

Analysis of the Molecular Dialogue Between Gray Mold (*Botrytis cinerea*) and Grapevine (*Vitis vinifera*) Reveals a Clear Shift in Defense Mechanisms During Berry Ripening

Jani Kelloniemi,¹ Sophie Trouvelot,¹ Marie-Claire Héloir,¹ Adeline Simon,² Bérengère Dalmais,² Patrick Frettinger,^{1,3} Agnès Cimerman,² Marc Fermaud,⁴ Jean Roudet,⁴ Sylvain Baulande,⁵ Christophe Bruel,³ Mathias Choquer,³ Linhdavanh Couvelard,⁵ Mathilde Duthieuw,⁵ Alberto Ferrarini,⁶ Victor Flors,⁷ Pascal Le Pêcheur,² Elise Loisel,⁴ Guillaume Morgant,² Nathalie Poussereau,³ Jean-Marc Pradier,² Christine Rascle,³ Lucie Trdá,¹ Benoit Poinssot,¹ and Muriel Viaud²

¹Université de Bourgogne, UMR 1347 Agroécologie, Pôle Interactions Plantes Micro-organismes–ERL CNRS 6300, 17 rue Sully, 21000 Dijon, France; ²INRA, UMR 1290 BIOGER, Avenue Lucien Brétignières, 78850 Grignon, France; ³UMR 5240 MAP, Université Lyon 1-CNRS-Bayer CropScience, Villeurbanne, France; ⁴INRA, UMR 1065 Santé et Agroécologie du Vignoble, 33882 Villenave d'Ornon, France; ⁵PartnerChip, 2 rue G. Crémieux, 91000 Evry, France; ⁶Università degli Studi di Verona, Dipartimento di Biotecnologie, Strada Le Grazie 15, 37134 Verona, Italy; ⁷University of Jaume I, Plant Physiology Section, CAMN, Castellón, 12071, Spain

Submitted 20 February 2015. Accepted 4 August 2015.

Mature grapevine berries at the harvesting stage (MB) are very susceptible to the gray mold fungus *Botrytis cinerea*, while veraison berries (VB) are not. We conducted simultaneous microscopic and transcriptomic analyses of the pathogen and the host to investigate the infection process developed by *B. cinerea* on MB versus VB, and the plant defense mechanisms deployed to stop the fungus spreading. On the pathogen side, our genome-wide transcriptomic data revealed that *B. cinerea* genes upregulated during infection of MB are enriched in functional categories related to necrotrophy, such as degradation of the plant cell wall, proteolysis, membrane transport, reactive oxygen species (ROS) generation, and detoxification. Quantitative-polymerase chain reaction on a set of representative genes related to virulence and microscopic observations further demonstrated that the infection is also initiated on VB but is stopped at the penetration stage. On the plant side, genome-wide transcriptomic analysis and metabolic data revealed a defense pathway switch during berry ripening. In response to *B. cinerea* inoculation, VB activated a burst of ROS, the salicylate-dependent defense pathway, the synthesis of the resveratrol phytoalexin, and cell-wall strengthening. On the contrary, in infected MB, the jasmonate-dependent pathway was activated, which did not stop the fungal necrotrophic process.

Grapevine is confronted by several severe diseases mainly caused by phytopathogenic fungi and oomycetes, including downy mildew (*Plasmopara viticola*), powdery mildew

Co-senior and co-corresponding authors: M. Viaud; Telephone +33 130 814 568; E-mail: viaud@versailles.inra.fr; and B. Poinssot; Telephone: +33 380 693 458; E-mail: benoit.poinssot@dijon.inra.fr

*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary figures and seven supplementary tables are published online.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2015.

(*Erysiphe necator*), and gray mold (*Botrytis cinerea*). Against these diseases, modern sustainable viticulture aims to limit chemical treatments by using alternative strategies. One of them is to trigger grapevine resistance by eliciting its innate immunity. Plant innate immunity is governed by two main defense pathways (Boller and Felix 2009). The first, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is based on recognition of evolutionarily conserved PAMPs or microbe- or damage-associated molecular patterns by receptors called pattern recognition receptors (Jones and Dangl 2006). The second, effector-triggered immunity (ETI) is activated after the detection of pathogen effectors by the plant (Böhm et al. 2014; Zipfel 2014). ETI is highly specific, depends on resistance genes, and is usually accompanied by a hypersensitive response (HR) manifesting as localized cell death at the point of infection (Jones and Dangl 2006). For some pathogens, like *B. cinerea*, the use of an ETI-based strategy cannot be considered, as no major resistant gene has been found in grapevine or in any other host species. Both PTI and ETI activate plant signaling events (Garcia-Brugger et al. 2006; Jeworutzki et al. 2010; Tsuda and Katagiri 2010) leading to a large-scale transcriptome reprogramming (Denoux et al. 2008; Pitzschke et al. 2009). Plant hormones such as salicylate (SA), jasmonate (JA), and ethylene (ET) take part in fine-tuning the defense responses (Koorneef and Pieterse 2008; López et al. 2008). In *Arabidopsis*, the consensus is that the SA-dependent signaling pathway is required for defense against biotrophs, while the JA and ET pathways are important against necrotrophs (Glazebrook 2005). One outcome of these defense signaling pathways is the production of antimicrobial secondary metabolites, such as phytoalexins (Hammerschmidt 1999), cell-wall reinforcements, and pathogenesis-related (PR) proteins such as beta-1,3 glucanases and chitinases (van Loon et al. 2006). In *Vitaceae* spp., the stilbene compounds resveratrol and its derivatives are the main phytoalexins produced via the phenylalanine-polymalonate pathway. The key enzymes are phenylalanine ammonia lyase (PAL) and stilbene synthase (STS), which condense one molecule of coumaroyl-CoA and three molecules

of malonyl-CoA to produce resveratrol (Coutos-Thévenot et al. 2001).

The development of anti-*Botrytis* strategies based on elicitation of plant immunity requires a better knowledge of the molecular interaction between this fungus and grape. In vineyards in spring, *B. cinerea* may infect young leaves and, more often, the grapevine inflorescences (Elmer and Michailides 2007; Martinez et al. 2005). Green berries and berries that begin to ripen, i.e., the veraison stage berries (VB), are not susceptible to *B. cinerea* (Deytieu-Belleau et al. 2009). On the other hand, mature berries (MB) are highly susceptible to the pathogen (Elmer and Michailides 2007; Kretschmer et al. 2007), thus causing substantial damages to the phenolic and organoleptic properties of wines (Ky et al. 2012).

In recent years, the polyphagous fungus *B. cinerea* has become one of the most extensively studied necrotrophic fungal pathogens (Dean et al. 2012). This ascomycete is able to kill and feed on host cells through the production of unspecific toxins, reactive oxygen species (ROS) and a battery of lytic enzymes (Choquer et al. 2007; van Kan 2006). In addition, several studies strongly suggest that *B. cinerea* and other necrotrophic fungi induce the HR as part of their infection strategy (Dickman et al. 2001; Govrin and Levine 2000; Van Baarlen et al. 2007). Even though studies on *B. cinerea* have been conducted using several host plants, the infection molecular program initiated on vineyard berries remains unknown. On the plant side, the known defense mechanisms activated in grapevine berries by *B. cinerea* are still scarce

(Bézier et al. 2002; Derckel et al. 1998; Iriti et al. 2004; Monteiro et al. 2003; Robert et al. 2002; Robinson et al. 1997; Salzman et al. 1998).

The aim of this study was to provide the first integrated view of the fungal and plant molecular events during the early process of infection of grape berries by *B. cinerea*. Taking advantage of the availability of the complete genome sequences of *B. cinerea* and *V. vinifera* (Amselem et al. 2011; Jaillon et al. 2007; Velasco et al. 2007), we analyzed the transcriptomes of both organisms during the early stages of VB and MB infection. Together with microscopic observations and quantification of defense metabolites, this wide genomic approach was used to answer several questions. What are the molecular mechanisms deployed by *B. cinerea* for successful infection on MB? Are they also deployed on berries at the veraison stage? What might be the defense mechanisms used by grapevine berries to block the *B. cinerea* infection process at the veraison stage?

RESULTS

Botrytis cinerea successfully infects mature berries but not berries at the veraison stage.

Observations in vineyards and in vitro experiments suggest that VB are not susceptible to *B. cinerea* infection, whereas MB are highly susceptible (Deytieu-Belleau et al. 2009; Elmer and Michailides 2007; Kretschmer et al. 2007). We verified this difference on *Vitis vinifera* cv. Marselan by performing infection time series experiments on VB and MB harvested from the

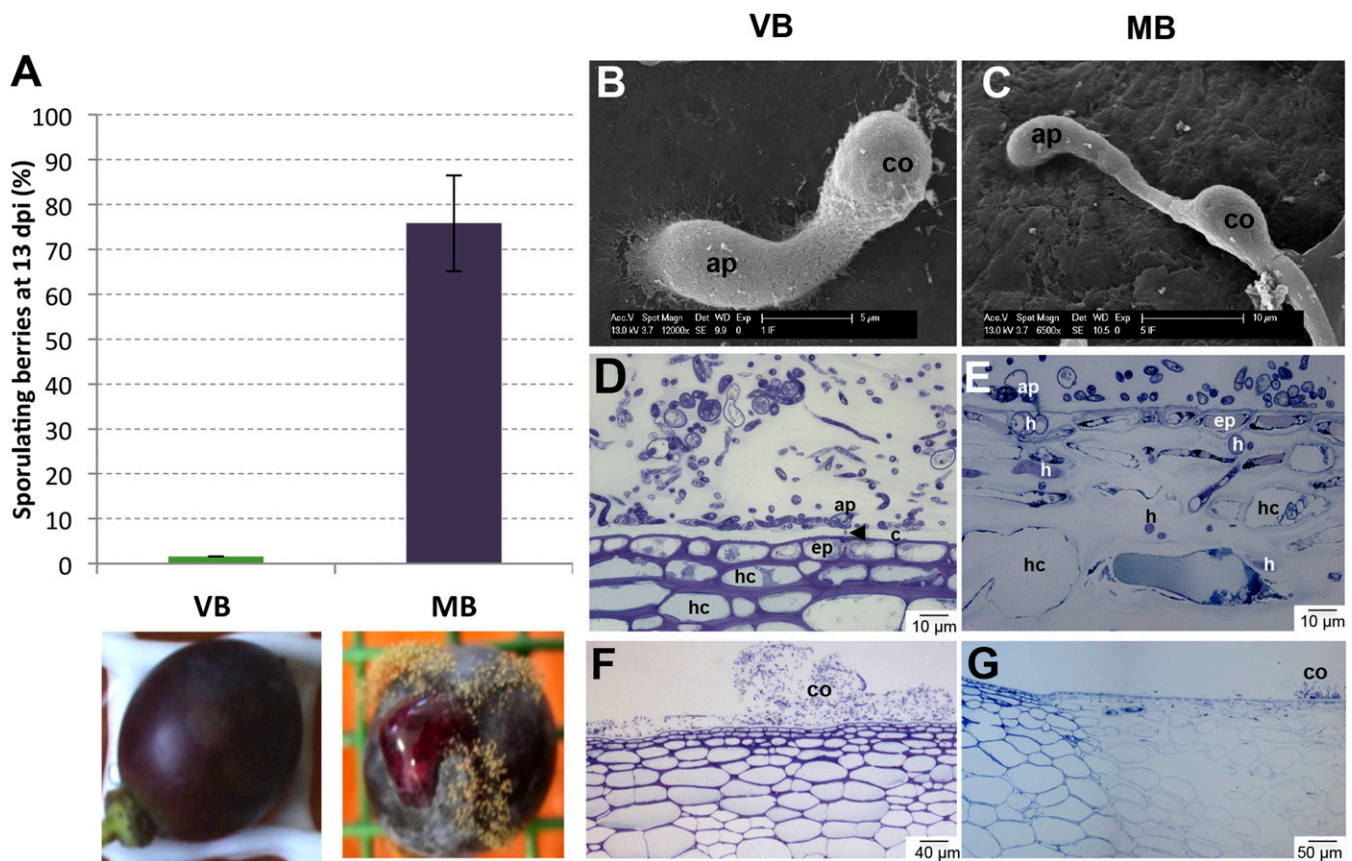


Fig. 1. Infection of grape berries by *Botrytis cinerea*. Berries at the veraison stage (VB) or mature berries (MB) were collected from the vineyards and inoculated with conidia of *B. cinerea*. **A**, Inoculation success as the percentage of berries with observed conidiophores at 13 days postinoculation and examples of infected VB (left) and MB (right). **B** and **C**, Scanning electronic micrographs of conidia (co) germination and appressoria (ap) formation on VB and MB at 24 h postinoculation (hpi). **D** to **G**, Micrographs of toluidine blue-stained vertical thin sections of inoculated areas in VB (**D** and **F**) and MB (**E** and **G**) at 48 hpi. The decoloration in **E** and **G** around the site of inoculation site is due to host cell degradation. c = cuticle, ep = epidermal cell, hc = hypodermal cell, and h = fungal hyphae. All micrographs in **B** through **G** are representative views of 15 different observation zones.

