Published in "Phytopathology doi: 10.1094/PHYTO-01-14-0007-R, 2014"
which should be cited to refer to this work.

# Differential responses of three grapevine cultivars to Botryosphaeria dieback

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## ABSTRACT

Botryosphaeria dieback is a fungal grapevine trunk disease which 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 cultivar '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 especially 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*: 2D gel electrophoresis, 'Chardonnay', 'Gewurztraminer', 'Mourvèdre', wood, Botryosphaeria dieback, brown stripe, proteome, phytoalexins, transcripts

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 hectolitre 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/BDA (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.

Botryosphaeria dieback is one of the main grapevine trunk diseases which are caused by several xylem-inhabiting fungi (6, 36, 45). Members of the Botryosphaeriaceae (6, 60) are the causal agents. These pathogens mainly attack the perennial organs of grapevine, causing wood discolourations as well as specific foliar symptoms (6), finally leading to premature plant death. A Botryosphaeria dieback-associated wood symptom that has been less considered till 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 being associated to 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 (34).

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, 33, 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 cv. 'Chardonnay' is less susceptible than cv. 'Gewurztraminer' and cv. 'Mourvèdre' to Botryospheria 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 the 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 the plants was inspected for the presence of brown stripe (Supplemental Figure 1), and samples consisting of the outer xylem (2-3 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 the analysis. 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 (1997) (35). Woody tissues used for protein, RNA and metabolites extractions were frozen in the field with liquid nitrogen and subsequently stored at −80 °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. (2014) (42). The powdered tissue was placed in microtubes (0.30 ± 0.01g 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 10000*g* for 5 min at 4 °C. The resultant pellet was washed once more with cold acetone. The pellet was sequentially rinsed at least 3 times with cold 80% acetone until the supernatant was colorless, then resuspended in 1.0 mL of cold 20% (w/v) trichloroacetic acid (TCA)/$H_2O$. The suspension was sonicated in a water bath at 4 °C for 10 min. After centrifugation, the pellets were sequentially washed twice with 20% (w/v) TCA/$H_2O$ and twice with 80% (v/v) acetone. This pellet was air-dried and the dry powder was resuspended in 0.7 mL dense sodium dodecyl sulphate (SDS) buffer [30% (w/v) sucrose, 2% (w/v) SDS, 0.1 M tris (hydroxymethyl) aminomethane (Tris) -HCl pH 8.0, 5% (v/v) 2-mercaptoethanol]. Then 0.7 mL of a 90% phenol solution (Sigma-Aldrich, St. Louis, MO, USA) was added, and the resulting mixture was vortexed for 30 s. The phenol phase, recovered by centrifugation at 10000*g* for 5 min at 4 °C, was separated in two aliquots. One of 0.7 mL was transferred to a 15 mL Falcon tube while an aliquot of 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 over-night at −20 °C. The precipitated proteins were

recovered by centrifugation, washed twice with cold 0.1 M ammonium acetate in methanol and twice with 80% (v/v) acetone. The final pellet was air-dried and stored at −80 °C. The pellet retrieved from the aliquot of 0.1 mL was dissolved in 100 $\mu$L of 7 M urea, 2 M thiourea, 4% (w/v) 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, USA) and bovine serum albumin (BSA) as standard. After quantification, protein samples were solubilised in a sample buffer consisting of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.5% (v/v) 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 $\mu$g of total protein fraction were diluted in a mixture containing sample buffer and 10% (v/v) glycerol to a final volume of 125 $\mu$L. IPG gel strips (ReadyStrip IPG, pH 4–7, 7 cm, Bio-Rad, Hercules, CA, USA) were actively rehydrated over-night at 20 °C with the mixture. Isoelectric focusing (IEF) was conducted at 20 °C in an IPGphor unit (Amersham Pharmacia, Sweden) as follows: a linear increase from 50 to 4000 V to give a total of 10000 V/h. Focused proteins were reduced and subsequently alkylated according to Görg et al. (1987) (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% (w/v) low melting point agarose, 0.15 M Bis-Tris/ 0.1 M HCl, and 0.2% (w/v) 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 $^{TM}$ Protein Staining Solution (Fermentas, USA) following the manufacturer's instructions.

**Image Analysis.** Digitized images at 36.6 $\mu$m resolution were obtained using the GS-800 scanner and Quantity One 4.6.2 software (Bio-Rad, Hercules, CA). Computerized 2D gel analysis, including spot detection and quantification, was performed using the PDQuest Basic 8.0.1 software (Bio-Rad, Hercules, CA, USA). The relative molecular mass was calibrated with internal protein markers (Precision Plus Protein Standards, Bio-Rad, Hercules, CA) after co-migration during the 2$^{nd}$ dimension. Quantification of detected protein spots was performed calculating the relative optical density×area (relative OD×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 were included in each analysis. Protein spots detected in at least 2 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×area ± standard deviation (SD) ($n$=3) values of each group were finally used to estimate relative expression level (relative OD×area %) of each protein spot among the groups. Differences among the means were evaluated by the Dunn's Multiple Comparison Test after that the null hypothesis (equal means) was rejected in the Kruskal-Wallis test, assuming a significance of $p \leq 0.05$. The relative expression ratio to the related control (ACC, ACG or ACM, see Table 1) in the other groups was also estimated. Values ≥|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 analysed by C18 reversed phase nanoHPLC on a nanoHPLC-Chip/MS system (Agilent Technologies, Palo Alto, USA)

coupled to an ion trap amaZon (Bruker Daltonics, Bremen, Germany) mass spectrometer. For tandem MS experiments, the system was operated in the data-dependant 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 *Vitis* genus, human keratins and trypsin, downloaded from National Center for Biotechnology Information nonredundant database (NCBInr, June 05, 2012) concatenated with reversed copies of all sequences (total 138416 entries). Database searching was carried out by using the following parameters: 2 missed cleavages; a parent and fragment mass tolerance of ± 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, USA). To minimize false positive identifications, results were subjected to very stringent Mascot and OMSSA filtering criteria as follows: 1) for the identification of proteins, all peptides are validated with both algorithms (Mascot and OMSSA); 2) for proteins identified with two peptides or more, OMSSA: -Log(E-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; 3) in the case of single peptide hits, OMSSA: -Log(E-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 to control and estimate 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 RT-PCR analysis of gene expression.** Total RNA was isolated from woody samples using a β-mercaptoethanol- and sodium azide-based protocole according to Magnin-Robert et al. (2014) (42). In total, 150 ng of total RNA were 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. (2002) (7). Expression of fifteen selected genes selected from proteomic results was tracked by quantitative Reverse-Transcripts Polymerase Chain Reaction using the primers reported in Supplemental Table 4, including the α-chain elongation factor 1 gene (*EF1-α*) 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 either 0.5 °C/s. Melting peaks were visualized for checking the specifity 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× or <0.5× 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 µl of the internal standard trans-4-hydroxystilbene (0.5 mg $ml^{-1}$). The mixture was shaken during 1 h at 40°C under 150 rpm in dark condition before centrifugation at 13000 g for 2 x 5 min. The supernatants were stored at -20°C until analysis.

**Quantification of Plant total phenolic compounds.** Total phenolics were determinated by using the Folin-Ciocalteau method (54) downscaled to 96-well-plate (E. Abou-Mansour, personal communication). An aliquot (30 µl) of appropriate dilution (woody tissues, 1:20 (v:v)) of methanolic extracts was mixed with 150 µl of Folin-Ciocalteau reagent (diluted by 10) and after 5 min of incubation at room temperature, 120 µl of sodium carbonate solution (10% w:v) were added. After incubation at room temperature (RT) for 2 h absorbance of the mixture was read against the prepared blank at 750 nm. Total phenolics were expressed as mg of gallic acid equivalents (GAE) per g 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, *trans*-vitisins A and B were extracted from lignified canes of Syrah (175 g) homogenized in 1 L of MeOH-$H_2O$ (7:3, v/v) for one hour at 40°C (Supplemental Figure 2). After filtration, MeOH was evaporated under vacuum and the aqueous residue adjusted to 100 ml with distilled water and extracted three times with 100 ml hexane, following three times with 100 ml ethyl acetate (EtOAc). Hexane extract was discarded and EtOAc

extracted evaporated and further purified by HPLC on a semi-preparative Ascentis C18 column (15 cm x 10 mm) 5 µm (Supelco, Bellefonte, PA). The structures were confirmed by $^{1}$H NMR (500 MHz, CD3OD) and $^{13}$C NMR (100 MHz, CD3OD) and ESI-MSn in accordance with Lin et al. (1992) (40) for *trans*-ε-viniferin, and Ito et al. (1999) (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 x 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 of 0.7 ml min$^{-1}$ and the injection of 60 µl. Spectral data for all peaks were accumulate 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 (). Although in lesser extent, these fungi (especially *Diplodia seriata*) as well as other fungal species non-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 (Supplemental Tables 5a, 6b and 6c).

