Syrah upon infection (Supplementary Fig. 3B). These results suggest that *B. cinerea* presence had limited influence on the Syrah transcriptome at the green stage of grape development.

Key biological processes activated or repressed due to infection (Fig. 4A and Supplementary Table S5) or cultivar (Fig. 4B and Supplementary Table S6) were determined by enrichment analyses of functional categories (P-value [?] 0.05) using FatiGO (Al-Shahrour, Díaz-Uriarte, and Dopazo 2004).

Several functional classes were upregulated in Trincadeira after infection: signaling, carbohydrate-related

(including trehalose, starch, and amino sugars metabolism), secondary metabolism (lignin metabolism, stilbenoid, and flavonoid biosynthesis), cell wall-related (xyloglucan modification), stress response (such as biotic and desiccation stress response, oxidative stress), phytoalexin biosynthesis, hormone signaling (mainly ethylene and jasmonate signaling) and lipid metabolism (oxylipin biosynthesis, glycerolipid, and α-linolenic acid metabolism). Moreover, several families of transcription factors were also found to be enriched in the set of genes that was upregulated in Trincadeira in response to infection (ZIM, WRKY, NAC, MYB, JAZ, ERF, and others). In contrast, an enrichment of the functional classes related to cell wall modification and photosynthesis was observed among the genes downregulated in Trincadeira infected samples (Fig. 4A).

Finally, only three functional categories were enriched in Syrah infected berries. These include, aquaporins, ethylene-mediated signaling pathway, and the ERF subfamily of transcription factors, all of them among the downregulated genes (Fig. 4A and Supplementary Table S5).

3.2.1 Genes involved in signaling pathways associated with defense are constitutively highly expressed in Syrah whereas in Trincadeira they are activated in response to infection

Categories of genes encoding R proteins, protein kinases, proteins involved in calcium signaling, ET-mediated signaling, and ZIM, JAZ, and AP2/ERF families of transcription factors are enriched in Syrah when comparing both cultivars at basal level. Expression of genes belonging to these categories was activated in Trincadeira only in response to *B. cinerea* infection (Fig. 4A and Supplementary Table S5). In detail,*B. cinerea* infection led to an increase of calcium signaling in Trincadeira, as suggested by the upregulation of many genes involved in calcium-sensing and signaling. Most of those genes were already highly expressed in Syrah when compared to Trincadeira before infection (e.g., calmodulin, calmodulin-binding proteins, and calcium-transporting ATPases) (Table 1). The same holds for the protein kinase functional category, with several up-regulated genes putatively encoding for protein kinases, receptor serine/threonine kinases, and for leucine-rich repeat receptor kinases, which appear to play central roles in signaling during pathogen recognition and plant defense mechanisms (Table 1 and Supplementary Table S3) (Afzal, Wood, and Lightfoot 2008; Torii 2004). Moreover, genes coding for GRAS transcription factors which have been previously associated with grapevine response to biotic stress (Grimplet et al. 2016) were upregulated in Trincadeira under infection and constitutively upregulated in Syrah (Table 1 and Supplementary Table S3).

Finally, the activation of defense-related genes in plants has been associated with different phytohormones, with JA and ET being essential for plant innate immune system against necrotrophic fungi (Anderson et al. 2004; S. AbuQamar, Moustafa, and Tran 2017). Among upregulated genes in Syrah before infection, when comparing with Trincadeira, were those involved in ET synthesis and jasmonates´ signaling; these genes were upregulated in Trincadeira under infection (Table 1 and Supplementary Table S3). Previous hormonal profiling performed in the same samples revealed the importance of jasmonates among other hormones in response to *B. cinerea* during early stages of ripening (Coelho et al. 2019). This study also validated by qPCR the present RNAseq data regarding hormonal metabolism.

3.2.2. Basal and under infection primary and secondary metabolism are strikingly different in between tolerant and susceptible cultivars

Functional enrichment analysis revealed a broad transcriptional contrast between primary and secondary metabolisms of the two cultivars before and under infection. Primary metabolism, in general, was activated in Trincadeira under infection (enrichment in amino sugar, tyrosine, nitrogen, and trehalose metabolisms, glycerolipid catabolism, and α-linolenic acid metabolism, as well as oxylipin biosynthesis and proteinase inhibitors functional classes) (Table 1 and Supplementary Table S3). Moreover, in Trincadeira, the infection led to the upregulation of genes involved in oxidative stress response, desiccation, temperature, and biotic stress response among others (Table 1 and Supplementary Table S3). On the other hand, urea cycle, photosynthesis, and steroid biosynthesis seem to be inhibited in Trincadeira, when comparing both infected cultivars (Table 1 and Supplementary Table S3). Interestingly, transcripts involved in the biosynthesis of the antioxidant α-tocopherol were upregulated in Syrah at basal level and downregulated in Trincadeira upon infection, in agreement with metabolomics data (Table 1 and Supplementary Table S3).

Concerning the cell wall metabolism, many genes encoding laccases, pectinesterases, and xyloglucan modifications were upregulated in Trincadeira under infection (Table 1). On the other hand, cellulose biosynthesis seems to be activated in Syrah, as suggested by the upregulation of several cellulose synthases when comparing both cultivars before and after infection. Furthermore, carbohydrate metabolism was also affected in Trincadeira by *B. cinerea* , mainly due to the upregulation of several genes encoding α- andβ- amylases and differently expressed genes coding sugar transporters (Table 1 and Supplementary Table S3).

Regarding secondary metabolism, many genes related to alkaloid metabolism and biosynthesis of taxol, terpenoids, and triterpenoids were downregulated in Trincadeira upon infection (Table 1 and Supplementary Table S3). On the other hand, anthocyanin biosynthesis was activated in Syrah pre-infection, however, is triggered by the fungus in Trincadeira, together with stilbenoid biosynthesis. Finally, phenylpropanoid metabolism was enriched in Syrah when comparing both infected cultivars. (Table 1 and Supplementary Table S3). In general, the data showed several genes involved in secondary metabolism already activated in Syrah at basal level and downregulated in Trincadeira upon *B. cinerea* infection.

3.3 Botrytis cinerea presents higher transcriptional reprogramming in the tolerant cultivar

Analysis of RNAseq showed that thousands of reads uniquely mapped to the fungus genome were detected in all infected samples (Supplementary Table S2). Few fungal reads were also detected in all control samples (Supplementary Table S2) confirming the natural and opportunistic presence of *B. cinerea* in the vineyards (S. F. AbuQamar, Moustafa, and Tran 2016). Considering in detail the number of *B. cinerea* genes expressed *in planta* , 531 different genes were Syrah-specific, 166 Trincadeira-specific, and 122 shared by both cultivars (Supplementary Table S7 and S8). PCA plot of all fungus normalized genes counts grouped all the control samples and showed a clear separation between infected cultivars, with the PC1 explaining 71.7% of the variability between control and infected samples (Supplementary Fig. S4).

As a necrotrophic pathogen, *B. cinerea* secrets a broad repertoire of virulence factors, triggering plant chlorosis and host cell death (Siewers et al. 2005). Several genes associated with virulence and growth were Syrah-specific (Table 2 and Supplementary Table S7). Fungal cell division appears to be promoted in the tolerant cultivar, as suggested by the expression of genes related to the cell cycle, cytoplasmic microtubule and actin cytoskeleton organization, and cellular amino acid biosynthetic process (Fig. 5 and Supplementary Table S7). Furthermore, the results showed a general activation of genes participating in ROS generation and/or oxidation-reduction processes, mainly in Syrah (Fig. 5 and Supplementary Table S7). In particular, the*BcNoxR (Bcin03g06840), a* major generator of ROS and essential for the development of sclerotia and full virulence (Li et al. 2016) (Table 2 and Supplementary Table S7). The same holds true for genes involved in signaling pathways (fungal protein kinases, calcium signaling), protein regulation (e.g., translation, protein folding, and phosphorylation), and protein transport (Table 2 and Supplementary Table S7). Several*B. cinerea* ribosomal proteins involved in translation mechanisms were expressed in both cultivars. Moreover, were expressed mainly in Syrah genes with a putative role in transcription regulation, several transcription factors (e.g., MYB, BZIP, NOT, TFIID, SRF, SFP1, and CP2, and zinc fingers), and genes involved in chromatin structure and modification (Fig. 5, Table 2, and Supplementary Table S7).

Additionally, many genes associated with fungal cellular degradation processes (e.g., autophagy, proteases, and ubiquitin-dependent protein catabolic process) and fungal cell wall organization (such as several carbohydrate-active enzymes (CAZymes) and chitin synthases) were detected as expressed in Syrah but not in Trincadeira (Fig.5 and Supplementary Table S7). CAZymes allow plant tissue colonization through host-cell wall modifications and release of carbohydrates for fungus consumption (Choquer et al. 2007; Blanco-Ulate et al. 2014). In detail, 46 and 16 annotated CAZymes were detected as expressed by *Botrytis cinerea* in Syrah and Trincadeira, respectively (Table 2 and Supplementary Table S7). Interestingly, several genes with a putative role in carbohydrate conversion were expressed mostly in Syrah berries. This includes, in particular, genes related to glycogen metabolism, glycolytic processes, TCA cycle, and trehalose biosynthesis (Table 2 and Supplementary Table S7). Fungal energy metabolism was also activated in the tolerant cultivar, as suggested by the expression of genes related to ATP synthesis and ATPase activity. Finally, several genes encoding players of lipid and fatty acid metabolism were also mainly expressed in Syrah

(Table 2 and Supplementary Table S7) together with fungal major facilitator superfamily (MFS) and sugar transporters. The expression of genes encoding ABC transporters as well as genes involved in the glyoxylate cycle were noticed in both infected grapes (Table 2 and Supplementary Table S7).