Bordeaux vineyards. The berries were thoroughly washed and were inoculated with conidia of the B05.10 *B. cinerea* strain and the infection process was followed at macro- and microscopic levels. Primary lesions were observed in MB between 48 to 72 h postinoculation (hpi). Spreading lesions were then observed between 72 and 96 hpi, with most of the MB bursting open at that point. Conidiophores became macroscopically visible between 96 and 120 hpi and sporulation was observed on 76 ± 11% of the MB at 13 days postinoculation (dpi) (Fig. 1A). In contrast, lesions were not observed on the VB over the 13-day observation period and fewer than 2% of the VB showed macroscopically visible sporulation at 13 dpi. Microscopic observations showed that conidia germination led to formation of unicellular appressoria between 16 and 24 hpi on both kinds of berries (Fig. 1B and C). Some multicellular appressoria, also called infection cushions, were also observed at 24 hpi and later (data not shown). Although conidia germination and appressoria formation proceeded similarly on the surface of VB and MB, clear differences were observed within and below the berry cuticle at the site of inoculation at 48 hpi. On VB, fungal penetration pegs passed through only up to two thirds of the cuticle and no mycelium colonization was detected under the cuticle (Fig. 1D). *B. cinerea* did not penetrate in the first epidermal cell layer and all the lower cell layers of the berry looked healthy (Fig. 1F). In contrast, inoculation of *B. cinerea* on MB resulted in appressorium penetration through the cuticle and mycelium colonization between the cuticle and the epidermal outer wall and, finally, between the different deeper cell layers (Fig. 1E). In the MB, the areas with successful invasion were lightly stained with toluidine blue, suggesting cell degradation by the necrotroph (Fig. 1G).

All together, these observations suggest that *B. cinerea* initiated a similar infection process both on MB and VB but failed to further colonize the berries at veraison stage. The infection and defense processes involved in the host-pathogen early stages of infection were further investigated by parallel transcriptomic analyses.

***B. cinerea* genes encoding virulence factors are upregulated during infection of both VB and MB.**

The transcriptome of *B. cinerea* at 16, 24, and 48 hpi after inoculation of MB was investigated using NimbleGen whole-genome oligonucleotide arrays (Amselem et al. 2011) and was compared with mycelium grown in vitro. Principal component analysis of the data indicated that the three in planta conditions are close to each other while the in vitro mycelium control condition (M) is clearly apart (Supplementary Fig. S1). Differential expression analysis performed using a gene analysis of variance (ANOVA)-based method (Simon and Biot 2010) revealed 3,001 differentially expressed (DE) genes (P value < 0.05, fold change > 2) whose expression patterns could be clustered into four main profiles (Fig. 2A; Supplementary Table S1). Functional enrichment analyses with defined sets of *B. cinerea* annotated genes (Amselem et al. 2011; Espino et al. 2010; Schumacher et al. 2014) were performed to reveal biological processes that are statistically overrepresented among each cluster (Supplementary Table S2).

Cluster 1 includes 1,344 genes that are upregulated at the three infection time points when compared with the in vitro growth, suggesting that they are involved in the infection process from the early stage of the time course (Fig. 2A). This first wave of in planta-upregulated genes is significantly enriched in genes encoding secreted proteases, such as the characterized aspartic proteases BcAP5, BcAP8, BcAP9, and BcAP14 (ten Have et al. 2010). There is also an enrichment in the genes encoding carbohydrate-active enzymes (Cantarel et al. 2009) corresponding to plant cell wall-degrading enzymes (CWDE) such as the endopolygalacturonase BcPG2 (BofuT4_P089000.1)

(Kars et al. 2005a) and the pectin methyl esterase BcPME2 (BofuT4_P021040) (Kars et al. 2005b). In total, 50 proteins in cluster 1, including proteases and CWDE, were previously identified in the secretome of *B. cinerea* (Espino et al. 2010). Genes in cluster 1 also suggest the activation of enzymes involved in the generation and detoxification of ROS. Notably, several genes encode ROS scavengers that protect the fungus against the oxidative burst, i.e., the superoxide dismutase BcSOD1 (BofuT4_P021610.1) (Rolke et al. 2004) and several peroxidases and enzymes belonging to the glutathione system (Heller and Tudzynski 2011). Lastly, genes involved in primary metabolism and respiration, such as mitochondrial carrier-encoding genes, are enriched to a lesser extent.

Cluster 2 includes 544 genes that are progressively upregulated between 24 and 48 hpi (Fig. 2A). This second wave of gene expression is mainly enriched in those encoding membrane transporters, both of the ATP-binding cassette (ABC) and the major facilitator family (MFS) types, including some predicted to allow sugar efflux. An enrichment was also observed for genes encoding proteases and ROS scavengers, such as BcGST1 (BofuT4_P103600.1) (Prins et al. 2000).

Cluster 3 includes 80 genes that are downregulated at 16 hpi but whose expression increases at 48 hpi. Finally, cluster 4 comprises 1,033 genes that are downregulated on MB (compared with the in vitro growth control), suggesting that these genes are involved in biological processes that are more important during saprophytic development than during infection. No functional category was significantly enriched in these two last clusters.

Gene-enrichment analysis, therefore, suggested that *B. cinerea* requires CWDE, proteases, ROS producers and scavengers, and membrane transporters to perform grape berry infection. In addition, genes encoding other known or suspected virulence factors were identified among the genes upregulated in planta. Notably, secondary metabolism gene clusters responsible for the biosynthesis of the botcinic acid and botrydial phytotoxins (*BcBoa* and *BcBot* genes, respectively) were identified. The *BcBoa6* gene encoding a polyketide synthase (PKS) acting as the key enzyme for botcinic acid synthesis and five other colocalized genes (*BcBoa3*, *BcBoa5*, *BcBoa11*, *BcBoa14*, and *BcBoa17*) (Dalmais et al. 2011) belong to cluster 2. The *BcBot1* gene that encodes a P450 monooxygenase essential for botrydial production (Siewers et al. 2005) and the *BcBot5* colocalized gene belong to cluster 3. Genes involved in abscisic acid (ABA) synthesis (Siewers et al. 2006) are part of clusters 2 (*BcAba1* and *BcAba4*) and 3 (*BcAba2*). Additionally, the *BcPks8* gene, encoding a PKS (Kroken et al. 2003), is significantly overexpressed from 16 hpi (cluster 1), as is the colocalized ABC transporter gene (BofuT4_P118990.1) that likely participates in the export of the polyketide. In addition, the data suggested that proteins with phytotoxic activities are produced during MB infection. The gene encoding the necrosis-and-ethylene-inducing proteins BcNEP1 (BofuT4_P070460.1) and BcNEP2 (BofuT4_P066710.1) (Schouten et al. 2008) and the ceratoplatenin family protein BcSPL2 (BofuT4_P000440.1) (Frías et al. 2011) are upregulated during infection (clusters 1 and 2).

The transcriptome of *B. cinerea* on VB at 16, 24, and 48 hpi could not be investigated using microarrays, due to the limited amount of fungal RNA compared with the plant RNA (below 20% of the total RNA extracted from infected tissues [data not shown]). To overcome this technical bottleneck, a quantitative polymerase chain reaction (qPCR) approach was used. We selected six genes encoding known virulence factors (*BcPgs2*, *BcBoa6*, *BcSod1*) or proteins expected to contribute to the infection program (*BcPme2*, *BcNep2*, *BcGst1*) among the genes identified as upregulated during the infection of MB (clusters 1 and 2) and investigated their expression on VB and MB that were harvested independently from those used for

microarrays (i.e., another year). qPCR confirmed that all six genes were upregulated on MB (Fig. 2B). Moreover, these genes were also shown to be upregulated at 16 and 24 hpi on VB. At 48 hpi, significant differences in the expression pattern appeared between VB and MB (except for *BcPg2*) that could be linked to the fact that the *B. cinerea* infection process is stopped in VB. Genes encoding the ROS scavengers BcSOD1 and BcGST1 are significantly overexpressed at 24 and 48 hpi on VB compared with MB, suggesting that the fungus encounters a stronger oxidative burst on VB.

In order to test whether the *B. cinerea* upregulated genes are essential for the infection process on grape berries, several corresponding knock-out (KO) mutants were inoculated on MB, and the disease progression was compared with the wild-

type (WT) strain. Conidial suspensions of the metabolite-deficient mutants *bcbot2Δ* (Pinedo et al. 2008), *bcboa6Δ*, *bcbot2Δ/bcboa6Δ* (Dalmais et al. 2011), *bcaba1Δ* (Siewers et al. 2004), and *bcpks8Δ* were inoculated on MB and the rate of 'berry bursting' due to tissue maceration was measured daily (Supplementary Table S3). The simple KO mutants were as virulent as the WT strain, while the *bcbot2Δ/bcboa6Δ* double mutant was partly impaired in its ability to induce burst of berries and to colonize them, as previously observed on other host tissues (Dalmais et al. 2011). Additionally, the *bcsod1Δ* mutant, which was previously shown to be impaired in the colonization of bean leaves (Rolke et al. 2004), was slightly reduced in infection on MB. In conclusion, although these genes are upregulated during the infection, the characterization of the

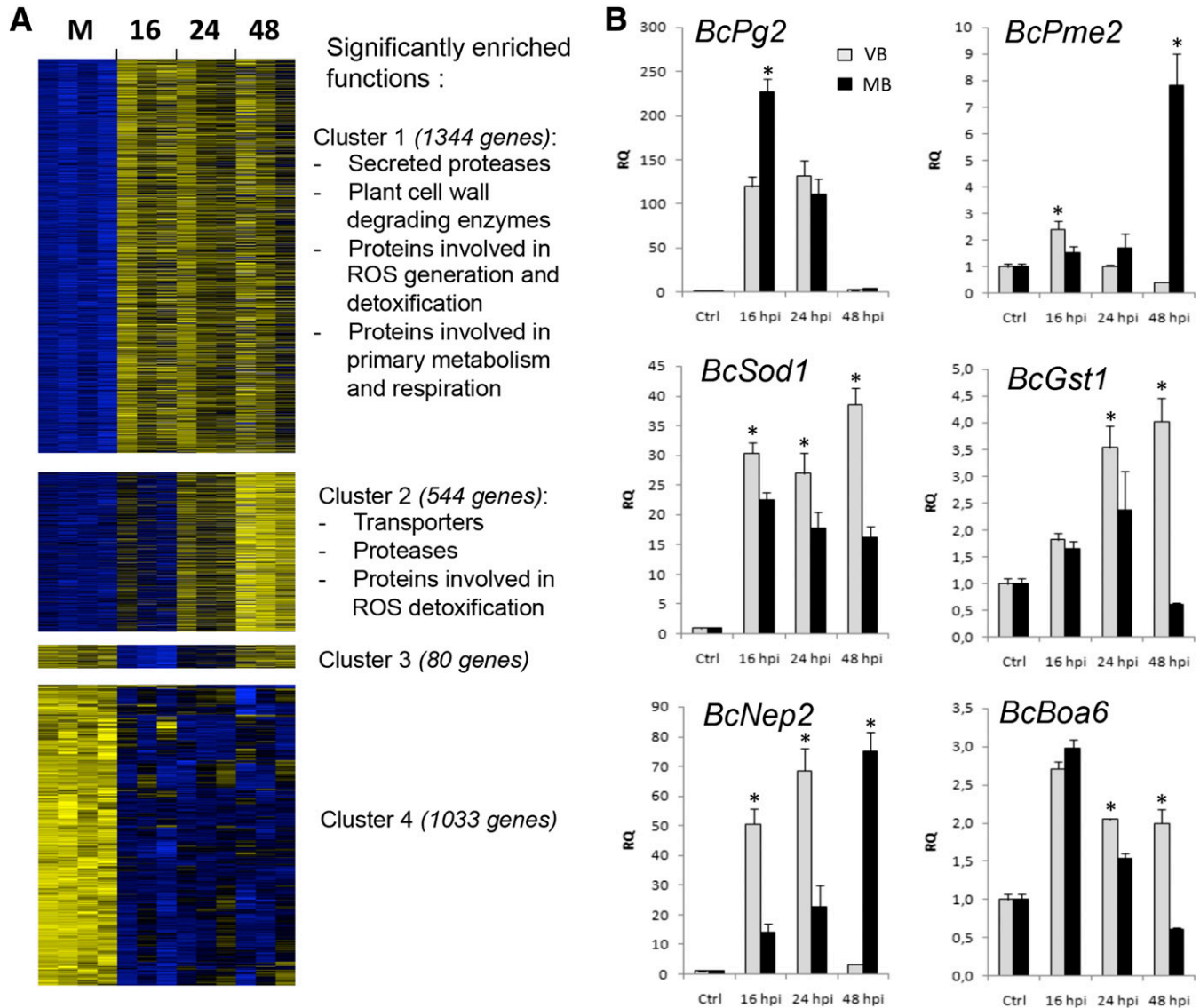


Fig. 2. Expression profiling of differentially expressed (DE) *Botrytis cinerea* genes during the early infection of mature grape berries (MB). **A**, Heatmap of the 3,001 DE genes ($P < 0.05$, fold-change > 2) during infection of MB by comparing NimbleGen arrays data at 16, 24, and 48 h postinoculation (hpi) and in vitro mycelium (M). Three biological experiments were realized per in planta condition and four repetitions were realized for the in vitro mycelium. Relative expressions (i.e., log₂-normalized intensities scaled by gene) of the genes in the four conditions were clustered and depicted by color scale, where shades of blue represent underexpressed genes and shades of yellow represent overexpressed genes. Enrichments among clusters were calculated using the lists of functionally annotated genes described by Amselem et al. (2011) and Schumacher et al. (2014) and a standard Fisher test. Only the lists with $P < 0.05$ and an odds ratio > 2 were considered as significantly overrepresented. **B**, Expression of six virulence-related genes in the veraison stage (VB) or MB. Gene expression levels were determined by quantitative polymerase chain reaction following the $2^{-\Delta\Delta CT}$ method, using the constitutively expressed actin gene *BcAct1* as a reference. RQ is the relative quantity calculated as gene expression at 16, 24, and 48 hpi, compared with in vitro mycelium (Ctrl). The error bar is the standard error of the mean of three biological replicates. Asterisks (*) indicate statistically significant difference between infected MB and VB samples ($P < 0.05$), using unpaired heteroscedastic Student's *t* test. Light gray and black columns represent VB and MB, respectively.

corresponding KO mutants did not reveal a major role in infection of grape berries.

