Cultivar- and Wood Area-Dependent Metabolomic Fingerprints of Grapevine Infected by *Botryosphaeria dieback*

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ABSTRACT

Botryosphaeria dieback is one of the most significant grapevine trunk diseases that affects the sustainability of the vineyards and provokes economic losses. The causal agents, *Botryosphaeriaceae* species, live in and colonize the wood of the perennial organs causing wood necrosis. Diseased vines show foliar symptoms, chlorosis, or apoplexy, associated to a characteristic brown stripe under the bark. According to the susceptibility of the cultivars, specific proteins such as PR-proteins and other defense-related proteins are accumulated in the brown stripe compared with the healthy woody tissues. In this study, we enhanced the characterization of the brown stripe and the healthy wood by obtaining a metabolite profiling for the three cultivars Chardonnay, Gewurztraminer,

and Mourvèdre to deeper understand the interaction between the *Botryosphaeria dieback* pathogens and grapevine. The study confirmed a specific pattern according to the cultivar and revealed significant differences between the brown stripe and the healthy wood, especially for phytochemical and lipid compounds. This is the first time that such chemical discrimination was made and that lipids were so remarkably highlighted in the interaction of *Botryosphaeriaceae* species and grapevine. Their role in the disease development is discussed.

Keywords: *Botryosphaeria dieback*, cultivar, FC-ICR-MS, grapevine trunk diseases, lipids, metabolomics, phytochemicals

Grapevine trunk diseases (GTDs), namely Esca, *Botryosphaeria*, and Eutypa diebacks are now considered as a major concern in viticulture. In France, a survey of 329 vineyards during the 2003 to 2008 period revealed that 54 to 100% of them were affected by Esca/*Botryosphaeria dieback*, depending on the region and year (Bruez et al. 2013). In 2008, about 2 to 8% of vines expressed symptoms (Bruez et al. 2013). In Spain, the incidence of GTDs was 10.5% in 2007 (Rubio and Garzón 2011) and reached 60 to 80% in old south Italian vineyards (Romanazzi 2009). About 13% of the French vineyard was considered unproductive in 2012, because of GTDs, and it reached 20% in Spain and 30% in Italy (Grosman and Doublet 2012). GTDs still extend worldwide and gradually degrade the vineyards, leading to huge economic impact due to yield decrease and vine death (Guerin-Dubrana et al. 2019). Costs for replacement of dead vines were indeed estimated to at least 1.5 billion dollars per year (Hofstetter et al. 2012). In California, overall costs associated to GTDs were estimated at more than \$260 million per year (for review, see Fontaine et al. 2016).

GTDs are caused by xylem-inhabiting fungi that colonize and degrade wood tissues, with subsequent symptoms on the aerial parts. Twenty-one different fungi of *Botryosphaeriaceae* species were associated to *Botryosphaeria dieback*; among with *Neofusicoccum*

parvum, *Botryosphaeria dothidea*, and *Diplodia seriata* (Urbez-Torres 2011; van Niekerk et al. 2006). Symptoms of *Botryosphaeria dieback* on the aerial parts consist in leaf intervein coloration or leaf wilting, bud necrosis or mortality, dead arms, shoot dieback, and bunch rot (Bertsch et al. 2013; Larignon et al. 2001; Spagnolo et al. 2014; Urbez-Torres 2011; van Niekerk et al. 2004). At the trunk level, the fungi cause perennial cankers, brown wood streaking, internal necrotic lesions, or a typical longitudinal orange/brown stripe located in the outer xylem, superficially and just beneath the bark (Fontaine et al. 2016; Larignon et al. 2001; Urbez-Torres 2011). This longitudinal stripe has the particularity to be undetectable before the vegetative season and is therefore associated with leaf symptoms (Larignon et al. 2001).

Although cultivars of *Vitis vinifera* L. could be ranked on the basis of the degree of GTD symptom expression (Bruez et al. 2013; Grosman and Doublet 2012; Murolo and Romanazzi 2014), none of them was found to be resistant. For example, Chardonnay is considered as less susceptible, Mourvèdre or Gewurztraminer susceptible, and Savagnin highly susceptible to Esca disease/*Botryosphaeria dieback* (Grosman and Doublet 2012). Despite active research, no effective and generalizable strategies of crop protection are now available (Mondello et al. 2018a, b), which makes further investigation necessary. Getting a good knowledge of the effects of the disease on the plant can help identify ways to limit its impact. Few years ago, a proteomic study was done to compare the response of woody tissues to *Botryosphaeria dieback* of the three cultivars Chardonnay, Mourvèdre, and Gewurztraminer, localized on the longitudinal brown stripe (Spagnolo et al. 2014). Accumulation of several proteins (PR proteins, and other stress- and defense-related proteins like polyphenol oxidase) were found inside the brown stripe wood of diseased vines compared with the neighboring apparent healthy wood of diseased or control vines.