**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 (Supplemental Tables 1, 2 and 3). Most of the identified proteins belonged to categories 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, 3B and 3C).

Considering the high number of total proteins identified, a selection was performed based on: i) the "identification percentage" (Supplemental Tables 1, 2 and 3), ii) the identification in two or three cultivars, and iii) their known direct or indirect involvement in stress responses, (Tables 2A, 2B and 2C). 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 2A). 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 a MLP-like protein 28 (s6014). The latter two proteins were also down regulated in BDC (Table 2A).

Nine out of the 10 proteins more abundant in BDC (s1013, s1117, s4119, s4120, and s4229)

belonged to the "defense and cell rescue" category (Table 2A). Among them were a polyphenol oxidase (s1013), two thaumatin-like (s1117), two gluthatione 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 (cysPEROX, s1117) gene expression and protein accumulation. Indeed, cysPEROX was over accumulated in BDC (Table 2A) 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 2A). In this sense, a significant up-regulation (500-fold) for *endoglu* gene expression was also observed in BDC compared to 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*-$\varepsilon$-viniferin, and the tetramer *tr*-vitisin B was observed especially in BDC compared to ACC (Table 3).

The 51 proteins less abundant in BDC than in ACC and ADC were included into 19 functional categories (Table 2A); the most represented were "defense and cell rescue" (7 proteins), "glycolysis/gluconeogenesis" (7 proteins) and "protein degradation" (5 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 2A). Correlating with the decreased

abundance of the correspondent protein in BDC, a down-regulation of the gene expression *IFRL4* (3-fold) 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 2B). 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 more accumulated (s1108, s1111, s2013, s3612, s4112 and s7325) belonged to the "defense and cell rescue" or "secondary metabolism" categories (Table 2B). 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 2B). No correlation was observed between protein abundance and gene expression in the case of cysPEROX and ANR (Table 2B, Fig. 4). The remaining four proteins were identified in s4112 (Table 2B): a 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 3). Moreover, stilbenic compounds were only detected in the diseased plants (Table 3). 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 2B). 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 2B). These proteins included: 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") a 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 2B). 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. A 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") a S-adenosylmethionine synthase 5 (SAMS; s6415; "defense and cell rescue") and a Caffeoyl-CoA O-methyltransferase (CCoAOMT; s4114; "secondary metabolism") (Table 2B). 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 a 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 2C).

In BDM, over regulation was observed for 14 proteins (s0001, s0201, s1302, s2306, s4203, s6202 and s6705, Table 2C). Five out of the 14 proteins belonged to the "defense and cell rescue" category (Table 2C) and among them were two thaumatin-like (s0201), a cysPEROX (s0201) and two PR-17 (s4203). Eleven out of these 14 proteins (s0201, s2306 and s4203) were

also more accumulated in ADM but with different abundance as compared to 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 2C). 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 endoglu (“defense and cell rescue”). An up-regulation was observed also for *endoglu* expression in ADM (240-fold) and in BDM (1000-fold) (Fig. 4). Regarding the total phenolic compounds, they were more present in BDM than in ADM and ACM while no difference was noted for stilbenic compounds except for *tr*-$\varepsilon$-viniferin, which was more abundant in BDM (Table 3).

Thirty proteins belonging to 12 categories were down regulated in BDM (Table 2C), the categories “protein destination” (eight proteins, especially HSP) and “defense and cell rescue” (seven proteins) being the most represented (Table 2C). An important down-regulation of the expression of *HSPCP* (encoding for a HSP chloroplastic; s1102-s1108-s0101) was observed for BDM (100-fold) and, with a weaker intensity, for ADM (20-fold) respect to ACM. These results corroborated with the protein accumulation (Table 2C). Finally, an epoxide hydrolase 2 (epoxH2; s1304; “metabolism of terpenoids and polyketides”) was less abundant in BDM while an up-regulation of *epoxH2* was therein (Fig. 4).

**Comparison of protein profiles from 'Chardonnay', 'Gewurztraminer' and 'Mourvèdre'.** Similar accumulation profiles were often observed for proteins shared by the three cultivars. A polyphenol oxydase chloroplastic-like isoform 1 (gi|147811887), a cysPEROX (gi|147789752), two thaumatin-like proteins (gi|2213852 and gi|8980665), a PR-17 (gi|147784683), and another homolog (gi|374431273) were over accumulated in the brown striped wood (BDC, BDG and BDM). On the contrary, an IFRL4 (gi|76559892), an SAMS 5 (gi|223635289), a 26.5 kDa HSP

mitochondrial (gi|225442975) and a MnSOD (gi|161778782) were therein less abundant.

Similar abundance profiles were usually observed also for proteins common to two cultivars. In both 'Chardonnay' (BDC) and 'Gewurztraminer' (BDG), a thaumatin-like (gi|33329390), a miraculin (gi|147828196), a GST5 (gi|158323772) and a stem-specific protein TSJT1 (gi|225432548) were over accumulated while a CHI (gi|158514257) and a cytosolic ascorbate peroxidase (gi|161778778) were down accumulated (BDC and ADG). Examples of similar accumulation profiles were also from proteins shared between 'Chardonnay' and 'Mourvèdre', especially in the brown striped wood (BDC and BDM). As observed for the GSTF9 (gi|225446791), an over accumulation was noted for an endoglu (gi|225441373); whereas, an L-ascorbate peroxidase 2 cytosolic (gi|225435177) and a MLP-like protein 28 (gi|225424272), were less abundant. However, examples of dissimilar abundance profiles were also noted. For instance, a major allergen Pru av 1 (gi|225431844) was down accumulated in 'Chardonnay' (BDC) and 'Mourvèdre' (BDM) but over accumulated in 'Gewurztraminer' (BDG). Still, a thioredoxin reductase 2-like (gi|225431669) was down accumulated in 'Chardonnay' (BDC) and over expressed in 'Gewurztraminer' (BDG).

Finally, some proteins were detected in only one cultivar. Proteins only identified in 'Chardonnay' were an osmotin-like protein (gi|1839046) and a HRp1 (gi|225456672), both of which were over expressed in BDC. A protein disulfide-isomerase A6 isoform 1 (gi|225450626) and a DNA damage-inducible protein 1 (gi|225462066) were also only found in Chardonnay but they were less abundant in BDC and ADC. Examples of specific proteins for 'Gewurztraminer' were an elicitor responsive protein 1 (gi|225449489) and an endo-1,3;1,4-beta-D-glucanase (gi|225436938); both were more accumulated in BDG. For 'Mourvèdre', an epoxH2 (gi|359496593) and a 1,3 beta glucanase (gi|6273716) were among the specific proteins.

**Comparison of gene profiles from 'Chardonnay', 'Gewurztraminer' and 'Mourvèdre'.** Gene expressions exhibiting the same behaviour in the three cultivars were observed. Expression

of *endoglu* was up-regulated in woody tissues of diseased plants; this gene had a stronger expression in 'Mourvèdre' than in 'Chardonnay' and 'Gewurztraminer'. For the three cultivars, the relative expression of *endoglu* was higher in BD than in AD (Fig. 4). Three other genes (*cysPEROX*, *glyxRed*, *GSHhyd* and *MnSOD*) showed similar expression in AD and BD as compared to their respective control (AC), although the abundance of these proteins was affected in woody tissues.

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*, *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 synthesised. 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 post-transcriptional regulation) (64). Moreover, the mRNAs are less stable than proteins (average half-life of 2.6-7 hours versus 46 hours). This can be one explanation of why at the t 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 *β*-1,3-glucanases (PR2), thaumatin-like and osmotin-like (PR-5), and PR-17 (62). The *β*-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 cv. '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 antimicrobial activity, acts as a signaling molecule by the activation of defense-related responses on *Vitis* cell: alkalinisation, 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 a 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, Nambeesen et al. (2012) (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 been 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 favoured with respect to that leading to flavonoids. Indeed, Vannozzi et al. (2012) (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 stress (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 behaviour 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 signalling function of epoxide hydrolase (47) from BDM to ADM, indicating a plant defense response to the disease emergence.

Considering the specific case of the cultivar 'Chardonnay', data of transcriptomic and proteomic studies are available from different organs of trunk disease-affected plants: leaves (36), (41), green stem (55), trunk, black streaked wood (42) and brown stripe (this study). Therefore, considering all these studies it seems that response in the different organs of trunk disease affected-plants have in common the activation of defence response while the antioxidant system, though ever involved, seems to be differentially perturbed depending from the protein species and the organ. The activation of defence 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 triosephosphate 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 to '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 up that changes observed in the wood of the three cultivars might also depend from a number of other factors 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, the 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, 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 for the two types of wood samples from diseased plants (asymptomatic and brown striped wood).

