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## Differential Responses of Three Grapevine Cultivars to *Botryosphaeria dieback*

Alessandro Spagnolo, Maryline Magnin-Robert, Tchilabalo Dilezitoko Alayi, Clara Cilindre, Christine Schaeffer-Reiss, Alain Van Dorsselaer, Christophe Clément, Philippe Larignon, Montserrat Ramirez-Suero, Julie Chong, Christophe Bertsch, Eliane Abou-Mansour, and Florence Fontaine

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### ABSTRACT

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*Botryosphaeria dieback* is a fungal grapevine trunk disease that represents a threat for viticulture worldwide due to the decreased production of affected plants and their premature death. This dieback is characterized by a typical wood discoloration called brown stripe. Herein, a proteome comparison of the brown striped wood from *Botryosphaeria dieback*-affected standing vines cultivars Chardonnay, Gewurztraminer, and Mourvèdre was performed. The transcript analysis for 15 targeted genes and the quantification of both total phenolics and specific stilbenes were also

performed. Several pathogenesis-related proteins and members of the antioxidant system were more abundant in the brown striped wood of the three cultivars, whereas other defense-related proteins were less abundant. Additionally, total phenolics and some specific stilbenes were more accumulated in the brown striped wood. Strongest differences among the cultivars concerned proteins of the primary metabolism, which looked to be particularly impaired in the brown striped wood of 'Chardonnay'. Low abundance of some proteins involved in defense response probably contributes to make global response insufficient to avoid the symptom development. The differential susceptibility of the three grapevine cultivars could be linked to the diverse expression of various proteins involved in defense response, stress tolerance, and metabolism.

*Additional keywords:* two-dimensional gel electrophoresis, phytoalexins.

Grapevine is one of the most important economic crops worldwide with a cultivated area representing more than 7.5 million hectare in the world and around 252 million hectoliter of wine produced in 2012 (FORECAST 2012). However, grapevine yield and quality are seriously compromised by infectious diseases caused by various fungi (14). Considering the heavy economic losses they cause worldwide, trunk diseases currently remain among the most important fungal affections of grapevine. For example, trunk disease incidence estimated over 6 years in 329 French vineyards reached values higher than 10% for esca/*Botryosphaeria dieback* and 25% for *Eutypa dieback* (9). Considering a replacement of only 1% of plant per year, the worldwide annual financial cost of it is without doubt in excess of 1.132 billion euros (28). Improving knowledge on trunk diseases is therefore urgently needed for the development of strategies to sustain worldwide viticulture.

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First and second authors contributed equally to this work.

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*Botryosphaeria dieback* is one of the main grapevine trunk diseases, which is caused by several xylem-inhabiting fungi (6,36, 45). Members of the family *Botryosphaeriaceae* (6,60) are the causal agents. These pathogens mainly attack the perennial organs of grapevine, causing wood discolorations as well as specific foliar symptoms (6), finally leading to premature plant death. A *Botryosphaeria dieback*-associated wood symptom that has been less considered until now is a brown stripe located in the outer xylem. This wood discoloration appears as a superficial, longitudinal orange/brown stripe just beneath the bark (Supplemental Figure 1), thus probably associated with both xylem and phloem flow. Unlike other grapevine trunk disease-related wood discolorations, brown stripe is not detectable before the vegetative season. It may extend from trunk until annual stems but is not retrievable in the roots. Furthermore, brown stripe is always associated with foliar symptoms (35).

Characterizing the impact of trunk diseases on grapevine physiology represents a key step for obtaining accurate knowledge on mechanisms that lead to disease development and the appearance of symptoms. Most knowledge concerns leaves and green stems (10,36,41,55,61) where the presence of the pathogenic fungi has not been reported. Apart from the accumulation of phenolic compounds and starch depletion in the wood (11,17,52), there is generally a lack of knowledge on the response of functional grapevine wood to trunk diseases. Recently, a study on black streaked and asymptomatic trunk wood has been performed

(42) but no information on the brown stripe developing under the bark is yet available. Grapevine trunk diseases appear especially complex since no grapevine species, neither cultivated varieties nor wild species, are known to be resistant (6,34,56). A disease susceptibility classification based on the percentage of foliar symptom expression was suggested for some cultivars (9), although it can vary with region and year (6). Nevertheless, it has been reported that 'Chardonnay' is less susceptible than 'Gewurztraminer' and 'Mourvèdre' to *Botryosphaeria dieback* and esca disease (25). By using a two-dimensional gel electrophoresis (2-DE)-based proteomic approach, the present study addresses a global overview of the protein signature in the brown striped trunk wood of grapevine cultivars Chardonnay (C), Gewurztraminer (G), and Mourvèdre (M). Moreover, analysis of transcripts coding for some identified proteins and quantification of targeted metabolites were performed as complement to the proteomic approach. Both total phenolic and stilbenic compounds were quantified in relation to their defense role and their involvement in the susceptibility level of some cultivars.

## MATERIALS AND METHODS

**Plant material.** Preliminary observations of brown stripe revealed that this symptom is especially present in the trunk of apoplectic or *Botryosphaeria dieback*-affected plants (diseased plants) (6). Consequently, three asymptomatic (control) and three diseased plants per cultivar were uprooted in July 2011 from three French vineyards, cultivated in a different location, of different age, and grafted on a different rootstock (Table 1) and considered for this study. Control plants did not show any trunk disease-related foliar symptom since at least 5 years. After removal of the bark, trunk of all of the plants was inspected for the presence of brown stripe, and samples consisting of the outer xylem (2 to 3 mm thick) were collected with a sterile chisel. Since brown stripe was detected only in the trunk of diseased plants, a total of three groups of samples per cultivar were collected: asymptomatic trunk wood from control (AC, asymptomatic control) and diseased (AD, asymptomatic diseased) plants, and brown striped trunk wood from diseased (BD, brown diseased) plants. Three biological replicates per group (i.e., three wood samples from three different plants) were carried out for all analyses. In order to verify the association of *Botryosphaeria dieback* agents with the brown stripe, all samples were also subjected to biological isolation-based screening as described by Larignon and Dubos (33). Woody tissues used for protein, RNA, and metabolite extractions were frozen in the field with liquid nitrogen and subsequently stored at  $-80^{\circ}\text{C}$ . Before each analysis, the amount of biological sample needed was ground to a fine powder in liquid nitrogen with a Mixer Mill MM 400 (Retsch, Haan, Germany).

**Protein extraction.** Total protein fraction of woody samples was isolated using a phenol-based procedure according to Magnin-Robert et al. (42). The powdered tissue was placed in microtubes ( $0.30 \pm 0.01$  g of powder per 2.0 ml microtubes) and then resuspended in 1.0 ml of cold acetone. After vortexing

thoroughly for 30 s, the tubes were centrifuged at  $10,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ . The resultant pellet was washed once more with cold acetone. The pellet was sequentially rinsed at least three times with cold 80% acetone until the supernatant was colorless, and then resuspended in 1.0 ml of cold 20% (wt/vol) trichloroacetic acid (TCA)/ $\text{H}_2\text{O}$ . The suspension was sonicated in a water bath at  $4^{\circ}\text{C}$  for 10 min. After centrifugation, the pellets were sequentially washed twice with 20% (wt/vol) TCA/ $\text{H}_2\text{O}$  and twice with 80% (vol/vol) acetone. This pellet was air-dried and the dry powder was resuspended in 0.7 ml of dense sodium dodecyl sulfate (SDS) buffer (30% [wt/vol] sucrose, 2% [wt/vol] SDS, 0.1 M tris (hydroxymethyl) aminomethane [Tris]-HCl, pH 8.0, 5% [vol/vol] 2-mercaptoethanol). Then, 0.7 ml of a 90% phenol solution (Sigma-Aldrich, St. Louis, MO) was added, and the resulting mixture was vortexed for 30 s. The phenol phase, recovered by centrifugation at  $10,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ , was separated in two aliquots. One aliquot (0.7 ml) was transferred to a 15 ml Falcon tube while another aliquot (0.1 ml) was placed in a 1.5 ml microtube. Further steps were followed in parallel. After addition of 5 volumes of cold 0.1 M ammonium acetate in methanol, proteins were precipitated from the phenol phase overnight at  $-20^{\circ}\text{C}$ . The precipitated proteins were recovered by centrifugation and washed twice with cold 0.1 M ammonium acetate in methanol and twice with 80% (vol/vol) acetone. The final pellet was air-dried and stored at  $-80^{\circ}\text{C}$ . The pellet retrieved from the 0.1 ml aliquot was dissolved in 100  $\mu\text{l}$  of 7 M urea, 2 M thiourea, 4% (wt/vol) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) and 60 mM 1,4-dithiothreitol (DTT) for protein quantification using the Pierce 660 nm Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA) and bovine serum albumin (BSA) as standard. After quantification, protein samples were solubilized in a sample buffer consisting of 7 M urea, 2 M thiourea, 4% (wt/vol) CHAPS, 0.5% (vol/vol) immobilized pH gradient (IPG) buffer 3-10, 60 mM DTT, and traces of bromophenol blue.

**Two-dimensional electrophoresis (2-DE).** For preparative 2-DE analysis, samples containing approximately 40  $\mu\text{g}$  of total protein fraction were diluted in a mixture containing sample buffer and 10% (vol/vol) glycerol to a final volume of 125  $\mu\text{l}$ . IPG gel strips (ReadyStrip IPG, pH 4 to 7, 7 cm, Bio-Rad, Hercules, CA) were actively rehydrated overnight at  $20^{\circ}\text{C}$  with the mixture. Isoelectric focusing (IEF) was conducted at  $20^{\circ}\text{C}$  in an IPGphor unit (Amersham Pharmacia, Sweden) as follows: a linear increase from 50 to 4,000 V to give a total of 10,000 V/h. Focused proteins were reduced and subsequently alkylated according to Görg et al. (24). IPG strips were then placed on the top of vertical slabs of polyacrylamide (12% T and 2.6% C) and sealed by a layer of 1% (wt/vol) low melting point agarose, 0.15 M Bis-Tris/0.1 M HCl, and 0.2% (wt/vol) SDS. Electrophoretic migration along the second dimension was performed using a Mini-Protean 3 Cell (Bio-Rad) under a voltage of 30 V for 20 min, followed by 150 V for 1.5 h. After completion of SDS-polyacrylamide gel electrophoresis (PAGE), gels were stained with colloidal Coomassie Brilliant Blue using the PageBlue Protein Staining Solution (Fermentas) following the manufacturer's instructions.

TABLE 1. Description of plant material and related groups of samples

Cultivar/rootstock	Vineyard age and location	Sample group <sup>z</sup>		
		Control plants		Diseased plants
		Asymptomatic wood	Asymptomatic wood	Brown striped wood
Chardonnay/41B	27 years – Avice (Epernay), France	ACC	ADC	BDC
Gewurztraminer/16-49C	24 years – Rouffach (Colmar), France	ACG	ADG	BDG
Mourvèdre/3309	15 years – Rodilhan (Nîmes), France	ACM	ADM	BDM

<sup>z</sup> ACC, asymptomatic control Chardonnay; ACG, asymptomatic control Gewurztraminer; ACM, asymptomatic control Mourvèdre; ADC, asymptomatic diseased Chardonnay; ADG, asymptomatic diseased Gewurztraminer; ADM, asymptomatic diseased Mourvèdre; BDC, brown diseased Chardonnay; BDG, brown diseased Gewurztraminer; and BDM, brown diseased Mourvèdre.