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

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Moreover, the differential of the susceptibility of the three cultivars could be linked to the diverse expression of proteins. In the present study, the same samples were analyzed by an ultra-high resolution method of metabolome analysis (Fourier transform-ion cyclotron resonance-mass spectrometry [FT-ICR-MS]), to obtain a metabolite profiling of these different woody areas, for the three cultivars, and to progress in the understanding of the impact of *Botryosphaeria dieback* in grapevine.

MATERIALS AND METHODS

Plant material. It was the same sampling as the one described in Spagnolo et al. (2014), i.e., Chardonnay, Gewurztraminer (white cultivars), and Mourvèdre (red cultivar). For each of them, three asymptomatic (control) and three *Botryosphaeria dieback* expressing vines, respectively, were uprooted in July 2011 from three French vineyards to collect samples. Control plants did not express any GTD-related foliar symptom since at least 5 years. Three groups of samples were collected per cultivar: asymptomatic white trunk wood from control (AC) and diseased (AD) plants, and brown striped trunk wood from diseased (SD) plants (Supplementary Fig. S1).

A total of nine samples corresponding to three biological replicates per group (i.e., three wood samples from three separated plants) were used for analysis. Raw wood samples collected in 2011 were immediately frozen in the field with liquid nitrogen and subsequently stored at -80°C until metabolomics analyses. In 2013, frozen wood samples were ground to a fine powder in liquid nitrogen with a Mixer Mill MM 400 (Retsch, Haan, Germany), and powders were then extracted for FT-ICR-MS metabolomics analyses.

FT-ICR-MS analysis. Prior to analysis, 15 mg of each sample were extracted with 1 ml of methanol (LC-MS grade, Fluka Analytical; Sigma-Aldrich, St. Louis, MO, U.S.A.) and sonicated for 30 min. After centrifugation ($25,000 \times g$, 10 min, room temperature), the supernatant was collected and rediluted in methanol (1/50 [vol/vol]). Ultra-high resolution mass spectra were acquired using an FT-ICR-MS (solariX, Bruker Daltonics GmbH, Bremen, Germany) equipped with a 12 Tesla superconducting magnet (MagneX Scientific Inc., Yarnton, GB) and an APOLO II ESI source (Bruker Daltonics GmbH) operated in the negative ionization mode. Samples were introduced into the microelectrospray source at a flow rate of 120 $\mu\text{l/h}$. Spectra were acquired with a time domain of 4 mega words over an m/z mass range of 100 to 1,000, and 300 scans were accumulated per sample.

Data analysis. Spectra were externally calibrated on clusters of arginine (methanol at 10 mg/liter). Further internal calibration was

performed for each sample by using a list of ubiquitous fatty acids and recurrent wine compounds, allowing mass accuracies of 0.1 ppm (Gugeon et al. 2009). After acquisition, all m/z detected were given an identification number (ID) in order to simplify further data processing. Data treatments were applied on the m/z lists and detailed in Supplementary Figure S2. First, m/z were reduced to values seen at least three times either in the whole data set or in the cultivar subsets. Remaining masses (m/z) coupled to their respective peak intensities were submitted to statistical analysis with the Perseus software (<http://maxquant.net/perseus/>), which performed hierarchical clustering (HCA) and multivariate analysis of variance (ANOVA) ($P < 0.05$) between groups of samples to get significant m/z (Supplementary Fig. S2, step a). m/z were also subjected to the NetCalc algorithm and in-house software tools to obtain elemental

Fig. 1. Characteristics of the three cultivar subsets. The global data file was divided into three cultivar subsets. Values on the top of the figure correspond to the number of raw m/z and assigned formulas for each cultivar. The structural categories were next defined by multidimensional stoichiometric constraints classification on the validated m/z subsets and relative proportions illustrated on pie plots. CH, Chardonnay; GW, Gewurztraminer; MV, Mourvèdre; and NM, nonmatching structures.