On the other hand, some virulence-related fungal genes were also detected in Trincadeira infected berries despite their more advanced state of infection (Fig.1 and Table 2), namely genes associated with sexual reproduction, fruit body formation, sporulation, and host colonization (Table 2). Moreover, a precursor of riboflavin, lipase 1, and a chitin deacetylase were identified only in Trincadeira and were described as important for *B. cinerea* infection strategy (Becker et al. 2010; Haile et al. 2020).

4 DISCUSSION

Gray mold is one of the most serious diseases affecting grapevines (Petrasch, Knapp, et al. 2019) and, even though recent studies focus on the molecular basis of *B. cinerea* pathogenicity (Kelloniemi et al. 2015; Srivastava et al. 2020; Zambounis et al. 2020), the processes behind necrotrophic infection of fruits at early ripening stages remain uncharted. Moreover, the combined analysis of both susceptible and tolerant green berries towards *Botrytis* infection has not been performed previously, leaving a gap in our understanding of the complex and temporal dynamics of *V. vinifera/B. cinerea* pathosystem. Previous to the present study, mechanisms involved in susceptibility of Trincadeira berries were analyzed considering omics approaches (Agudelo-Romero et al. 2015). Late green (EL33) and veraison (EL35) berries with grey mold symptoms evidenced a reprogramming of carbohydrate and lipid metabolisms with a putative involvement of jasmonic acid, ethylene, polyamines, and auxins (Agudelo-Romero et al. 2015). In this study, we confirmed that this metabolic reprogramming occurs even at an earlier stage of berry ripening (EL32) in the susceptible variety. In contrast, the tolerant Syrah variety remained largely unaffected and thereby presents the opportunity to investigate early infection stages without and with minimized contributions of related to necrotrophic damage of the berries. Analysis of the fungal transcriptome indicates that *B. cinerea* is in a more virulent stage of interaction with the tolerant variety, revealing new mechanisms associated with this fungal infection.

4.1 Pre-activated defenses in Syrah are likely to be responsible for its resilience against Botrytis cinerea attack

Transcriptome and metabolome analyses revealed that Syrah metabolism was minimally modulated by *Botrytis cinerea* infection, suggesting that tolerance is mainly due to basal defenses. Interestingly, and even though no genes for complete resistance (R genes) to *B. cinerea*have been identified in plants, functional category enrichment analysis revealed that R proteins and protein kinases were constitutively upregulated in Syrah, while their expression was triggered in Trincadeira by infection. The same was true for genes related to Ca^{2+} mediated signaling, JA signaling pathway, and ET biosynthesis. Calcium signaling modulates the regulation of protein kinases, and SA, ET, and JA metabolism (Lecourieux, Ranjeva, and Pugin 2006), which are frequent participants in plant response to *B. cinerea* infection (Agudelo-Romero et al. 2015; Peng, Coelho et al. 2019; Haile et al. 2020) and might likewise be important for basal tolerance. Furthermore, plasma membrane Ca^{2+} ATPases appear to be important components of receptor-mediated signaling for plant immune responses and development (Frei dit Frey et al. 2012).

The regulation of transcription is known to be paramount for an effective plant defense (Moore, Loake, and Spoel 2011). Several genes coding for transcription factors (ZIM, JAZ, ERF, AP2, WRKY, NAC) were highly expressed in Syrah healthy berries and upregulated in Trincadeira under infection. Orthologous genes of three transcription factors (*WRKY33* , *BOS1* , and *MIC2*) that influence immune responses in *A. thaliana* (Mengiste et al. 2003; Birkenbihl, Diezel, and Somssich 2012; Dobón et al. 2015) were also noticed in this interaction and might contribute to the basal resistance of Syrah. The same holds true for genes belonging to the GRAS family of transcription factors, which have been involved in plant response to biotic stress (Grimplet et al. 2016).

Omics data underscored a distinct reprogramming of metabolic pathways between cultivars. As previously reported for a more advanced green stage, EL33 (Agudelo-Romero et al. 2015), the primary metabolism was activated in Trincadeira in response to *Botrytis* infection albeit photosynthesis appeared to be inhibited.

This is a typical response to biotic stress, putatively compensating for activation of defense-related pathways and/or feedback regulation mediated by sugar signals (reviewed by Rojas et al. 2014). Additionally, the expression of genes involved in carbohydrate metabolism and also fatty acid metabolism are known to affect downstream defense responses against fungal pathogens (Rojas et al. 2014; Agudelo-Romero et al. 2015; Xiao and Kachroo 2019). Transcript and metabolite analyses also indicated that Syrah may better cope with oxidative stress induced by *B. cinerea,* namely due to accumulation of the antioxidant α-tocopherol In fact, a recent study showed that the absence of α-tocopherol in *A. thaliana* leaf chloroplasts may delay plant defense activation against *B. cinerea* through enhanced lipid peroxidation (Cela et al. 2018).

Transcriptomic data showed that Trincadeira green berries respond to infection by up-regulating genes involved in lipid metabolism (e.g. α-linolenic acid metabolism). Interestingly, high content in long-chain saturated hexacosanoic acid (cerotic acid) and of a triterpenoid of the ursolic/ oleanolic acid family were observed in Syrah at basal level when compared to Trincadeira. Moreover, genes involved in triterpenoid biosynthesis (coding for β-amyrin synthases) were upregulated in Syrah at basal level. Therefore, the basal tolerance observed in Syrah may rely on the pre-activated lipid-related defenses. Ursolic and oleanolic acids are commonly found in epicuticular waxes of plants and in grapes in particular (Pensec et al. 2014). These compounds also showed antifungal properties in apple (Shu et al. 2019) and very-long-chain fatty acids, such as hexacosanoic acid have been associated with plant defense (Raffaele, Leger, and Roby 2009).

Many genes involved in phenylpropanoid and flavonoid pathways were upregulated in Syrah at basal level (Fig. 4 and Table 1). Metabolomic data already showed a higher constitutive presence of the phenylpropanoids *trans* -4-hydroxycinnamic acid and epigallocatechin in Syrah (Fig. 2 and Fig. 3). Epigallocatechin is a precursor of epigallocatechin-3-gallate that is known for its antioxidant properties and has been suggested to promote jasmonic acid signaling in *A. thaliana* , increasing the resistance to *B. cinerea* (Hong et al. 2015).

The putative and positive metabolic markers involved in Syrah basal tolerance also included the volatiles 2-ethylfuran, hexanal, and (E, E)-2,4-Hexadien-1-al. Although studies addressing the role of plant volatiles during necrotrophic infection are scarce, Utto and colleagues (2008) showed that hexanal reduces postharvest infection of tomatoes by*B. cinerea.* Also, 2-ethylfuran has been reported to prevent downy mildew symptoms in grapevine leaves (Lazazzara et al. 2018). Volatiles are indeed involved in resistance to fungal pathogens and they can even contribute to resistance-related phenotypes of neighboring receiver plants (Quintana-Rodriguez et al. 2015; Pierik, Ballaré, and Dicke 2014). Furthermore, the volatiles benzaldehyde and decanal were accumulated in Trincadeira upon infection and might be used, once validated, as markers of an advanced *B. cinerea* infection stage.

4.2 Successful defense in Syrah putatively induces wide activation of specific signaling pathways and carbohydrate metabolism in Botrytis cinerea

The *Botrytis cinerea*transcriptome *in planta was* addressed in a few species, such as*A. thaliana* (W. Zhang et al. 2019), cucumber (Kong et al. 2015), kiwifruit (Zambounis et al. 2020), tomato, and others (Srivastava et al. 2020). However, the molecular mechanisms associated with successful*B. cinerea* infection during the early stages of fruit ripening are unknown. In grapevine, the fungal transcriptome was explored at flowering stage (Haile et al. 2017), berry mature stage (Kelloniemi et al. 2015; Haile et al. 2020), and fungal quiescence on hard green berries (Haile et al. 2020). Haile and colleagues (2020) proposed a basal metabolic activity during quiescence with only 289 fungal genes expressed in hard green berries in contrast with the large number of active genes found in this study (Supplementary Table S2).

In general, green fruits are reported as resistant to infection by*B. cinerea,* which remains quiescent until the onset of ripening. However, we previously reported green berries of the highly susceptible cultivar Trincadeira exhibiting heavy symptoms of infection (Agudelo Romero et al. 2015, Coelho et al 2019). In this study, we confirmed that the fungus is active in Trincadeira but, more surprisingly, it seems highly virulent and far from quiescent in the Syrah cultivar (Fig. 6). From the 653 *B. cinerea* genes expressed in Syrah green berries, many were virulence-related (Table 2, Supplemental Tables S7-S8) and only seven genes were in common

with the quiescent state described by Haille et al. (2020) (Supplementary Table S9).

Moreover, transcriptomic analysis showed a ratio of 13/1000 and 57/1000 of *B. cinerea* to *V. vinifera* reads in Trincadeira and Syrah respectively, indicating a higher transcriptional effort of the necrotrophic fungus to proliferate and overcome Syrah's basal defenses during infection. The abundance of pathogen transcripts seems to be partially related to fungal biomass and virulence (Blanco-Ulate et al. 2014; Haile et al. 2020; W. Zhang et al. 2019). Our results support the presence of a highly virulent fungus in green fruits of the tolerant cultivar; such high virulence might be related to the natural variation of pathogen strains (Siewers et al. 2005; Choquer et al. 2007). Moreover, in Trincadeira green berries only 288 fungal genes were detected, even though symptoms of infection were clear (Fig. 1D, Fig. 6). It can be expected that fungal virulence in this cultivar was also higher before the development of symptoms indicating that the fungus may lose virulence-associated mechanisms when the infection was successful. Similar temporal transcriptional dynamics were observed in the white-rot fungus *Obba rivulosa,* where a higher level of virulence associated gene expression was detected at early stages of wood colonization, after which the majority of those genes revealed reduced expression (Marinović et al. 2018).