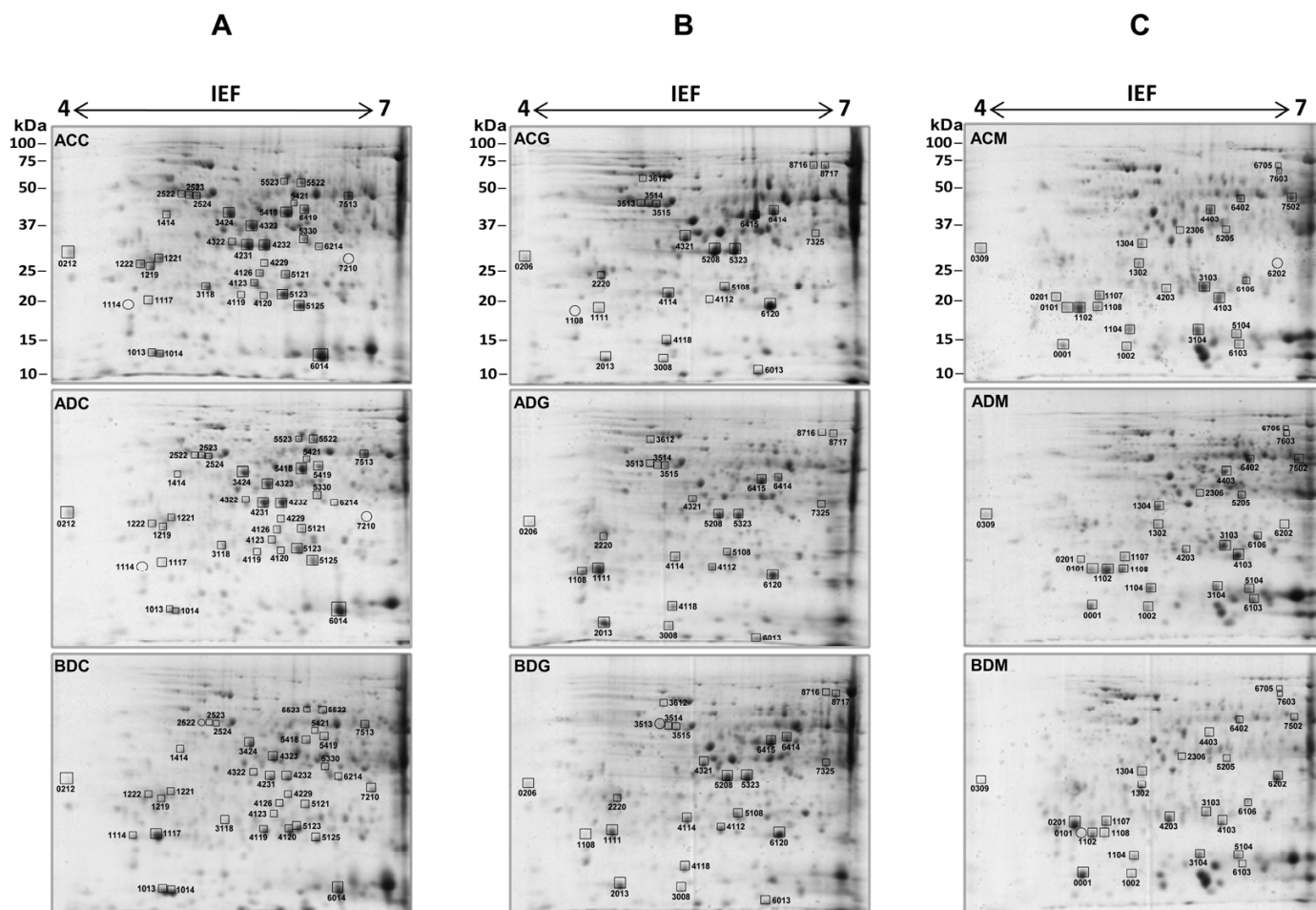
**Image analysis.** Digitized images at 36.6  $\mu\text{m}$  resolution were obtained using the GS-800 scanner and Quantity One 4.6.2 software (Bio-Rad). Computerized 2D gel analysis, including spot detection and quantification, was performed using the PDQuest Basic 8.0.1 software (Bio-Rad). The relative molecular mass was calibrated with internal protein markers (Precision Plus Protein Standards, Bio-Rad) after co-migration during the 2nd dimension. Quantification of detected protein spots was performed by calculating the relative optical density (OD)  $\times$  area in the gels. Normalization was set up according to the total spot density. Three different image analyses (one for each cultivar) were performed. Since three biological repetitions per group were considered for the 2-DE approach, a total of nine gel images per cultivar was included in each analysis. Protein spots detected in at least two biological repetitions of a given group were considered for analysis and compared in all the groups. Among the differentially expressed protein spots, 36 from Chardonnay, 24 from Gewurztraminer, and 26 from Mourvèdre (Fig. 1) were subjected to in-gel trypsin digestion followed by nanoLC-MS/MS analysis.

The mean relative OD  $\times$  area  $\pm$  standard deviation (SD) ( $n = 3$ ) values of each group were finally used to estimate relative expression level (relative OD  $\times$  area %) of each protein spot among the groups. Differences among the means were evaluated by Dunn's multiple comparison test and after that the null hypothesis (equal means) was rejected in the Kruskal-Wallis test, assuming a sig-

nificance of  $P \leq 0.05$ . The relative expression ratio to the related control (ACC, ACG, or ACM) (Table 1) in the other groups was also estimated. Values  $\geq 2$  were discussed.

**Protein identification by mass spectrometry.** Protein spots of interest were excised manually and submitted to in-gel digestion. Reduction, alkylation, and tryptic in-gel digestion were performed as previously described (55). Tryptic digests were analyzed by C18 reversed phase nanoHPLC on a nanoHPLC-Chip/MS system (Agilent Technologies, Palo Alto, CA) coupled to an ion trap amaZon (Bruker Daltonics, Bremen, Germany) mass spectrometer. For tandem MS experiments, the system was operated in the data-dependent mode using 6 MS/MS events. The complete system was fully controlled by Hystar 3.2 (Bruker Daltonics).

Mass data collected during nanoLC-MS/MS were processed, converted into ".mgf" files with DataAnalysis 4.0 (Bruker Daltonics), and interpreted using the MASCOT 2.3.02 algorithm (Matrix Science, London, UK) and Open Mass Spectrometry Search Algorithm (OMSSA). Searches were performed without any molecular weight or isoelectric point restrictions against an in-house generated protein database composed of protein sequences of the genus *Vitis*, human keratins, and trypsin, downloaded from National Center for Biotechnology Information nonredundant database (NCBI, June 05, 2012) concatenated with reversed copies of all sequences (total 138,416 entries). Database searching was carried out by using the following parameters: two missed cleavages; a parent and fragment mass tolerance



**Fig. 1.** Map of the identified protein spots quantitatively differentially expressed in the asymptomatic trunk wood of control (AC) or diseased (AD) plants and in the brown striped one (BD) of diseased plants in each analysis: **A**, Chardonnay (ACC, ADC, and BDC), **B**, Gewurztraminer (ACG, ADG, and BDG), and **C**, Mourvèdre (ACM, ADM, and BDM). Isoelectric focusing (IEF) was performed on precast dry polyacrylamide 7 cm length gels ReadyStrip IPG (pH 4 to 7). The relative molecular mass (kDa) was calibrated with standard protein markers (Prestained SDS-PAGE Standards, Bio-Rad) after co-second dimensional electrophoresis. Only spots detected in at least two biological replicates were chosen for identification (indicated with a square). Spots that were not detected in any gel of the same group are indicated with a circle.

of  $\pm 0.25$  Da; carbamidomethyl, N-terminal acetylation, oxidized methionine as variable modifications.

Mascot and OMSSA results were loaded into the Scaffold 3 software (Proteome Software, Portland, OR). To minimize false positive identifications, results were subjected to very stringent Mascot and OMSSA filtering criteria as follows: (i) for the identification of proteins, all peptides are validated with both algorithms (Mascot and OMSSA); (ii) for proteins identified with two peptides or more, OMSSA:  $-\text{Log}(E\text{-value})$  scores are greater

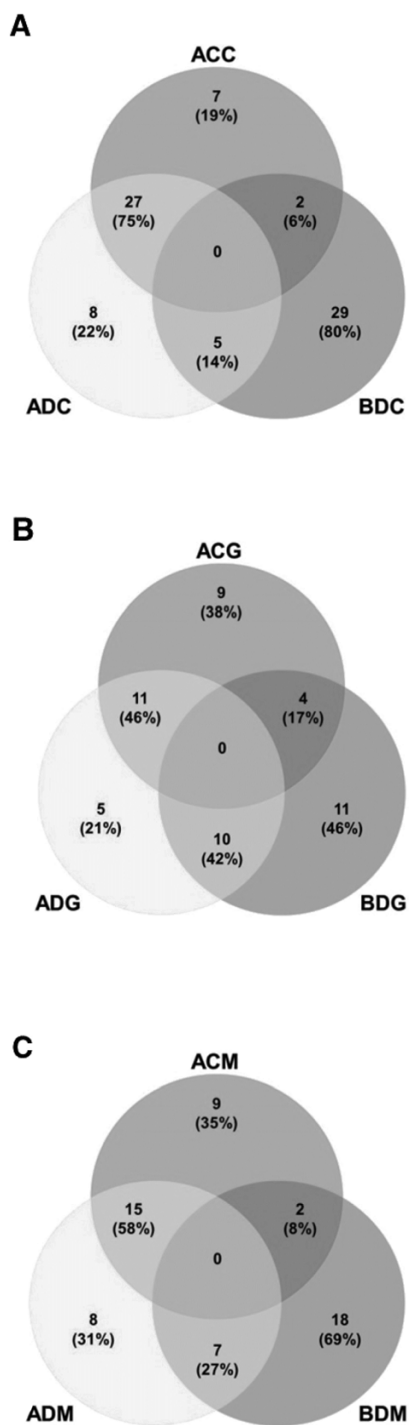
than 8.5, and Mascot: ion scores must be greater than both the associated identity scores (the 95% Mascot significance threshold) and 30; (iii) in the case of single peptide hits, OMSSA:  $-\text{Log}(e\text{-value})$  scores are higher than 9, and for Mascot: ion minus identity scores greater than 5 and unique peptide ion scores greater than 30. The target-decoy database search allowed control and estimated the false positive identification rate of our study (19). Thus, the final catalogue of proteins presents an estimated false positive rate below 1%. A list of all identified proteins with Mascot and OMSSA is provided in Supplemental Tables 1, 2, and 3.

**Functional classification of identified proteins.** A functional classification of the identified proteins was performed by using GenomeNet Database Resources (<http://www.genome.jp/kegg>) or according to their role described in the literature. Highest percentages of similar protein spot expression between two groups of samples were observed in the three cultivars when the asymptomatic wood from control and diseased vines was considered (Fig. 2); these values were 46% (Gewurztraminer), 58% (Mourvèdre), and 75% (Chardonnay). Most of the differences of expression among the spots from each cultivar selected for identification were quantitative, thus more or less abundant depending on the group of sample. However, some qualitative differences (presence/absence) were also observed such as for spots s1114, s2522, and s7210 in Chardonnay, s1108 and s3513 in Gewurztraminer, and s0101 and s6202 in Mourvèdre (Fig. 1).