Strongest differences among the three cultivars concerned especially 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.



## ACKNOWLEDGEMENTS

This research was financed by the national CPER (Contrat de Projet État-Région) program - task E1 altesca and CASDAR (Compte d'Affectation Spéciale au Développement Agricole et Rural). Proteomic studies were supported by the CNRS, the "Agence Nationale de la Recherche"

(ANR) and the “Région Alsace”. The authors thank Marine Rondeau for her help provided during the technical improvements of transcripts analysis. Dr. Laurence Mercier of the company Moët & Chandon is thanked for making available the vineyard used as the experimental plot in this study.

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**Table 1.** Description of plant material and related groups of samples.

| cultivar/rootstock | vineyard age and location | sample group | | |
|---|---|---|---|---|
| | | control plants | diseased plants | |
| | | asymptomatic wood | asymptomatic wood | brown striped wood |
| Chardonnay/41B | 27 years – Avize (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 |

Abbreviations: 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; BDM, Brown Diseased Mourvèdre.

**Table 2A.** 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 cultivar 'Chardonnay' (C).

| Spot[a] | ratio to ACC [b] | | Matched Protein [c] | Accession number [d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADC | BDC | | | | | |
| 1114 | --- | * | osmotin-like protein [*Vitis vinifera*] | gi\|1839046 | 23.86 | 26.70 | defense and cell rescue |
| 1114 | --- | * | thaumatin-like protein [*Vitis vinifera*] | gi\|33329390 | 23.86 | 26.70 | defense and cell rescue |
| 7210 | --- | * | GEM-like protein 5 [*Vitis vinifera*] | gi\|225468805 | 31.54 | 4.21 | transcription |
| 7210 | --- | * | glucan endo-1,3-beta-glucosidase [*Vitis vinifera*] | gi\|225441373 | 36.66 | 37.10 | defense and cell rescue |
| 1117 | 0.9 | 24.8 | 2-Cys peroxiredoxin [*Vitis vinifera*] | gi\|147789752 | 30.19 | 41.80 | defense and cell rescue |
| 1117 | 0.9 | 24.8 | thaumatin-like protein [*Vitis vinifera*] | gi\|8980665 | 24.35 | 11.90 | defense and cell rescue |
| 1117 | 0.9 | 24.8 | VVTL1 [*Vitis vinifera*] | gi\|2213852 | 23.95 | 50.50 | defense and cell rescue |
| 4119 | 1.2 | 11.6 | miraculin [*Vitis vinifera*] | gi\|147828196 | 22.42 | 19.20 | defense and cell rescue |
| 4119 | 1.2 | 11.6 | glutathione S-transferase 5 [*Vitis vinifera*] | gi\|158323772 | 24.85 | 54.60 | defense and cell rescue |
| 4119 | 1.2 | 11.6 | glutathione S-transferase F9 [*Vitis vinifera*] | gi\|225446791 | 24.89 | 54.60 | defense and cell rescue |
| [illegible] | 1.2 | 11.6 | pathogenesis-related protein 17 [*Vitis pseudoreticulata*] | gi\|147784683 | 25.31 | 26.10 | defense and cell rescue |
| [illegible] | 1.0 | 6.3 | hypersensitive-induced response protein 1 isoform 3[*Vitis vinifera*] | gi\|225456672 | 32.07 | 55.30 | defense and cell rescue |
| [illegible] | 1.0 | 4.1 | polyphenol oxidase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|147811887 | 66.97 | 9.15 | secondary metabolism |
| 1222 | 1.0 | 2.1 | 14-3-3 protein [*Vitis vinifera*] | gi\|359492889 | 29.33 | 57.30 | signal transduction |
| [illegible] | 2.0 | 14.6 | stem-specific protein TSJT1 [*Vitis vinifera*] | gi\|225432548 | 25.23 | 41.90 | defense and cell rescue |
| [illegible] | 8.2 | 0.2 | glutamate decarboxylase 1 [*Vitis vinifera*] | gi\|225466257 | 55.21 | 15.20 | amino acid metabolism |
| [illegible] | 8.2 | 0.2 | enolase [*Vitis vinifera*] | gi\|225455555 | 48.09 | 52.10 | glycolysis/ gluconeogenesis |
| [illegible] | 3.0 | 1.7 | 60S acidic ribosomal protein P0 [*Vitis vinifera*] | gi\|225449110 | 34.26 | 26.60 | translation |
| 021 | 2.3 | 0.6 | uncharacterized protein LOC100232885 [*Vitis vinifera*] | gi\|225447003 | 18.40 | 75.70 | unknown protein |
| [illegible] | 2.2 | 1.9 | TPA: isoflavone reductase-like protein 5 [*Vitis vinifera*] | gi\|76559894 | 33.87 | 48.00 | secondary metabolism |
| [illegible] | 1.5 | 0.5 | T-complex protein 1 subunit beta [*Vitis vinifera*] | gi\|225459806 | 57.31 | 27.10 | protein destination |
| [illegible] | 1.4 | 0.5 | actin-depolymerizing factor 1-like [*Vitis vinifera*] | gi\|225439733 | 16.01 | 18.70 | cell growth and death |
| [illegible] | 1.4 | 0.5 | ATP-dependent Clp protease proteolytic subunit-related protein 4, chloroplastic-like [*Vitis vinifera*] | gi\|296085709 | 18.71 | 12.40 | protein degradation |
| [illegible] | 1.4 | 0.5 | proteasome subunit beta type-1[*Vitis vinifera*] | gi\|225453909 | 24.61 | 10.80 | protein degradation |
| [illegible] | 1.4 | 0.5 | actin-depolymerizing factor 2-like isoform 1 [*Vitis vinifera*] | gi\|225449595 | 15.94 | 25.20 | cell growth and death |
| [illegible] | 0.9 | 0.5 | elongation factor 1-beta 1 [*Vitis vinifera*] | gi\|296083911 | 25.53 | 22.80 | translation |
| [illegible] | 0.9 | 0.5 | eukaryotic translation initiation factor 3 subunit F [*Vitis vinifera*] | gi\|147856131 | 38.91 | 33.30 | translation |
| [illegible] | 0.7 | 0.5 | glutamine synthetase nodule isozyme isoform 1[*Vitis vinifera*] | gi\|147768273 | 39.13 | 42.90 | energy metabolism |
| [illegible] | 0.7 | 0.5 | peroxidase 12-like [*Vitis vinifera*] | gi\|359493149 | 39.17 | 8.06 | defense and cell rescue |
| [illegible] | 0.7 | 0.5 | probable protein disulfide-isomerase A6 isoform 1[*Vitis vinifera*] | gi\|225450626 | 39.25 | 23.00 | protein processing in e.r. |
| [illegible] | 0.6 | 0.4 | actin-7 [*Vitis vinifera*] | gi\|225431585 | 41.71 | 69.20 | cell growth and death |
| 1219 | 0.6 | 0.4 | 14-3-3 protein [*Vitis vinifera*] | gi\|226295432 | 28.63 | 14.30 | signal transduction |
| 5522 | 0.6 | 0.4 | 2,3-bisphosphoglycerate-independent phosphoglycerate mutase isoform 1 [*Vitis vinifera*] | gi\|225439064 | 61.07 | 22.00 | glycolysis/ gluconeogenesis |
| 5522 | 0.6 | 0.4 | pyruvate decarboxylase isozyme 1 [*Vitis vinifera*] | gi\|225443847 | 62.41 | 26.20 | glycolysis/ gluconeogenesis |
| 5522 | 0.6 | 0.4 | glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform [*Vitis vinifera*] | gi\|225452196 | 59.14 | 4.65 | pentose phosphate pathway |
| 5522 | 0.6 | 0.4 | d-3-phosphoglycerate dehydrogenase, chloroplastic-like [*Vitis vinifera*] | gi\|225428898 | 62.54 | 45.40 | energy metabolism |
| 3118 | 0.7 | 0.3 | triosephosphate isomerase, cytosolic isoform 1[*Vitis vinifera*] | gi\|225434935 | 27.33 | 27.60 | glycolysis/ gluconeogenesis |
| 3118 | 0.7 | 0.3 | proteasome subunit alpha type-2-B [*Vitis vinifera*] | gi\|225423722 | 25.57 | 24.70 | protein degradation |
| 5125 | 1.4 | 0.3 | 26.5 kDa heat shock protein, mitochondrial [*Vitis vinifera*] | gi\|225442975 | 26.30 | 17.20 | protein destination/ processing in e.r. |
| 5125 | 1.4 | 0.3 | flavoprotein wrbA isoform 1 [*Vitis vinifera*] | gi\|225461209 | 21.72 | 42.40 | energy metabolism |
| 5125 | 1.4 | 0.3 | manganese superoxide dismutase [*Vitis vinifera*] | gi\|161778782 | 25.27 | 11.80 | defense and cell rescue |