Among the *Botrytis cinerea* virulence- and growth-related genes expressed in Syrah were noticed chitin synthases (*BcCHSIIIa and BcCHSIV*), genes involved in germination (*Bcg3, Bac* and*Bmp1*), conidia regulators (*Bmp3 and Bcsak1*), resistance to cyclosporin A *(Bcp1)* (Choquer et al. 2007) and elicitors of hypersensitive response such as *BcSpl1* (Frías, González, and Brito 2011). Moreover, many genes putatively associated with signaling (e.g., protein phosphorylation and calcium-mediated) and transcription factors were expressed only in Syrah and might be novel and important elements of pathogenesis. Additionally, the expression in Syrah of many genes involved in chromatin structure and modification reenforce the putative association between epigenetic mechanisms and virulence, as previously reported (Breen et al. 2016).

The fungal transcriptome results suggested that transcriptional and protein synthesis activities were carried out during infection especially in Syrah green berries, including the expression of several genes coding for regulators of transcription, ribosomal related, protein folding and protein phosphorylation. Such profile was observed during a quiescent *Botrytis cinerea* infection and in response to phytoalexins in grapevine (Zheng et al. 2011; Haile et al. 2020). Plants typically trigger an oxidative burst at the early stages of infection, generating several ROS to counteract pathogen invasion. However, as a necrotrophic fungus, *B. cinerea* can take advantage of that and even produce its own ROS (Hua et al. 2018). Several fungal key players in oxidative stress were expressed mainly in Syrah, such as*BcNoxR* essential for virulence (Hua et al. 2018), the generator of H_2O_2 (*Bcsod1*), peroxidases, and others. Interestingly, polyamine metabolism, which is associated with responses to stresses and ROS scavenging (Fortes et al. 2019) was also activated in the fungus infecting Syrah (*Bcin14g01740.1* ,*Bcin11g03520.1* , *Bctpo5,* and *Bcspe2*) and might have novel roles during *B. cinerea* infection.

Fungal necrotrophic infection also relies on the secretion of enzymes to exploit and disassemble cell wall polysaccharides and use them as the main energy source (Kong et al. 2015). Actually, several CAZymes involved in such processes were expressed by the fungus mainly in Syrah with glycosyltransferases and glycoside hydrolases being the most representative classes. Moreover, the expression of ABC and MFS transporters, together with several genes putatively encoding peptidases in Syrah might suggest an attempt of the fungus to mitigate the action of host defense. Indeed, in *A. thaliana, B. cinerea* ABC transporters were shown to be essential for tolerance against camalexin (Stefanato et al. 2009) whereas MFS transporters provided tolerance to glucosinolate-breakdown products and were required for pathogenicity (Vela-Corcía et al. 2019). Furthermore, genes involved in fungal exocytosis (e.g., vesicle-mediated transport) were also expressed mainly in Syrah; such active transport is crucial for virulence and typically involved in the growth or exudation of fungal toxins to the intercellular space (W. Zhang et al. 2019). Moreover, genes involved in autophagy processes were expressed in Syrah and might have an important role during early infection characterized by nutritional limitations, since it is a process of recycling unnecessary or dysfunctional cellular components with great influence on conidial germination and virulence (Liu et al. 2019).

During infection, the fungus converts plant hexoses and fructose into mannitol, which together with trehalose,

are the most common fungal storage carbohydrates (Dulermo et al. 2009; Solomon, Waters, and Oliver 2007). Interestingly, fungal carbohydrate metabolism was active in Syrah, as suggested by the expression of genes involved in glycogen metabolism, glycolytic processes, and trehalose biosynthesis. Similar results were described during sunflower infection (Dulermo et al. 2009) and suggests the targeting of glucose into the TCA cycle. Moreover, fungal lipid and fatty acid metabolisms appear to be active *in planta* during interaction with the tolerant cultivar, as suggested by the expression of genes coding for of acetyl-CoA dehydrogenase and carboxylases, long-chain acyl-CoA synthetase 7, and others. The association between lipid and carbon metabolisms involving glyoxylate and TCA cycles was previously hypothesized as fundamental for early fungus development and host invasion before having access to host nutrients (Z.-Y. Wang et al. 2003). On the other hand, several genes associated with gluconeogenesis (e.g., pyruvate carboxylase, a pyruvate kinase, two glucose-6-phosphate, and others) were expressed only in Trincadeira and are likely associated with fungal proliferation after successful infection. Since *B. cinerea* was only capable of successful infection in Trincadeira, these results may be important to understand the dynamics of proliferation and infection strategies of the fungus. In particular, they provide insights into how fungal and plant carbohydrate metabolisms are balanced with both fungal and plant defensive strategies.

A comparative meta-analysis was performed in order to retrieve differences between our data and the *Botrytis cinerea*transcriptome during infection of ripening berries as described by Haile et al. (2020). This study integrated transcriptome and metabolome data to investigate the crosstalk between the plant and the fungus during pathogen quiescence and egression. It is noteworthy to mention the limitations of comparing both studies since different pathogenic infection stages were considered. Nevertheless, the comparison revealed a broad repertoire of expressed transcripts (Supplemental Table S9), which is indicative of high genome plasticity and transcriptional flexibility (Hua et al. 2018; W. Zhang et al. 2019; Soltis et al. 2020). In fact, this plasticity may contribute to the aptitude of*Botrytis cinerea* to infect a wide number of plant species. Common functional classes were also observed among the expressed genes, such as redox processes, ATP-related, or protein folding classes. In both studies, several genes coding for ribosomal proteins were also expressed suggesting that ribosomes and translation may play a fundamental function in the infection process. Plant ribosomal proteins have been recently associated with biotic stress responses (Cruz et al. 2019), but their role in pathogenesis remains intriguing. Moreover, an uncharacterized secreted protein *(Bcin15g00100)* was expressed in all the samples analyzed in both studies, deserving special attention since the orthologous in *Blumeria graminis* was associated with virulence (Pliego et al. 2013).

5 CONCLUSIONS

Understanding grey mold disease processes and early host/pathogen interactions is crucial to disclose new disease control and efficient management strategy models. Our study revealed a contrasting response between Syrah and Trincadeira cultivars, underlining the importance of studying cultivars with different susceptible/tolerance levels and specifically at a stage of developing grapes that is generally thought be low or even non-susceptible. Syrah was barely affected by *B. cinerea* infection at the green stage of development, eventually as indicated in this study due to pre-activated defensive mechanisms. The observed resilience against the necrotrophic fungus was likely based on specific signaling pathways, hormonal regulation and activation of secondary metabolism. In contrast, Trincadeira was severely affected by*B. cinerea* and a broad reprogramming of primary and secondary metabolisms was observed, putatively regulated by jasmonate- and ethylene- mediated signaling pathways and several transcription factors of the ZIM/JAZ, NAC, MYB, ERF and GRAS families. This study also suggested promising metabolic markers of tolerance against grey mold disease at early stages, including 2-ethylfuran, a compound from the ursolic/ oleanolic acid family, hexanal, (E, E)-2,4-Hexadien-1-al, cinnamic acid, shikimic acid and hexacosanoic acid, phenylacetaldehyde, and epigallocatechin.

Regarding the fungus, an opposite scenario was found, with higher transcriptional activity shown during infection of the tolerant cultivar. The study put in evidence the plasticity of the pathogen´s transcriptome, revealing several genes related to virulence and fungal growth, signaling, carbohydrate and lipid metabolism expressed during infection of Syrah green berries, which might be important for early stages of infection at

which the fungus is thought to be arrested in its interaction with Syrah. Nevertheless, different genes related to growth and virulence were also detected only in Trincadeira and might be important to understand the late stages of regulatory mechanisms behind necrotrophic fungus proliferation after successful infection. Altogether, and since only few genes have been described previously to be involved in pathogenicity (Hua et al. 2018), the newly identified putative elements of virulence might be targeted for functional characterization and to develop new and efficient control strategies.*Botrytis cinerea* is a fungus that infects several plant species worldwide and the knowledge gathered in this *in vivo* pathogen interaction study may provide valuable hints to be translated to other plant species.

6 ACCESSION NUMBERS

RNA-seq data is available at the NCBI SRA database under PRJNA611792 accession number.

7 ACKNOWLEDGMENTS

This research was supported by Fundação para a Ciência e Tecnologia (FCT) through the research project "GrapeInfectomics" (PTDC/ASP-HOR/28485/2017). This article/publication is based upon work from COST Action CA 17111 INTEGRAPE, supported by COST (European Cooperation in Science and Technology). FS and D.P. were recipients of fellowships from BioSys PhD programme PD65-2012 (PD/BD/114385/2016 and PB/BD/130976/2017, respectively). Work additionally supported by UIDB/04046/2020 Centre grant, to BioISI and from FCT, Portugal. The authors declare no conflict of interest.

8 SUPPORTING INFORMATION

Supplementary Figure S1 - Principal component analysis (PCA) of metabolic profiles of *Botrytis cinerea* infected and control berries of *V. vinifera* cv. Trincadeira and cv. Syrah at the green developmental stage (EL32).

Supplementary Figure S2 - Multidimensional scaling (MDS) of RNA-seq analysis with V. vinifera mapped reads of *Botrytis cinerea* infected and control berries of *V. vinifera* cv. Trincadeira and cv. Syrah at the green developmental stage (EL32).

Supplementary Figure S3 - Venn diagrams of *Vitis vinifera* differentially expressed genes. (A) Differentially expressed genes in response to the cultivar in control and infected berries. (B) Differentially expressed genes in response to the infection in Syrah and Trincadeira cultivars.

Supplementary Figure S4 - Principal component analysis (PCA) of*Botrytis cinerea* genes expressed *in planta* comparing the constitutive and upon infection transcriptome of both Syrah and Trincadeira cultivars at the green developmental stage (EL32).

Supplementary Table S1 - Normalized responses of all detected profiled volatile and soluble metabolites from green berries of*Vitis vinifera* cv. Syrah and cv. Trincadeira that were either non-infected or infected with *Botrytis cinerea* .