**RNA extraction and real-time reverse-transcripts polymerase chain reaction (RT-PCR) analysis of gene expression.** Total RNA was isolated from woody samples using a  $\beta$ -mercaptoethanol- and sodium azide-based protocol according to Magnin-Robert et al. (42). In total, 150 ng of total RNA was reverse-transcribed using the Verso SYBR 2-step QRT ROX enzyme (ABgene, Surrey, UK) according to the manufacturer's protocol. PCR conditions were those described by Bézier et al. (7). Expression of 15 selected genes selected from proteomic results was tracked by quantitative RT-PCR using the primers reported in Supplemental Table 4, including the  $\alpha$ -chain elongation factor 1 gene (*EF1- $\alpha$* ) and ubiquitin carrier protein E2 (*UBE2*), which were used as the internal standard to normalize the starting template of cDNA. Reactions were carried out in a real-time PCR detector Chromo 4 apparatus (Bio-Rad) using the following thermal profile: 15 s at 95°C (denaturation) and 1 min at 60°C (annealing/extension) for 40 cycles. Melting curve assay was performed from 65 to 95°C at 0.5°C/s. Melting peaks were visualized for checking the specificity of each amplification. Results correspond to the means of the independent experiments. They were expressed relatively to the control corresponding to a fixed value of 1. Control samples consisted of asymptomatic trunk wood from control (AC) for each cultivar. The analyzed genes were considered significantly up- or down-regulated when changes of their expression was  $>2\times$  or  $<0.5\times$ , respectively.

**Extraction of plant phenolic compounds.** Methanolic extracts were prepared from 50 mg of powdered woody tissues mixed with 1 ml of methanol (MeOH) and 25  $\mu\text{l}$  of the internal standard trans-4-hydroxystilbene (0.5 mg  $\text{ml}^{-1}$ ). The mixture was shaken for 1 h at 40°C under 150 rpm in dark condition before two centrifugations at  $13,000 \times g$  for 5 min. The supernatants were stored at  $-20^\circ\text{C}$  until analysis.

**Quantification of plant total phenolic compounds.** Total phenolics were determined by using the Folin-Ciocalteu method (54) downscaled to 96-well plate (E. Abou-Mansour, *personal communication*). An aliquot (30  $\mu\text{l}$ ) of appropriate dilution of methanolic extracts (1:20, vol/vol) was mixed with 150  $\mu\text{l}$  of Folin-Ciocalteu reagent (diluted by 10) and after 5 min of incubation at room temperature, 120  $\mu\text{l}$  of sodium carbonate solution (10% wt/vol) was added. After incubation at room temperature for 2 h, absorbance of the mixture was read against the prepared blank at 750 nm. Total phenolics were expressed as

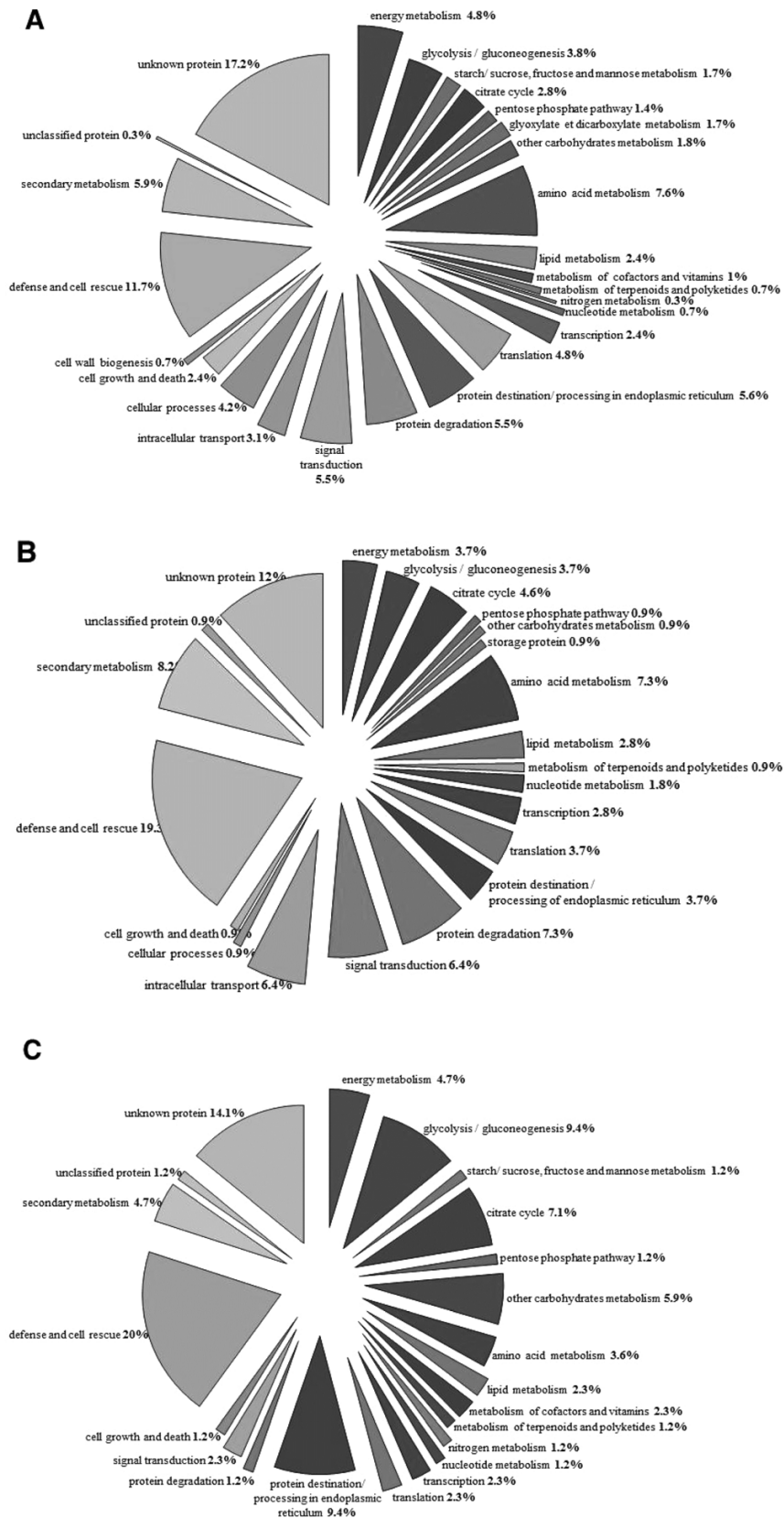


**Fig. 2.** Venn diagrams showing number and percentage of protein spots with similar expression ( $|\text{ratio of relative OD} \times \text{area\%}| < 2$ ,  $p < 0.05$ ) in the asymptomatic trunk wood of control (AC) or diseased (AD) plants and in the brown striped one (BD) of diseased plants in each analysis: cultivars **A**, Chardonnay (ACC, ADC, and BDC), **B**, Gewurztraminer (ACG, ADG, and BDG), and **C**, Mourvèdre (ACM, ADM, and BDM).

milligram of gallic acid equivalents (GAE) per gram of plant tissues.

**Quantification of stilbenes.** Standards such as *trans*-piceid, *trans*-resveratrol, and *trans*-pterostilbene were purchased from

Extrasynthèse (Genay, France) (Supplemental Figure 2). The *trans*-piceatannol, *trans*-*ε*-viniferin, and *trans*-vitisins A and B were extracted from lignified canes of Syrah (175 g) homogenized in 1 liter of MeOH-H<sub>2</sub>O (7:3, vol/vol) for 1 h at 40°C. After



**Fig. 3.** Functional classification of total proteins identified from cultivars **A**, Chardonnay, **B**, Gewurztraminer, and **C**, Mourvèdre using the GenomeNet Database Resources website (<http://www.genome.jp/kegg>) and reports in the literature.

filtration, MeOH was evaporated under vacuum and the aqueous residue adjusted to 100 ml with distilled water and extracted three times with 100 ml of hexane and then three times with 100 ml of ethyl acetate (EtOAc). Hexane extract was discarded and EtOAc was evaporated and further purified by HPLC on a semi-preparative Ascentis C18 column (15 cm × 10 mm) 5 μm (Supelco, Bellefonte, PA). The structures were confirmed by <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) and ESI-MSn in accordance with Lin et al. (40) for *trans-ε*-viniferin, and Ito et al. (30) for *trans*-vitisins A and B. Stilbenes analysis was performed by HPLC-DAD. The separation was achieved on MN Nucleosil C-18 column 5 μm (250 × 4 mm). The mobile phase consisted of water/formic acid (0.5%) (solvent A) and acetonitrile (solvent B). The linear gradient started with 5% of B for 5 min and increased to 55% within 25 min, reaching 80% at 28 min and 100% at 32 min. The flow rate was 0.7 ml min<sup>-1</sup> for the injection of 60 μl. Spectral data for all peaks were accumulated in the range between 220 and 600 nm. Chromatograms were recorded at 320 nm for quantification; all samples were injected three times. Stilbenes quantification was achieved by correlating the area

ratios of compounds versus the corresponding ratios of internal standard.

## RESULTS

**Detection of Botryosphaeria dieback agents within the brown striped wood.** Results of biological isolation showed that Botryosphaeriaceae species likely are abundantly present in the brown striped wood (Supplemental Table 5). Although in lesser extent, these fungi (especially *Diplodia seriata*) as well as other fungal species not involved in grapevine trunk diseases were also isolated from the apparently healthy wood of diseased plants. On the contrary, no fungi were isolated from the wood of control plants wherein brown stripe was not detected.

**Differences in protein abundance between asymptomatic and brown striped wood.** The nanoLC-MS/MS analysis allowed the identification of 290, 109, and 85 single protein species (53) for 'Chardonnay', 'Gewurztraminer', and 'Mourvèdre', respectively. In most cases, more than one protein was identified in the same spot. Most of the identified proteins belonged to categories

TABLE 2. Identified proteins differentially expressed in the asymptomatic trunk wood of control (AC) and diseased (AD) plants and in the brown striped one of diseased (BD) plants of cultivar Chardonnay (C)