**Table 2A. Continued**

| Spot[a] | ratio to ACC [b] | | Matched Protein [c] | Accession number.[d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADC | BDC | | | | | |
| 5121 | 0.7 | 0.3 | triosephosphate isomerase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|225427917 | 34.66 | 52.20 | glycolysis/ gluconeogenesis |
| 5121 | 0.7 | 0.3 | cytosolic ascorbate peroxidase [*Vitis vinifera*] | gi\|161778778 | 27.98 | 17.40 | defense and cell rescue |
| 5330 | 0.7 | 0.3 | cysteine synthase [*Vitis vinifera*] | gi\|359487832 | 36.52 | 5.56 | amino acid metabolism |
| 5330 | 0.7 | 0.3 | glyoxylate reductase isoform 2 [*Vitis vinifera*] | gi\|297743258 | 34.29 | 10.90 | glyoxylate and dicarboxylate metabolism |
| 5330 | 0.7 | 0.3 | malate dehydrogenase, cytoplasmic [*Vitis vinifera*] | gi\|225438145 | 35.49 | 11.10 | citrate cycle |
| 5330 | 0.7 | 0.3 | malate dehydrogenase, mitochondrial [*Vitis vinifera*] | gi\|225461618 | 36.77 | 33.90 | citrate cycle |
| 5330 | 0.7 | 0.3 | probable fructose-bisphosphate aldolase 3, chloroplastic [*Vitis vinifera*] | gi\|225424114 | 42.88 | 20.90 | glycolysis/ gluconeogenesis |
| 5330 | 0.7 | 0.3 | auxin-induced protein PCNT115 isoform 1 [*Vitis vinifera*] | gi\|225433674 | 37.52 | 18.80 | signal transduction |
| 5330 | 0.7 | 0.3 | thioredoxin reductase 2-like [*Vitis vinifera*] | gi\|225431669 | 39.52 | 16.60 | nucleotide metabolism |
| 5330 | 0.7 | 0.3 | malate dehydrogenase [*Vitis vinifera*] | gi\|225443845 | 36.78 | 49.10 | citrate cycle |
| 5330 | 0.7 | 0.3 | malate dehydrogenase, chloroplastic [*Vitis vinifera*] | gi\|225457407 | 43.59 | 33.90 | citrate cycle |
| 4232 | 1.3 | 0.2 | TPA: isoflavone reductase-like protein 4 [*Vitis vinifera*] | gi\|76559892 | 33.82 | 52.30 | secondary metabolism |
| 4126 | 1.0 | 0.2 | hydroxyacylglutathione hydrolase cytoplasmic [*Vitis vinifera*] | gi\|225448353 | 28.65 | 26.70 | other carbohydrates metabolism |
| [illegible] | 0.9 | 0.2 | polyphenol oxidase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|147811887 | 66.97 | 9.15 | secondary metabolism |
| 2524 | 0.7 | 0.2 | mitochondrial-processing peptidase subunit alpha [*Vitis vinifera*] | gi\|225445041 | 54.45 | 31.20 | protein degradation |
| 423 | 0.6 | 0.2 | RecName: Full=Chalcone--flavonone isomerase 2 | gi\|147843260 | 34.27 | 12.20 | translation |
| 4119 | 0.5 | 0.2 | eukaryotic initiation factor 4A-3-like [*Vitis vinifera*] | gi\|225464928 | 46.43 | 37.10 | translation |
| [illegible] | 1.1 | 0.1 | RecName: Full=S-adenosylmethionine synthase 5 Short=MAT 5 | gi\|223635289 | 42.78 | 64.20 | defense and cell rescue |
| 3721 | 1.0 | 0.1 | formamidase isoform 1 [*Vitis vinifera*] | gi\|225438970 | 49.72 | 36.10 | glyoxylate and dicarboxylate metabolism |
| 412[illegible] | 0.8 | 0.1 | stem-specific protein TSJT1[*Vitis vinifera*] | gi\|225461387 | 27.92 | 14.90 | defense and cell rescue |
| 4[illegible] | 0.8 | 0.1 | proteasome subunit beta type-6 [*Vitis vinifera*] | gi\|225445670 | 24.98 | 11.60 | protein degradation |
| 4[illegible]2 | 0.8 | 0.1 | RecName: Full=Chalcone--flavonone isomerase 2 | gi\|158514257 | 25.12 | 20.90 | secondary metabolism |
| [illegible] | 0.8 | 0.1 | L-ascorbate peroxidase 2, cytosolic [*Vitis vinifera*] | gi\|225435177 | 27.54 | 38.00 | other carbohydrates metabolism |
| 6014 | 0.4 | 0.1 | major allergen Pru av 1 [*Vitis vinifera*] | gi\|225431844 | 17.11 | 15.80 | defense and cell rescue |
| 6014 | 0.4 | 0.1 | MLP-like protein 28 [*Vitis vinifera*] | gi\|225424272 | 17.18 | 43.00 | defense and cell rescue |
| [illegible] | 1.7 | 0.0 | clathrin light chain 2-like [*Vitis vinifera*] | gi\|147766743 | 34.92 | 20.10 | intracellular transport |
| [illegible] | 1.7 | 0.0 | hexokinase-2, chloroplastic [*Vitis vinifera*] | gi\|225457987 | 44.76 | 21.20 | glycolysis/ gluconeogenesis |
| [illegible] | 1.7 | 0.0 | tubulin alpha chain [*Vitis vinifera*] | gi\|225429189 | 49.54 | 60.90 | intracellular transport |
| [illegible] | 1.7 | 0.0 | tubulin beta-1 chain [*Vitis vinifera*] | gi\|225426414 | 50.04 | 21.80 | intracellular transport |
| [illegible] | 1.7 | 0.0 | ATP synthase subunit beta, mitochondrial-like [*Vitis vinifera*] | gi\|147838606 | 59.61 | 42.90 | energy metabolism |
| 1414 | 0.1 | 1.5 | DNA damage-inducible protein 1[*Vitis vinifera*] | gi\|225462066 | 45.06 | 15.40 | protein processing in endoplasmic reticulum |
| 1414 | 0.1 | 1.5 | transaldolase isoform 1 [*Vitis vinifera*] | gi\|225425280 | 48.09 | 12.70 | glycolysis/ gluconeogenesis |