Fig. 2. Comparison of the three cultivar data subsets. Venn diagram of the distribution of specific formulas for the three cultivars (top panel) (CH, Chardonnay; GW, Gewurztraminer; and MV, Mourvèdre) and multidimensional stoichiometric constraints classification structural categories distribution of the 2,612 common formulas (bottom panel). NM, nonmatching structures.

Fig. 3. Comparison of AC, AD, and SD sample types. For each comparison, Venn diagrams at the top of the illustration indicate the number of differentially accumulated m/z , common/specific to the three cultivars. The common m/z were next validated (number indicated in the middle part of the figure) and submitted to search for identification (MassTriX). Resulting numbers of annotated m/z are indicated in the bottom Venn diagrams. AC, white wood of asymptomatic vines; AD, white wood of diseased vines; SD, brown stripe of diseased vines; CH, Chardonnay; GW, Gewurztraminer; MV, Mourvèdre; and NM, nonmatching structures.

chemical formula (Tziotis et al. 2011) validated by setting van Krevelen (VK) chemical constraints ($O/C \text{ ratio} \leq 1$; double bound equivalent (DBE): $0 < DBE/C < 5$ $H/C \text{ ratio} \leq 2n + 2$ and aromaticity index (IA): $-20 < IA < 0.7$; element counts: $C \leq 100$, $H \leq 200$, $O \leq 80$, $n \leq 3$, $S \leq 3$, and $P \leq 1$) applied to exclude rare or impossible formulas (Supplementary Fig. S2, step b). Elemental formulas were next classified into the main putative chemical functional categories (lipids, peptides, amino sugars, carbohydrates, nucleotides, and phytochemicals/aromatic compounds) using the multidimensional stoichiometric constraints classification (MSCC) described by Rivas-Ubach et al. (2018), based on the $C/H/O/N/P$ stoichiometric ratios (Supplementary Fig. S2, step c). We used the short term “phytochemicals” for “phytochemicals/aromatic compounds,” which corresponds to secondary metabolites. Proportions of resulting predicted classified and nonclassified (not matching: NM) categories are graphically represented on pie plots. m/z values were also fed into MassTriX software (<http://masstrix3.helmholtz-muenchen.de/masstrix3/>) for annotation requests using KEGG, HMDB, and LipidMaps databases, and *V. vinifera* as preferred organism (1 ppm tolerance value) (Supplementary Fig. S2, step d). Compounds identified as isotopologues (^{13}C , ^{15}N , and ^{18}O) already listed helped for category revision when necessary (MSCC, Supplementary Fig. S2, step e) but were discarded from output lists and further discussions. In pairwise comparisons, the most

TABLE 1. List of the 23 top m/z accumulated in Chardonnay AD samples compared with AC samples^a