VB and MB differ in their transcriptome reprogramming during *B. cinerea* infection.

Grapevine Nimblegen arrays were used both to investigate the transcriptome of infected MB (simultaneously to *B. cinerea* transcriptome described above) and to compare it with the transcriptome of infected VB. When compared with the basal expression level (0 hpi), the numbers of *V. vinifera* genes significantly deregulated (induced or repressed) by the infection were higher in MB than in VB (P value < 0.05 , fold change > 2) (Fig. 3A). A total of 3,025 DE genes were found in MB, whereas only 159 DE genes were found in VB (Fig. 3B). About 90% of the DE genes were found at both 24 and 48 hpi in VB, whereas a large increase in DE genes was observed from 24 to 48 hpi in MB. Only nine DE genes were found to be commonly regulated in both the inoculated VB and MB at both 24 and 48 hpi (Fig. 3B; Supplementary Table S4). Five of them were predicted to encode very similar germin-like proteins (GLPs) classified as VvGLP4 and VvGLP4-like according to Godfrey et al. (2007). Furthermore, 23 common genes showed a delayed induction in MB as they were found to be significantly

deregulated in VB already at 24 hpi but at 48 hpi in MB (Fig. 3B). Notable genes in that group were another four VvGLP4-like genes, VvGLP3, five terpene synthase-encoding genes, and two genes encoding nitrilase and nitrile hydratases putatively involved in indole 3-acetic acid synthesis. The VvGLP transcripts were among the most highly induced genes in the inoculated VB skin tissue at 24 and 48 hpi (Fig. 3C). Interestingly, the SA-marker gene VvPR1 was the most induced in inoculated VB data, whereas transcripts of different enzymes of the JA biosynthesis (phospholipase, lipase, allene oxide synthase, jasmonate *O*-methyltransferase) were induced in MB data.

Hierarchical clustering of the DE genes among all conditions resulted in 15 clusters that were subsequently put through gene ontology (GO) analysis (Fig. 3C). The DE genes upregulated strongly in infected VB grouped into clusters 3, 4, 5, and 7. Cluster 3 is enriched in genes encoding extracellular proteins (21 in total). Twelve of them encode GLPs, including the ones mentioned above and five encode PR proteins (two PR1s, a PR3 endochitinase, and a PR5 thaumatin-like). Cluster 5 is also enriched in genes encoding extracellular proteins (aspartic proteinase and polygalacturonase inhibitor) and receptor kinases (a cysteine-rich receptor like kinase, a leucine-rich

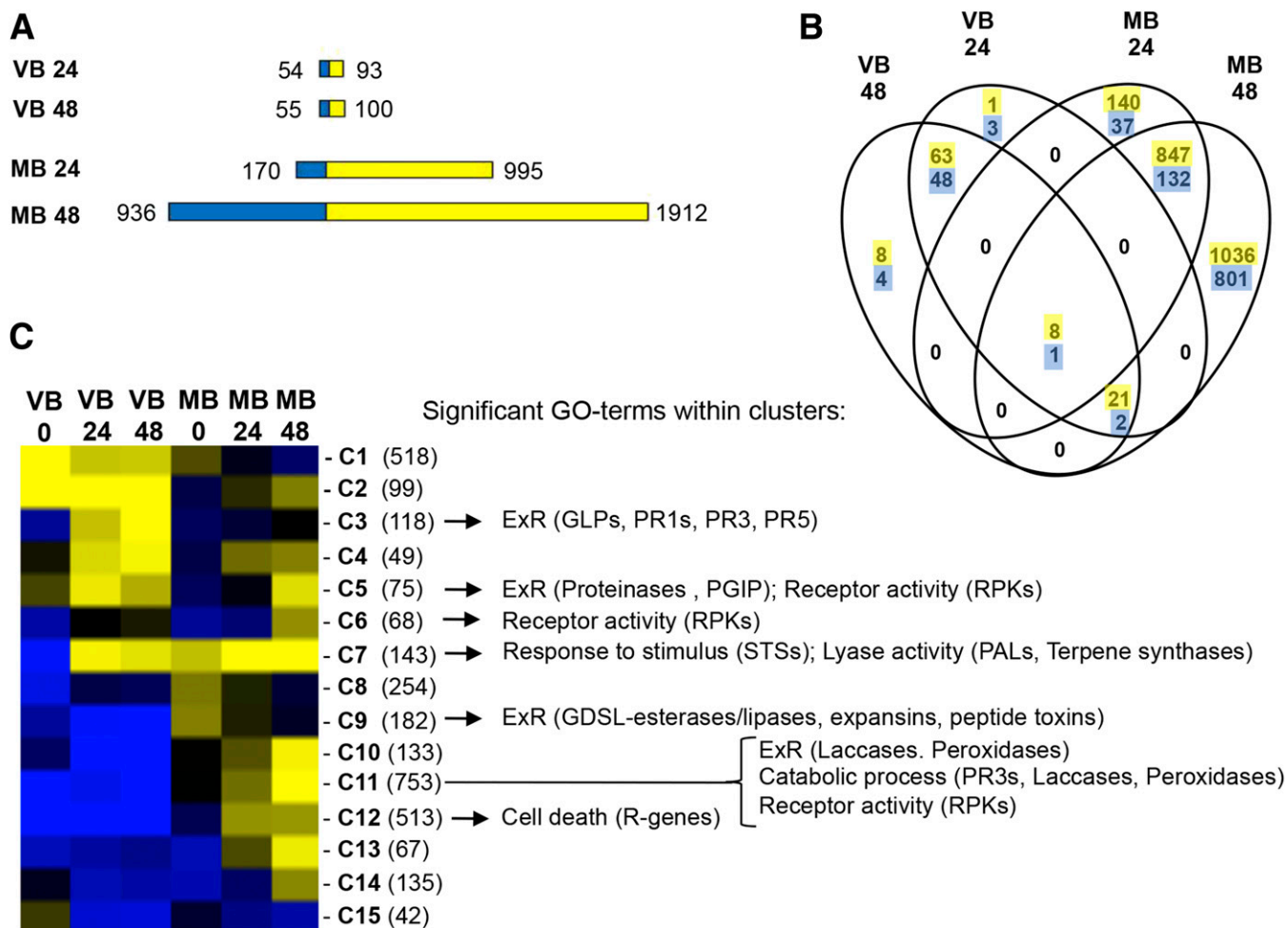


Fig. 3. Analysis of the transcriptomic data of veraison-stage berries (VB) and mature grape berries (MB) infected by *Botrytis cinerea*. **A**, Numbers of differentially expressed (DE) genes ($P < 0.05$, fold-change > 2) either upregulated (yellow) or downregulated (blue) during infection. **B**, Venn diagram showing the number of DE genes unique to or common within the four conditions. The top and bottom numbers correspond to the up- and downregulated genes, respectively. **C**, Hierarchical clustering of the DE genes using log₂-transformed averages of the normalized fluorescence intensities of the three biological replicates per modality. The number of genes in each cluster (C1 to C15) is in parentheses. ExR = extracellular region, GLP = germin-like protein, PR = pathogenesis-related, PGIP = polygalacturonidase-inhibiting protein, RPK = receptor-like protein kinase, STS = stilbene synthase, PAL = phenylalanine ammonia lyase, and R = resistance.

repeat [LRR]-receptor-like S/T protein kinase, a putative receptor kinase). Cluster 7 groups genes highly induced during *B. cinerea* infection not only in VB but also in MB. It is enriched in genes encoding proteins belonging to the ‘response to stimulus’ GO group, such as putative STS (29 copies), and to the ‘lyase activity’ GO group, such as putative PAL (seven copies) and terpene synthases (five copies). The DE genes downregulated in VB were found in clusters 1, 9, 10, 14, and 15. Among them, only cluster 9 is enriched in genes encoding extracellular proteins, such as GDSL esterase/lipases and expansins.

Clusters 6, 10, 11, 12, 13, and 14 contain DE genes that are upregulated in infected MB but show little expression in inoculated VB. Clusters 6 and 11 are enriched in genes belonging to the ‘receptor activity’ GO group, with many of them encoding cysteine-rich receptor-like kinases. Cluster 11 is also enriched in genes encoding extracellular proteins and proteins with oxidoreductase activity, including laccase-like proteins (30), peroxidases (11), and cytokinin dehydrogenases (five). Lastly, cluster 12 is enriched in genes associated with cell death. A total of 44 such genes were found and all of them were putative R protein–encoding genes with toll interleukin 1 receptor-coiled coil nucleotide binding site LRR-domains, many of them resembling either *RPP13*, which confers downy mildew resistance in *A. thaliana* or *N* of *Nicotiana glutinosa*, granting resistance to *Tobacco mosaic virus*.

In conclusion, the whole transcriptome analysis of infected VB versus infected MB suggested that different defense mechanisms

could be involved. These differences were further investigated by qPCR and by quantification of the corresponding metabolites.

ROS are rapidly produced in inoculated VB.

In the *Botrytis*-induced grapevine transcriptomes, genes encoding H₂O₂-producing VvGLPs (Godfrey et al. 2007) were expressed both earlier and more strongly in VB than in MB. Subsequent qPCR on independent berry samples infected by *B. cinerea* indicated that genes encoding the putative superoxide generating NADPH-oxidase VvRbohD (Dubreuil-Maurizi et al. 2010; Gauthier et al. 2014) and VvGLP3 are induced on VB at 24 and 48 hpi, respectively (Fig. 4A). To confirm the suspected increased amounts of H₂O₂ in infected VB, 3,3-diaminobenzidine (DAB) staining of berry peel samples were performed at 72 hpi (Fig. 4B through G). Numerous spots of reddish-brown DAB precipitates were observed in inoculated areas of VB (Fig. 4B). Furthermore, colocalization of DAB-stained single epidermal cells and pathogen appressoria were observed (Fig. 4D and F). In comparison, no evidence of H₂O₂ could be observed on MB, in which *B. cinerea* had developed more multicellular appressoria (Fig. 4C, E, and G).

The SA/JA hormonal balance differs between *Botrytis*-infected VB and MB.