**Table 2B.** 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 cultivar 'Gewurztraminer' (G).

| Spot[a] | ratio to ACG [b] | | Matched Protein [c] | Accession number [d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADG | BDG | | | | | |
| 1108 | * | * | thaumatin-like protein [*Vitis vinifera*] | gi\|33329390 | 23.86 | 26.70 | defense and cell rescue |
| 1108 | * | * | VVTL1 [*Vitis vinifera*] | gi\|2213852 | 23.95 | 42.80 | defense and cell rescue |
| 4112 | 12.0 | 40.3 | endo-1,3;1,4-beta-D-glucanase [*Vitis vinifera*] | gi\|225436938 | 26.08 | 5.02 | cell growth and death |
| 4112 | 12.0 | 40.3 | pathogenesis-related protein 17 [*Vitis pseudoreticulata*] | gi\|374431273 | 25.26 | 40.70 | defence and cell rescue |
| 4112 | 12.0 | 40.3 | miraculin [*Vitis vinifera*] | gi\|147828196 | 22.42 | 17.20 | defense and cell rescue |
| 4112 | 12.0 | 40.3 | glutathione S-transferase 5 [*Vitis vinifera*] | gi\|158323772 | 24.85 | 52.30 | defense and cell rescue |
| 4112 | 12.0 | 40.3 | stem-specific protein TSJT1 [*Vitis vinifera*] | gi\|225432548 | 25.23 | 42.80 | defense and cell rescue |
| 4112 | 12.0 | 40.3 | proteasome subunit beta type-6 [*Vitis vinifera*] | gi\|225445670 | 24.98 | 14.20 | protein degradation |
| 1111 | 13.2 | 31.8 | RNA-binding protein 8A [*Vitis vinifera*] | gi\|225431497 | 22.29 | 15.30 | transcription |
| 1111 | 13.2 | 31.8 | hypothetical protein VITISV_025619 [*Vitis vinifera*] 2-Cys peroxiredoxin [*Vitis vinifera*] | gi\|147789752 | 30.16 | 22.00 | defense and cell rescue |
| [illegible] | 13.2 | 31.8 | thaumatin-like protein [*Vitis vinifera*] | gi\|8980665 | 24.35 | 11.90 | defense and cell rescue |
| [illegible] | 3.8 | 12.9 | malate dehydrogenase, mitochondrial [*Vitis vinifera*] | gi\|225461618 | 36.77 | 23.90 | citrate cycle |
| [illegible] | 3.8 | 12.9 | probable fructose-bisphosphate aldolase 3, chloroplastic [*Vitis vinifera*] | gi\|225424114 | 42.88 | 17.80 | glycolysis/ gluconeogenesis |
| 7325 | 3.8 | 12.9 | auxin-induced protein PCNT115 isoform 1 [*Vitis vinifera*] | gi\|225433674 | 37.52 | 31.10 | signal transduction |
| [illegible] | 3.8 | 12.9 | thioredoxin reductase 2-like [*Vitis vinifera*] | gi\|225431669 | 39.52 | 15.50 | nucleotide metabolism |
| [illegible] | 3.8 | 12.9 | malate dehydrogenase [*Vitis vinifera*] | gi\|225443845 | 36.86 | 23.60 | citrate cycle |
| [illegible] | 3.8 | 12.9 | major allergen Pru av 1 [*Vitis vinifera*] | gi\|225431844 | 17.11 | 9.49 | defense and cell rescue |
| [illegible] | 3.8 | 12.9 | glyceraldehyde-3-phosphate dehydrogenase, cytosolic [*Vitis vinifera*] | gi\|297733609 | 37.43 | 29.20 | glycolysis/ gluconeogenesis |
| 752 | 3.8 | 12.9 | RecName: Full=Peroxidase 4; Flags: Precursor [*Vitis vinifera*] | gi\|223635590 | 34.04 | 7.48 | defence and cell rescue |
| [illegible] | 3.8 | 12.9 | succinyl-CoA ligase [ADP-forming] subunit alpha-1, mitochondrial-like [*Vitis vinifera*] | gi\|225457502 | 34.86 | 14.90 | citrate cycle |
| [illegible] | 3.8 | 12.9 | anthocyanidin reductase [*Vitis vinifera*] | gi\|359496568 | 36.71 | 17.80 | secondary metabolism |
| [illegible] | 3.8 | 12.9 | fructose-bisphosphate aldolase cytoplasmic isozyme [*Vitis vinifera*] | gi\|225440976 | 38.61 | 24.90 | glycolysis/ gluconeogenesis |
| [illegible] | 3.8 | 12.9 | UPF0160 protein MYG1, mitochondrial-like [*Vitis vinifera*] | gi\|225426621 | 38.25 | 35.50 | unclassified protein |
| [illegible] | 2.0 | 3.2 | NAD-dependent malic enzyme 62 kDa isoform, mitochondrial [*Vitis vinifera*] | gi\|225453250 | 69.32 | 6.08 | pyruvate metabolism |
| 2012 | 1.3 | 2.8 | polyphenol oxidase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|147811887 | 66.97 | 6.32 | secondary metabolism |
| 2012 | 1.3 | 2.8 | elicitor-responsive protein 1[*Vitis vinifera*] | gi\|225449489 | 17.41 | 14.90 | defence and cell rescue |
| [illegible] | 19.6 | 0.3 | superoxide dismutase [Cu-Zn], chloroplastic [*Vitis vinifera*] | gi\|147789545 | 21.69 | 46.70 | defence and cell rescue |
| [illegible] | 0.3 | 1.2 | uncharacterized protein LOC100232885 [*Vitis vinifera*] | gi\|225447003 | 18.40 | 81.50 | unknown protein |
| [illegible] | 0.5 | 1.0 | proteasome subunit alpha type-2-B [*Vitis vinifera*] | gi\|225423722 | 25.57 | 42.60 | protein degradation |
| [illegible] | 0.5 | 1.0 | RecName: Full=Chalcone--flavonone isomerase 2 | gi\|158514257 | 25.06 | 36.30 | secondary metabolism |
| [illegible] | 0.5 | 1.0 | triosephosphate isomerase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|225427917 | 34.66 | 34.90 | glycolysis/ gluconeogenesis |
| 5108 | 0.5 | 1.0 | cytosolic ascorbate peroxidase [*Vitis vinifera*] | gi\|161778778 | 27.98 | 12.60 | defense and cell rescue |
| 6120 | 1.0 | 0.5 | 26.5 kDa heat shock protein, mitochondrial [*Vitis vinifera*] | gi\|225442975 | 26.30 | 8.58 | protein destination |
| 6120 | 1.0 | 0.5 | flavoprotein wrbA isoform 1 [*Vitis vinifera*] | gi\|225461209 | 21.72 | 37.90 | energy metabolism |
| 6120 | 1.0 | 0.5 | manganese superoxide dismutase [*Vitis vinifera*] | gi\|161778782 | 25.27 | 6.58 | defense and cell rescue |
| 6120 | 1.0 | 0.5 | putative transcription factor [*Vitis vinifera*] | gi\|14582465 | 16.68 | 14.10 | transcription |
| 6013 | 0.7 | 0.5 | superoxide dismutase [Cu-Zn] isoform 2 [*Vitis vinifera*] | gi\|225451120 | 15.26 | 29.60 | defence and cell rescue |
| 6013 | 0.7 | 0.5 | glycine-rich RNA-binding protein GRP1A-like [*Vitis vinifera*] | gi\|359475330 | 16.31 | 54.90 | defence and cell rescue |
| 8716 | 0.4 | 0.5 | transketolase, chloroplastic-like [*Vitis vinifera*] | gi\|359490179 | 78.84 | 25.00 | pentose phosphate pathway |
| 2220 | 0.8 | 0.4 | 14-3-3 protein [*Vitis vinifera*] | gi\|226295432 | 28.63 | 10.70 | signal transduction |
| 4118 | 0.8 | 0.4 | hypothetical protein VITISV_023716 [*Vitis vinifera*] type II peroxiredoxin C [*Vitis vinifera*] | gi\|147781540 | 17.24 | 64.20 | defence and cell rescue |
| 4118 | 0.8 | 0.4 | actin-depolymerizing factor 2-like isoform 1 [*Vitis vinifera*] | gi\|225449595 | 15.94 | 16.50 | cell growth and death |

**Table 2B. Continued**

| Spot[a] | ratio to ACG [b] | | Matched Protein [c] | Accession number [d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADG | BDG | | | | | |
| 5323 | 0.7 | 0.4 | dihydroflavonol-4-reductase [*Vitis vinifera*] | gi\|147805693 | 35.76 | 3.68 | secondary metabolism |
| 3515 | 1.0 | 0.3 | ATP synthase subunit beta, mitochondrial-like [*Vitis vinifera*] | gi\|147838606 | 59.91 | 18.20 | energy metabolism |
| 3515 | 1.0 | 0.3 | mitochondrial-processing peptidase subunit alpha [*Vitis vinifera*] | gi\|225445041 | 54.45 | 26.50 | protein degradation |
| 5208 | 0.6 | 0.3 | TPA: isoflavone reductase-like protein 4 [*Vitis vinifera*] | gi\|76559892 | 33.82 | 70.50 | secondary metabolism |
| 6414 | 0.5 | 0.3 | eukaryotic initiation factor 4A-3-like [*Vitis vinifera*] | 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 [*Vitis vinifera*] | gi\|1707959 | 39.30 | 4.78 | amino acid metabolism |
| 4321 | 0.4 | 0.3 | glutelin type-A 1 [*Vitis vinifera*] | gi\|225435090 | 38.31 | 31.20 | storage protein |
| 4321 | 0.4 | 0.3 | adenosine kinase 2 [*Vitis vinifera*] | gi\|225449018 | 37.72 | 67.40 | nucleotide metabolism |
| 4321 | 0.4 | 0.3 | cysteine synthase isoform 2 [*Vitis vinifera*] | gi\|225451235 | 34.35 | 30.50 | amino acid metabolism |
| 4321 | 0.4 | 0.3 | malate dehydrogenase, cytoplasmic [*Vitis vinifera*] | gi\|225438145 | 35.49 | 6.33 | citrate cycle |
| 4114 | 0.3 | 0.3 | RecName: Full=Caffeoyl-CoA O-methyltransferase [*Vitis vinifera*] | gi\|225428851 | 27.22 | 59.10 | secondary metabolism |
| 3514 | 1.0 | 0.2 | tubulin alpha chain [*Vitis vinifera*] | gi\|225429189 | 49.54 | 54.70 | intracellular transport |
| [illegible] | 0.5 | 0.2 | transketolase, chloroplastic-like [*Vitis vinifera*] | gi\|359490179 | 78.84 | 32.40 | pentose phosphate pathway |
| [illegible] | 0.9 | --- | hsp70-binding protein 1-like [*Vitis vinifera*] | gi\|225440422 | 43.30 | 21.70 | protein destination |