Supplementary Table S2 - Summary of RNA-seq sequencing and mapping statistics. SY, Syrah; Tr, Trincadeira; C, Mock inoculated (Control); I, *B. cinerea* inoculated (Infected). 1-5 indicate the biological replicates.

Supplementary Table S3 - List of *Vitis vinifera* genes differentially expressed comparing the constitutive and upon infection transcriptome of both cultivars and, individually, for each variety (Infected vs. Control). Functional classification from Grimplet et al., BMC Res Notes 2012.

Supplementary Table S4 - Summary of grapevine DEGs for each comparison with FDR [?] 0.05 and log2 $|FC| > 1.0$.

Supplementary Table S5 - Functional enrichment analysis in*Botrytis cinerea* infection-responsive transcripts in green berries. Functional categories significantly over-represented (0.05 Benjamini-Hochberg ad-

justed p-value in a Fisher's exact test) within each expression profile. Functional classification from Grimplet et al., BMC Res Notes 2012. All DEGs (FDR [?] 0.05 and log2 |FC| > 1.0) were used for the functional enrichment.

Supplementary Table S6 - Functional enrichment analysis in Syrah-responsive transcripts in green berries. Functional categories significantly over-represented (0.05 Benjamini-Hochberg adjusted p-value in a Fisher's exact test) within each expression profile. Functional classification from Grimplet et al., BMC Res Notes 2012.

Supplementary Table S7 - List of *Botrytis cinerea* genes expressed *in planta* comparing the constitutive and upon infection transcriptome of both Syrah and Trincadeira cultivars. Functional classification from Petrasch et al., 2019. C, Mock inoculated (Control); I, B. cinerea inoculated (Infected). 1-5 indicate the biological replicates.

Supplementary Table S8 – Summary of *B. cinerea* gene count. B. cinerea genes were considered expressed in planta if normalized gene count was equal to zero in all three control replicates and higher than twenty in all three infected replicates. C, Mock inoculated (Control); I, *B. cinerea* inoculated (Infected). 1-5 indicate the biological replicates.

Supplementary Table S9 – Metanalysis of the *Botrytis cinerea* genes expressed *in planta,* at green Syrah and Trincadeira infected berries compared with *Botrytis cinerea* genes expressed in Haile et al., 2020 (hard-green berry during quiescent infection, pre-egressed ripe berry, and egression on ripe berry). Functional classification from Petrasch et al., 2019.

9 REFERENCES

AbuQamar, Synan F., Khaled Moustafa, and Lam-Son P. Tran. 2016. "'Omics' and Plant Responses to Botrytis Cinerea." *Frontiers in Plant Science* 7. https://doi.org/10.3389/fpls.2016.01658.

AbuQamar, Synan, Khaled Moustafa, and Lam Son Tran. 2017. "Mechanisms and Strategies of Plant Defense against Botrytis Cinerea." *Critical Reviews in Biotechnology* 37 (2): 262–74. https://doi.org/10.1080/07388551.2016.1271767.

Afzal, Ahmed J., Andrew J. Wood, and David A. Lightfoot. 2008. "Plant Receptor-like Serine Threonine Kinases: Roles in Signaling and Plant Defense." *Molecular Plant-Microbe Interactions: MPMI* 21 (5): 507–17. https://doi.org/10.1094/MPMI-21-5-0507.

Agudelo-Romero, Patricia, Cristina Bortolloti, Maria Salome Pais, Antonio Fernandez Tiburcio, and Ana Margarida Fortes. 2013. "Study of Polyamines during Grape Ripening Indicate an Important Role of Polyamine Catabolism." *Plant Physiology and Biochemistry* 67 (June): 105–19. https://doi.org/10.1016/j.plaphy.2013.02.024.

Agudelo-Romero, Patricia, Alexander Erban, Cecilia Rego, Pablo Carbonell-Bejerano, Teresa Nascimento, Lisete Sousa, Jose M. Martinez-Zapater, Joachim Kopka, and Ana Margarida Fortes. 2015. "Transcriptome and Metabolome Reprogramming in Vitis Vinifera Cv. Trincadeira Berries upon Infection with Botrytis Cinerea." *Journal of Experimental Botany* 66 (7): 1769–85. https://doi.org/10.1093/jxb/eru517.

Alkan, Noam, and Ana M. Fortes. 2015. "Insights into Molecular and Metabolic Events Associated with Fruit Response to Post-Harvest Fungal Pathogens." *Frontiers in Plant Science* 6. https://doi.org/10.3389/fpls.2015.00889.

Al-Shahrour, Fatima, Ramon Diaz-Uriarte, and Joaquin Dopazo. 2004. "FatiGO: A Web Tool for Finding Significant Associations of Gene Ontology Terms with Groups of Genes." *Bioinformatics (Oxford, England)* 20 (4): 578–80. https://doi.org/10.1093/bioinformatics/btg455.

Anders, Simon, Paul Theodor Pyl, and Wolfgang Huber. 2015. "HTSeq—a Python Framework to Work with High-Throughput Sequencing Data." *Bioinformatics* 31 (2): 166–69. https://doi.org/10.1093/bioinformatics/btu638.

Anderson, Jonathan P., Ellet Badruzsaufari, Peer M. Schenk, John M. Manners, Olivia J. Desmond, Christina Ehlert, Donald J. Maclean, Paul R. Ebert, and Kemal Kazan. 2004. "Antagonistic Interaction between Abscisic Acid and Jasmonate-Ethylene Signaling Pathways Modulates Defense Gene Expression and Disease Resistance in Arabidopsis." *The Plant Cell* 16 (12): 3460–79. https://doi.org/10.1105/tpc.104.025833.

Andrews, Simon. 2010. "FastQC: A Quality Control Tool for High Throughput Sequence Data." 2010. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Armijo, Grace, Rudolf Schlechter, Mario Agurto, Daniela Munoz, Constanza Nunez, and Patricio Arce-Johnson. 2016. "Grapevine Pathogenic Microorganisms: Understanding Infection Strategies and Host Response Scenarios." *Frontiers in Plant Science* 7. https://doi.org/10.3389/fpls.2016.00382.

Becker, Jeffrey M., Sarah J. Kauffman, Melinda Hauser, Liyin Huang, Molly Lin, Susan Sillaots, Bo Jiang, Deming Xu, and Terry Roemer. 2010. "Pathway Analysis of Candida Albicans Survival and Virulence Determinants in a Murine Infection Model." *Proceedings of the National Academy of Sciences of the United States of America* 107 (51): 22044–49. https://doi.org/10.1073/pnas.1009845107.

Birkenbihl, Rainer P., Celia Diezel, and Imre E. Somssich. 2012. "Arabidopsis WRKY33 Is a Key Transcriptional Regulator of Hormonal and Metabolic Responses toward Botrytis Cinerea Infection." *Plant Physiology* 159 (1): 266–85. https://doi.org/10.1104/pp.111.192641.

Blanco-Ulate, Barbara, Abraham Morales-Cruz, Katherine C. H. Amrine, John M. Labavitch, Ann L. T. Powell, and Dario Cantu. 2014. "Genome-Wide Transcriptional Profiling of Botrytis Cinerea Genes Targeting Plant Cell Walls during Infections of Different Hosts." *Frontiers in Plant Science* 5: 435. https://doi.org/10.3389/fpls.2014.00435.

Breen, James, Luis Alejandro Jose Mur, Anushen Sivakumaran, Aderemi Akinyemi, Michael James Wilkinson, and Carlos Marcelino Rodriguez Lopez. 2016. "Botrytis Cinerea Loss and Restoration of Virulence during in Vitro Culture Follows Flux in Global DNA Methylation." *BioRxiv* , August, 059477. https://doi.org/10.1101/059477.

Burdziej, Aleksandra, Cezary Paczkowski, Agnès Destrac-Irvine, Tristan Richard, Stéphanie Cluzet, and Anna Szakiel. 2019. "Triterpenoid Profiles of the Leaves of Wild and Domesticated Grapevines." *Phytochemistry Letters* 30 (April): 302–8. https://doi.org/10.1016/j.phytol.2019.02.009.

Cela, Jana, John K. S. Tweed, Anushen Sivakumaran, Michael R. F. Lee, Luis A. J. Mur, and Sergi Munné-Bosch. 2018. "An Altered Tocopherol Composition in Chloroplasts Reduces Plant Resistance to Botrytis Cinerea." *Plant Physiology and Biochemistry: PPB* 127 (June): 200–210. https://doi.org/10.1016/j.plaphy.2018.03.033.

Choquer, Mathias, Elisabeth Fournier, Caroline Kunz, Caroline Levis, Jean-Marc Pradier, Adeline Simon, and Muriel Viaud. 2007. "Botrytis Cinerea Virulence Factors: New Insights into a Necrotrophic and Polyphageous Pathogen." *FEMS Microbiology Letters* 277 (1): 1–10. https://doi.org/10.1111/j.1574-6968.2007.00930.x.

Coelho, João, Marilia Almeida-Trapp, Diana Pimentel, Flávio Soares, Pedro Reis, Cecília Rego, Axel Mithöfer, and Ana Margarida Fortes. 2019. "The Study of Hormonal Metabolism of Trincadeira and Syrah Cultivars Indicates New Roles of Salicylic Acid, Jasmonates, ABA and IAA during Grape Ripening and upon Infection with Botrytis Cinerea." *Plant Science* 283 (June): 266–77. https://doi.org/10.1016/j.plantsci.2019.01.024.

Conde, Artur, Flávio Soares, Richard Breia, and Hernâni Gerós. 2018. "Postharvest Dehydration Induces Variable Changes in the Primary Metabolism of Grape Berries." *Food Research International* 105 (March): 261–70. https://doi.org/10.1016/j.foodres.2017.11.052.

Coombe, B. G. 1995. "Growth Stages of the Grapevine: Adoption of a System for Identifying Grapevine Growth Stages." *Australian Journal of Grape and Wine Research* 1 (2): 104–10.

https://doi.org/10.1111/j.1755-0238.1995.tb00086.x.