Microarray results suggested that several JA marker genes were upregulated in the inoculated MB, whereas SA marker genes were upregulated in VB only. Transcript expression

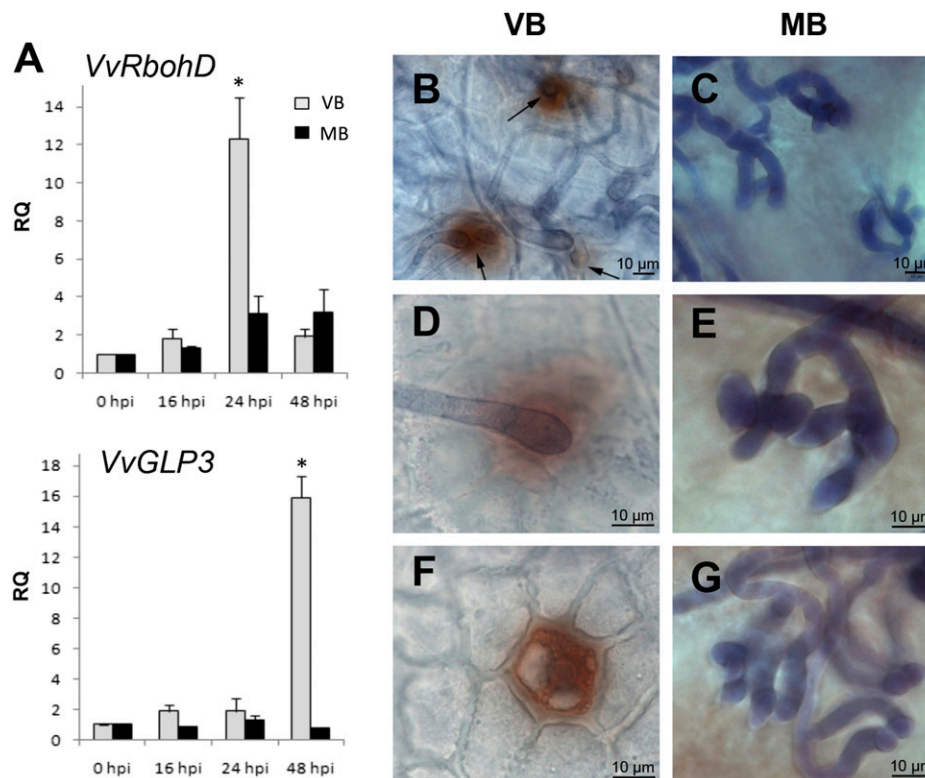


Fig. 4. Reactive oxygen species in *Botrytis cinerea*-infected veraison stage berries (VB) and mature grape berries (MB). **A**, Expression of genes encoding the NADPH oxidase VvRbohD that produces O₂⁻ and the germin-like protein VvGLP3 that is a superoxide dismutase converting O₂⁻ into H₂O₂. Gene expression levels were determined by quantitative polymerase chain reaction following the 2^{-ΔΔCT} method, using the constitutively expressed gene *VvATP16* as a reference. RQ is the relative quantity calculated as gene expression of inoculated samples compared with mock-inoculated samples at the same time point and compared with time 0, set as 1. The error bar is the standard error of the mean of three biological replicates. Asterisks (*) indicate statistically significant differences between infected MB and VB samples ($P < 0.05$), using unpaired heteroscedastic Student's *t* test. Light gray and black columns represent VB and MB, respectively. **B to G**, 3,3-Diaminobenzidine staining (brown precipitate) to detect H₂O₂ production on inoculated berries at 72 h postinoculation (hpi). On VB (**B**, **D**, and **F**), many appressoria (arrows) coincided with brown-stained single epidermal cells directly under them (**F** is a deeper optical section of **D**), in contrast to MB samples (**C**, **E**, and **G**), in which this colocalization was never observed.

measured by qPCR confirmed the specific induction of the SA marker gene *VvPR1* in VB, whereas the JA marker gene *VvJAZ1* was specifically induced in MB (Fig. 5A). To confirm these results, the amounts of free SA and JA-isoleucine (JA-Ile) were quantified by liquid chromatography-mass spectrometry (LC-MS) in VB and MB peel samples during *B. cinerea* infection. Although the SA level in VB was not significantly induced by infection, its basal level (at 0 hpi) was around 10-fold higher than in MB (Fig. 5B). Regarding JA-Ile, its initial amount in MB was approximately 10-fold higher than in VB and it significantly increased after *B. cinerea* inoculation.

Production of resveratrol is highly induced in infected VB.

Microarray data also suggested that two defense-related parts of the plant phenylpropanoid pathway, the ones leading to stilbenes (resveratrol and its derivatives) and lignin production, were induced in inoculated VB. Using qPCR, we confirmed that *VvPAL* and *VvSTS* were strongly expressed in inoculated VB at 16 and 24 hpi (Fig. 6A). This expression pattern fitted with the quantification of resveratrol; its amount had quadrupled from the basal level to approximately 65 µg per gram of dry weight at 48 hpi in VB, while its amount decreased 2.5-fold during the same time period in MB (Fig. 6B).

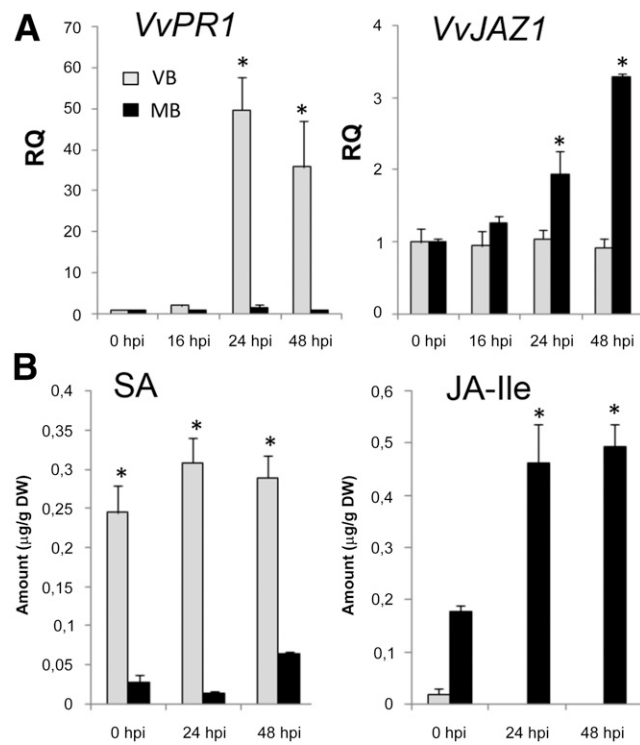


Fig. 5. Salicylic acid (SA)- and jasmonic acid (JA)-dependent pathways in *Botrytis cinerea*-infected veraison stage berries (VB) and mature grape berries (MB). **A**, Expression of the SA marker gene *VvPR1* and the JA marker gene *VvJAZ1* encoding the jasmonate ZIM-domain protein 1. Gene expression levels were determined by quantitative polymerase chain reaction following the $2^{-\Delta\Delta CT}$ method, using the constitutively expressed gene *VvATP16* as a reference. RQ is the relative quantity calculated as transcript amount in inoculated samples compared with mock-inoculated samples at the same time point and compared with time 0, set as 1. The error bar is the standard error of the mean of three biological replicates. Light gray and black columns represent VB and MB, respectively. **B**, Amounts of SA and JA-isoleucine quantified by liquid chromatography-mass spectrometry. The error bar is the standard error of the mean of three biological replicates. Asterisks (*) indicate statistically significant difference between infected MB and VB samples ($P < 0.05$), using unpaired heteroscedastic Student's *t* test.

VB reinforce cell walls after contact with *B. cinerea*.

The penetration of the *B. cinerea* infectious hyphae through the cuticle of VB was blocked by 48 hpi (Fig. 1F), suggesting that secondary cell-wall strengthening might have been activated in the plant. In order to test this hypothesis, two grapevine genes encoding putative enzymes of the monolignol pathway were tested by qPCR: i) the *p*-coumarate 3-hydroxylase (*VvC3H*) and ii) the cinnamoyl CoA reductase (*VvCCR*). Transcripts of *VvCCR* and *VvC3H* were specifically upregulated in response to *B. cinerea* on VB while they were negatively regulated in inoculated MB (Fig. 7A). The expression of these lignin-associated genes positively correlated with amounts of the corresponding metabolites (caffeic acid, ferulic acid, and chlorogenic acid) that specifically accumulated in VB (Fig. 7B).

In tomato and bean, plant cell-wall strengthening can also result from increased cross-linking of pre-existing extensins in the cell wall in response to fungal elicitors (Bradley et al. 1992; Brady and Fry 1997). An orthology-based search led to the identification of a grapevine gene encoding a proline-rich extensin-like protein (*VvEXT*), and both the microarray data and qPCR experiments from independent biological experiments showed that this gene was highly upregulated in the VB (Fig. 7A). All together, the transcript and metabolite quantifications indicate that specific cell-wall reinforcement is activated in VB

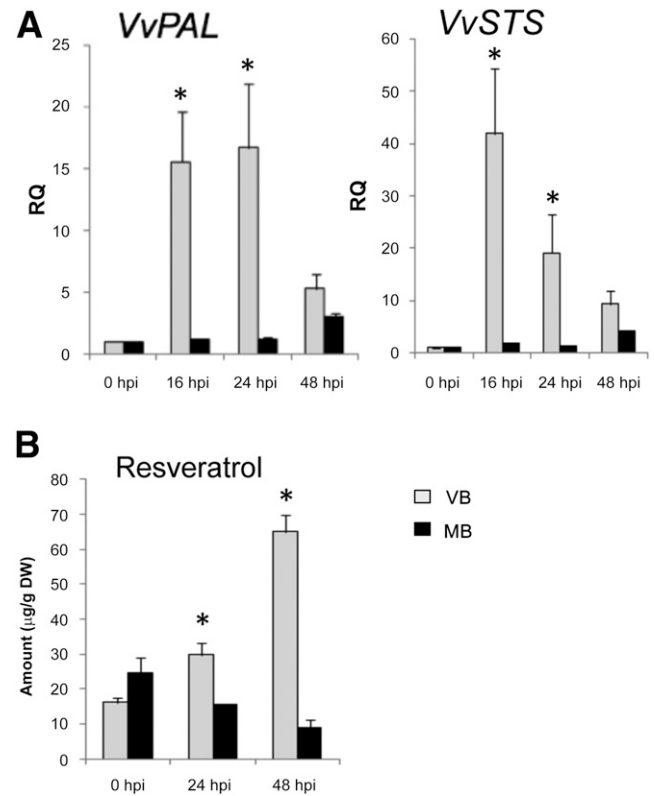


Fig. 6. Resveratrol-related pathway in *Botrytis cinerea*-infected veraison stage berries (VB) and mature grape berries (MB). **A**, Expression of the phenylalanine lyase (*VvPAL*) and the stilbene synthase (*VvSTS*) genes leading to the production of resveratrol. Gene expression levels were determined by quantitative polymerase chain reaction following the $2^{-\Delta\Delta CT}$ method, using the constitutively expressed gene *VvATP16* as a reference. RQ is the relative quantity calculated as gene expression of inoculated samples compared with mock-inoculated samples at the same time point and compared with time 0, set as 1. The error bar is the standard error of the mean of three biological replicates. Light gray and black columns represent VB and MB, respectively. **B**, Amounts of resveratrol quantified by liquid chromatography-mass spectrometry. Asterisks (*) indicate statistically significant differences between infected MB and VB samples ($P < 0.05$), using unpaired heteroscedastic Student's *t* test.

during *B. cinerea* infection. Microscopic analysis further indicated that papillae (thickenings of the cell wall) were formed beneath the appressoria in VB (Fig. 7C). In comparison, no papillae could be detected during early fungal infection on MB (Fig. 7D).

DISCUSSION

Despite the important economic impact of gray mold disease on grapevine, the details of infection of *B. cinerea* on this host have remained largely unknown. Our study provides the first overview of the *B. cinerea* transcriptome during the early stages of infection, i.e., penetration of the cuticle and necrosis of the first plant cells of mature berries (MB). When compared with the transcriptome of in vitro mycelium, 1,888 *B. cinerea* genes were overexpressed during penetration and early colonization of MB (clusters 1 and 2) and 80 genes were first downregulated and then upregulated (cluster 3). Functional annotation of these upregulated genes confirmed that plant CWDE, proteases, ROS

producers and scavengers, phytotoxic proteins, and secondary metabolites, such as botrydial and botcinic acid, are significant components of the *B. cinerea* infection machinery. From the previously characterized virulence factors, we investigated the botrydial and botcinic acid biosynthesis enzymes (Dalmais et al. 2011) and the superoxide dismutase SOD1 (Rolke et al. 2004). Also, a new putative virulence factor, i.e., PKS8, was studied. However, none of the corresponding KO mutants (*bcbot2Δ*, *bcboa6Δ*, *bcaba1Δ*, *bcsod1Δ*, and *bcpsk8Δ*) showed a significant virulence defect on the harvested MB. Only the *bcbot2Δ/bcboa6Δ* double mutant was partly impaired, confirming the redundant roles of the phytotoxins (Dalmais et al. 2011). Redundancy between virulence factors may be the reason for the lack of a significant decrease of virulence of the other single KO mutants. Although the pathogenicity test we used indicated that none of the tested genes were essential for grape berry infection, these genes may contribute to the infection process. *B. cinerea* genes upregulated during the infection of sunflower leaves (48 hpi) were also enriched in those encoding