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**Table 2C.** 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 cultivar 'Mourvèdre' (M).

| Spot[a] | ratio to ACM[b] | | Matched Protein [c] | Accession number [d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADM | BDM | | | | | |
| 6202 | * | * | pyridoxal kinase-like [*Vitis vinifera*] | gi\|296087176 | 37.76 | 14.00 | metabolism of cofactors and vitamins |
| 6202 | * | * | 1,3 beta glucanase [*Vitis vinifera*] | gi\|6273716 | 13.35 | 91.80 | cell growth and death |
| 6202 | * | * | glucan endo-1,3-beta-glucosidase [*Vitis vinifera*] | gi\|225441373 | 36.66 | 51.50 | defense and cell rescue |
| 4203 | 47.7 | 30.5 | glutathione S-transferase F9 [*Vitis vinifera*] | gi\|225446791 | 24.89 | 63.40 | defense and cell rescue |
| 4203 | 47.7 | 30.5 | hypothetical protein VITISV_038846 [*Vitis vinifera*] pathogenesis-related protein 17 [*Vitis pseudoreticulata*] | gi\|147784683 | 25.31 | 37.60 | defense and cell rescue |
| 4203 | 47.7 | 30.5 | pathogenesis-related protein 17 [*Vitis pseudoreticulata*] | gi\|374431273 | 25.26 | 44.70 | defence and cell rescue |
| 0201 | 2.3 | 25.8 | 2-Cys peroxiredoxin [*Vitis vinifera*] | gi\|147789752 | 30.19 | 8.42 | defense and cell rescue |
| 0201 | 2.3 | 25.8 | thaumatin-like protein [*Vitis vinifera*] | gi\|8980665 | 24.35 | 11.90 | defense and cell rescue |
| [illegible] | 2.3 | 25.8 | VVTL1 [*Vitis vinifera*] | gi\|2213852 | 23.95 | 50.50 | defense and cell rescue |
| [illegible] | 2.3 | 25.8 | RNA-binding protein 8A [*Vitis vinifera*] | gi\|225431497 | 22.29 | 7.92 | transcription |
| [illegible] | 6.0 | 12.0 | probable nitronate monooxygenase [*Vitis vinifera*] | gi\|225462874 | 36.53 | 56.60 | energy metabolism |
| 0001 | 1.6 | 4.0 | polyphenol oxidase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|147811887 | 66.97 | 9.15 | secondary metabolism |
| [illegible] | 1.4 | 3.9 | transketolase, chloroplastic-like [*Vitis vinifera*] | gi\|359490179 | 78.84 | 30.00 | pentose phosphate pathway |
| [illegible] | 1.3 | 2.3 | chorismate mutase, chloroplastic [*Vitis vinifera*] | gi\|225452920 | 36.40 | 14.70 | amino acid metabolism |
| [illegible] | 7.6 | 1.8 | malate dehydrogenase, cytoplasmic [*Vitis vinifera*] | gi\|225438145 | 35.49 | 8.43 | citrate cycle |
| [illegible] | 7.6 | 1.8 | malate dehydrogenase, mitochondrial [*Vitis vinifera*] | gi\|225461618 | 36.77 | 15.10 | citrate cycle |
| 5201 | 7.6 | 1.8 | probable fructose-bisphosphate aldolase 3, chloroplastic [*Vitis vinifera*] | gi\|225424114 | 42.88 | 9.67 | glycolysis/ gluconeogenesis |
| 5205 | 7.6 | 1.8 | putative fructokinase-5-like [*Vitis vinifera*] | gi\|225459906 | 34.92 | 72.30 | glycolysis/ gluconeogenesis |
| [illegible] | 7.6 | 1.8 | malate dehydrogenase, chloroplastic [*Vitis vinifera*] | gi\|225457407 | 43.59 | 48.70 | citrate cycle |
| [illegible] | 2.0 | 1.1 | argininosuccinate synthase, chloroplastic-like [*Vitis vinifera*] | gi\|225456274 | 55.16 | 7.04 | amino acid metabolism |
| [illegible] | 2.0 | 1.1 | biotin carboxylase 1, chloroplastic-like [*Vitis vinifera*] | gi\|225445664 | 57.32 | 4.57 | lipid metabolism |
| [illegible] | 2.0 | 0.6 | 18.2 kDa class I heat shock protein [*Vitis vinifera*] | gi\|147785904 | 18.15 | 12.50 | protein destination |
| 7603 | 0.8 | 0.5 | NADP-dependent malic enzyme [*Vitis vinifera*] | gi\|225445108 | 65.26 | 8.80 | pyruvate metabolism |
| [illegible] | 0.8 | 0.5 | heat shock protein STI-like [*Vitis vinifera*] | gi\|359497489 | 36.32 | 42.30 | protein destination |
| [illegible] | 0.7 | 0.5 | ATPase subunit 1 [*Vitis vinifera*] | gi\|164685652 | 44.27 | 8.47 | energy metabolism |
| [illegible] | 0.7 | 0.5 | uncharacterized aminotransferase y4uB-like [*Vitis vinifera*] | gi\|225464809 | 57.62 | 12.10 | signal transduction |
| [illegible] | 0.7 | 0.5 | elongation factor 1-alpha-like [*Vitis vinifera*] | gi\|225435233 | 49.32 | 22.10 | translation |
| [illegible] | 0.6 | 0.5 | 23.6 kDa heat shock protein, mitochondrial isoform 1 [*Vitis vinifera*] | gi\|225466111 | 23.72 | 36.10 | protein destination |
| 6106 | 1.2 | 0.4 | L-ascorbate peroxidase 2, cytosolic [*Vitis vinifera*] | gi\|225435177 | 27.54 | 52.80 | Other carbohydrates metabolism |
| 6106 | 1.2 | 0.4 | enolase [*Vitis vinifera*] | gi\|225455555 | 48.09 | 24.90 | glycolysis/ gluconeogenesis |
| 4103 | 0.9 | 0.4 | 26.5 kDa heat shock protein, mitochondrial [*Vitis vinifera*] | gi\|225442975 | 26.30 | 17.20 | protein destination |
| 4103 | 0.9 | 0.4 | flavoprotein wrbA isoform 1 [*Vitis vinifera*] | gi\|225461209 | 21.72 | 24.60 | energy metabolism |
| 4103 | 0.9 | 0.4 | manganese superoxide dismutase [*Vitis vinifera*] | gi\|161778782 | 17.03 | 9.74 | defense and cell rescue |
| 4103 | 0.9 | 0.4 | glutathione S-transferase F9 [*Vitis vinifera*] | gi\|225446793 | 24.95 | 13.50 | defense and cell rescue |
| 1104 | 0.9 | 0.4 | peroxiredoxin-2B [*Vitis vinifera*] | gi\|225445188 | 17.24 | 39.50 | defense and cell rescue |

**Table 2C. continued**

| Spot[a] | ratio to ACM [b] | | Matched Protein [c] | Accession number [d] | Mw [e] | Coverage % [f] | Category [g] |
|---|---|---|---|---|---|---|---|
| | ADM | BDM | | | | | |
| 3104 | 0.6 | 0.4 | 22.0 kDa heat shock protein [*Vitis vinifera*] | gi\|225459900 | 21.11 | 48.90 | protein destination |
| 3104 | 0.6 | 0.4 | uncharacterized N-acetyltransferase p20 [*Vitis vinifera*] | gi\|225432712 | 20.18 | 27.20 | uncharacterizied protein |
| 6103 | 6.3 | 0.3 | major allergen Pru av 1 [*Vitis vinifera*] | gi\|225431844 | 17.11 | 17.70 | defense and cell rescue |
| 6103 | 6.3 | 0.3 | MLP-like protein 28 [*Vitis vinifera*] | gi\|225424272 | 17.18 | 58.30 | defense and cell rescue |
| 1002 | 1.3 | 0.3 | regulator of ribonuclease-like protein 2 [*Vitis vinifera*] | gi\|225430941 | 17.75 | 13.30 | transcription |
| 1002 | 1.3 | 0.3 | 18.2 kDa class I heat shock protein [*Vitis vinifera*] | gi\|225449290 | 17.03 | 15.90 | protein destination |
| 1002 | 1.3 | 0.3 | superoxide dismutase [Cu-Zn], chloroplastic [*Vitis vinifera*] | gi\|147789545 | 21.69 | 41.50 | defence 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 [*Vitis vinifera*] | gi\|76559892 | 33.82 | 17.20 | secondary metabolism |
| 0309 | 1.4 | 0.2 | uncharacterized protein LOC100232885 [*Vitis vinifera*] | gi\|225447003 | 18.40 | 66.50 | unknown protein |
| 1304 | 1.7 | 0.2 | fructokinase-2 [*Vitis vinifera*] | gi\|225433918 | 35.18 | 8.48 | glycolysis/gluconeogenesis |
| 1304 | 1.7 | 0.2 | epoxide hydrolase 2 [*Vitis vinifera*] | gi\|359496593 | 34.90 | 15.20 | metabolism of terpenoids and polyketides |
| [illegible] | 0.6 | 0.1 | triosephosphate isomerase, chloroplastic-like isoform 1 [*Vitis vinifera*] | gi\|225427917 | 34.66 | 41.70 | glycolysis/gluconeogenesis |
| [illegible] | 0.6 | 0.1 | ras-related protein RABA1d [*Vitis vinifera*] | gi\|147772737 | 9.74 | 23.00 | signal transduction |
| [illegible] | 0.5 | 0.1 | small heat shock protein, chloroplastic [*Vitis vinifera*] | gi\|225455238 | 25.01 | 42.30 | protein destination |
| 1105 | 0.8 | 0.01 | small heat shock protein, chloroplastic [*Vitis vinifera*] | gi\|225455238 | 25.01 | 36.60 | protein destination |
| 0105 | 0.4 | | small heat shock protein, chloroplastic [*Vitis vinifera*] | gi\|225455238 | 25.01 | 28.60 | protein destination |

[a] Spot code as reported in Figures 1 and 4.