ID	P	t test	Formula	Cat.	m/z	Annotations
6398	0.0001	-6.119	$C_7H_{13}NO_5$	As	190.072108	2-Amino-3,7-dideoxy-D-threo-hept-6-ulosonic acid
10556	0.0015	-4.668	$C_{18}H_{14}O_8$	Ph	357.061607	5,3'-Dihydroxy-4',5'-dimethoxy-6,7-methylenedioxyisoflavone
7059	0.0004	-4.473	$C_9H_{14}N_2O_5$	Pp	229.082987	Aspartyl-L-proline; (2S)-1-[(2S)-2-amino-3-carboxy-propanoyl]pyrrolidine-2-carboxylic acid
10808	0.0001	-4.363	$C_{24}H_{46}O_2$	Lp	365.342494	(15Z)-Tetracosenoic acid; nervonic acid
6580	<0.0001	-4.254	$C_{11}H_{12}N_2O_2$	Pp	203.082615	L-Tryptophan; tryptophan; (S)- α -amino- β -(3-indolyl)-propionic acid
6805	0.0025	-4.207	$C_9H_{17}NO_5$	Lp	218.103393	Pantothenate; pantothenic acid; (R)-pantothenate
8781	0.0005	-4.134	$C_{19}H_{38}O_2$	Lp	297.279901	Nonadecanoic acid; nonadecylic acid
9077	0.0008	-4.130	$C_{18}H_{28}O_4$	Lp	307.191489	5-O-methylembelin
8030	0.0003	-3.952	$C_{17}H_{34}O_2$	Lp	269.248606	Methyl palmitate
8390	<0.0001	-3.942	$C_{12}H_{15}NO_7$	Ph	284.077572	Buchananine
11523	0.0001	-3.938	$C_{22}H_{16}O_7$	Ph	391.082331	Hosloppin
6352	0.0030	-3.901	$C_8H_{13}NO_4$	Lp	186.077178	6-Acetamido-3-oxohexanoate
6569	0.0017	-3.895	$C_9H_{17}NO_4$	Lp	202.108462	L-Acetylcarnitine (see KEGG C02571); (3S)-3-acetyloxy-4-trimethylammonio-butanoate
9192	0.0006	-3.89	$C_{20}H_{40}O_2$	Lp	311.295532	Phytanate; phytanic acid
7693	0.0024	-3.803	$C_{11}H_{20}N_2O_5$	Pp	259.129924	L- γ -Glutamyl-L-isoleucine
10447	0.0009	-3.763	$C_{20}H_{32}O_5$	Lp	351.2177	Prostaglandin H2; (5Z,13E)-(15S)-9 α ,11 α -epidioxo-15-hydroxyprosta-5,13-dienoate; (5Z,13E,15S)-9 α ,11 α -epidioxo-15-hydroxyprosta-5,13-dienoate
6364	0.0038	-3.732	$C_9H_{16}O_4$	Lp	187.097585	Azelaic acid; nonanedioic acid; 1,7-heptanedicarboxylic acid
10007	0.0015	-3.731	$C_{22}H_{44}O_2$	Lp	339.326849	Docosanoic acid; docosanoate; behenic acid
8715	0.0053	-3.72	$C_{18}H_{32}O_3$	Lp	295.227864	(9Z,12Z)-(8S)-Hydroxyoctadeca-9,12-dienoic acid; laetiseric acid; 8-hydroxylinoleic acid
7362	0.0027	-3.69	$C_{13}H_{24}O_4$	Lp	243.160178	1,11-Undecanedicarboxylic acid; tridecanedioic acid
6674	0.0009	-3.661	$C_{11}H_{14}O_4$	Ph	209.08194	Sinapyl alcohol; sinapoyl alcohol
7602	0.0008	-3.639	$C_{16}H_{32}O_2$	Lp	255.232949	Hexadecanoic acid; hexadecanoate; hexadecylic acid; palmitic acid; palmitate; cetyllic acid
8073	0.0035	-3.624	$C_{16}H_{32}O_3$	Lp	271.227874	(R)-3-Hydroxy-hexadecanoic acid; (3R)-3-hydroxyhexadecanoic acid

^a Selection was made using Perseus applying t test (FDR = 0.001 and $S_0 = 1$) and components are ranked according to their t test statistic values. AC, white wood of asymptomatic vines; AD, white wood of diseased vines. ID, component identity; P , t test associated P value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

TABLE 2. List of the 39 top *m/z* accumulated or depleted in Gewurztraminer AD samples compared with AC samples (negative or positive test statistic values)^a