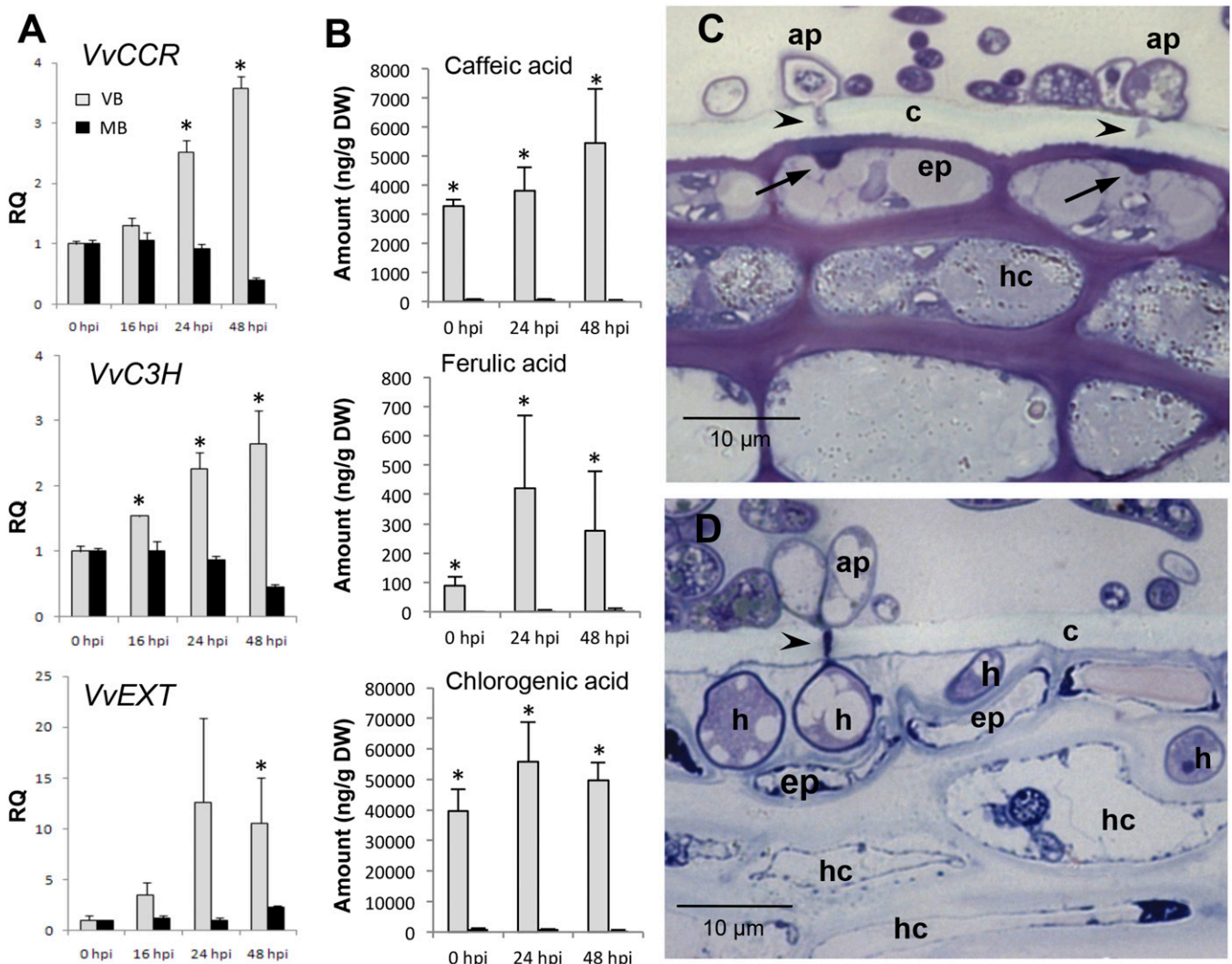


Fig. 7. Cell-wall reinforcement in *Botrytis cinerea* infected-veraison stage berries (VB) and mature grape berries (MB). **A**, Expression of genes encoding the cinnamoyl-CoA reductase (*VvCCR*) and *p*-coumarate 3-hydroxylase (*VvC3H*) that are involved in lignin biosynthesis and the extensin-like protein (*VvEXT*). Gene expression levels were determined by quantitative polymerase chain reaction following the $2^{-\Delta\Delta CT}$ method, using the constitutively expressed gene *VvATP16* as a reference. RQ is the relative quantity calculated as gene expression of inoculated samples compared with mock-inoculated samples at the same time point and compared with time 0, set as 1. Light gray and black columns represent VB and MB, respectively. **B**, Amounts of caffeic, ferulic, and chlorogenic acid quantified by liquid chromatography-mass spectrometry. The error bar is the standard error of the mean of three biological replicates. Asterisks (*) indicate statistically significant differences between infected MB and VB samples ($P < 0.05$), using unpaired heteroscedastic Student's *t* test. **C** and **D**, Penetration pegs (arrowheads) from appressoria were stopped by papillae formation (arrows) in VB (**C**) but not in MB (**D**). ap = appressorium, c = cuticle, ep = epidermis, hc = hypodermal cell, and h = hyphae.

CWDE and sugar-type MFS transporters (Amselem et al. 2011), suggesting that, in both interactions, a metabolism shift occurs to degrade and feed on the host tissues. Other transcriptomic studies investigating the interaction between *B. cinerea* and some of its host plants were not designed to compare in vitro versus in planta fungal gene expression. Nevertheless, comparison of the lists of the 200 most highly expressed *B. cinerea* genes in the array-based transcriptome of infected bean leaves (*Phaseolus vulgaris*, 48 hpi) (Schumacher et al. 2012), and the RNAseq-based transcriptome analysis of infected lettuce (*Lactuca sativa*, 12, 24, and 48 hpi) (De Cremer et al. 2013) and infected tomato leaves (*Solanum lycopersicoides*, 24 and 48 hpi) (Smith et al. 2014), allowed us to identify a number of genes that were also highlighted in our study (i.e., those encoding the aspartic protease AP8, SOD1, and the secondary metabolism gene clusters responsible for botrydial and botcinic acid synthesis). Finally, a study published by Blanco-Ulate et al. (2014) demonstrated that *B. cinerea* expresses a common set of CWDE when infecting tomato, grapevine fruits, or lettuce but may also attack more unusual host wall polysaccharide substrates with specific enzymes, depending on the host tissue. All together, these results and ours suggest that some common proteolytic, oxidative, and phytotoxic activities of *B. cinerea* are similarly induced on different hosts, while other virulence-related functions would be crucial only in some hosts. Recent examples of the latter are the catabolism of D-galacturonic acid (Zhang and van Kan 2013) and arabinan (Nafisi et al. 2014).

One of the main objectives of this study was to investigate why the grape berries at the veraison stage (VB) are highly resistant to *B. cinerea*, while the MB are highly susceptible. Both our microscopic observations and expression analyses of a set of six fungal genes related to virulence, suggested that *B. cinerea* initiated its infection program both on VB and on MB. Germinated conidia formed appressoria, and the expression of the *BcPg2*, *BcPme2*, *BcNep2*, *BcSod1*, *BcGst1*, and *BcBoa6* genes was induced at 16 and 24 hpi. Our data also demonstrated that further infection in VB is halted by cell-wall reinforcement and formation of papillae just underneath the appressoria. Gene expression, microscopic, and metabolite analyses indicated that this defense process is based on localized H₂O₂ production and lignin synthesis. In grapevine, the production of O₂^{•-} could be explained by the upregulation of the *VvRbohD* gene, while its conversion into H₂O₂ could rely on the upregulation of the *VvGlp3* gene (Godfrey et al. 2007). Genetic evidence for Rboh function in the pathogen-induced oxidative burst has been provided by knocking down the *Rboh* gene in different plant species. Double mutants of *Arabidopsis AtrbohD* and *AtrbohF* genes show greatly reduced ROS production in response to infection by avirulent *Pseudomonas syringae* pv. *tomato* DC3000 and *Hyaloperonospora parasitica* (Torres et al. 2002). Similarly, *NbRboh*-silenced *Nicotiana benthamiana* plants show a reduced oxidative burst and disease resistance to *Phytophthora infestans* (Yoshioka et al. 2003). The production of H₂O₂ and lignin at the site of *B. cinerea* inoculation was also observed in green tomatoes, which are resistant to gray mold (Cantu et al. 2009). Moreover, the tomato *sitiens* mutant, which is highly resistant to *B. cinerea*, shows a primed H₂O₂ accumulation, *PR1* overexpression, and cell-wall reinforcement in the epidermal cells (Asselbergh et al. 2007). In *Arabidopsis* leaves infected with *B. cinerea*, the microarray data analysis also revealed a clear induction of a cluster of genes involved in the lignin biosynthesis at 24 and 48 hpi (Ferrari et al. 2007). The fact that the *AtRbohD* mutant is unable to make any H₂O₂ or lignin deposition provides genetic evidence that lignin synthesis needs the RbohD-mediated H₂O₂ production (Denness et al. 2011). Our results indicate that the localized H₂O₂ production by SOD-like activities (*VvGLP3*) (Godfrey et al. 2007) is linked to lignification processes that block the penetration of the appressorium by papillae formation.

Grape berries infected with *B. cinerea* are known to activate the production of stilbenes (Jeandet et al. 1995), including the resveratrol phytoalexin that inhibits *B. cinerea* growth (Schouten et al. 2002; Zheng et al. 2011). Our results are consistent with those of Bavaresco et al. (1997) showing that infected VB but not MB activate the production of resveratrol. We also confirmed that the genes involved in the biosynthesis (*STS* and *PAL*) are upregulated in inoculated berries, as previously suggested by semiquantitative PCR (Kretschmer et al. 2007). In the same way, transgenic grapevine plantlets overexpressing the *STS* gene are more resistant to *B. cinerea* (Coutos-Thévenot et al. 2001). In *Arabidopsis*, the *PAD3* gene, encoding a cytochrome P450 involved in synthesis of the camalexin phytoalexin, was significantly induced during *B. cinerea* infection (Ferrari et al. 2007; Zhou et al. 1999). Camalexin induces programmed cell death in *B. cinerea* (Shlezinger et al. 2011) and, consequently, the *Arabidopsis pad3* mutant is more susceptible to *B. cinerea* (Galletti et al. 2008; Rowe et al. 2010). All together, these data suggest that phytoalexin production is a common plant defense pathway involved in resistance against this necrotrophic pathogen.

As suggested by the presented transcriptomic data and metabolite analysis, the SA pathway might also participate in basal resistance in VB. This observation contradicts the common dogma in *A. thaliana*, in which the SA- and the JA/ET- pathways are associated with defense against biotrophic and necrotrophic pathogens, respectively (Glazebrook 2005). If *Arabidopsis* mutants impaired in JA (*coi1*, *aos1*) and ET (*ein2*) are more susceptible to *B. cinerea*, it's interesting to note that transgenic plants overexpressing the bacterial *nahG* gene (which inhibits the accumulation of SA) are also more susceptible to *B. cinerea* (Ferrari et al. 2003, 2007; Rowe et al. 2010). Moreover, SA or its analog benzothiadiazole (BTH) induces resistance to *B. cinerea* in *Arabidopsis*, tomato, or sunflower (Audenaert et al. 2002; De Meyer et al. 1999; Dmitriev et al. 2003; Ferrari et al. 2003; Murphy et al. 2000). Last, the treatment of grapevine berries with BTH triggers protection against *B. cinerea* (Iriti et al. 2005), and the SA marker gene *VvPR1* was shown to be upregulated when grapevine leaves were treated with *B. cinerea* elicitors (Repka 2006). In the resistant VB, the SA-dependent marker gene *VvPR1* was significantly induced during *B. cinerea* infection, whereas *VvJAZ1*, which is a marker of the JA-dependent pathway, was not. Similarly the amount of JA-Ile was not significantly induced during *B. cinerea* infection on VB.

In *Arabidopsis*, tomato, or *N. benthamiana* plant leaves, a finely tuned cross-talk network of signals involving JA, SA, but also ABA seems to be important for resistance to *B. cinerea* (Asselbergh et al. 2007; Ferrari et al. 2003, 2007; Glazebrook 2005; Rowe et al. 2010). Quantification of the ABA amount by LC-MS showed a similar slight decrease during *B. cinerea* infection on VB and MB (Supplementary Fig. S2). Thus, on VB, only the activation of the SA-dependent pathway is correlated to resistance against *B. cinerea*.

In conclusion, the data obtained both on the fungal and the plant sides demonstrated that a true interaction occurs between VB and *B. cinerea* and that grapevine possesses efficient defense mechanisms to block gray mold disease at the phenologic VB stage. These efficient defense mechanisms involve localized H₂O₂ production, activation of SA-dependent defense genes, and genes involved in stilbene and lignin biosynthesis. Hence, there is an accumulation of caffeic, ferulic, and chlorogenic acids, allowing the formation of papillae, and of resveratrol. Together, these mechanical and chemical defenses block *B. cinerea* from invading the VB skin epidermal layer. Interestingly, while many defense mechanisms are activated upon *B. cinerea* inoculation on VB, our microarray data demonstrated that, in inoculated MB, a

very different transcriptome is activated. Part of the difference was caused by activation of the JA-dependent defense pathway. Despite this activation at 24 hpi, MB are highly susceptible to gray mold disease. It is well-known that ripening involves significant changes in the physiological and biochemical properties of grape berries (Dai et al. 2013) and therefore influences the course of infection by pathogens. The relation between fruit ripening and susceptibility to *B. cinerea* has been mainly investigated in tomato. Modifications of the tomato cell wall during ripening contribute to susceptibility to gray mold (Cantu et al. 2008). Transcriptomic data even suggested that *B. cinerea* would induce the expression of tomato genes involved in ripening in order to favor its development (Cantu et al. 2009). Our study seems to indicate that, in grapevine, ripening is associated with a drastic shift in defense mechanisms against gray mold from a SA-dependent pathway at VB to a JA-dependent one in MB.

MATERIALS AND METHODS

Grape berry inoculations.

All VB and MB used in this study belong to the cultivar Marselan and were collected from the same experimental INRA vineyard near Bordeaux between 2009 and 2013 (Supplementary Table S5). VB were sampled from clusters showing approximately 10% of the berries having changed color (beginning of veraison, stage 35, according to Eichorn and Lorenz [1977]). Visually healthy berry bunches were randomly collected from a total of 25 cultivation rows. Individual berries were washed for 45 min under running tap water supplemented with three drops per liter of Tween 80 every 5 min, followed by 15 min of washing under running deionized water.