[b] Ratio of spot expression values (relative OD*area%) in asymptomatic (ADC, ADG or ADM) and brown striped (BDC, BDG or BDM) wood of diseased plants to the related control (ACC, ACG or ACM). Values indicating over or down expression (ratio ≥ |2|) are highlighted in yellow or grey, 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.

[c] Protein identified via the MASCOT and OMSSA search engines against in house made database from NCBInr database.

[d] Accession No. of the matched protein as reported in the NCBI database.

[e] Molecular mass (kDa).

[f] Percentage of the protein sequence covered by the matching peptides.

[g] functional category retrieved from GenomeNet Database Resources website (http:www.genome.jp/kegg) or in literature.

**Table 3.** Total Phenolic (TP) and stilbenes compounds concentrations in asymptomatic trunk wood of control (AC) or diseased (AD) plants and in the brown striped one of diseased (BD) plants from 'Chardonnay' (C), 'Gewurztraminer' (G) and 'Mourvèdre' (M).

| Sample | TP (mg GAE $g^{-1}$ FW)* | Stilbenes compounds (µg $g^{-1}$ FW) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *tr*-piceids | *tr*-resveratrol | *tr*-ε-viniferin | *tr*-vitisin A | *tr*-vitisin B | *tr*-piceatannol |
| ACC | 0.431±0.023 ab | 0.015±0.010 a | 11.92±10.05 a | nd a | nd a | 0.007±0.003 a | nd a |
| ADC | 0.426±0.057 a | 0.034±0.009 b | 35.26±11.35 ab | nd a | nd a | 0.004±0.004 a | nd a |
| BDC | 1.057±0.139 b | 0.311±0.053 b | 162.9±61.30 b | 0.782±0.312 b | nd a | 0.12±0.007 a | nd a |
| ACG | 0.123±0.007 a | nd a | nd a | nd a | nd a | nd a | nd a |
| ADG | 0.227±0.048 ab | 0.080±0.006 a | 0.099±0.055 ab | 0.058±0.040 ab | 0.081±0.058 a | 0.007±0.003 ab | 0.008±0.006 b |
| BDG | 1.093±0.261 b | 0.045±0.017 a | 1.207±0.332 b | 3.485±1.116 b | 42.49±19.49 b | 0.133±0.054 a | 0.128±0.042 b |
| ACM | 0.299±0.042 a | 0.133±0.032 a | 104.8±17.08 a | 0.046±0.009 a | nd a | nd a | nd a |
| ADM | 0.394±0.044 ab | 0.080±0.019 a | 264.4±74.57 a | 0.034±0.013 ab | nd a | 0.078±0.009 b | nd a |
| BDM | 0.687±0.070 b | 0.134±0.018 a | 297.6±24.03 a | 1.418±0.318 b | nd a | nd a | nd a |

a-b: Lower case letters indicate significant difference (α = 0.05) for the concentrations found for each cultivar (Dunn's multiple comparison test, $P \leq 0.05$); nd: not-detected. * Total phenolics were expressed as mg of gallic acid equivalents (GAE) per g of plant tissues.

# FIGURE CAPTION

**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 4–7). The relative molecular mass (kDa) was calibrated with standard protein markers (Prestained SDS-PAGE Standards, Bio-Rad, USA) 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.

**Fig. 2.** Venn diagrams showing number and percentage of protein spots with similar expression (|ratio of relative OD×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: **A**) 'Chardonnay' (ACC, ADC and BDC), **B**) 'Gewurztraminer' (ACG, ADG and BDG) and **C**) 'Mourvèdre' (ACM, ADM and BDM).

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

**Fig. 4.** Relative expression of 15 selected genes in the asymptomatic (AD) and in the brown striped (BD) wood of diseased vines cv. 'Chardonnay' (C), cv. 'Gewurztraminer' (G) and cv. 'Mourvèdre' (M). The colour scale bars represent the ratio values corresponding to the mean of

three independent experiments. Genes overexpressed appear in shades of red, with expression level higher than 30 in bright red, while those repressed appear in shades of blue, with intensity lower than 0.1 in dark blue (white = no change in gene expression compared to control). Gene expression was considered as significantly up- or down-regulated to the 1 × control, when changes in relative expression were > 2× or < 0.5×, respectively. [a] selected genes whose expressions are significantly induced or repressed in woody tissues of diseased vines compared to their respective controls (C, G or M) at $p \leq 0.05$.

Supplemental Table 1

http://www.scientificsocieties.org/PHYTOXtras/PHYTO-01-14-0007-R_SupplementalTable2.xls

Supplemental Table 2

http://www.scientificsocieties.org/PHYTOXtras/PHYTO-01-14-0007-R_SupplementalTable3.xls

Supplemental Table 3

http://www.scientificsocieties.org/PHYTOXtras/PHYTO-01-14-0007-R_SupplementalTable4.xls