Spot <sup>t</sup>	Ratio to ACC <sup>u</sup>		Matched protein <sup>v</sup>	Accession number <sup>w</sup>	Mw <sup>x</sup>	Coverage % <sup>y</sup>	Category <sup>z</sup>
	ADC	BDC					
1114	---	*	Osmotin-like protein ( <i>Vitis vinifera</i> )	gi 1839046	23.86	26.70	Defense and cell rescue
1114	---	*	Thaumatococin-like protein ( <i>V. vinifera</i> )	gi 33329390	23.86	26.70	Defense and cell rescue
7210	---	*	GEM-like protein 5 ( <i>V. vinifera</i> )	gi 225468805	31.54	4.21	Transcription
7210	---	*	Glucan endo-1,3-beta-glucosidase ( <i>V. vinifera</i> )	gi 225441373	36.66	37.10	Defense and cell rescue
1117	0.9	24.8	2-Cys peroxiredoxin ( <i>V. vinifera</i> )	gi 147789752	30.19	41.80	Defense and cell rescue
1117	0.9	24.8	Thaumatococin-like protein ( <i>V. vinifera</i> )	gi 8980665	24.35	11.90	Defense and cell rescue
1117	0.9	24.8	VVTL1 ( <i>V. vinifera</i> )	gi 2213852	23.95	50.50	Defense and cell rescue
4119	1.2	11.6	Miraculin ( <i>V. vinifera</i> )	gi 147828196	22.42	19.20	Defense and cell rescue
4119	1.2	11.6	Glutathione S-transferase 5 ( <i>V. vinifera</i> )	gi 158323772	24.85	54.60	Defense and cell rescue
4119	1.2	11.6	Glutathione S-transferase F9 ( <i>V. vinifera</i> )	gi 225446791	24.89	54.60	Defense and cell rescue
4119	1.2	11.6	Pathogenesis-related protein 17 ( <i>Vitis pseudoreticulata</i> )	gi 147784683	25.31	26.10	Defense and cell rescue
4229	1.0	6.3	Hypersensitive-induced response protein 1 isoform 3 ( <i>V. vinifera</i> )	gi 225456672	32.07	55.30	Defense and cell rescue
1013	1.0	4.1	Polyphenol oxidase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 147811887	66.97	9.15	Secondary metabolism
1222	1.0	2.1	14-3-3 protein ( <i>V. vinifera</i> )	gi 359492889	29.33	57.30	Signal transduction
4120	2.0	14.6	Stem-specific protein TSJT1 ( <i>V. vinifera</i> )	gi 225432548	25.23	41.90	Defense and cell rescue
7513	8.2	0.2	Glutamate decarboxylase 1 ( <i>V. vinifera</i> )	gi 225446257	55.21	15.20	Amino acid metabolism
7513	8.2	0.2	Enolase ( <i>V. vinifera</i> )	gi 225455555	48.09	52.10	Glycolysis/gluconeogenesis
4322	3.0	1.7	60S acidic ribosomal protein P0 ( <i>V. vinifera</i> )	gi 225449110	34.26	26.60	Translation
0212	2.3	0.6	Uncharacterized protein LOC100232885 ( <i>V. vinifera</i> )	gi 225447003	18.40	75.70	Unknown protein
6214	2.2	1.9	TPA: isoflavone reductase-like protein 5 ( <i>V. vinifera</i> )	gi 76559894	33.87	48.00	Secondary metabolism
5523	1.5	0.5	T-complex protein 1 subunit beta ( <i>V. vinifera</i> )	gi 225459806	57.31	27.10	Protein destination
5123	1.4	0.5	Actin-depolymerizing factor 1-like ( <i>V. vinifera</i> )	gi 225439733	16.01	18.70	Cell growth and death
5123	1.4	0.5	ATP-dependent Clp protease proteolytic subunit-related protein 4, chloroplastic-like ( <i>V. vinifera</i> )	gi 296085709	18.71	12.40	Protein degradation
5123	1.4	0.5	Proteasome subunit beta type-1 ( <i>V. vinifera</i> )	gi 225453909	24.61	10.80	Protein degradation
5123	1.4	0.5	Actin-depolymerizing factor 2-like isoform 1 ( <i>V. vinifera</i> )	gi 225449595	15.94	25.20	Cell growth and death
1221	0.9	0.5	Elongation factor 1-beta 1 ( <i>V. vinifera</i> )	gi 296083911	25.53	22.80	Translation
1221	0.9	0.5	Eukaryotic translation initiation factor 3 subunit F ( <i>V. vinifera</i> )	gi 147856131	38.91	33.30	Translation
4323	0.7	0.5	Glutamine synthetase nodule isozyme isoform 1 ( <i>V. vinifera</i> )	gi 147768273	39.13	42.90	Energy metabolism
4323	0.7	0.5	Peroxidase 12-like ( <i>V. vinifera</i> )	gi 359493149	39.17	8.06	Defense and cell rescue
4323	0.7	0.5	Probable protein disulfide-isomerase A6 isoform 1 ( <i>V. vinifera</i> )	gi 225450626	39.25	23.00	Protein processing in e.r.
3424	0.6	0.4	Actin-7 ( <i>V. vinifera</i> )	gi 225431585	41.71	69.20	Cell growth and death
1219	0.6	0.4	14-3-3 protein ( <i>V. vinifera</i> )	gi 226295432	28.63	14.30	Signal transduction
5522	0.6	0.4	2,3-bisphosphoglycerate-independent phosphoglycerate mutase isoform 1 ( <i>V. vinifera</i> )	gi 225439064	61.07	22.00	Glycolysis/gluconeogenesis
5522	0.6	0.4	Pyruvate decarboxylase isozyme 1 ( <i>V. vinifera</i> )	gi 225443847	62.41	26.20	Glycolysis/gluconeogenesis

(Continued on next page)

<sup>t</sup> Spot code as reported in Figure 1.

<sup>u</sup> Ratio of spot expression values (relative OD\*area%) in asymptomatic (ADC, asymptomatic diseased Chardonnay) and brown striped (BDC, brown diseased Chardonnay) wood of diseased plants to the related control (ACC, asymptomatic control Chardonnay). Values indicating over or down expression (ratio ≥ |2|) are highlighted in light or dark gray, respectively. Values were replaced by an asterisk or a dashed line when the spot was not detected in the control or in the sample from diseased plant, respectively.

<sup>v</sup> Protein identified via the MASCOT and OMSSA search engines against in house made database from NCBI database.

<sup>w</sup> Accession number of the matched protein as reported in the NCBI database.

<sup>x</sup> Molecular mass (kDa).

<sup>y</sup> Percentage of the protein sequence covered by the matching peptides.

<sup>z</sup> Functional category retrieved from GenomeNet Database Resources website (<http://www.genome.jp/kegg>) or in literature. e.r. indicates endoplasmic reticulum.

shared by the three cultivars. Nevertheless, proteins of the “glyoxylate and dicarboxylate metabolism” and the “cell wall biogenesis” were only identified in ‘Chardonnay’. No protein involved in “cellular processes” or “intracellular transport” was identified in ‘Mourvèdre’, while no protein belonging to the “starch/sucrose metabolism” or “metabolism of cofactors and vitamins” was identified in ‘Gewurztraminer’. The category “storage proteins” was solely found in ‘Gewurztraminer’. Apart from the proteins with unknown function (unknown protein), most proteins were involved in defense responses (“defense and cell rescue”) and represented 11.7, 19.3, and 20.0% of total proteins identified in ‘Chardonnay’, ‘Gewurztraminer’, and ‘Mourvèdre’, respectively (Fig. 3A, B, and C).

Considering the high number of total proteins identified, a selection was performed based on the following: (i) the identification percentage, (ii) the identification in two or three cultivars, and (iii) their known direct or indirect involvement in stress responses (Tables 2, 3, and 4). Differences of protein spot abundance between asymptomatic and brown striped wood were more marked in ‘Chardonnay’ and ‘Mourvèdre’ than in ‘Gewurztraminer’ (Fig. 2).

‘Chardonnay’. In ADC, a glutamate decarboxylase (s7513; “amino acid metabolism”), an enolase (s7513; “glycolysis/gluconeogenesis”), a 60S acidic ribosomal protein P0 (s4322; “translation”) and a TPA: isoflavone reductase-like protein 5 (s6214; “secondary metabolism”) were over accumulated (Table 2). Instead, a lower abundance was observed for four proteins: a DNA damage-inducible protein 1 (s1414; “protein processing in endoplasmic reticulum”) and a transaldolase isoform 1 (s1414; “glycolysis/ gluconeogenesis”), and two other proteins belonging to the “defense and cell rescue” category, namely a major allergen Pru av 1 and an MLP-like protein 28 (s6014). The latter two proteins were also down regulated in BDC (Table 2).

Nine out of the ten proteins more abundant in BDC (s1013, s1117, s4119, s4120, and s4229) belonged to the “defense and cell rescue” category (Table 2). Among them were a polyphenol oxidase (s1013), two thaumatin-like (s1117), two glutathione-S-transferases (GST; s4119), a pathogenesis-related protein 17 (PR-17; s4119) and a hypersensitive-response induced protein 1 isoform 3 (HRP1; s4229). In this sense, an up-regulation of the gene *HRP1* expression was also observed in BDC (Fig. 4). In contrast, no correlation was observed between 2-Cys peroxiredoxin

TABLE 2. (Continued from previous page)

Spot <sup>t</sup>	Ratio to ACC <sup>u</sup>		Matched protein <sup>v</sup>	Accession number <sup>w</sup>	Mw <sup>x</sup>	Coverage % <sup>y</sup>	Category <sup>z</sup>
	ADC	BDC					
5522	0.6	0.4	Glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform ( <i>V. vinifera</i> )	gi 225452196	59.14	4.65	Pentose phosphate pathway
5522	0.6	0.4	D-3-phosphoglycerate dehydrogenase, chloroplastic-like ( <i>V. vinifera</i> )	gi 225428898	62.54	45.40	Energy metabolism
3118	0.7	0.3	Triosephosphate isomerase, cytosolic isoform 1 ( <i>V. vinifera</i> )	gi 225434935	27.33	27.60	Glycolysis/ gluconeogenesis
3118	0.7	0.3	Proteasome subunit alpha type-2-B ( <i>V. vinifera</i> )	gi 225423722	25.57	24.70	Protein degradation
5125	1.4	0.3	26.5 kDa heat shock protein, mitochondrial ( <i>V. vinifera</i> )	gi 225442975	26.30	17.20	Protein destination/ processing in e.r.
5125	1.4	0.3	Flavoprotein wrba isoform 1 ( <i>V. vinifera</i> )	gi 225461209	21.72	42.40	Energy metabolism
5125	1.4	0.3	Manganese superoxide dismutase ( <i>V. vinifera</i> )	gi 161778782	25.27	11.80	Defense and cell rescue
5121	0.7	0.3	Triosephosphate isomerase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 225427917	34.66	52.20	Glycolysis/gluconeogenesis
5121	0.7	0.3	Cytosolic ascorbate peroxidase ( <i>V. vinifera</i> )	gi 161778778	27.98	17.40	Defense and cell rescue
5330	0.7	0.3	Cysteine synthase ( <i>V. vinifera</i> )	gi 359487832	36.52	5.56	Amino acid metabolism
5330	0.7	0.3	Glyoxylate reductase isoform 2 ( <i>V. vinifera</i> )	gi 297743258	34.29	10.90	Glyoxylate and dicarboxylate metabolism
5330	0.7	0.3	Malate dehydrogenase, cytoplasmic ( <i>V. vinifera</i> )	gi 225438145	35.49	11.10	Citrate cycle
5330	0.7	0.3	Malate dehydrogenase, mitochondrial ( <i>V. vinifera</i> )	gi 225461618	36.77	33.90	Citrate cycle
5330	0.7	0.3	Probable fructose-bisphosphate aldolase 3, chloroplastic ( <i>V. vinifera</i> )	gi 225424114	42.88	20.90	Glycolysis/gluconeogenesis
5330	0.7	0.3	Auxin-induced protein PCNT115 isoform 1 ( <i>V. vinifera</i> )	gi 225433674	37.52	18.80	Signal transduction
5330	0.7	0.3	Thioredoxin reductase 2-like ( <i>V. vinifera</i> )	gi 225431669	39.52	16.60	Nucleotide metabolism
5330	0.7	0.3	Malate dehydrogenase ( <i>V. vinifera</i> )	gi 225443845	36.78	49.10	Citrate cycle
5330	0.7	0.3	Malate dehydrogenase, chloroplastic ( <i>V. vinifera</i> )	gi 225457407	43.59	33.90	Citrate cycle
4232	1.3	0.2	TPA: isoflavone reductase-like protein 4 ( <i>V. vinifera</i> )	gi 76559892	33.82	52.30	Secondary metabolism
4126	1.0	0.2	Hydroxyacylglutathione hydrolase cytoplasmic ( <i>V. vinifera</i> )	gi 225448353	28.65	26.70	Other carbohydrates metabolism
1014	0.9	0.2	Polyphenol oxidase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 147811887	66.97	9.15	Secondary metabolism
2524	0.7	0.2	Mitochondrial-processing peptidase subunit alpha ( <i>V. vinifera</i> )	gi 225445041	54.45	31.20	Protein degradation
4231	0.6	0.2	Recname: Full=Chalcone--flavonone isomerase 2	gi 147843260	34.27	12.20	Translation
5419	0.5	0.2	Eukaryotic initiation factor 4A-3-like ( <i>V. vinifera</i> )	gi 225464928	46.43	37.10	Translation
5418	1.1	0.1	Recname: Full=S-adenosylmethionine synthase 5 Short=MAT 5	gi 223635289	42.78	64.20	Defense and cell rescue
5421	1.0	0.1	Formamidase isoform 1 ( <i>V. vinifera</i> )	gi 225438970	49.72	36.10	Glyoxylate and dicarboxylate metabolism
4123	0.8	0.1	Stem-specific protein TSJT1 ( <i>V. vinifera</i> )	gi 225461387	27.92	14.90	Defense and cell rescue
4123	0.8	0.1	Proteasome subunit beta type-6 ( <i>V. vinifera</i> )	gi 225445670	24.98	11.60	Protein degradation
4123	0.8	0.1	Recname: Full=Chalcone--flavonone isomerase 2	gi 158514257	25.12	20.90	Secondary metabolism
4123	0.8	0.1	L-ascorbate peroxidase 2, cytosolic ( <i>V. vinifera</i> )	gi 225435177	27.54	38.00	Other carbohydrates metabolism
6014	0.4	0.1	Major allergen Pru av 1 ( <i>V. vinifera</i> )	gi 225431844	17.11	15.80	Defense and cell rescue
6014	0.4	0.1	MLP-like protein 28 ( <i>V. vinifera</i> )	gi 225424272	17.18	43.00	Defense and cell rescue
2522	1.7	0.0	Clathrin light chain 2-like ( <i>V. vinifera</i> )	gi 147766743	34.92	20.10	Intracellular transport
2522	1.7	0.0	Hexokinase-2, chloroplastic ( <i>V. vinifera</i> )	gi 225457987	44.76	21.20	Glycolysis/gluconeogenesis
2522	1.7	0.0	Tubulin alpha chain ( <i>V. vinifera</i> )	gi 225429189	49.54	60.90	Intracellular transport
2523	1.7	0.0	Tubulin beta-1 chain ( <i>V. vinifera</i> )	gi 225426414	50.04	21.80	Intracellular transport
2523	1.7	0.0	ATP synthase subunit beta, mitochondrial-like ( <i>V. vinifera</i> )	gi 147838606	59.61	42.90	Energy metabolism
1414	0.1	1.5	DNA damage-inducible protein 1 ( <i>V. vinifera</i> )	gi 225462066	45.06	15.40	Protein processing in e.r.
1414	0.1	1.5	Transaldolase isoform 1 ( <i>V. vinifera</i> )	gi 225425280	48.09	12.70	Glycolysis/gluconeogenesis