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	<i>m/z</i>	Annotations
6371	0.0328	-2.951	C ₇ H ₁₁ NO ₅	As	188.056451	N-Acetyl-L-glutamate; N-acetyl-L-glutamic acid
9942	0.0106	-2.909	C ₁₆ H ₁₈ O ₈	Ph	337.092839	1-Caffeoyl-4-deoxyquinic acid
12506	0.0112	-2.901	C ₂₁ H ₃₇ O ₇ P	Lp	431.220428	PA(18:3(6Z,9Z,12Z)/0:0); 1-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3P
8729	0.0291	-2.901	C ₁₄ H ₁₉ NO ₆	Lp	296.113986	Phenethylamine glucuronide (see KEGG C03033); phenylethylamino)oxane-2-carboxylic acid
13981	0.0157	-2.855	C ₂₁ H ₂₄ O ₁₃	Ph	483.114396	Diospyrin
8447	0.0089	-2.852	C ₁₂ H ₁₇ NO ₇	Lp	286.093239	Volkenin
6414	0.0087	-2.768	C ₇ H ₁₂ O ₆	Cb	191.056114	Quinate; quinic acid; kinic acid; chinic acid; L-quinic acid; L-quinic acid; (-)-quinic acid
13994	0.0102	-2.743	C ₂₂ H ₄₅ O ₉ P	Lp	483.272897	PG(16:0/0:0); 1-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
17801	0.0277	-2.732	C ₄₂ H ₇₉ O ₁₃ P	Lp	821.518937	PI(13:0/20:1(11Z)); 1-tridecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-myo-inositol)
17738	0.0158	-2.727	C ₄₃ H ₇₅ O ₁₂ P	Lp	813.492787	PI(P-16:0/18:4(6Z,9Z,12Z,15Z)); 1-(1Z-hexadecenyl)-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phospho-(1'-myo-inositol) [1Z-alkenyl,2-acylglycerophosphoinositols]
15389	0.0280	-2.715	C ₂₅ H ₃₄ O ₁₄	Lp	557.187735	7-Epiloganin tetraacetate
16418	0.0169	-2.714	C ₃₆ H ₆₅ O ₈ P	Lp	655.434639	PA(13:0/20:3(8Z,11Z,14Z)); 1-tridecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3P
17357	0.0202	-2.710	C ₄₃ H ₇₉ O ₈ P	Lp	753.544006	PA(18:1(9Z)/22:2(13Z,16Z)); 1-(9Z-octadecenoyl)-2-(13Z,16Z-docosadienoyl)-glycero-3P
17171	0.0200	-2.691	C ₃₉ H ₆₉ O ₁₀ P	Lp	727.455621	PG(13:0/20:4(5Z,8Z,11Z,14Z)); 1-tridecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-glycero-3-phospho-(1'-sn-glycerol)
12566	0.0137	-2.682	C ₂₁ H ₃₉ O ₇ P	Lp	433.236085	Oleoylglycerone phosphate
17364	0.0213	-2.680	C ₄₃ H ₈₁ O ₈ P	Lp	755.560008	PA(18:0/22:2(13Z,16Z)); 1-octadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3P
17284	0.0259	-2.663	C ₄₀ H ₇₃ O ₁₀ P	Lp	743.487155	PG(16:0/18:3(6Z,9Z,12Z)); 1-hexadecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)
17301	0.0213	-2.652	C ₄₀ H ₇₅ O ₁₀ P	Lp	745.502826	PG(16:0/18:2(9Z,12Z)); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)
11978	0.0140	-2.626	C ₁₉ H ₃₉ O ₇ P	Lp	409.236062	1-Palmitoylglycerol 3P; 1-hexadecanoyl-sn-glycero-3P
16981	0.0219	-2.625	C ₃₇ H ₇₁ O ₁₀ P	Lp	705.471462	PG(12:0/19:1(9Z)); 1-dodecanoyl-2-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-sn-glycerol)
11190	0.0055	-2.624	C ₂₁ H ₁₆ O ₇	Ph	379.082660	Diphyllin
17699	0.0168	-2.620	C ₄₁ H ₇₇ O ₁₃ P	Lp	807.503427	PI(16:0/16:1(9Z)) (see KEGG C00626); 1-hexadecanoyl-2-(9Z-hexadecenoyl)-sn-glycero-3-phosphoinositol
16538	0.0188	-2.617	C ₃₇ H ₆₇ O ₈ P	Lp	669.450309	PA(14:0/20:3(8Z,11Z,14Z)); 1-tetradecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3P
17118	0.0280	-2.614	C ₃₈ H ₇₅ O ₁₀ P	Lp	721.502885	PG(16:0/16:0); 1,2-dihexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
16562	0.0201	-2.573	C ₃₇ H ₆₉ O ₈ P	Lp	671.466004	PA(16:0/18:2(9Z,12Z)) (see KEGG C00416); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3P
16702	0.0097	-2.572	C ₃₈ H ₆₇ O ₈ P	Lp	681.45028	PA(13:0/22:4(7Z,10Z,13Z,16Z)); 1-tridecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P
15365	0.0233	-2.537	C ₂₅ H ₃₂ O ₁₄	Ph	555.172014	7-Dehydrologenin tetraacetate

(Continued on next page)

^a Selection was made using Perseus applying *t* test (FDR = 0.03 and *S*₀ = 1) and components are ranked according to their *t* test statistic values. If multiple MassTrix annotations for the same formula were obtained, only the two first ones are listed. AC, white wood of asymptomatic vines; AD, white wood of diseased vines; ID, component identity; *P*, *t* test associated *P* value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and *m/z*, measured mass value.