Cruz, Marco Tulio Solano de la, Jacel Adame-García, Josefat Gregorio-Jorge, Verónica Jiménez-Jacinto, Leticia Vega-Alvarado, Lourdes Iglesias-Andreu, Esteban Elías Escobar-Hernández, and Mauricio Luna-Rodríguez. 2019. "Increase in Ribosomal Proteins Activity: Translational Reprogramming in Vanilla Planifolia Jacks., against Fusarium Infection." *BioRxiv* , June, 660860. https://doi.org/10.1101/660860.

Dalio, Ronaldo, Diogo Magalhães, and Marcos Machado. 2017. "PAMPs, PRRs, Effectors and R-Genes Associated with Citrus–Pathogen Interactions." *Annals of Botany* 119: 749–74.

Dethloff, Frederik, Alexander Erban, Isabel Orf, Jessica Alpers, Ines Fehrle, Olga Beine-Golovchuk, Stefanie Schmidt, Jens Schwachtje, and Joachim Kopka. 2014. "Profiling Methods to Identify Cold-Regulated Primary Metabolites Using Gas Chromatography Coupled to Mass Spectrometry." *Methods in Molecular Biology (Clifton, N.J.)*1166: 171–97. https://doi.org/10.1007/978-1-4939-0844-8_14.

Dobón, Albor, Juan Vicente Canet, Javier García-Andrade, Carlos Angulo, Lutz Neumetzler, Staffan Persson, and Pablo Vera. 2015. "Novel Disease Susceptibility Factors for Fungal Necrotrophic Pathogens in Arabidopsis." *PLOS Pathogens* 11 (4): e1004800. https://doi.org/10.1371/journal.ppat.1004800.

Dulermo, Thierry, Christine Rascle, Gaetan Chinnici, Elisabeth Gout, Richard Bligny, and Pascale Cotton. 2009. "Dynamic Carbon Transfer during Pathogenesis of Sunflower by the Necrotrophic Fungus Botrytis Cinerea: From Plant Hexoses to Mannitol." *New Phytologist* 183 (4): 1149–62. https://doi.org/10.1111/j.1469-8137.2009.02890.x.

Fortes, Ana M., Patricia Agudelo-Romero, Marta S. Silva, Kashif Ali, Lisete Sousa, Federica Maltese, Young H. Choi, et al. 2011. "Transcript and Metabolite Analysis in Trincadeira Cultivar Reveals Novel Information Regarding the Dynamics of Grape Ripening." *BMC Plant Biology* 11 (1): 149. https://doi.org/10.1186/1471-2229-11-149.

Fortes, Ana Margarida, Patricia Agudelo-Romero, Diana Pimentel, and Noam Alkan. 2019. "Transcriptional Modulation of Polyamine Metabolism in Fruit Species Under Abiotic and Biotic Stress." *Frontiers in Plant Science* 10. https://doi.org/10.3389/fpls.2019.00816.

Frei dit Frey, Nicolas, Malick Mbengue, Mark Kwaaitaal, Lisette Nitsch, Denise Altenbach, Heidrun Häweker, Rosa Lozano-Duran, et al. 2012. "Plasma Membrane Calcium ATPases Are Important Components of Receptor-Mediated Signaling in Plant Immune Responses and Development1[C][W][OA]." *Plant Physiology* 159 (2): 798–809. https://doi.org/10.1104/pp.111.192575.

Frías, Marcos, Celedonio González, and Nélida Brito. 2011. "BcSpl1, a Cerato-Platanin Family Protein, Contributes to Botrytis Cinerea Virulence and Elicits the Hypersensitive Response in the Host." *The New Phytologist* 192 (2): 483–95. https://doi.org/10.1111/j.1469-8137.2011.03802.x.

González-Domínguez, Elisa, Tito Caffi, Nicola Ciliberti, and Vittorio Rossi. 2015. "A Mechanistic Model of Botrytis Cinerea on Grapevines That Includes Weather, Vine Growth Stage, and the Main Infection Pathways." *PloS One* 10 (10): e0140444. https://doi.org/10.1371/journal.pone.0140444.

Gourgues, M., A. Brunet-Simon, M.-H. Lebrun, and C. Levis. 2004. "The Tetraspanin BcPls1 Is Required for Appressorium-Mediated Penetration of Botrytis Cinerea into Host Plant Leaves." *Molecular Microbiology*51 (3): 619–29.

Grimplet, Jérôme, Patricia Agudelo-Romero, Rita T. Teixeira, Jose M. Martinez-Zapater, and Ana M. Fortes. 2016. "Structural and Functional Analysis of the GRAS Gene Family in Grapevine Indicates a Role of GRAS Proteins in the Control of Development and Stress Responses." *Frontiers in Plant Science* 7. https://doi.org/10.3389/fpls.2016.00353.

Grimplet, Jérôme, John Van Hemert, Pablo Carbonell-Bejerano, José Díaz-Riquelme, Julie Dickerson, Anne Fennell, Mario Pezzotti, and José M. Martínez-Zapater. 2012. "Comparative Analysis of Grapevine Whole-

Genome Gene Predictions, Functional Annotation, Categorization and Integration of the Predicted Gene Sequences." *BMC Research Notes*5 (1): 213. https://doi.org/10.1186/1756-0500-5-213.

Haile, Zeraye Mehari, Giulia Malacarne, Stefania Pilati, Paolo Sonego, Marco Moretto, Domenico Masuero, Urska Vrhovsek, Kristof Engelen, Elena Baraldi, and Claudio Moser. 2020. "Dual Transcriptome and Metabolic Analysis of Vitis Vinifera Cv. Pinot Noir Berry and Botrytis Cinerea During Quiescence and Egressed Infection." *Frontiers in Plant Science* 10. https://doi.org/10.3389/fpls.2019.01704.

Haile, Zeraye Mehari, Stefania Pilati, Paolo Sonego, Giulia Malacarne, Urska Vrhovsek, Kristof Engelen, Paul Tudzynski, Michela Zottini, Elena Baraldi, and Claudio Moser. 2017. "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." *Plant, Cell & Environment* 40 (8): 1409–28. https://doi.org/10.1111/pce.12937.

Hong, Young-Shick, Clara Cilindre, Gérard Liger-Belair, Philippe Jeandet, Norbert Hertkorn, and Philippe Schmitt-Kopplin. 2011. "Metabolic Influence of Botrytis Cinerea Infection in Champagne Base Wine." *Journal of Agricultural and Food Chemistry* 59 (13): 7237–45. https://doi.org/10.1021/jf200664t.

Hua, Li, Chen Yong, Zhang Zhanquan, Li Boqiang, Qin Guozheng, and Tian Shiping. 2018. "Pathogenic Mechanisms and Control Strategies of Botrytis Cinerea Causing Post-Harvest Decay in Fruits and Vegetables." *Food Quality and Safety* 2 (3): 111–19. https://doi.org/10.1093/fqsafe/fyy016.

Hummel, Jan, Nadine Strehmel, Joachim Selbig, Dirk Walther, and Joachim Kopka. 2010. "Decision Tree Supported Substructure Prediction of Metabolites from GC-MS Profiles." *Metabolomics* 6 (2): 322–33. https://doi.org/10.1007/s11306-010-0198-7.

Jaillon, Olivier, Jean-Marc Aury, Benjamin Noel, Alberto Policriti, Christian Clepet, Alberto Casagrande, Nathalie Choisne, et al. 2007. "The Grapevine Genome Sequence Suggests Ancestral Hexaploidization in Major Angiosperm Phyla." *Nature* 449 (7161): 463–67. https://doi.org/10.1038/nature06148.

Kan, Jan A. L. Van, Joost H. M. Stassen, Andreas Mosbach, Theo A. J. Van Der Lee, Luigi Faino, Andrew D. Farmer, Dimitrios G. Papasotiriou, et al. 2017. "A Gapless Genome Sequence of the Fungus Botrytis Cinerea." *Molecular Plant Pathology* 18 (1): 75–89. https://doi.org/10.1111/mpp.12384.

Kelloniemi, Jani, Sophie Trouvelot, Marie-Claire Héloir, Adeline Simon, Bérengère Dalmais, Patrick Frettinger, Agnès Cimerman, et al. 2015. "Analysis of the Molecular Dialogue Between Gray Mold (Botrytis Cinerea) and Grapevine (Vitis Vinifera) Reveals a Clear Shift in Defense Mechanisms During Berry Ripening." *Molecular Plant-Microbe Interactions* 28 (11): 1167–80. https://doi.org/10.1094/MPMI-02-15-0039-R.

Kim, Daehwan, Ben Langmead, and Steven L. Salzberg. 2015. "HISAT: A Fast Spliced Aligner with Low Memory Requirements." *Nature Methods* 12 (4): 357–60. https://doi.org/10.1038/nmeth.3317.

Kong, Weiwen, Nan Chen, Tingting Liu, Jing Zhu, Jingqi Wang, Xiaoqing He, and Yi Jin. 2015. "Large-Scale Transcriptome Analysis of Cucumber and Botrytis Cinerea during Infection." *PLOS ONE* 10 (11): e0142221. https://doi.org/10.1371/journal.pone.0142221.

Kopka, Joachim, Nicolas Schauer, Stephan Krueger, Claudia Birkemeyer, Björn Usadel, Eveline Bergmüller, Peter Dörmann, et al. 2005. "GMD@CSB.DB: The Golm Metabolome Database." *Bioinformatics (Oxford, England)* 21 (8): 1635–38. https://doi.org/10.1093/bioinformatics/bti236.

Lazazzara, Valentina, Christoph Bueschl, Alexandra Parich, Ilaria Pertot, Rainer Schuhmacher, and Michele Perazzolli. 2018. "Downy Mildew Symptoms on Grapevines Can Be Reduced by Volatile Organic Compounds of Resistant Genotypes." *Scientific Reports* 8 (1): 1618. https://doi.org/10.1038/s41598-018-19776-2.