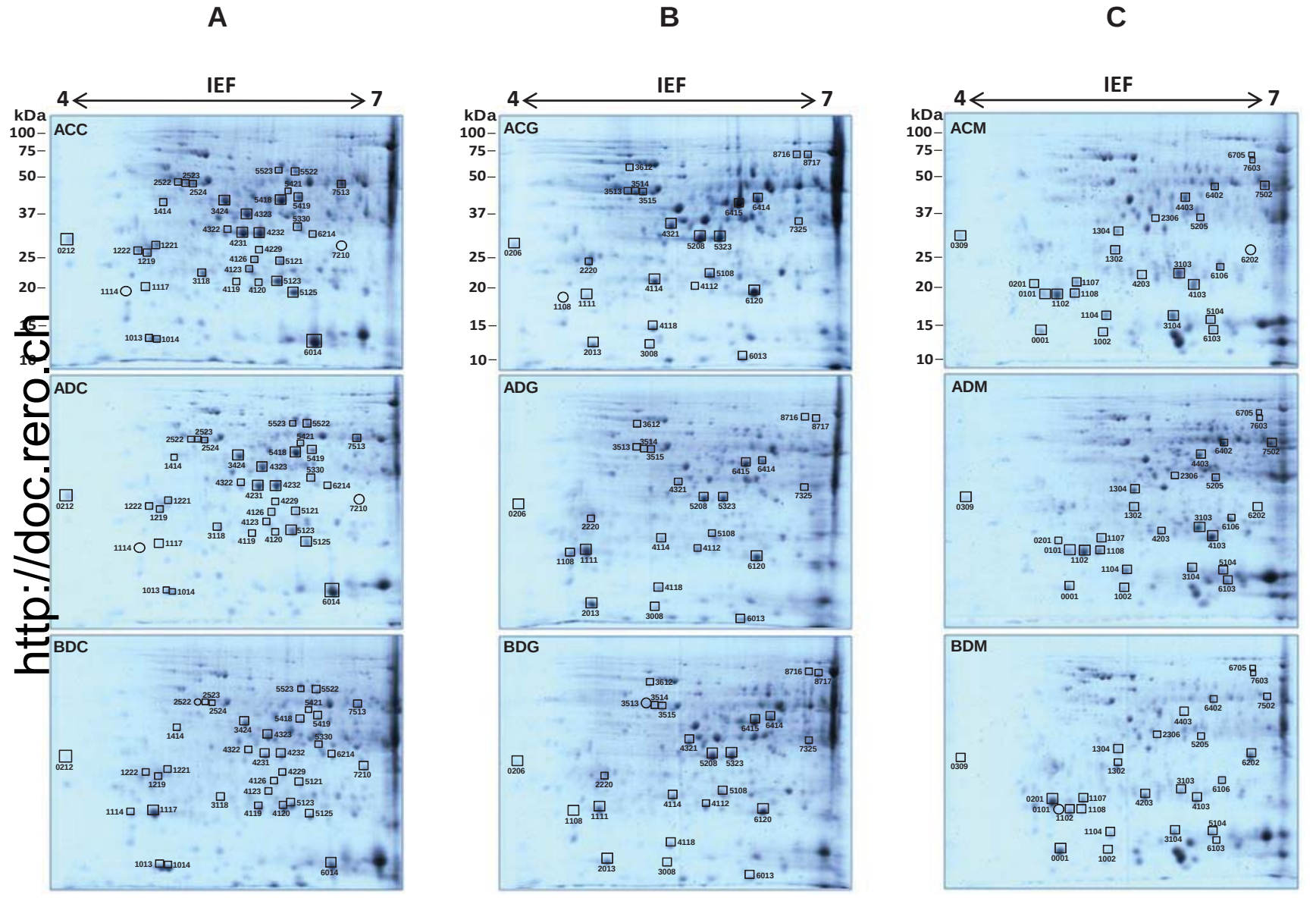




**Fig. 1, Spagnolo et al., *Phytopathology***

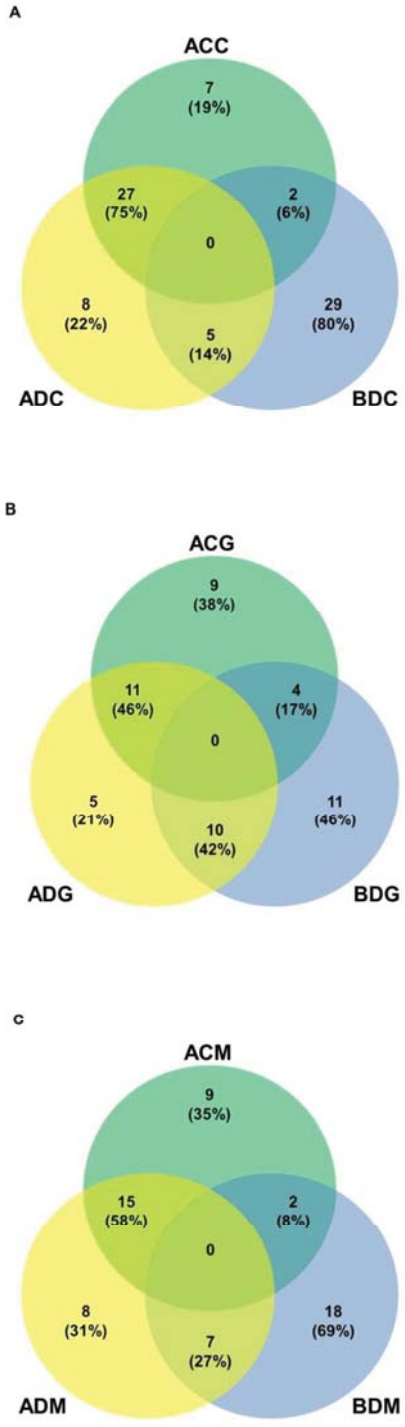


**Fig. 2, Spagnolo et al., *Phytopathology***

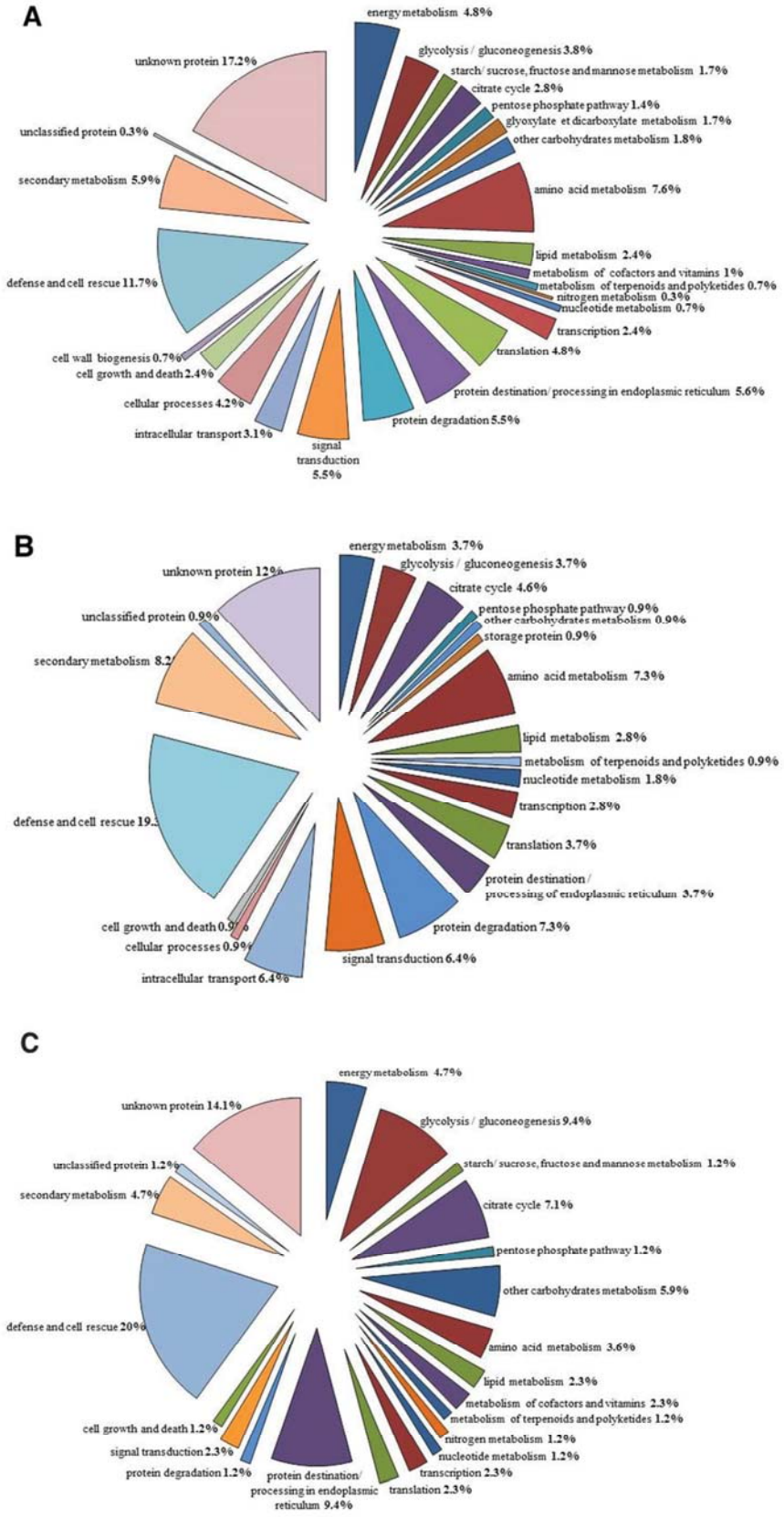


**Fig. 3, Spagnolo et al., *Phytopathology***

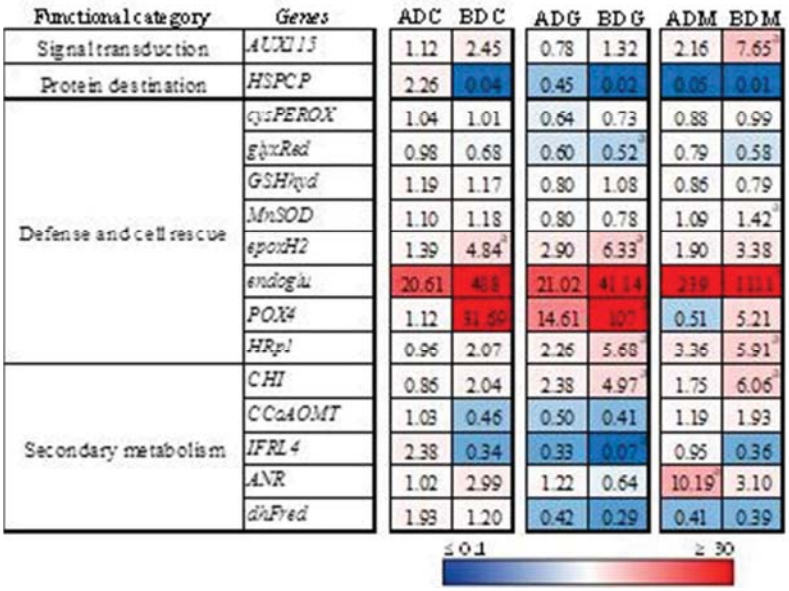


**Fig. 4. Spagnolo et al., *Phytopathology***