(cysPEROX, s1117) gene expression and protein accumulation. Indeed, cysPEROX was over accumulated in BDC (Table 2) while the *cysPEROX* gene expression was similar to control (Fig. 4). Three proteins were detected in only BDC, namely an osmotin-like and a thaumatin-like (s1114), and a glucan endo-1,3-beta-glucosidase (endoglu; s7210) (Table 2). In this sense, a significant up-regulation (500-fold) for *endoglu* gene expression was also observed in BDC compared with ACC (Fig. 4). In addition to the high abundance of proteins associated to defense response, an accumulation of the glycosylated stilbenes, *tr*-piceids, the monomer *tr*-resveratrol, the dimer *tr*- $\epsilon$ -viniferin, and the tetramer *tr*-vitisin B was observed especially in BDC compared with ACC (Table 5).

The 51 proteins less abundant in BDC than in ACC and ADC were included in 19 functional categories (Table 2); the most represented were “defense and cell rescue” (seven proteins), “glycolysis/gluconeogenesis” (seven proteins) and “protein degradation” (five proteins). Five proteins (s2522 and 2523), namely a clathrin light chain 2-like (“cellular processes”), a hexokinase-2, chloroplastic (“glycolysis/gluconeogenesis”), a tubulin alpha chain (“intracellular transport”), a tubulin beta-1 chain (“intracellular transport”) and an ATP synthase subunit beta, mitochondrial-like (“energy metabolism”) were detected in ACC and ADC but not in BDC. Among the 51 proteins were also a 14-3-3 protein (s1219; “signal transduction”), a glyoxylate reductase isoform 2 (glyxRed; s5330; “glyoxylate metabolism”), a hydroxyacylglutathione hydrolase cytoplasmic (GSHhyd; s4126; “other carbohydrates metabolism”), an auxin-induced protein PCNT115 (AUX115; s5330; “signal transduction”), and an isoflavone reductase-like protein 4 (IFRL4; s4232; “secondary metabolism”)

(Table 2). Correlating with the decreased abundance of the correspondent protein in BDC, a down-regulation of the gene expression *IFRL4* (threefold) was also therein observed (Fig. 4). Instead, no correlation between the protein abundance and the gene expression was observed for *glyxRed*, *GSHhyd*, and *AUX115*.

“*Gewurztraminer*”. In ADG, 15 proteins including a cytosolic ascorbate peroxidase (s5108; “defense and cell rescue”), a chalcone isomerase (CHI; s5108; “secondary metabolism”), and a proteasome subunit alpha type-2-B (s5108; “protein degradation”) were less abundant while a superoxide dismutase (SOD), [Cu-Zn] chloroplastic (s3008; “defense and cell rescue”) was more abundant (Table 3). No positive correlation was observed between gene expression and protein accumulation in ADG. In fact, CHI protein accumulation was lower in ADG than in ACC, while *CHI* expression was slightly up-regulated (Fig. 4).

In BDG, 13 out of the 26 proteins were more accumulated (s1108, s1111, s2013, s3612, s4112, and s7325) belonged to the “defense and cell rescue” or “secondary metabolism” categories (Table 3). Except for an elicitor-responsive protein 1 and a polyphenol oxidase (s2013), the other 11 proteins were also more abundant in ADG, although in a lesser extent. A peroxidase 4 (POX4; s7325), a hypothetical protein homolog to cysPEROX (s1111), three thaumatin-like (s1108 and s1111), a major allergen Pru av 1 (s7325), and an anthocyanidin reductase (ANR; s7325) were among them. *POX4* expression was induced in ADG (15-fold) and in BDG (100-fold) (Fig. 4) and correlated positively with the protein abundance in the two samples (Table 3). No correlation was observed between protein abundance and gene expression in the case of cysPEROX and ANR (Table 3, Fig. 4). The remaining four proteins were identified in s4112 (Table 3): a

TABLE 3. Identified proteins differentially expressed in the asymptomatic trunk wood of control (AC) and diseased (AD) plants and in the brown striped one of diseased (BD) plants of cultivar Gewurztraminer (G)

Spot <sup>1</sup>	Ratio to ACG <sup>2</sup>		Matched protein <sup>v</sup>	Accession number <sup>w</sup>	Mw <sup>x</sup>	Coverage % <sup>y</sup>	Category <sup>z</sup>
	ADG	BDG					
1108	*	*	Thaumatococcoside synthase ( <i>Vitis vinifera</i> )	gi 33329390	23.86	26.70	Defense and cell rescue
1108	*	*	VVTL1 ( <i>V. vinifera</i> )	gi 2213852	23.95	42.80	Defense and cell rescue
4112	12.0	40.3	Endo-1,3;1,4-beta-D-glucanase ( <i>V. vinifera</i> )	gi 225436938	26.08	5.02	Cell growth and death
4112	12.0	40.3	Pathogenesis-related protein 17 ( <i>Vitis pseudoreticulata</i> )	gi 374431273	25.26	40.70	Defense and cell rescue
4112	12.0	40.3	Miraculin ( <i>V. vinifera</i> )	gi 147828196	22.42	17.20	Defense and cell rescue
4112	12.0	40.3	Glutathione S-transferase 5 ( <i>V. vinifera</i> )	gi 158323772	24.85	52.30	Defense and cell rescue
4112	12.0	40.3	Stem-specific protein TSJT1 ( <i>V. vinifera</i> )	gi 225432548	25.23	42.80	Defense and cell rescue
4112	12.0	40.3	Proteasome subunit beta type-6 ( <i>V. vinifera</i> )	gi 225445670	24.98	14.20	Protein degradation
1111	13.2	31.8	RNA-binding protein 8A ( <i>V. vinifera</i> )	gi 225431497	22.29	15.30	Transcription
1111	13.2	31.8	Hypothetical protein VITISV_025619 ( <i>V. vinifera</i> ) 2-Cys peroxidase ( <i>V. vinifera</i> )	gi 147789752	30.16	22.00	Defense and cell rescue
1111	13.2	31.8	Thaumatococcoside synthase ( <i>V. vinifera</i> )	gi 8980665	24.35	11.90	Defense and cell rescue
7325	3.8	12.9	Malate dehydrogenase, mitochondrial ( <i>V. vinifera</i> )	gi 225461618	36.77	23.90	Citrate cycle
7325	3.8	12.9	Probable fructose-bisphosphate aldolase 3, chloroplastic ( <i>V. vinifera</i> )	gi 225424114	42.88	17.80	Glycolysis/gluconeogenesis
7325	3.8	12.9	Auxin-induced protein PCNT115 isoform 1 ( <i>V. vinifera</i> )	gi 225433674	37.52	31.10	Signal transduction
7325	3.8	12.9	Thioredoxin reductase 2-like ( <i>V. vinifera</i> )	gi 225431669	39.52	15.50	Nucleotide metabolism
7325	3.8	12.9	Malate dehydrogenase ( <i>V. vinifera</i> )	gi 225443845	36.86	23.60	Citrate cycle
7325	3.8	12.9	Major allergen Pru av 1 ( <i>V. vinifera</i> )	gi 225431844	17.11	9.49	Defense and cell rescue
7325	3.8	12.9	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic ( <i>V. vinifera</i> )	gi 297733609	37.43	29.20	Glycolysis/gluconeogenesis
7325	3.8	12.9	Recname: Full=Peroxidase 4; Flags: Precursor ( <i>V. vinifera</i> )	gi 223635590	34.04	7.48	Defense and cell rescue
7325	3.8	12.9	Succinyl-coa ligase (ADP-forming) subunit alpha-1, mitochondrial-like ( <i>V. vinifera</i> )	gi 225457502	34.86	14.90	Citrate cycle
7325	3.8	12.9	Anthocyanidin reductase ( <i>V. vinifera</i> )	gi 359496568	36.71	17.80	Secondary metabolism

(Continued on next page)

<sup>1</sup> Spot code as reported in Figure 1.

<sup>2</sup> Ratio of spot expression values (relative OD\*area%) in asymptomatic (ADG, asymptomatic diseased Gewurztraminer) and brown striped (BDG, brown diseased Gewurztraminer) wood of diseased plants to the related control (ACG, asymptomatic control Gewurztraminer). Values indicating over or down expression (ratio  $\geq |2|$ ) are highlighted in light or dark gray, respectively. Values were replaced by an asterisk or a dashed line when the spot was not detected in the control or in the sample from diseased plant, respectively.

<sup>v</sup> Protein identified via the MASCOT and OMSSA search engines against in house made database from NCBI database.

<sup>w</sup> Accession number of the matched protein as reported in the NCBI database.