Lecourieux, David, Raoul Ranjeva, and Alain Pugin. 2006. "Calcium in Plant Defence-Signalling Pathways." *New Phytologist* 171 (2): 249–69. https://doi.org/10.1111/j.1469-8137.2006.01777.x.

Li, Hua, Zhanquan Zhang, Chang He, Guozheng Qin, and Shiping Tian. 2016. "Comparative Proteomics Reveals the Potential Targets of BcNoxR, a Putative Regulatory Subunit of NADPH Oxidase of Botrytis Cinerea." *Molecular Plant-Microbe Interactions®* 29 (12): 990–1003. https://doi.org/10.1094/MPMI-11-16-0227-R.

Liu, Yue, Jiane-Kang Liu, Gui-Hua Li, Ming-Zhe Zhang, Ying-Ying Zhang, Yuan-Yuan Wang, Jie Hou, Song Yang, Jiao Sun, and Qing-Ming Qin. 2019. "A Novel Botrytis Cinerea-specific Gene BcHBF1 Enhances Virulence of the Grey Mould Fungus via Promoting Host Penetration and Invasive Hyphal Development." *Molecular Plant Pathology* 20 (5): 731–47. https://doi.org/10.1111/mpp.12788.

Lommen, Arjen. 2009. "MetAlign: Interface-Driven, Versatile Metabolomics Tool for Hyphenated Full-Scan Mass Spectrometry Data Preprocessing." *Analytical Chemistry* 81 (8): 3079–86. https://doi.org/10.1021/ac900036d.

Luedemann, Alexander, Katrin Strassburg, Alexander Erban, and Joachim Kopka. 2008. "TagFinder for the Quantitative Analysis of Gas Chromatography–Mass Spectrometry (GC-MS)-Based Metabolite Profiling Experiments." *Bioinformatics (Oxford, England)* 24 (5): 732–37. https://doi.org/10.1093/bioinformatics/btn023.

Marinović, Mila, Maria Victoria Aguilar-Pontes, Miaomiao Zhou, Otto Miettinen, Ronald P. de Vries, Miia R. Mäkelä, and Kristiina Hildén. 2018. "Temporal Transcriptome Analysis of the White-Rot Fungus Obba Rivulosa Shows Expression of a Constitutive Set of Plant Cell Wall Degradation Targeted Genes during Growth on Solid Spruce Wood." *Fungal Genetics and Biology* , Basidiomycete research in the post-genomic era, 112 (March): 47–54. https://doi.org/10.1016/j.fgb.2017.07.004.

Mbengue, Malick, Olivier Navaud, Rémi Peyraud, Marielle Barascud, Thomas Badet, Rémy Vincent, Adelin Barbacci, and Sylvain Raffaele. 2016. "Emerging Trends in Molecular Interactions between Plants and the Broad Host Range Fungal Pathogens Botrytis Cinerea and Sclerotinia Sclerotiorum." *Frontiers in Plant Science* 7. https://doi.org/10.3389/fpls.2016.00422.

Mengiste, Tesfaye, Xi Chen, John Salmeron, and Robert Dietrich. 2003. "The BOTRYTIS SUSCEPTIBLE1 Gene Encodes an R2R3MYB Transcription Factor Protein That Is Required for Biotic and Abiotic Stress Responses in Arabidopsis." *The Plant Cell* 15 (11): 2551–65. https://doi.org/10.1105/tpc.014167.

Monteiro, Sara, Mahmoud Barakat, Maria A. Piçarra-Pereira, Artur R. Teixeira, and Ricardo B. Ferreira. 2003. "Osmotin and Thaumatin from Grape: A Putative General Defense Mechanism against Pathogenic Fungi." *Phytopathology* 93 (12): 1505–12. https://doi.org/10.1094/PHYTO.2003.93.12.1505.

Moore, John W., Gary J. Loake, and Steven H. Spoel. 2011. "Transcription Dynamics in Plant Immunity." *The Plant Cell* 23 (8): 2809–20. https://doi.org/10.1105/tpc.111.087346.

Ono, Erika, Kazuyuki Mise, and Yoshitaka Takano. 2020. "RLP23 Is Required for Arabidopsis Immunity against the Grey Mould Pathogen Botrytis Cinerea." *Scientific Reports* 10 (1): 13798. https://doi.org/10.1038/s41598-020-70485-1.

Peng, Hui, Tianbao Yang, and Wayne M. Jurick Ii. 2014. "Calmodulin Gene Expression in Response to Mechanical Wounding and Botrytis Cinerea Infection in Tomato Fruit." *Plants* 3 (3): 427–41. https://doi.org/10.3390/plants3030427.

Pensec, Flora, Cezary Paczkowski, Marta Grabarczyk, Agnieszka Woźniak, Mélanie Bénard-Gellon, Christophe Bertsch, Julie Chong, and Anna Szakiel. 2014. "Changes in the Triterpenoid Content of Cuticular Waxes during Fruit Ripening of Eight Grape (Vitis Vinifera) Cultivars Grown in the Upper Rhine Valley." *Journal of Agricultural and Food Chemistry* 62 (32): 7998–8007. https://doi.org/10.1021/jf502033s.

Petrasch, Stefan, Steven J. Knapp, Jan A. L. van Kan, and Barbara Blanco-Ulate. 2019. "Grey Mould of Strawberry, a Devastating Disease Caused by the Ubiquitous Necrotrophic Fungal Pathogen Botrytis Cinerea." *Molecular Plant Pathology* 0 (0). https://doi.org/10.1111/mpp.12794.

Petrasch, Stefan, Christian J. Silva, Saskia D. Mesquida-Pesci, Karina Gallegos, Casper van den Abeele, Victor Papin, Francisco J. Fernandez-Acero, Steven J. Knapp, and Barbara Blanco-Ulate. 2019. "Infection Strategies Deployed by Botrytis Cinerea, Fusarium Acuminatum, and Rhizopus Stolonifer as a Function of Tomato Fruit Ripening Stage." *Frontiers in Plant Science* 10. https://doi.org/10.3389/fpls.2019.00223.

Pierik, Ronald, Carlos L. Ballare, and Marcel Dicke. 2014. "Ecology of Plant Volatiles: Taking a Plant Community Perspective." *Plant, Cell & Environment* 37 (8): 1845–53. https://doi.org/10.1111/pce.12330.

Pliego, Clara, Daniela Nowara, Giulia Bonciani, Dana M. Gheorghe, Ruo Xu, Priyanka Surana, Ehren Whigham, et al. 2013. "Host-Induced Gene Silencing in Barley Powdery Mildew Reveals a Class of Ribonuclease-Like Effectors." *Molecular Plant-Microbe Interactions(r)* 26 (6): 633–42. https://doi.org/10.1094/MPMI-01-13-0005-R.

Quintana-Rodriguez, Elizabeth, Adan T. Morales-Vargas, Jorge Molina-Torres, Rosa M. Adame-Alvarez, Jorge A. Acosta-Gallegos, and Martin Heil. 2015. "Plant Volatiles Cause Direct, Induced and Associational Resistance in Common Bean to the Fungal Pathogen Colletotrichum Lindemuthianum." *Journal of Ecology* 103 (1): 250–60. https://doi.org/10.1111/1365-2745.12340.

Robinson, Mark D., Davis J. McCarthy, and Gordon K. Smyth. 2010. "EdgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data." *Bioinformatics* 26 (1): 139–40. https://doi.org/10.1093/bioinformatics/btp616.

Shu, Chang, Handong Zhao, Wenxiao Jiao, Bangdi Liu, Jiankang Cao, and Weibo Jiang. 2019. "Antifungal Efficacy of Ursolic Acid in Control of Alternaria Alternata Causing Black Spot Rot on Apple Fruit and Possible Mechanisms Involved." *Scientia Horticulturae* 256 (October): 108636. https://doi.org/10.1016/j.scienta.2019.108636.

Siewers, Verena, Muriel Viaud, Daniel Jimenez-Teja, Isidro G. Collado, Christian Schulze Gronover, Jean-Marc Pradier, Bettina Tudzynski, and Paul Tudzynski. 2005. "Functional Analysis of the Cytochrome P450 Monooxygenase Gene Bcbot1 of Botrytis Cinerea Indicates That Botrydial Is a Strain-Specific Virulence Factor." *Molecular Plant-Microbe Interactions: MPMI* 18 (6): 602–12. https://doi.org/10.1094/MPMI-18-0602.

Solomon, Peter S., Ormonde D. C. Waters, and Richard P. Oliver. 2007. "Decoding the Mannitol Enigma in Filamentous Fungi." *Trends in Microbiology* 15 (6): 257–62. https://doi.org/10.1016/j.tim.2007.04.002.

Soltis, Nicole E., Celine Caseys, Wei Zhang, Jason A. Corwin, Susanna Atwell, and Daniel J. Kliebenstein. 2020. "Pathogen Genetic Control of Transcriptome Variation in the Arabidopsis Thaliana – Botrytis Cinerea Pathosystem." *Genetics* 215 (1): 253–66. https://doi.org/10.1534/genetics.120.303070.

Srivastava, Dhruv Aditya, Gulab Chand Arya, Eswari PJ Pandaranayaka, Ekaterina Manasherova, Dov Prusky, Yigal Elad, Omer Frenkel, and Arye Harel. 2020. "Transcriptome Profiling Data of Botrytis Cinerea Infection on Whole Plant Solanum Lycopersicum." *Molecular Plant-Microbe Interactions(r)* , June. https://doi.org/10.1094/MPMI-05-20-0109-A.

Strehmel, Nadine, Jan Hummel, Alexander Erban, Katrin Strassburg, and Joachim Kopka. 2008. "Retention Index Thresholds for Compound Matching in GC-MS Metabolite Profiling." *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 871 (2): 182–90. https://doi.org/10.1016/j.jchromb.2008.04.042.