The *B. cinerea* inoculum was produced by growing strain B05-10 (Quidde et al. 1998) on solid medium (1.5% malt extract, 0.5% glucose, 0.1% tryptone, 0.1% casein hydrolysate, 0.1% yeast extract, and 0.02% tRNA), first, for 5 days in the dark, followed by 5 days under black light (300 to 400 nm) to promote fungal sporulation (OSRAM L36-73; 10-h day and 14-h night).

For microscopic and transcriptomic analysis, the equatorial area of each berry was inoculated with three inoculum droplets spotted in a triangle, each of 10 μ l with 5,000 conidia, in potato dextrose broth (PDB) (1 g of potato starch per liter, 5 g of dextrose per liter; Duchefa Biochimie). The inoculated berries were kept in clear plastic boxes in 100% relative humidity in a growth chamber with fluorescent light tubes (CONVIRON ATC26; T = 20°C, light intensity 75 μ E s⁻¹ m⁻², 12-h day and 12-h night). The 0, 24, and 48 hpi peel samples (without pulp, i.e., exocarp) were collected 2 h before the onset of the dark period and the 16-hpi samples 2 h after the onset of the light period. The exocarp of the inoculated area (diameter of 5 mm) from 60 berries was used per RNA sample. Triplicate biological experiments were realized with VB and MB and were repeated in independent years. After sampling for microscopic and transcriptomic analyses, remaining berries were observed every day and the disease symptoms quantified until 13 dpi. The rate of berries bursting and the percentage of berries showing *Botrytis* conidiophores were measured on 180 berries per modality (triplicate of 60 mock- or *Botrytis*-inoculated berries).

For the pathotests of the *B. cinerea* KO mutants, 18 mature berries were inoculated with 5,000 conidia in 10 μ l of PDB and were incubated at 16°C. The rate of bursting and sporulation was measured daily. The experiments were repeated three times with berries harvested between 2011 and 2013.

Microscopy of *B. cinerea* mycelium development on berries tissues.

Berries exocarp segments (2 \times 7 mm) were excised from inoculation points (three segments per berry) and were fixed

immediately in 2.5% glutaraldehyde (in 0.1 M sodium-cacodylate buffer, pH 7.2) for 20 to 25 min under vacuum, and then, overnight at 4°C with gentle rotation. Samples were twice washed (10 min) in the same buffer and were postfixed in 1% osmium tetroxide (OsO₄) in the same buffer for 1 h at 4°C. Then, samples were washed in cacodylate buffer, were dehydrated in a graded ethanol series, and were treated with propylene oxide. Dehydrated samples were subsequently embedded in Epon (Merck, Darmstadt, Germany) and were sectioned using a Reichert Ultracut E microtome (Leica, Reuil-Malmaison, France) with glass knife (Diatome, Bienne, Switzerland). The semithin (0.5 μ m) sections were stained with 1% aqueous toluidine blue (in 1% sodium tetraborate) and were examined under a bright-field light microscope (Leica).

For scanning microscopy, berries exocarp disks (7 mm in diameter) were excised and were fixed with 2% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) for 1 h under vacuum, and then, overnight at 4°C with gentle rotation. Disks were washed three times in phosphate buffer, were dehydrated in a graded ethanol series at 4°C, critical point dried (Critical Point Dryer, Emscope CDP750; Ashford, England), and were coated with gold. Samples were observed with a scanning electron microscope (Philips XL-30 ESEM LaB6).

For subcellular localization of H₂O₂, berries inoculated by *B. cinerea* or mock-inoculated (PDB medium) were harvested at 72 hpi and were then infiltrated by 1 mg of DAB-HCl per milliliter. For this step, disposable 5-ml syringes, fitted with 0.45 mm diameter needles, were used in order to inject DAB droplets into the exocarp and the mesocarp of the detached berries. Three injections were performed, dispatched all around the fruit. Absorption and polymerization of DAB was confirmed by dark staining (reddish-brown) of the vascular and wound tissues. After 5 h at 25°C, 7 mm diameter pieces were excised in the exocarp, were put overnight in absolute methanol, and were then transferred into chloral hydrate solution (2.5 g/ml) overnight at room temperature to clarify the tissues. The berry disks were then mounted on microscope slides in 50% glycerol (aqueous solution), with the epidermis uppermost. H₂O₂ was visualized as a reddish-brown coloration.

RNA preparation.

RNA was extracted from grape tissues and from *B. cinerea* mycelium with a protocol modified from Reid et al. (2006). Pre-warmed (60°C) extraction buffer consisting of 300 mM Tris-HCl (pH 8), 25 mM EDTA, 2 M NaCl, 1 mM aurintricarboxylic acid, 2% hexadecyl-trimethyl-ammonium bromide, 2% polyvinylpyrrolidone, 0.05% spermidine trihydrochloride, and 2% β -mercaptoethanol was added to frozen berry peel powder at 15 ml/g and was shaken vigorously. Samples were incubated in a 60°C water bath for 10 min and shaken every 2 min. An equal volume of chloroform/3-methyl-1-butanol (24:1) was added and samples were mixed vigorously, followed by centrifugation at 3,500 \times g for 15 min at 4°C. The water phase was collected and subjected to a repeated chloroform extraction. Nucleic acids in the water phase were precipitated in 0.3 M sodium acetate (pH 5.2) and by adding 0.6 vol of isopropyl alcohol. After 1 h at -20°C, the precipitates were pelleted by centrifugation at 3,500 \times g for 30 min at 4°C, and the pellet was dissolved in 1 ml of TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA). LiCl was added to 2.5 M and large RNAs were precipitated overnight at 4°C and were pelleted by centrifugation at 20 000 \times g for 30 min at 4°C. The pellet was washed with 70% ethanol and was dissolved in water. Remaining traces of DNA were removed by DNaseI treatment (DNA-free; Invitrogen, Life Technologies, Saint-Aubin, France) and the RNA quality was analyzed using a RNA 6000 nano kit (Agilent Technologies, Waldbronn, Germany).

B. cinerea array hybridization and data analysis.

The complete transcriptome of *B. cinerea* was investigated both during the infection of grape berries (12, 24, and 48 hpi) or after 48 h of *in vitro* mycelium development on synthetic medium (per liter: 20 g of glucose, 2 g of NaNO₃, 1 g of KH₂PO₄, 0.5 g of MgSO₄, 7 H₂O, 0.5 g of KCl, 0.01 g of FeSO₄, 7 H₂O) with the same light conditions as for the infected berries. Synthesis of double-stranded cDNA, Cy3-labeling, and hybridization on microarrays were done by PartnerChip (Evry, France), using the procedures established by NimbleGen (Roche, Mannheim, Germany) and the reagents from Invitrogen. NimbleGen 4-plex arrays with 62,478 60-mer specific probes covering all the 20,885 predicted gene models of *B. cinerea* (Amselem et al. 2011) were used. Details of experiments and raw values are available in the Gene Expression Omnibus database (GEO) under the entry GSE65559. Data processing, quality controls, and differential expression analysis were performed using ANAIS methods (Simon and Biot 2010). DE genes were identified using one-way ANOVA with the false discovery rate (FDR) test for multiple tests correction of ANOVA *P* values. Transcripts with a corrected *P* value < 0.05 and more than a twofold change in transcript level were considered as significantly differentially expressed. The expression patterns for the DE genes were clustered with Genesis software (Sturn et al. 2002). Gene set enrichment analyses (Fisher's exact test) were further performed on lists of annotated genes (Amselem et al. 2011).

Grape array hybridization and data analysis.

The cDNA synthesis, labeling, hybridization, and washing steps were performed according to the NimbleGen Arrays User's Guide (version 3.2). Each sample was hybridized to a NimbleGen microarray 090818 Vitis exp HX12 (Roche, NimbleGen), which contains probes targeted to 29,549 predicted grapevine genes, representing 98.6% of the genes predicted from the V1 annotation of the 12x grapevine genome. Each microarray was scanned using an Axon GenePix 4400A (Molecular Devices, Sunnyvale, CA, U.S.A.) at 532 nm (Cy3 absorption peak) and GenePix Pro7 software (Molecular Devices). Images were analyzed using NimbleScan v2.5 software (Roche), which produces pair files containing the raw signal intensity data for each probe and calls files with normalized expression data derived from the average of the intensities of the four probes for each gene. All microarray expression data are available in the GEO under the entry GSE65969. The statistically significant transcripts from the grapevine microarray data were detected using ANAIS with the FDR test for multiple tests correction of ANOVA *P* values (Simon and Biot 2010). Transcripts with a fold change greater than 2 and *P* value < 0.05 were considered significant. Hierarchical clustering of significant berry transcripts was done with MeV software (Saeed et al. 2006). The GO analysis of the individual gene clusters was done with agriGO, with the Fisher's exact test and the FDR test as chosen statistical tests (*P* < 0.05) (Du et al. 2010).

qPCR.

For the qPCR, cDNA was synthesized using Superscript III reverse transcription (Invitrogen), oligo(dT) primers, and 1.5 µg of DNA-free total RNA, according to the manufacturer's instructions. The 5-µl reactions (2 µl of 1:80 diluted cDNA, 2.5 µl GoTaq qPCR master mix [Promega, Madison, WI, U.S.A.], and a final primer concentration of 500 nM) were run in a LightCycler480 (Roche) using a thermal cycling profile of 95°C for 15 min, 40 to 50 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Each assay was tested in a dissociation protocol to ensure a single amplicon was produced. The primer pair

efficiency was taken into account by using the NO values of the LinRegPCR program (Ruijter et al. 2009) in relative induction value calculations. *VvATP16* and *BcAct1* were used as the reference genes for *V. vinifera* and *B. cinerea*, respectively (Gamm et al. 2011; Simon et al. 2013). All primers and corresponding gene identifiers can be found in Supplementary Table S6 and in other previous publications (Trdá et al. 2014; Schumacher et al. 2015). Statistical significance was calculated with a Student's heteroscedastic *t* test considering each technical replicate as an individual sample (*n* = 9).

B. cinerea mutagenesis.

The *B. cinerea* gene *BcPks8* (Kroken et al. 2003) was deleted by homologous recombination. Pairs of primers 5F and 5R were used to amplify regions of about 1 kb in the 5' and in the 3' regions of the target gene and the hygromycin resistance gene (*hph*) (Supplementary Table S7). Then, a KO cassette consisting of the 5' region of *BcPks8*, the resistance gene and the 3' region of *BcPks8* was generated with the Gene KO kit obtained from the Fungal Genetic Stock Center (Colot et al. 2006). Protoplasts from the B05.10 strain were prepared and transformed as previously described (Simon et al. 2013). They were then plated in molten osmotically stabilized medium agar containing 100 µg of hygromycin per milliliter (Invitrogen). Transformants were purified by single-spore cultures. The screenings for gene inactivation events were done by PCR, using the primers located outside the cassette flanking regions (verif-5 and verif-3) and primers located inside the resistance gene *hph*. Additionally, the genetic purity of the KO transformants was checked by the absence of the WT allele (primers Fint and Rint). Information about published *B. cinerea* KO mutants used in this study is available at INRA's *Botrytis cinerea* mutants database.

Grape metabolites.

To determine changes in JA, SA, ferulic acid, caffeic acid, and chlorogenic acid levels upon inoculation, peel discs from the mock-infected or infected grapevine berries were collected and were frozen immediately in liquid nitrogen. Before extraction, a mixture of internal standards containing 100 ng of dihydrojasmonic acid, d6-SA, and propyl-paraben was added. Freeze-dried tissue (0.05 g) was homogenized in 2.5 ml of ultrapure water; after centrifugation (5,000 × g, 40 min), the supernatant was recovered acidified and partitioned against diethylether as described by Flors et al. (2008). The dried organic phase was resuspended in 1 ml of a water/methanol (90:10) solution and an aliquot was injected into the high-performance liquid chromatography (HPLC) system. Analyses were carried out using a Waters Alliance 2690 HPLC system (Milford, MA, U.S.A.) with nucleosil ODS reversed-phase column (100 × 2 mm i.d.; 5 µm) (Scharlab, Barcelona, Spain). The chromatographic system was interfaced to a Quatro LC (quadrupole-hexapole-quadrupole). Compound quantities were compared with their respective standard curves for all metabolites. Quantifications were carried out with Mass Lynx (v 1.4, Micromass) software using the internal standards as reference for extraction recovery and the standard curves as quantifiers.

The resveratrol phytoalexin was quantified in an aliquot of the previously described aqueous extract. The chromatography was carried out in the same conditions used for hormonal measurements using gradients of water and methanol, both phases containing 0.01% of HCOOH, as described by Flors et al. (2008). The mass spectrometer was programmed to detect transitions from *m/z* 227.1 to *m/z* 143.1 for resveratrol. Relative retention time was 10.06 for resveratrol. All compounds were quantified using a calibration curve built with their respective standards.

ACKNOWLEDGMENTS

We are grateful to the Agence National de la Recherche (ANR) and to the Comité National des Interprofessions des Vins à Appellation d'Origine (CNIV) for their financial support (grant PCS-08-GENO-148 SafeGrape). We also thank the Bordeaux experimental INRA vineyard of Château Couhins for providing the harvested berries, P. Tudzynski (University of Muenster, Germany) for providing the *B. cinerea bcsod1* and *bcaba1* mutants, and the Servicios Centrales de Instrumentación Científica de la Universitat Jaume I for the use of its facilities and its technical advices. J. Kelloniemi was financially supported by the "Conseil Regional de Bourgogne" (Dinos-Agrale 12, grant 2010-9201AAO050S5542). We also wish to thank the Microscopy Center INRA/uB of the DimaCell platform and, more particularly, A. Bonnotte for her technical assistance with SEM.