<sup>x</sup> Molecular mass (kDa).

<sup>y</sup> Percentage of the protein sequence covered by the matching peptides.

<sup>z</sup> Functional category retrieved from GenomeNet Database Resources website (<http://www.genome.jp/kegg>) or in literature.

miraculin, a GST5 and a PR-17 and a stem-specific protein TSJT1. Phenolic and mono-, di-, and tetramer stilbenic compounds were also more abundant in diseased (ADG, ABG) than in control plants, only the glucosylated piceid being less abundant (Table 5). Moreover, stilbenic compounds were only detected in the diseased plants (Table 5). Additionally, a malate dehydrogenase (s7325; “citrate cycle”), a AUX115 (s7325; “signal transduction”), and an endo-1,3;1,4-beta-D-glucanase (s4112; “cell growth and death”) were identified (Table 3). No correlation between *AUX115* gene expression and AUX115 protein level was revealed (Fig. 4).

Twenty-three proteins were less abundant in BDG (s2220, s3513, s3514, s3515, s4114, s4118, s4321, s5208, s5323, s6013, s6120, s6414, and s6415) (Table 3). These proteins included the following: a 14-3-3 protein (s2220; “signal transduction”), an ATP synthase subunit beta, a mitochondrial-like (s3515; “energy metabolism”), a manganese superoxide dismutase (MnSOD; s6120; “defense and cell rescue”), an IFRL4 (s5208; “secondary metabolism”), and a 26.5 kDa heat shock protein (HSP) mitochondrial (s6120; “protein destination”). A positive correlation was observed between *IFRL4* gene expression and the abundance of IFRL4 in the woody tissues of diseased plants (Fig. 4 and Table 3). Moreover, transcript analysis revealed no significant perturbation of *MnSOD* expression in tested sample (Fig. 4), while a decline in the abundance of this protein in the same sample was recorded. An hsp70-binding protein 1-like (s3513; “protein destination”) was not detected in BDG. Some of these proteins were less accumulated also in ADG, e.g., a malate

dehydrogenase, cytoplasmic (s4321; “citrate cycle”), an S-adenosylmethionine synthase 5 (SAMS; s6415; “defense and cell rescue”), and a Caffeoyle-CoA O-methyltransferase (CCoAOMT; s4114; “secondary metabolism”) (Table 3). The expression of the gene encoding for CCoAOMT was down-regulated in ADG (2-fold) and BDG (2.4-fold) in comparison to ACC (Fig. 4), which confirmed the decline of protein abundance.

**‘Mourvèdre’.** In ADM, some proteins such as an MLP-like protein 28 (s6103; “defense and cell rescue”), a major allergen Pru av 1 (s6103; “defense and cell rescue”), and a 18.2 kDa class I HSP (s5104; “protein destination”) were more abundant (s5104, s5205, s6103, and s6402) (Table 4).

In BDM, over regulation was observed for 14 proteins (s0001, s0201, s1302, s2306, s4203, s6202, and s6705) (Table 4). Five out of the fourteen proteins belonged to the “defense and cell rescue” category (Table 4) and among them were two thaumatin-like (s0201), a cysPEROX (s0201), and two PR-17 (s4203). Eleven out of these fourteen proteins (s0201, s2306, and s4203) were also more accumulated in ADM but with different abundance compared with BDM. Among them were a probable nitronate monooxygenase (s2306; “energy metabolism”), a GSTF9 (s4203; “defense and cell rescue”), and two PR proteins (s4203; “defense and cell rescue”). As observed in ‘Chardonnay’ and ‘Gewurztraminer’, no correlation between *cysPEROX* gene expression and accumulation of the corresponding protein was noted (Fig. 4, Table 4). Three proteins were detected in only ADM and BDM (s6202): a pyridoxal kinase-like (“metabolism of cofactors and vitamins”), a 1,3 beta glucanase (“cell growth and death”), and an

TABLE 3. (Continued from previous page)

Spot <sup>t</sup>	Ratio to ACG <sup>u</sup>		Matched protein <sup>v</sup>	Accession number <sup>w</sup>	Mw <sup>x</sup>	Coverage % <sup>y</sup>	Category <sup>z</sup>
	ADG	BDG					
7325	3.8	12.9	Fructose-bisphosphate aldolase cytoplasmic isozyme ( <i>V. vinifera</i> )	gi 225440976	38.61	24.90	Glycolysis/gluconeogenesis
7325	3.8	12.9	UPF0160 protein MYG1, mitochondrial-like ( <i>V. vinifera</i> )	gi 225426621	38.25	35.50	Unclassified protein
3612	2.0	3.2	NAD-dependent malic enzyme 62 kDa isoform, mitochondrial ( <i>V. vinifera</i> )	gi 225453250	69.32	6.08	Pyruvate metabolism
2013	1.3	2.8	Polyphenol oxidase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 147811887	66.97	6.32	Secondary metabolism
2013	1.3	2.8	Elicitor-responsive protein 1 ( <i>V. vinifera</i> )	gi 225449489	17.41	14.90	Defense and cell rescue
3008	19.6	0.3	Superoxide dismutase (Cu-Zn), chloroplastic ( <i>V. vinifera</i> )	gi 147789545	21.69	46.70	Defense and cell rescue
0206	0.3	1.2	Uncharacterized protein LOC100232885 ( <i>V. vinifera</i> )	gi 225447003	18.40	81.50	Unknown protein
5108	0.5	1.0	Proteasome subunit alpha type-2-B ( <i>V. vinifera</i> )	gi 225423722	25.57	42.60	Protein degradation
5108	0.5	1.0	Recname: Full=Chalcone--flavonone isomerase 2	gi 158514257	25.06	36.30	Secondary metabolism
5108	0.5	1.0	Triosephosphate isomerase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 225427917	34.66	34.90	Glycolysis/gluconeogenesis
5108	0.5	1.0	Cytosolic ascorbate peroxidase ( <i>V. vinifera</i> )	gi 161778778	27.98	12.60	Defense and cell rescue
6120	1.0	0.5	26.5 kDa heat shock protein, mitochondrial ( <i>V. vinifera</i> )	gi 225442975	26.30	8.58	Protein destination
6120	1.0	0.5	Flavoprotein wrba isoform 1 ( <i>V. vinifera</i> )	gi 225461209	21.72	37.90	Energy metabolism
6120	1.0	0.5	Manganese superoxide dismutase ( <i>V. vinifera</i> )	gi 161778782	25.27	6.58	Defense and cell rescue
6120	1.0	0.5	Putative transcription factor ( <i>V. vinifera</i> )	gi 14582465	16.68	14.10	Transcription
6013	0.7	0.5	Superoxide dismutase (Cu-Zn) isoform 2 ( <i>V. vinifera</i> )	gi 225451120	15.26	29.60	Defense and cell rescue
6013	0.7	0.5	Glycine-rich RNA-binding protein GRP1A-like ( <i>V. vinifera</i> )	gi 359475330	16.31	54.90	Defense and cell rescue
8716	0.4	0.5	Transketolase, chloroplastic-like ( <i>V. vinifera</i> )	gi 359490179	78.84	25.00	Pentose phosphate pathway
2220	0.8	0.4	14-3-3 protein ( <i>V. vinifera</i> )	gi 226295432	28.63	10.70	Signal transduction
4118	0.8	0.4	Hypothetical protein VITISV_023716 ( <i>V. vinifera</i> ) type II peroxiredoxin C ( <i>V. vinifera</i> )	gi 147781540	17.24	64.20	Defense and cell rescue
4118	0.8	0.4	Actin-depolymerizing factor 2-like isoform 1 ( <i>V. vinifera</i> )	gi 225449595	15.94	16.50	Cell growth and death
5323	0.7	0.4	Dihydroflavonol-4-reductase ( <i>V. vinifera</i> )	gi 147805693	35.76	3.68	Secondary metabolism
3515	1.0	0.3	ATP synthase subunit beta, mitochondrial-like ( <i>V. vinifera</i> )	gi 147838606	59.91	18.20	Energy metabolism
3515	1.0	0.3	Mitochondrial-processing peptidase subunit alpha ( <i>V. vinifera</i> )	gi 225445041	54.45	26.50	Protein degradation
5208	0.6	0.3	TPA: isoflavone reductase-like protein 4 ( <i>V. vinifera</i> )	gi 76559892	33.82	70.50	Secondary metabolism
6414	0.5	0.3	Eukaryotic initiation factor 4A-3-like ( <i>V. vinifera</i> )	gi 225464928	46.43	32.00	Translation
6415	0.5	0.3	Recname: Full=S-adenosylmethionine synthase 5 Short=MAT 5	gi 223635289	42.78	67.00	Defense and cell rescue
6415	0.5	0.3	Glutamine synthetase cytosolic isozyme 2 ( <i>V. vinifera</i> )	gi 1707959	39.30	4.78	Amino acid metabolism
4321	0.4	0.3	Glutelin type-A 1 ( <i>V. vinifera</i> )	gi 225435090	38.31	31.20	Storage protein
4321	0.4	0.3	Adenosine kinase 2 ( <i>V. vinifera</i> )	gi 225449018	37.72	67.40	Nucleotide metabolism
4321	0.4	0.3	Cysteine synthase isoform 2 ( <i>V. vinifera</i> )	gi 225451235	34.35	30.50	Amino acid metabolism
4321	0.4	0.3	Malate dehydrogenase, cytoplasmic ( <i>V. vinifera</i> )	gi 225438145	35.49	6.33	Citrate cycle
4114	0.3	0.3	Recname: Full=Caffeoyle-coa O-methyltransferase ( <i>V. vinifera</i> )	gi 225428851	27.22	59.10	Secondary metabolism
3514	1.0	0.2	Tubulin alpha chain ( <i>V. vinifera</i> )	gi 225429189	49.54	54.70	Intracellular transport
8717	0.5	0.2	Transketolase, chloroplastic-like ( <i>V. vinifera</i> )	gi 359490179	78.84	32.40	Pentose phosphate pathway
3513	0.9	---	Hsp70-binding protein 1-like ( <i>V. vinifera</i> )	gi 225440422	43.30	21.70	Protein destination

TABLE 4. Identified proteins differentially expressed in the asymptomatic trunk wood of control (AC) and diseased (AD) plants and in the brown striped one of diseased (BD) plants of cultivar Mourvèdre (M)