Szakiel, Anna, Cezary Paczkowski, Flora Pensec, and Christophe Bertsch. 2012. "Fruit Cuticular Waxes as a Source of Biologically Active Triterpenoids." *Phytochemistry Reviews* 11 (2): 263–84. https://doi.org/10.1007/s11101-012-9241-9.

Torii, Keiko U. 2004. "Leucine-Rich Repeat Receptor Kinases in Plants: Structure, Function, and Signal Transduction Pathways." *International Review of Cytology* 234: 1–46. https://doi.org/10.1016/S0074-7696(04)34001-5.

Torres, Miguel Angel, Jonathan D. G. Jones, and Jeffery L. Dangl. 2006. "Reactive Oxygen Species Signaling in Response to Pathogens." *Plant Physiology* 141 (2): 373–78. https://doi.org/10.1104/pp.106.079467.

Utto, Weerawate, A. John Mawson, and John E. Bronlund. 2008. "Hexanal Reduces Infection of Tomatoes by Botrytis Cinerea Whilst Maintaining Quality." *Postharvest Biology and Technology* 47 (3): 434–37. https://doi.org/10.1016/j.postharvbio.2007.08.008.

Vallarino, José G., Alexander Erban, Ines Fehrle, Alisdair R. Fernie, Joachim Kopka, and Sonia Osorio. 2018. "Acquisition of Volatile Compounds by Gas Chromatography-Mass Spectrometry (GC-MS)." *Methods in Molecular Biology (Clifton, N.J.)* 1778: 225–39. https://doi.org/10.1007/978-1-4939-7819-9_16.

Vela-Corcía, David, Dhruv Aditya Srivastava, Avis Dafa-Berger, Neta Rotem, Omer Barda, and Maggie Levy. 2019. "MFS Transporter from Botrytis Cinerea Provides Tolerance to Glucosinolate-Breakdown Products and Is Required for Pathogenicity." *Nature Communications* 10 (1): 1–11. https://doi.org/10.1038/s41467-019-10860-3.

Veloso, Javier, and Jan A. L. van Kan. 2018. "Many Shades of Grey in Botrytis–Host Plant Interactions." *Trends in Plant Science* 23 (7): 613–22. https://doi.org/10.1016/j.tplants.2018.03.016.

Viret, Olivier, Markus Keller, V. Gunta Jaudzems, and F. Mary Cole. 2004. "Botrytis Cinerea Infection of Grape Flowers: Light and Electron Microscopical Studies of Infection Sites." *Phytopathology* 94 (8): 850–57. https://doi.org/10.1094/PHYTO.2004.94.8.850.

Wang, Xuli, Nan Jiang, Jinling Liu, Wende Liu, and Guo-Liang Wang. 2014. "The Role of Effectors and Host Immunity in Plant–Necrotrophic Fungal Interactions." *Virulence* 5 (7): 722–32. https://doi.org/10.4161/viru.29798.

Wang, Zheng-Yi, Christopher R. Thornton, Michael J. Kershaw, Li Debao, and Nicholas J. Talbot. 2003. "The Glyoxylate Cycle Is Required for Temporal Regulation of Virulence by the Plant Pathogenic Fungus Magnaporthe Grisea." *Molecular Microbiology* 47 (6): 1601–12. https://doi.org/10.1046/j.1365-2958.2003.03412.x.

Xiao, Xueqiong, and Aardra Kachroo. 2019. "Plant Defences against Fungal Attack: Perception and Signal Transduction." In *ELS* , 1–8. American Cancer Society. https://doi.org/10.1002/9780470015902.a0003438.pub3.

Zambounis, Antonios, Ioannis Ganopoulos, Dimitris Valasiadis, Lefkothea Karapetsi, and Panagiotis Madesis. 2020. "RNA Sequencing-Based Transcriptome Analysis of Kiwifruit Infected by Botrytis Cinerea." *Physiological and Molecular Plant Pathology* 111 (August): 101514. https://doi.org/10.1016/j.pmpp.2020.101514.

Zhang, Lisha, Ilona Kars, Bert Essenstam, Thomas W. H. Liebrand, Lia Wagemakers, Joyce Elberse, Panagiota Tagkalaki, Devlin Tjoitang, Guido van den Ackerveken, and Jan A. L. van Kan. 2014. "Fungal Endopolygalacturonases Are Recognized as Microbe-Associated Molecular Patterns by the Arabidopsis Receptor-Like Protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1." *Plant Physiology* 164 (1): 352–64. https://doi.org/10.1104/pp.113.230698.

Zhang, Wei, Jason A Corwin, Daniel Harrison Copeland, Julie Feusier, Robert Eshbaugh, David E Cook, Suzi Atwell, and Daniel J Kliebenstein. 2019. "Plant–Necrotroph Co-Transcriptome Networks Illuminate a Metabolic Battlefield." Edited by Kenichi Tsuda and Ian T Baldwin.*ELife* 8 (May): e44279. https://doi.org/10.7554/eLife.44279.

Zheng, Chuanlin, Mathias Choquer, Bing Zhang, Hui Ge, Songnian Hu, Huiqin Ma, and Shangwu Chen. 2011. "LongSAGE Gene-Expression Profiling of Botrytis Cinerea Germination Suppressed by Resveratrol, the Major Grapevine Phytoalexin." *Fungal Biology* 115 (9): 815–32. https://doi.org/10.1016/j.funbio.2011.06.009.

10 TABLES

Table 1- Selected grapevine differentially expressed genes in susceptible and tolerant cultivars (FDR [?] 0.05 and log2 |FC| > 1.5). Complete dataset in Supplementary Table S3.

Unique ID	log Fold change	log Fold change
	Syrah Infected/ Syrah Control	Trincadeira Infected/
Biotic stress response and secondary metabolism	*Biotic stress response and secondary metabolism*	*Biotic stress response*
VIT_00s0266g00070		-3.34
VIT_01s0010g02930		4.14
VIT_03s0038g04390		5.89
VIT_04s0210g00120		
VIT_05s0020g03200		
VIT_05s0077g01690		7.06
VIT_06s0004g05310		-1.95
VIT_06s0004g07650		-1.51
VIT_09s0002g00220		2.76
VIT_10s0003g03650		
VIT_11s0016g05010		4.03
VIT_12s0034g00130		3.76
VIT_12s0035g01000		10.3
VIT_13s0064g00340		
VIT_14s0068g01920		2.56
VIT_14s0081g00770		
VIT_16s0039g01280		3.95
VIT_16s0039g01870		4.47
VIT_16s0100g00090		4.88
VIT_16s0100g01070		6.48
VIT_16s0100g01190		6.89
VIT_18s0041g00920		4.96
VIT_18s0117g00370		
Oxidative stress	*Oxidative stress*	
VIT_04s0008g03600		
VIT_04s0079g00690		11.96
VIT_08s0040g00920		4.96
VIT_10s0003g00390		4.04
VIT_16s0039g01410		
Signaling, Transcription factors and Kinases	*Signaling, Transcription factors and Kinases*	*Signaling, Transcripti*
VIT_00s0425g00030		
VIT_00s0463g00020		1.78
VIT_02s0033g00390		7.92
VIT_06s0004g04990		
VIT_06s0061g01400		5.16
VIT_07s0031g01710		4.22
VIT_08s0007g03630		
VIT_08s0007g08750		3.24
VIT_08s0032g01220		3.16
VIT_12s0142g00800		
VIT_19s0014g04040		6.95
VIT_19s0014g04940		2.22
Hormonal metabolism	*Hormonal metabolism*	*Hormonal metabolism*
VIT_00s0253g00150		2.57

Unique ID	log Fold change	log Fold change
VIT_03s0063g01820		4.81
VIT_04s0008g02230		2.87
VIT_04s0008g05760		3.17
VIT_05s0049g00510		2.33
VIT_09s0002g09140		
VIT_10s0003g00590		4.31
VIT_10s0003g03800		2.95
VIT_11s0016g00660		5.56
VIT_11s0016g00670		2.56
VIT_11s0016g00710		2.13
VIT_15s0046g02220		7.12
VIT_16s0013g00980	-1.59	
Carbohydrate metabolism	*Carbohydrate metabolism*	*Carbohydrate metabol*
VIT_00s0181g00180		-1.71
VIT_02s0154g00110		3.18
VIT_05s0020g03140		5.57
VIT_05s0077g00840		3.60
VIT_07s0005g01680		3.75
VIT_07s0005g02220		-1.80
VIT_14s0030g00220		-2.20
VIT_14s0030g00300		
VIT_14s0060g00760		4.67
VIT_14s0066g00810		3.15
VIT_17s0000g01820		3.24
VIT_17s0119g00150		7.65
Cell wall metabolism	*Cell wall metabolism*	*Cell wall metabolism*
VIT_00s2620g00010		2.75
VIT_01s0127g00870		-1.72
VIT_01s0137g00240		-2.39
VIT_06s0009g02590		11.05
VIT_07s0005g01030		
VIT_08s0007g08330		7.19
VIT_08s0040g01340		7.20
VIT_11s0052g01180		3.33
VIT_12s0059g01010		
VIT_18s0122g00690		9.65
Lipid metabolism	Lipid metabolism	Lipid metabolism
VIT_04s0079g00790		2.52
VIT_06s0004g01500		2.26
VIT_07s0005g01240		3.47
VIT_07s0141g00060		3.68
VIT_09s0002g01080		2.33
VIT_13s0067g01120		2.12
VIT_14s0066g01670		6.80
VIT_16s0022g01120		
VIT_16s0022g01150		

Table 2- Selected *in planta* detected *Botrytis cinerea* transcripts. Genes were only considered present if normalized gene count (RPKM) was equal to zero in all three control replicates and higher than zero in all

three infected replicates. Complete dataset in Supplementary Table S7.