LITERATURE CITED

- Amselem, J., Cuomo, C. A., van Kan, J. A., Viaud, M., Benito, E. P., Couloux, A., Coutinho, P. M., de Vries, R. P., Dyer, P. S., Fillinger, S., Fournier, E., Gout, L., Hahn, M., Kohn, L., Lapalu, N., Plummer, K. M., Pradier, J. M., Quévillon, E., Sharon, A., Simon, A., ten Have, A., Tudzynski, B., Tudzynski, P., Wincker, P., Andrew, M., Anthouard, V., Beever, R. E., Beffa, R., Benoit, I., Bouzid, O., Brault, B., Chen, Z., Choquer, M., Collémare, J., Cotton, P., Danchin, E. G., Da Silva, C., Gautier, A., Giraud, C., Giraud, T., Gonzalez, C., Grossetete, S., Guildener, U., Henrissat, B., Howlett, B. J., Kodira, C., Kretschmer, M., Lappartient, A., Leroch, M., Levis, C., Mauceli, E., Neuvéglise, C., Oeser, B., Pearson, M., Poulain, J., Poussereau, N., Quesneville, H., Rascle, C., Schumacher, J., Ségurens, B., Sexton, A., Silva, E., Sirven, C., Soanes, D. M., Talbot, N. J., Templeton, M., Yandava, C., Yarden, O., Zeng, Q., Rollins, J. A., Lebrun, M. H., and Dickman, M. 2011. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet. 7:e1002230.
- Asselbergh, B., Curvers, K., Franca, S. C., Audenaert, K., Vuylsteke, M., Van Breusegem, F., and Höfte, M. 2007. Resistance to *Botrytis cinerea* in *sitiens*, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. Plant Physiol. 144:1863-1877.
- Audenaert, K., De Meyer, G. B., and Höfte, M. M. 2002. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. Plant Physiol. 128:491-501.
- Bavaresco, L., Petegolli, D., Cantu, E., Fregoni, M., Chiusa, G., and Trevisan, M. 1997. Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis 36:77-83.
- Bézier, A., Lambert, B., and Baillieul, F. 2002. Cloning of a grapevine *Botrytis*-responsive gene that has homology to the tobacco hypersensitivity-related *hsr203J*. J. Exp. Bot. 53:2279-2280.
- Blanco-Ulate, B., Morales-Cruz, A., Amrine, K. C., Labavitch, J. M., Powell, A. L., and Cantu, D. 2014. Genome-wide transcriptional profiling of *Botrytis cinerea* genes targeting plant cell walls during infections of different hosts. Front. Plant Sci. 5:435.
- Böhm, H., Albert, I., Fan, L., Reinhard, A., and Nürnberger, T. 2014. Immune receptor complexes at the plant cell surface. Curr. Opin. Plant Biol. 20:47-54.
- Boller, T., and Felix, G. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60:379-406.
- Bradley, D. J., Kjellbom, P., and Lamb, C. J. 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell 70:21-30.
- Brady, J. D., and Fry, S. C. 1997. Formation of di-isodityrosine and loss of isodityrosine in the cell walls of tomato cell-suspension cultures treated with fungal elicitors or H₂O₂. Plant Physiol. 115: 87-92.
- Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. 2009. The carbohydrate-active enzymes database (CAZY): An expert resource for glycogenomics. Nucleic Acids Res. 37:D233-D238.
- Cantu, D., Blanco-Ulate, B., Yang, L., Labavitch, J. M., Bennett, A. B., and Powell, A. L. 2009. Ripening-regulated susceptibility of tomato fruit to *Botrytis cinerea* requires NOR but not RIN or ethylene. Plant Physiol. 150:1434-1449.
- Cantu, D., Vicente, A. R., Greve, L. C., Dewey, F. M., Bennett, A. B., Labavitch, J. M., and Powell, A. L. 2008. The intersection between cell wall disassembly, ripening, and fruit susceptibility to *Botrytis cinerea*. Proc. Natl. Acad. Sci. U.S.A. 105:859-864.
- Choquer, M., Fournier, E., Kunz, C., Levis, C., Pradier, J. M., Simon, A., and Viaud, M. 2007. *Botrytis cinerea* virulence factors: New insights into a necrotrophic and polyphageous pathogen. FEMS Microbiol. Lett. 277:1-10.
- Colot, H. V., Park, G., Turner, G. E., Ringelberg, C., Crew, C. M., Litvinkova, L., Weiss, R. L., Borkovich, K. A., and Dunlap, J. C. 2006. A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. Proc. Natl. Acad. Sci. U.S.A. 103:10352-10357.
- Coutos-Thévenot, P., Poinsot, B., Bonomelli, A., Yean, H., Breda, C., Buffard, D., Esnault, R., Hain, R., and Boulay, M. 2001. In vitro tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase *Vst1* gene under the control of a pathogen-inducible PR 10 promoter. J. Exp. Bot. 52:901-910.
- Dai, Z. W., Léon, C., Feil, R., Lunn, J. E., Delrot, S., and Gomès, E. 2013. Metabolic profiling reveals coordinated switches in primary carbohydrate metabolism in grape berry (*Vitis vinifera* L.), a non-climacteric fleshy fruit. J. Exp. Bot. 64:1345-1355.
- Dalmats, B., Schumacher, J., Moraga, J., LE Pêcheur, P., Tudzynski, B., Collado, I. G., and Viaud, M. 2011. The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. Mol. Plant Pathol. 12: 564-579.
- De Cremer, K., Mathys, J., Vos, C., Froenicke, L., Michelmore, R. W., Cammue, B. P., and De Coninck, B. 2013. RNAseq-based transcriptome analysis of *Lactuca sativa* infected by the fungal necrotroph *Botrytis cinerea*. Plant Cell Environ. 36:1992-2007.
- De Meyer, G., Capieau, K., Audenaert, K., Buchala, A., Métraux, J. P., and Höfte, M. 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Mol. Plant-Microbe Interact. 12: 450-458.
- Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G. D. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414-430.
- Denness, L., McKenna, J. F., Segonzac, C., Wormit, A., Madhou, P., Bennett, M., Mansfield, J., Zipfel, C., and Hamann, T. 2011. Cell wall damage-induced lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process in *Arabidopsis*. Plant Physiol. 156:1364-1374.
- Denoux, C., Galletti, R., Mammarella, N., Gopalan, S., Werck, D., De Lorenzo, G., Ferrari, S., Ausubel, F. M., and Dewdney, J. 2008. Activation of defense response pathways by OGs and Flg22 elicitors in *Arabidopsis* seedlings. Mol. Plant 1:423-445.
- Derckel, J. P., Audran, J. P., Haye, B., Lambert, B., and Legendre, L. 1998. Characterization, induction by wounding and salicylic acid, and activity against *Botrytis cinerea* of chitinases and Beta-1,3-glucanases of ripening grape berries. Physiol. Plant. 104:56-64.
- Deytieu-Belleau, C., Geny, L., Roudet, J., Mayet, V., Donèche, B., and Fermaud, M. 2009. Grape berry skin features related to ontogenic resistance to *Botrytis cinerea*. Eur. J. Plant Pathol. 125:551-563.
- Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T., and French, R. 2001. Abrogation of disease development in plants expressing animal antiapoptotic genes. Proc. Natl. Acad. Sci. U.S.A. 98:6957-6962.
- Dmitriev, A., Tena, M., and Jorin, J. 2003. Systemic acquired resistance in sunflower (*Helianthus annuus* L.). Tsitol. Genet. 37:9-15.
- Du, Z., Zhou, X., Ling, Y., Zhang, Z., and Su, Z. 2010. agriGO: A GO analysis toolkit for the agricultural community. Nucleic Acids Res. 38: W64-W70.
- Dubreuil-Maurizi, C., Trouvelot, S., Frettinger, P., Pugin, A., Wendehenne, D., and Poinsot, B. 2010. β -Aminobutyric acid primes an NADPH oxidase-dependent reactive oxygen species production during grapevine-triggered immunity. Mol. Plant-Microbe Interact. 23: 1012-1021.
- Eichorn, K. W., and Lorenz, D. H. 1977. Phänologische entwicklungsstadien der rebe. Nachrichtenbl. Deut. Pflanzenschutzd. 29:119-120.
- Elmer, P. A. G., and Michailides, T. J. 2007. Epidemiology of *Botrytis cinerea* in orchard and vine crops. Pages 243-272. in: *Botrytis: Biology, Pathology and Control*. Y. Elad, B. Williamson, P. Tudzynski, and N. Delen, eds. Springer, Dordrecht, The Netherlands.
- Espino, J. J., Gutiérrez-Sánchez, G., Brito, N., Shah, P., Orlando, R., and González, C. 2010. The *Botrytis cinerea* early secretome. Proteomics 10: 3020-3034.
- Ferrari, S., Galletti, R., Denoux, C., De Lorenzo, G., Ausubel, F. M., and Dewdney, J. 2007. Resistance to *Botrytis cinerea* induced in *Arabidopsis* by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiol. 144:367-379.