Spot <sup>1</sup>	Ratio to ACM <sup>a</sup>		Matched protein <sup>v</sup>	Accession number <sup>w</sup>	Mw <sup>x</sup>	Coverage % <sup>y</sup>	Category <sup>z</sup>
	ADM	BDM					
6202	*	*	Pyridoxal kinase-like ( <i>Vitis vinifera</i> )	gi 296087176	37.76	14.00	Metabolism of cofactors and vitamins
6202	*	*	1,3 beta glucanase ( <i>V. vinifera</i> )	gi 6273716	13.35	91.80	Cell growth and death
6202	*	*	Glucan endo-1,3-beta-glucosidase ( <i>V. vinifera</i> )	gi 225441373	36.66	51.50	Defense and cell rescue
4203	47.7	30.5	Glutathione S-transferase F9 ( <i>V. vinifera</i> )	gi 225446791	24.89	63.40	Defense and cell rescue
4203	47.7	30.5	( <i>Vitis pseudoreticulata</i> )	gi 147784683	25.31	37.60	Defense and cell rescue
4203	47.7	30.5	Pathogenesis-related protein 17 ( <i>Vitis pseudoreticulata</i> )	gi 374431273	25.26	44.70	Defense and cell rescue
0201	2.3	25.8	2-Cys peroxidoxin ( <i>V. vinifera</i> )	gi 147789752	30.19	8.42	Defense and cell rescue
0201	2.3	25.8	Thaumatin-like protein ( <i>V. vinifera</i> )	gi 8980665	24.35	11.90	Defense and cell rescue
0201	2.3	25.8	VVTL1 ( <i>V. vinifera</i> )	gi 2213852	23.95	50.50	Defense and cell rescue
0201	2.3	25.8	RNA-binding protein 8A ( <i>V. vinifera</i> )	gi 225431497	22.29	7.92	Transcription
2306	6.0	12.0	Probable nitronate monoxygenase ( <i>V. vinifera</i> )	gi 225462874	36.53	56.60	Energy metabolism
0001	1.6	4.0	Polyphenol oxidase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 147811887	66.97	9.15	Secondary metabolism
6705	1.4	3.9	Transketolase, chloroplastic-like ( <i>V. vinifera</i> )	gi 359490179	78.84	30.00	Pentose phosphate pathway
1302	1.3	2.3	Chorismate mutase, chloroplastic ( <i>V. vinifera</i> )	gi 225452920	36.40	14.70	Amino acid metabolism
5205	7.6	1.8	Malate dehydrogenase, cytoplasmic ( <i>V. vinifera</i> )	gi 147783145	35.49	8.43	Citrate cycle
5205	7.6	1.8	Malate dehydrogenase, mitochondrial ( <i>V. vinifera</i> )	gi 225461618	36.77	15.10	Citrate cycle
5205	7.6	1.8	Probable fructose-bisphosphate aldolase 3, chloroplastic ( <i>V. vinifera</i> )	gi 225424114	42.88	9.67	Glycolysis/gluconeogenesis
5205	7.6	1.8	Putative fructokinase-5-like ( <i>V. vinifera</i> )	gi 225459906	34.92	72.30	Glycolysis/gluconeogenesis
5205	7.6	1.8	Malate dehydrogenase, chloroplastic ( <i>V. vinifera</i> )	gi 225457407	43.59	48.70	Citrate cycle
6402	2.0	1.1	Argininosuccinate synthase, chloroplastic-like ( <i>V. vinifera</i> )	gi 225456274	55.16	7.04	Amino acid metabolism
6402	2.0	1.1	Biotin carboxylase 1, chloroplastic-like ( <i>V. vinifera</i> )	gi 225445664	57.32	4.57	Lipid metabolism
5104	2.0	0.6	18.2 kDa class I heat shock protein ( <i>V. vinifera</i> )	gi 147785904	18.15	12.50	Protein destination
7603	0.8	0.5	NADP-dependent malic enzyme ( <i>V. vinifera</i> )	gi 225445108	65.26	8.80	Pyruvate metabolism
7603	0.8	0.5	Heat shock protein STI-like ( <i>V. vinifera</i> )	gi 359497489	36.32	42.30	Protein destination
7502	0.7	0.5	Atpase subunit 1 ( <i>V. vinifera</i> )	gi 164685652	44.27	8.47	Energy metabolism
7502	0.7	0.5	Uncharacterized aminotransferase y4ub-like ( <i>V. vinifera</i> )	gi 225464809	57.62	12.10	Signal transduction
7502	0.7	0.5	Elongation factor 1-alpha-like ( <i>V. vinifera</i> )	gi 225435233	49.32	22.10	Translation
1107	0.6	0.5	23.6 kDa heat shock protein, mitochondrial isoform 1 ( <i>V. vinifera</i> )	gi 225466111	23.72	36.10	Protein destination
6106	1.2	0.4	L-ascorbate peroxidase 2, cytosolic ( <i>V. vinifera</i> )	gi 225435177	27.54	52.80	Other carbohydrates metabolism
6106	1.2	0.4	Enolase ( <i>V. vinifera</i> )	gi 225455555	48.09	24.90	Glycolysis/gluconeogenesis
4103	0.9	0.4	26.5 kDa heat shock protein, mitochondrial ( <i>V. vinifera</i> )	gi 225442975	26.30	17.20	Protein destination
4103	0.9	0.4	Flavoprotein wrba isoform 1 ( <i>V. vinifera</i> )	gi 225461209	21.72	24.60	Energy metabolism
4103	0.9	0.4	Manganese superoxide dismutase ( <i>V. vinifera</i> )	gi 161778782	17.03	9.74	Defense and cell rescue
4103	0.9	0.4	Glutathione S-transferase F9 ( <i>V. vinifera</i> )	gi 225446793	24.95	13.50	Defense and cell rescue
1104	0.9	0.4	Peroxiredoxin-2B ( <i>V. vinifera</i> )	gi 225445188	17.24	39.50	Defense and cell rescue
3104	0.6	0.4	22.0 kDa heat shock protein ( <i>V. vinifera</i> )	gi 225459900	21.11	48.90	Protein destination
3104	0.6	0.4	Uncharacterized N-acetyltransferase p20 ( <i>V. vinifera</i> )	gi 225432712	20.18	27.20	Uncharacterized protein
6103	6.3	0.3	Major allergen Pru av 1 ( <i>V. vinifera</i> )	gi 225431844	17.11	17.70	Defense and cell rescue
6103	6.3	0.3	MLP-like protein 28 ( <i>V. vinifera</i> )	gi 225424272	17.18	58.30	Defense and cell rescue
1002	1.3	0.3	Regulator of ribonuclease-like protein 2 ( <i>V. vinifera</i> )	gi 225430941	17.75	13.30	Transcription
1002	1.3	0.3	18.2 kDa class I heat shock protein ( <i>V. vinifera</i> )	gi 225449290	17.03	15.90	Protein destination
1002	1.3	0.3	Superoxide dismutase (Cu-Zn), chloroplastic ( <i>V. vinifera</i> )	gi 147789545	21.69	41.50	Defense and cell rescue
4403	0.8	0.3	Recname: Full=S-adenosylmethionine synthase 5 Short=MAT 5	gi 223635289	42.77	64.20	Defense and cell rescue
1304	1.7	0.2	TPA: isoflavone reductase-like protein 4 ( <i>V. vinifera</i> )	gi 76559892	33.82	17.20	Secondary metabolism
0309	1.4	0.2	Uncharacterized protein LOC100232885 ( <i>V. vinifera</i> )	gi 225447003	18.40	66.50	Unknown protein
1304	1.7	0.2	Fructokinase-2 ( <i>V. vinifera</i> )	gi 225433918	35.18	8.48	Glycolysis/gluconeogenesis
1304	1.7	0.2	Epoxide hydrolase 2 ( <i>V. vinifera</i> )	gi 359496593	34.90	15.20	Metabolism of terpenoids and polyketides
3103	0.6	0.1	Triosephosphate isomerase, chloroplastic-like isoform 1 ( <i>V. vinifera</i> )	gi 225427917	34.66	41.70	Glycolysis/gluconeogenesis
3103	0.6	0.1	Ras-related protein RABA1d ( <i>V. vinifera</i> )	gi 147772737	9.74	23.00	Signal transduction
1102	0.5	0.1	Small heat shock protein, chloroplastic ( <i>V. vinifera</i> )	gi 225455238	25.01	42.30	Protein destination
1108	0.8	0.01	Small heat shock protein, chloroplastic ( <i>V. vinifera</i> )	gi 225455238	25.01	36.60	Protein destination
0101	0.4		Small heat shock protein, chloroplastic ( <i>V. vinifera</i> )	gi 225455238	25.01	28.60	Protein destination

<sup>1</sup> Spot code as reported in Figure 1.

<sup>a</sup> Ratio of spot expression values (relative OD\*area%) in asymptomatic (ADM, asymptomatic diseased Mourvèdre) and brown striped (BDM, brown diseased Mourvèdre) wood of diseased plants to the related control (ACM, asymptomatic control Mourvèdre). Values indicating over or down expression (ratio  $\geq$  |2|) are highlighted in light or dark gray, respectively. Values were replaced by an asterisk or a dashed line when the spot was not detected in the control or in the sample from diseased plant, respectively.

<sup>v</sup> Protein identified via the MASCOT and OMSSA search engines against in house made database from NCBI database.

<sup>w</sup> Accession number of the matched protein as reported in the NCBI database.

<sup>x</sup> Molecular mass (kDa).

<sup>y</sup> Percentage of the protein sequence covered by the matching peptides.

<sup>z</sup> Functional category retrieved from GenomeNet Database Resources website (<http://www.genome.jp/kegg>) or in literature.



Three genes presented similar expression in 'Mourvèdre' and 'Gewurztraminer'. The *HRp1* was up-regulated in AD and BD of these two cultivars, but only in BD for 'Chardonnay'. The *dhFred* and *HSPCP* were down-regulated in AD and BD of both 'Mourvèdre' and 'Gewurztraminer'. In the case of *HSPCP*, the accumulation and the repression of this transcript was respectively observed in ADC and BDC (Fig. 4). Three other genes exhibited similar expression profiles in 'Chardonnay' and 'Mourvèdre'; *epoxH2*, *CHI*, and *POX4* were only up-regulated in BD. No gene with similar expression profiles in 'Chardonnay' and 'Gewurztraminer' was observed.

Four genes (*AUX115*, *CCoAOMT*, *IFRL4*, and *ANR*) showed differential expression pattern in the three cultivars (Fig. 4). For example, *IFRL4* transcripts were down-regulated in BD of the three cultivars and in ADG, similar to control in ADM, and up-regulated in ADC (Fig. 4).

## DISCUSSION

**Correlation between RNA transcript and protein levels.** The result of qRT-PCR analysis showed poor correlation between the transcript and the protein expressions of seven genes (*cysPEROX*, *glyxRed*, *GSHhyd*, *MnSOD*, *epoxH2*, *CHI*, and *ANR*) of the selected candidates. Other studies on grapevine have already shown the indirect correlation between mRNA level and protein abundance (42,55). Moreover, in almost every organism that has been examined to date, steady-state transcript abundance only partially predicts the protein level (16,26). This data demonstrates a substantial role for regulatory processes occurring after that mRNA is synthesized. The cellular concentrations of proteins correlate with the abundances of their corresponding mRNAs, but not strongly (40% of protein variation can be explained by mRNAs abundance and 60% by posttranscriptional regulation) (64). Moreover, the mRNAs are less stable than proteins (average half-life of 2.6 to 7 h versus 46 h). This can be one explanation of why at the time of sampling it is possible to observe no direct correlation between the regulation of a given gene and the abundance of the related protein.