Unique ID	RPKMs	RPKMs	RPKMs
	Syrah Infected Inf2	Syrah Infected Inf3	Syrah Infe Inf4
Virulence and Growth	*Virulence and Growth*	*Virulence and Growth*	*Virulence*
Bcin01g01520.1	1037	949.4	409.4
Bcin01g04560.1	175.1	240.5	622.3
Bcin01g05060.1	69.1	189.7	245.4
Bcin01g08260.1			
Bcin02g01260.1	451.0	619.3	267.1
Bcin02g04870	822.0	376.3	324.5
Bcin02g08170.1	90.7	186.9	53.7
Bcin02g08280.1	100.4	137.9	237.9
Bcin03g00500.1	314.3	2158	372.3
Bcin03g00750.1	982.1	224.8	387.7
Bcin03g01480.1	520.8	357.6	616.9
Bcin03g03390.1	2136	2933	2024
Bcin03g03880.1	301.1	206.8	356.7
Bcin04g03690.1	165.8	227.8	392.9
Bcin04g05630.1	171.4	470.7	203.0
Bcin05g00730.1	5988	2530	1909
Bcin05g01450.1	1022	468.3	1009
Bcin05g05530.1	347.3	238.5	411.3
Bcin05g06320.2			
Bcin05g07640.1	214.3	588.7	1015
Bcin06g02460.1	384.0	527.3	454.8
Bcin06g04460.1	651.9	1342	1158
Bcin07g03340.1	216.1	296.8	255.9
Bcin07g04950.1	226.6	311.2	536.9
Bcin08g03620.1	93.2	64.0	55.2
Bcin09g03570.1	555.8	763.3	658.3
Bcin09g06140.1	53.0	218.4	62.8
Bcin10g00740.1	422.1	579.6	499.9
Bcin10g01180.1	1757	1689	624.5
Bcin10g05640.1			
Bcin11g01720.1	825.0	679.8	1172.6
Bcin11g05430.1	243.4	334.3	576.6
Bcin12g01370.1			
Bcin14g01550.1	367.2	168.1	145.0
Bcin15g02590.1	57.3	118.0	67.9
Bcin15g03610.1	146.5	201.2	173.6
Bcin16g03140.1	715.9	983.1	565.3
Signalling			
Bcin01g00930.1	340.6	233.9	605.1
Bcin03g05990.1	691.9	950.2	819.5
Bcin09g02390.1	287.1	131.4	566.7
Bcin10g04140.1	310.6	426.5	183.9
Bcin11g02950.1	81.5	559.7	96.5
Bcin11g04070.1	301.1	310.1	89.1
Bcin14g03860.1	91.0	249.9	215.5

Unique ID	RPKMs	RPKMs	RPKMs
Bcin15g03580.1	403.5	69.3	59.7
Bcin16g01130.1	377.5	172.8	149.1
Protein biosynthesis and regulation	*Protein biosynthesis and regulation*	*Protein biosynthesis and regulation*	*Protein bi*
Bcin02g06250.1	824.6	377.5	325.6
Bcin02g06900.1	716.6	2952	848.8
Bcin03g05960.1	2454	842.8	2180.7
Bcin03g06970.1			
Bcin07g01170.1	3936	2703	4662
Bcin12g00420.1	444.3	305.1	1052
Bcin12g03890.1	178.2	489.4	211.0
Carbohydrate metabolism	*Carbohydrate metabolism*	*Carbohydrate metabolism*	*Carbohydr*
Bcin01g06740.1	97.8	268.7	115.9
Bcin01g07270.1	230.9	317.1	547.0
Bcin01g09950.1			
Bcin01g10310.1	330.2	113.4	97.8
Bcin02g01650.1	492.3	225.3	194.4
Bcin02g08340.1	538.8	739.9	638.1
Bcin07g00940.3	112.7	51.6	44.5
Bcin08g00740.1	649.5	446.0	769.3
Bcin09g00150.1	415.3	380.2	983.9
Bcin10g01500.1			
Bcin11g05700.1	835.3	382.4	989.3
Bcin12g02300.1	986.4	1083	467.3
Bcin15g02270.1	680.3	373.7	805.8
Bcin15g03620.1	229.8	473.4	136.1
Lipid metabolism	*Lipid metabolism*	*Lipid metabolism*	*Lipid meta*
Bcin01g00440.1	212.0	194.1	83.7
Bcin02g00210.1			
Bcin04g00760.1	301.2	413.7	178.4
Bcin04g01780.1			
Bcin07g06960.1	291.3	120.0	172.5
Bcin09g02790.1	480.4	659.7	948.3
Bcin16g03180.1	261.8	1078	1240
Cell Wall metabolism	*Cell Wall metabolism*	*Cell Wall metabolism*	*Cell Wall*
Bcin01g03390.1	62.6	85.9	222.3
Bcin01g03790.2	46.5	63.9	55.1
Bcin02g06930.1			
Bcin03g00640.1	1904	1664	205.1
Bcin04g01290.1	968.6	532.0	229.4
Bcin04g03120.1	561.4	154.2	531.9
Bcin06g07010.1	1540	705.0	3648
Bcin07g04810.1	91.9	126.2	108.8
Bcin08g00910.1	578.6	397.3	342.7
Bcin08g02140.1	162.7	148.9	385.4
Bcin09g01110.1	1051	206.3	1245.4
Bcin10g02280.1	563.6	193.5	834.5
Bcin10g05590.1	218.6	100.1	86.3
Bcin10g06130.1	461.6	633.9	273.4
Bcin11g04800.1			
Bcin12g05360.1			

Unique ID	RPKMs	RPKMs	RPKMs
Bcin13g04610.1	99.7	136.9	118.1
Bcin15g00810.1	458.3	1048	542.8

11 Figure Legends

Figure 1 - Clusters of *Vitis vinifera cv.* (A) Syrah and (B) Trincadeira grapes naturally infected with grey mold (*Botrytis cinerea)* at green developmental stage (EL32). (C) Magnification of (A). (D) Magnification of (B): fungal sporulation was observed in infected Trincadeira clusters.

Figure 2 - Analysis of infection- and cultivar-responsive metabolites from *Botrytis cinerea* infected and control berries of *V. vinifera* cv. Trincadeira and cv. Syrah at the green developmental stage (EL32).

Soluble and volatile metabolites that were significantly increased or decreased in at least one comparison (response ratio [?] 1.5 and p-value [?] 0.05, Student's t-test) with main pathways tagged. Response ratios were $\log_2$-transformed and hierarchically clustered using Euclidean distance and complete linkage. Asterisks indicate statistical significance comparing to the control (Student's t-test: * p-value [?] 0.05; ** p-value [?] 0.01; *** p-value [?] 0.001).

Figure 3 - Analysis of potential positive metabolic markers of *B. cinerea* infection at green stage (EL32) of development of *V. vinifera* cv. Trincadeira and cv. Syrah.

Metabolites selected were either significantly increased after infection at one or both cultivars (response ratio [?]1.5 and p-value [?] 0.05, Student's t-test) or only detected in infected berries. Square brackets indicate metabolites that were identified/ classified only by mass spectral match. Grey boxes indicate that the respective metabolites were not detectable.

Figure 4 - Enriched functional subcategories (A) in *Botrytis cinerea* infection-responsive transcripts (Infection vs Control) and (B) in Syrah-responsive transcripts (Syrah vs Trincadeira) (adjusted p-value [?] 0.05). Circles' size represents the number of genes ($\log_{10}$). A complete dataset in Supplemental Table S5 and S6.

Figure 5 – Biological processes associated with fungal *in planta* expressed genes. Bar graph are the top 30 represented GOs sorted by number. Labels on the top of each bar indicate the number of genes that matched that particular enriched category. The complete dataset is presented in Supplemental Table S7.

Figure 6 – General representation of plant colonization by *Botrytis cinerea* in tolerant and susceptible cultivars (A) Syrah's photosynthetic tissues are shown in green and represent healthy and green tissues with activated basal defenses (largely unchanged by the infection) namely the expression of genes coding for R proteins and secondary metabolism related. On the other hand, the fungus enters an active virulence program involving among others the release of cutinases and proteases; (B) Trincadeira's photosynthetic tissues are shown in bordeaux color and represent infected cells with defenses largely activated by fungal presence namely the expression of genes involved in cell wall, hormonal, primary and secondary metabolisms. Opposite behavior is noticed in the fungus by presenting limiting transcriptional activity but related with a more advanced stage of infection namely the expression of genes involved in sporulation. Solid green or bordeaux lines indicate living tissue, and dashed lines indicate dead tissue.

Fungal and plant cells are not proportionally scaled. Ap, appressorium; C, conidia.

Plant genes: *Beta amyrin synthase –BAS; Cinnamyl alcohol dehydrogenase – CAD;R protein disease resistance; R protein L6; Sugar transporter ERD6 like 3- ERD6 like 3; Glutathione S-transferase 25–GST; Receptor serine/threonine kinase –RSTK; Calmodulin binding protein –CBP; Cellulose synthase CSLA09– CSLA09; Cellulose synthase CSLA04- CSLA04; Pectinesterase family –PE family;*Polygalacturonase - *PG1; Pathogenesis protein 10–PR-10; Cationic peroxidase; Stilbene synthase –STS; Chitin-inducible gibberellin-responsive protein 1–CIGR1; Allene oxide synthase- AOS* ; *Galactinol synthase.*

Fungal genes: *mitogen-activated protein kinase- mp1; transcription factor MBZ1- BczipA; Mitogen activated protein kinase spm1- Bmp3; Mitogen activated protein kinase hog1- Bcsak1; Superoxide dismutase [Cu-Zn]- Bcsod1; MFS transporter ptIL- MFS;Sporulation protein kinase pit- Bcime2; cutinase gene palindrome-binding protein- Bcwcl2; Pro-apoptotic serine protease NMA111- Bcnma; signaling mucin MSB2- Bcmsb2.*