- Ferrari, S., Plotnikova, J. M., De Lorenzo, G., and Ausubel, F. M. 2003. *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. *Plant J.* 35:193-205.
- Flors, V., Ton, J., van Doorn, R., Jakab, G., García-Agustín, P., and Mauch-Mani, B. 2008. Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant J.* 54:81-92.
- Frías, M., González, C., and Brito, N. 2011. BcSpl1, a cerato-platanin family protein, contributes to *Botrytis cinerea* virulence and elicits the hypersensitive response in the host. *New Phytol.* 192:483-495.
- Galletti, R., Denoux, C., Gambetta, S., Dewdney, J., Ausubel, F. M., De Lorenzo, G., and Ferrari, S. 2008. The AtrbohD-mediated oxidative burst elicited by oligogalacturonides in *Arabidopsis* is dispensable for the activation of defense responses effective against *Botrytis cinerea*. *Plant Physiol.* 148:1695-1706.
- Gamm, M., Héloir, M. C., Kelloniemi, J., Poinssot, B., Wendehenne, D., and Adrian, M. 2011. Identification of reference genes suitable for qRT-PCR in grapevine and application for the study of the expression of genes involved in pterostilbene synthesis. *Mol. Genet. Genomics* 285:273-285.
- García-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D., and Pugin, A. 2006. Early signaling events induced by elicitors of plant defenses. *Mol. Plant-Microbe Interact.* 19:711-724.
- Gauthier, A., Trouvelot, S., Kelloniemi, J., Frettinger, P., Wendehenne, D., Daire, X., Joubert, J. M., Ferrarini, A., Delledonne, M., Flors, V., and Poinssot, B. 2014. The sulfated laminarin triggers a stress transcriptome before priming the SA- and ROS-dependent defenses during grapevine's induced resistance against *Plasmopara viticola*. *PLoS One* 9:e88145.
- Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205-227.
- Godfrey, D., Able, A. J., and Dry, I. B. 2007. Induction of a grapevine germin-like protein (VvGLP3) gene is closely linked to the site of *Erysiphe necator* infection: A possible role in defense? *Mol. Plant-Microbe Interact.* 20:1112-1125.
- Govrin, E. M., and Levine, A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr. Biol.* 10:751-757.
- Hammerschmidt, R. 1999. Phytoalexins: What have we learned after 60 years? *Annu. Rev. Phytopathol.* 37:285-306.
- Heller, J., and Tudzynski, P. 2011. Reactive oxygen species in phytopathogenic fungi: Signaling, development and disease. *Annu. Rev. Phytopathol.* 49:369-390.
- Iriti, M., Rossoni, M., Borgo, M., and Faoro, F. 2004. Benzothiadiazole enhances resveratrol and anthocyanin biosynthesis in grapevine, meanwhile improving resistance to *Botrytis cinerea*. *J. Agric. Food Chem.* 52:4406-4413.
- Iriti, M., Rossoni, M., Borgo, M., Ferrara, L., and Faoro, F. 2005. Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: Primary versus secondary metabolism. *J. Agric. Food Chem.* 53:9133-9139.
- Jaillon, O., Aury, J. M., Noel, B., Pollicriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Huguency, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lechamy, A., Scarpelli, C., Artiguenave, F., Pè, M. E., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A. F., Weissenbach, J., Quéter, F., and Wincker, P.; French-Italian Public Consortium for Grapevine Genome Characterization. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:463-467.
- Jeandet, P., Bessis, R., Sbaghi, M., and Meunier, P. 1995. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *J. Phytopathol.* 143:135-139.
- Jeworutzki, E., Roelfsema, M. R., Anshütz, U., Krol, E., Elzenga, J. T., Felix, G., Boller, T., Hedrich, R., and Becker, D. 2010. Early signaling through the *Arabidopsis* pattern recognition receptors FLS2 and EFR involves Ca-associated opening of plasma membrane anion channels. *Plant J.* 62:367-378.
- Jones, J. D., and Dangl, J. L. 2006. The plant immune system. *Nature* 444:323-329.
- Kars, I., Krooshof, G. H., Wagemakers, L., Joosten, R., Benen, J. A., and van Kan, J. A. 2005a. Necrotizing activity of five *Botrytis cinerea* endopolygalacturonases produced in *Pichia pastoris*. *Plant J.* 43:213-225.
- Kars, I., McCalman, M., Wagemakers, L., and VAN Kan, J. A. 2005b. Functional analysis of *Botrytis cinerea* pectin methylesterase genes by PCR-based targeted mutagenesis: Bcpme1 and Bcpme2 are dispensable for virulence of strain B05.10. *Mol. Plant Pathol.* 6:641-652.
- Koornneef, A., and Pieterse, C. M. 2008. Cross talk in defense signaling. *Plant Physiol.* 146:839-844.
- Kretschmer, M., Kassemeyer, H. H., and Hahn, M. 2007. Age-dependent grey mould susceptibility and tissue-specific defence gene activation of grapevine berry skins after infection by *Botrytis cinerea*. *J. Phytopathol.* 155:258-263.
- Kroken, S., Glass, N. L., Taylor, J. W., Yoder, O. C., and Turgeon, B. G. 2003. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proc. Natl. Acad. Sci. U.S.A.* 100:15670-15675.
- Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Geny, L., Rey, P., Donèche, B., and Teissedre, P. L. 2012. Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. *Aust. J. Grape Wine Res.* 18:215-226.
- López, M. A., Bannenberg, G., and Castresana, C. 2008. Controlling hormone signaling is a plant and pathogen challenge for growth and survival. *Curr. Opin. Plant Biol.* 11:420-427.
- Martinez, F., Dubos, B., and Fermaud, M. 2005. The role of saprotrophy and virulence in the population dynamics of *Botrytis cinerea* in vineyards. *Phytopathology* 95:692-700.
- Monteiro, S., Barakat, M., Piçarra-Pereira, M. A., Teixeira, A. R., and Ferreira, R. B. 2003. Osmotin and thaumatin from grape: A putative general defense mechanism against pathogenic fungi. *Phytopathology* 93:1505-1512.
- Murphy, A. M., Holcombe, L. J., and Carr, J. P. 2000. Characteristics of salicylic acid-induced delay in disease caused by a necrotrophic fungal pathogen in tobacco. *Physiol. Mol. Plant Pathol.* 57:47-54.
- Nafisi, M., Stranne, M., Zhang, L., van Kan, J. A., and Sakuragi, Y. 2014. The endo-arabinanase BcAra1 is a novel host-specific virulence factor of the necrotic fungal phytopathogen *Botrytis cinerea*. *Mol. Plant-Microbe Interact.* 27:781-792.
- Pinedo, C., Wang, C. M., Pradier, J. M., Dalmais, B., Choquer, M., Le Pêcheur, P., Morgant, G., Collado, I. G., Cane, D. E., and Viaud, M. 2008. Sesquiterpene synthase from the botrydial biosynthetic gene cluster of the phytopathogen *Botrytis cinerea*. *ACS Chem. Biol.* 3:791-801.
- Pitzschke, A., Schikora, A., and Hirt, H. 2009. MAPK cascade signalling networks in plant defence. *Curr. Opin. Plant Biol.* 12:421-426.
- Prins, T. W., Wagemakers, L., Schouten, A., and van Kan, J. A. 2000. Cloning and characterization of a glutathione S-transferase homologue from the plant pathogenic fungus *Botrytis cinerea*. *Mol. Plant Pathol.* 1:169-178.
- Quidde, T., Osbourn, A. E., and Tudzynski, P. 1998. Detoxification of α -tomatine by *Botrytis cinerea*. *Physiol. Mol. Plant Pathol.* 52:151-165.
- Reid, K. E., Olsson, N., Schlosser, J., Peng, F., and Lund, S. T. 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. *BMC Plant Biol.* 6:27.
- Repka, V. 2006. Early defence responses induced by two distinct elicitors derived from a *Botrytis cinerea* in grapevine leaves and cell suspensions. *Biol. Plant.* 50:94-106.
- Robert, N., Roche, K., Lebeau, Y., Breda, C., Boulay, M., Esnault, R., and Buffard, D. 2002. Expression of grapevine chitinase genes in berries and leaves infected by fungal or bacterial pathogens. *Plant Sci.* 162:389-400.
- Robinson, S. P., Jacobs, A. K., and Dry, I. B. 1997. A class IV chitinase is highly expressed in grape berries during ripening. *Plant Physiol.* 114:771-778.
- Rolke, Y., Liu, S., Quidde, T., Williamson, B., Schouten, A., Weltring, K. M., Siewers, V., Tenberge, K. B., Tudzynski, B., and Tudzynski, P. 2004. Functional analysis of H₂O₂-generating systems in *Botrytis cinerea*: The major Cu-Zn-superoxide dismutase (BCSOD1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. *Mol. Plant Pathol.* 5:17-27.
- Rowe, H. C., Walley, J. W., Corwin, J., Chan, E. K., Dehesh, K., and Kliebenstein, D. J. 2010. Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in *Botrytis cinerea* pathogenesis. *PLoS Pathog.* 6:e1000861.
- Ruijter, J. M., Ramakers, C., Hoogaars, W. M., Karlen, Y., Bakker, O., van den Hoff, M. J., and Moorman, A. F. 2009. Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res.* 37:e45.

- Saeed, A. I., Bhagabati, N. K., Braisted, J. C., Liang, W., Sharov, V., Howe, E. A., Li, J., Thiagarajan, M., White, J. A., and Quackenbush, J. 2006. TM4 microarray software suite. *Methods Enzymol.* 411:134-193.
- Salzman, R. A., Tikhonova, I., Bordelon, B. P., Hasegawa, P. M., and Bressan, R. A. 1998. Coordinate accumulation of antifungal proteins and hexoses constitutes a developmentally controlled defense response during fruit ripening in grape. *Plant Physiol.* 117:465-472.
- Schouten, A., van Baarlen, P., and van Kan, J. A. 2008. Phytotoxic Nep1-like proteins from the necrotrophic fungus *Botrytis cinerea* associate with membranes and the nucleus of plant cells. *New Phytol.* 177:493-505.
- Schouten, A., Wagemakers, L., Stefanato, F. L., van der Kaaij, R. M., and van Kan, J. A. 2002. Resveratrol acts as a natural antifungicide and induces self-intoxication by a specific laccase. *Mol. Microbiol.* 43:883-894.
- Schumacher, J., Pradier, J. M., Simon, A., Traeger, S., Moraga, J., Collado, I. G., Viaud, M., and Tudzynski, B. 2012. Natural variation in the *VELVET* gene *bcvell* affects virulence and light-dependent differentiation in *Botrytis cinerea*. *PLoS One* 7:e47840.
- Schumacher, J., Simon, A., Cohrs, K. C., Traeger, S., Porquier, A., Dalmais, B., Viaud, M., and Tudzynski, B. 2015. The VELVET complex in the gray mold fungus *Botrytis cinerea*: Impact of BcLAE1 on differentiation, secondary metabolism, and virulence. *Mol. Plant-Microbe Interact.* 28:659-674.
- Schumacher, J., Simon, A., Cohrs, K. C., Viaud, M., and Tudzynski, P. 2014. The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. *PLoS Genet.* 10:e1004040.
- Shlezinger, N., Minz, A., Gur, Y., Hatam, I., Dagdas, Y. F., Talbot, N. J., and Sharon, A. 2011. Anti-apoptotic machinery protects the necrotrophic fungus *Botrytis cinerea* from host-induced apoptotic-like cell death during plant infection. *PLoS Pathog.* 7:e1002185.
- Siewers, V., Kokkelink, L., Smedsgaard, J., and Tudzynski, P. 2006. Identification of an abscisic acid gene cluster in the grey mold *Botrytis cinerea*. *Appl. Environ. Microbiol.* 72:4619-4626.
- Siewers, V., Smedsgaard, J., and Tudzynski, P. 2004. The P450 monooxygenase BcABA1 is essential for abscisic acid biosynthesis in *Botrytis cinerea*. *Appl. Environ. Microbiol.* 70:3868-3876.
- Siewers, V., Viaud, M., Jimenez-Teja, D., Collado, I. G., Gronover, C. S., Pradier, J. M., Tudzynski, B., and Tudzynski, P. 2005. Functional analysis of the cytochrome P450 monooxygenase gene *bcbot1* of *Botrytis cinerea* indicates that botrydial is a strain-specific virulence factor. *Mol. Plant-Microbe Interact.* 18:602-612.
- Simon, A., and Biot, E. 2010. ANAIS: Analysis of NimbleGen arrays interface. *Bioinformatics* 26:2468-2469.
- Simon, A., Dalmais, B., Morgant, G., and Viaud, M. 2013. Screening of a *Botrytis cinerea* one-hybrid library reveals a Cys2His2 transcription factor involved in the regulation of secondary metabolism gene clusters. *Fungal Genet. Biol.* 52:9-19.
- Smith, J. E., Mengesha, B., Tang, H., Mengiste, T., and Bluhm, B. H. 2014. Resistance to *Botrytis cinerea* in *Solanum lycopersicoides* involves widespread transcriptional reprogramming. *BMC Genomics* 15:334.
- Sturn, A., Quackenbush, J., and Trajanoski, Z. 2002. Genesis: Cluster analysis of microarray data. *Bioinformatics* 18:207-208.
- ten Have, A., Espino, J. J., Dekkers, E., Van Sluyter, S. C., Brito, N., Kay, J., González, C., and van Kan, J. A. 2010. The *Botrytis cinerea* aspartic proteinase family. *Fungal Genet. Biol.* 47:53-65.
- Torres, M. A., Dangel, J. L., and Jones, J. D. 2002. *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc. Natl. Acad. Sci. U.S.A.* 99:517-522.
- Trdá, L., Fernandez, O., Boutrot, F., Héloir, M. C., Kelloniemi, J., Daire, X., Adrian, M., Clément, C., Zipfel, C., Dorey, S., and Poinssot, B. 2014. The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. *New Phytol.* 201:1371-1384.
- Tsuda, K., and Katagiri, F. 2010. Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Curr. Opin. Plant Biol.* 13:459-465.
- Van Baarlen, P., Woltering, E. J., Staats, M., and Van Kan, J. A. L. 2007. Histochemical and genetic analysis of host and non-host interactions of *Arabidopsis* with three *Botrytis* species: An important role for cell death control. *Mol. Plant Pathol.* 8:41-54.
- van Kan, J. A. 2006. Licensed to kill: The lifestyle of a necrotrophic plant pathogen. *Trends Plant Sci.* 11:247-253.
- van Loon, L. C., Rep, M., and Pieterse, C. M. 2006. Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* 44:135-162.
- Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., Pindo, M., Fitzgerald, L. M., Vezzulli, S., Reid, J., Malacarne, G., Iliev, D., Coppola, G., Wardell, B., Micheletti, D., Macalma, T., Facci, M., Mitchell, J. T., Perazzolli, M., Eldredge, G., Gatto, P., Oyzerski, R., Moretto, M., Gutin, N., Stefanini, M., Chen, Y., Segala, C., Davenport, C., Demattè, L., Mraz, A., Battilana, J., Stormo, K., Costa, F., Tao, Q., Si-Ammour, A., Harkins, T., Lackey, A., Perbost, C., Taillon, B., Stella, A., Soloviyev, V., Fawcett, J. A., Sterck, L., Vandepoele, K., Grando, S. M., Toppo, S., Moser, C., Lanchbury, J., Bogden, R., Skolnick, M., Sgaramella, V., Bhatnagar, S. K., Fontana, P., Gutin, A., Van de Peer, Y., Salamini, F., and Viola, R. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS One* 2:e1326.
- Yoshioka, H., Numata, N., Nakajima, K., Katou, S., Kawakita, K., Rowland, O., Jones, J. D., and Doke, N. 2003. *Nicotiana benthamiana* gp91phox homologs NbrbohA and NbrbohB participate in H₂O₂ accumulation and resistance to *Phytophthora infestans*. *Plant Cell* 15:706-718.
- Zhang, L., and van Kan, J. A. 2013. *Botrytis cinerea* mutants deficient in D-galacturonic acid catabolism have a perturbed virulence on *Nicotiana benthamiana* and *Arabidopsis*, but not on tomato. *Mol. Plant Pathol.* 14:19-29.
- Zheng, C., Choquer, M., Zhang, B., Ge, H., Hu, S., Ma, H., and Chen, S. 2011. LongSAGE gene-expression profiling of *Botrytis cinerea* germination suppressed by resveratrol, the major grapevine phytoalexin. *Fungal Biol.* 115:815-832.
- Zhou, N., Tootle, T. L., and Glazebrook, J. 1999. *Arabidopsis* PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *Plant Cell* 11:2419-2428.
- Zipfel, C. 2014. Plant pattern-recognition receptors. *Trends Immunol.* 35:345-351.

AUTHOR-RECOMMENDED INTERNET RESOURCES

Fungal Genetic Stock Center database: <http://www.fgsc.net/clones.html>
 INRA's *Botrytis cinerea* mutants database: <http://botbioger.versailles.inra.fr/botmut/index.html>