**Specific changes in protein abundance from the brown striped wood.** A number of proteins included in the "defense and cell rescue" category were over expressed in the brown striped wood (BDC, BDG, and BDM). Among them were some pathogenesis-related (PR) proteins such as  $\beta$ -1,3-glucanases (PR2), thaumatin-like and osmotin-like (PR-5), and PR-17 (62). The  $\beta$ -1,3-glucanases are abundant in plants and play key roles in cell division, trafficking of materials through plasmodesmata, and in withstanding abiotic stresses. These proteins also defend plants against fungal pathogens either alone or in association with other antifungal proteins (5). Members of the PR-5 family are also known to accumulate to high levels in response to biotic stress (10,23) and to have antifungal (44) and anti-oomycete (13) activities. No specific property has so far been described for PR-17 (62) but the induction of a *PR-17* gene (called *NtPR27-like*) was described in grapevine leaves in response to *E. lata* infection (10). Over accumulation of PR2, PR5, and PR17 was also observed in the black streaked wood of apoplectic and in esca proper-affected grapevines of 'Chardonnay' (42), being therefore associated to the high rate of inoculum of trunk disease agents. Additionally, a polyphenol oxidase (PPO) chloroplastic isoform 1 was more abundant in the brown striped wood of the three cultivars. PPO is wound-inducible and is involved in plant resistance (58) through the production of phytoalexins, phenols, and lignins (15,57). Our results also showed an accumulation of phenolic and some stilbene compounds in the brown stripe for the three cultivars. In this way, the up-regulation of *POX4* observed in 'Gewurztraminer', at both protein and transcript level, suggests the synthesis of several resveratrol oligomers in the presence of hydrogen peroxide (38). Resveratrol, in addition to its classical antimi-

crobial activity, acts as a signaling molecule by the activation of defense-related responses on *Vitis* cell: alkalization, mild elevation of reactive oxygen species (ROS), and PR-5 and PR-10 transcripts accumulation (12). A faster and stronger accumulation of some PR proteins and stilbene compounds was also observed in the leaves of *Vitis vinifera* cultivars less susceptible to esca disease (32). However, in our study the simultaneous low abundance of other proteins involved in defense response in the brown striped wood of two or three cultivars was also noted. It was the case of an SAMS 5 shared by all cultivars and an MLP-like 28 identified in 'Chardonnay' and 'Mourvèdre'. Similar decreasing of SAMS abundance was observed in the trunk wood and in green stems of esca proper-affected vines (42,55). The SAMS produces the S-adenosylmethionine (SAM), leading to the biosynthesis of polyamines and ethylene (50,59), known to be involved in plant defense response. In this way, it was also suggested that the SAMS have a role in the intrinsic resistance capability of the *Erysiphe necator*- and *Plasmopara viticola*-resistant grapevine cultivar (Regent) (21). Moreover, many studies suggested the existence of complex crosstalk between ethylene and polyamines synthesis pathways (37,46). Thus, Nambesee et al. (46) suggested a negative effect of increased polyamines levels on ethylene synthesis and/or signaling, which leads to higher susceptibility of plant to fungal pathogen. In grapevine, the polyamines catabolism contributes to the resistance plant state through modulation of immune response (27). The decrease of the SAMS 5 abundance in the brown stripe suggests that polyamines- and/or ethylene-mediated defense response may have a role in preventing symptom emergence. Based on similarities in amino acid sequences, the major latex protein (MLP) represent one of the three distinct groups related to the PR10 family and confirm the role of MLP in pathogen defense responses (48). The MLP, identified in phloem (65), was reported as down regulated in grapevine leaves in response to phytoplasma infection (43). A direct role of MLP on the fungal agents of Botryosphaeria dieback might therefore be hypothesized.

A decline of abundance for three enzymes of the phenylpropanoid pathway was also observed: an *IFRL4* shared in the three cultivars, a *CHI* in both 'Chardonnay' and 'Gewurztraminer', and a *dhFred* in 'Gewurztraminer'. Altogether, these results suggest that the phenylpropanoid pathway which leads to the accumulation of stilbenes was favored with respect to that leading to flavonoids. Indeed, Vannozzi et al. (63) observed diametrically opposed regulation of stilbene synthase genes (stilbenic compounds pathway) and chalcone synthase genes (flavonoid pathway) in grapevine in response to stress (UV-C exposition, downy mildew inoculation) suggesting that flow of carbon between these two competing metabolic pathways is tightly regulated.

Regarding proteins involved in stress tolerance, the trend was a low accumulation in the brown striped wood for the three cultivars. Small HSPs (smHSPs) play an important chaperone role in maintaining cellular functions when plants are subjected to a variety of stresses (2). Their differential level in Pierce's disease (PD)-resistant and PD-susceptible grapevine genotypes supports the idea that smHSPs might be implicated in resistance (66). In our study, the down regulation of smHSPs (at protein and transcript level), which is in agreement with the finding in black streaked wood (42), suggests that these proteins are likely related to some cellular dysfunctions leading to the external disease expression. Similarly, all the isoforms of the SOD identified in this study were down regulated in the brown striped wood. SOD is an enzyme known to take part in the antioxidant system. Additionally, an *epoxH2* identified in 'Mourvèdre' was found to be down regulated in BDM. The substrate specificity and regulatory behavior of the plant soluble epoxide hydrolases argue for a primary function of this enzyme in host defense and growth (47). Since several toxins produced by grapevine trunk disease

agents are characterized by the presence of epoxides in their chemical structure (1,4), a role of this enzyme in the detoxification process of these compounds could be hypothesized. The abundance in ADM could be interpreted as the result of cell signaling function of epoxide hydrolase (47) from BDM to ADM, indicating a plant defense response to the disease emergence.

Considering the specific case of Chardonnay, data of transcriptomic and proteomic studies are available from different organs of trunk disease-affected plants: leaves (36,41), green stems (55), black streaked trunk wood (42), and brown stripe (this study). Considering all these studies it seems that a similar defense response is triggered in the different organs of trunk disease-affected plants. For the antioxidant system, it seems to be differentially differentially perturbed depending from the protein species and the organ. The activation of defense response in woody tissues (trunk disease agents found) as well as in green stems and leaves (trunk disease agents not found) reinforces the hypothesis of the translocation of fungal toxic metabolites from woody tissues to the foliage through the xylem flow for explaining the development of related foliar symptoms (6).

**Differential responses according to the cultivar.** As observed for proteins specifically regulated in the brown striped wood, different processes represented in additional categories were regulated according to the cultivar. Several proteins involved in primary metabolism and energy were differentially expressed in the three cultivars. In 'Chardonnay', enzymes of the glycolysis pathway such as a hexokinase-2 chloroplastic, a phosphoglycerate mutase, and a triose phosphate isomerase cytosolic were less abundant in BDC. Conversely, a fructose-bisphosphate aldolase cytoplasmic isozyme and a cytosolic glyceraldehyde-3-phosphate dehydrogenase were more accumulated in 'Gewurztraminer' (BDG). Two enzymes taking part in the citrate cycle, namely a mitochondrial malate dehydrogenase and a succinyl-CoA ligase, were also identified. The first, common to the three cultivars, was less abundant in 'Chardonnay' (BDC) and more abundant in 'Gewurztraminer' (BDG), whereas the second, only identified in 'Gewurztraminer', was over regulated in BDG. Furthermore, a glucose-6-phosphate dehydrogenase and a glyxRed, which are respectively the rate-limiting step of the oxidative pentose phosphate pathway and an enzyme of the glyoxylate metabolism (3), were less accumulated in 'Chardonnay' (BDC). These findings provide the indication that a different perturbation of the glycolysis and citrate cycle pathways probably occurred in the three cultivars, with a strong impairment in 'Chardonnay' and an over regulation in 'Gewurztraminer'.

Proteins differentially accumulated in the three cultivars also include members of the antioxidant system such as GSTs and peroxiredoxins. GSTs perform diverse catalytic as well as non-catalytic roles in the detoxification of xenobiotics such as toxins, for preventing oxidative damage (22). The two isoforms of GSTF9 identified in this study showed different profiles of abundance. One, exclusively identified in 'Mourvèdre', was down accumulated in BDM while the other, present in both 'Chardonnay' and 'Gewurztraminer', was over accumulated in BDC and BDG. Another GST, namely a GST5, was over expressed in the brown striped wood of both 'Chardonnay' (BDC) and 'Gewurztraminer' (BDG). Peroxiredoxins are a family of peroxidases found in all organisms and represent central elements of the antioxidant defense system (18). In this study, a cysPEROX common to the three cultivars and a peroxiredoxin-2B found only in 'Mourvèdre' were respectively more and less abundant in the brown striped wood. Therefore, a slightly different perturbation of the antioxidant system, although related only to the protein species, was observed in 'Mourvèdre' compared with 'Chardonnay' and 'Gewurztraminer'.

Amino acid metabolism was also perturbed but different proteins were implicated in each cultivar. A glutamate decarboxylase, which catalyses the synthesis of gamma aminobutyrate (GABA)

from glutamate (EC 4.1.1.15), was identified in 'Chardonnay' (high abundance in ADC and low abundance in BDC); a glutamine synthetase was identified in only 'Gewurztraminer' (low abundance in BDG) while a chorismate mutase and an arginino-succinate synthase, respectively involved in the phenylalanine and tyrosine biosynthesis and urea cycle, were identified in only 'Mourvèdre' (high abundance in ADM and BDM). The over accumulation of glutamate decarboxylase in ADC could be linked to the cytosolic acidification and increase of cytosolic calcium which often accompany biotic and abiotic stresses (20,31). The accumulation of GABA was observed in symptomatic leaves of esca-affected vines (39) as well as in botrytized grape berries (29) and has been reported as resulting from the plant response to several types of stress ([39] and references therein). The over accumulation of glutamate decarboxylase in ADC could indicate the involvement of this enzyme in earlier plant response. Over expression of arginino-succinate synthase (EC 6.3.4.5) could be linked to the production of nitric oxide (8), which might be directly toxic to invading microbes, affect the redox status of the cell, and together with ROS, trigger the hypersensitive response (HR) and other defense-related processes (51,67).

Finally, a 14-3-3 protein was differentially abundant in 'Chardonnay' (accumulation in BDC) and 'Gewurztraminer' (low abundance in BDG), but absent in 'Mourvèdre'. The 14-3-3 proteins function as regulators of a wide range of target proteins in all eukaryotes and accumulate in response to abiotic and biotic stresses in plants (49). Their involvement in defense response is likely related to the regulation of defense-related genes and proteins as well as to their participation in signal transduction pathways.

It is important to point out that changes observed in the wood of the three cultivars might also depend on a number of other factors such as rootstock, vine age, location and related climate, and soil characteristics. On the other hand, there are indications that among the different factors playing a role in the incidence of grapevine trunk diseases, cultivar seems to be a major one (9).

**Conclusions.** The high rate of Botryosphaeriaceae biological isolation from the brown stripe confirms the association of this symptom with Botryosphaeria dieback agents. In response to these pathogens and/or their toxic metabolites, our results show the abundance of PR proteins (PR-2, PR-5, and PR-17) and members of the antioxidant system (GST5, cysPEROX) in the brown striped wood of the three cultivars. Additionally, total phenolics and some specific stilbenes were more accumulated in the brown striped wood. However, the low abundance of other proteins involved in defense response (SAMS, IFRL4, smHSPs, and SOD) probably contributes to make global response insufficient to avoid the development of brown stripe as well as foliar symptoms. Proteins down regulated in the brown striped wood or over regulated in the asymptomatic one could be regarded as one limiting factor involved in the development of the disease. Protein abundance seemed to be more related to the nature of the sample (asymptomatic or brown striped wood) in 'Chardonnay' and 'Mourvèdre', whereas for 'Gewurztraminer' it seemed to be linked to the presence of foliar symptoms, being more similar to the two types of wood samples from diseased plants (asymptomatic and brown striped wood).

Strongest differences among the three cultivars concerned proteins of the primary metabolism, which looked to be particularly impaired in 'Chardonnay' (BDC). In 'Gewurztraminer' (BDG), the glycolysis and citrate cycle pathways seemed to be over regulated while a deficiency of the antioxidant system and an over regulation of some amino acid metabolism appeared to occur in 'Mourvèdre' (BDM). The different susceptibility of the three cultivars could be explicated, at least in part, by the diverse expression of various proteins involved in defense, stress tolerance, and metabolism. Validation of these findings using complementary approaches could be carried out in the future.

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