TABLE 2. (Continued from previous page)

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	<i>m/z</i>	Annotations	
16853	0.0227	−2.535	C ₃₉ H ₆₇ O ₈ P	Lp	693.450342	PA(14:1(9Z)/22:4(7Z,10Z,13Z,16Z)); 1-(9Z-tetradecenoyl)-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P	PA(18:1(9Z)/18:4(6Z,9Z,12Z,15Z)); 1-(9Z-octadecenoyl)-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3P
16436	0.0280	−2.533	C ₃₆ H ₆₇ O ₈ P	Lp	657.450318	PA(13:0/20:2(11Z,14Z)); 1-tridecanoyl-2-(11Z,14Z-eicosadienoyl)-glycero-3P	PA(14:1(9Z)/19:1(9Z)); 1-(9Z-tetradecenoyl)-2-(9Z-nonadecenoyl)-glycero-3P
17113	0.0046	−2.524	C ₃₃ H ₃₈ O ₁₈	Ph	721.199196	6'''-(3-Hydroxy-3-methylglutaroyl)isoviolanthin	
16871	0.0241	−2.523	C ₃₉ H ₆₉ O ₈ P	Lp	695.466015	PA(16:0/20:4(5Z,8Z,11Z,14Z)); 1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3P	PA(14:0/22:4(7Z,10Z,13Z,16Z)); 1-tetradecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P
16337	0.0177	−2.516	C ₃₅ H ₆₉ O ₈ P	Lp	647.465942	PA(16:0/16:0) (see KEGG C00416); 1,2-dihexadecanoyl-rac-glycero-3P	PA(16:0/16:0)[rac]; 1,2-dihexadecanoyl-sn-glycero-3P
16924	0.0289	−2.505	C ₃₉ H ₇₃ O ₈ P	Lp	699.497294	PA(18:0/18:2(9Z,12Z)) (see KEGG C00416); 1-octadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3P	PA(18:1(11Z)/18:1(11Z)) (see KEGG C00416); 1,2-di(11Z-octadecenyl)-rac-glycero-3P
15250	0.0086	−2.503	C ₂₈ H ₅₅ O ₈ P	Lp	549.35616	PA(13:0/12:0); 1-tridecanoyl-2-dodecanoyl-glycero-3P	
16184	0.0092	−2.481	C ₃₄ H ₆₅ O ₈ P	Lp	631.434712	PA(12:0/19:1(9Z)); 1-dodecanoyl-2-(9Z-nonadecenoyl)-glycero-3P	PA(13:0/18:1(9Z)); 1-tridecanoyl-2-(9Z-octadecenyl)-glycero-3P
13509	0.0092	−2.480	C ₂₁ H ₃₉ O ₉ P	Lp	465.225763	Dolichyl b-D-glucosyl phosphate (see KEGG C01246); [3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-(3,7,11-trimethyldodeca-6,10-dienoxy)phosphinic acid	
17172	0.0276	−2.470	C ₄₁ H ₇₇ O ₈ P	Lp	727.528589	PA(16:0/22:2(13Z,16Z)); 1-hexadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3P	PA(16:1(9Z)/22:1(11Z)); 1-(9Z-hexadecenoyl)-2-(11Z-docosenyl)-glycero-3P
12745	0.0225	−2.463	C ₂₀ H ₂₄ O ₉ S	Ph	439.106482	Hallactone B	R replaced by H in 3,5,7,9,11,13,15,17-octaooxo-eicosanoyl-[acp]
6773	0.0115	+3.849	C ₁₀ H ₁₉ NO ₄	Lp	216.124136	O-Propanoylcarnitine; O-propionylcarnitine	Propionylcarnitine (see KEGG C03017); 3-propanoyloxy-4-trimethylammonio-butanoate

TABLE 3. List of the 24 top annotated m/z accumulated in Chardonnay SD samples compared with AC samples^a

ID	P	t test	Formula	Cat	m/z	Annotations	
7635	<0.0001	-7.962	C ₁₄ H ₁₀ O ₅	Ph	257.045540	Gentisin; 1,7-dihydroxy-3-methoxyxanthone	Isogentisin; 1,3-dihydroxy-7-methoxyxanthone
11523	0.0001	-6.967	C ₂₂ H ₁₆ O ₇	Ph	391.082331	Hosloppin	8-C-p-Hydroxybenzylkaempferol
11098	<0.0001	-6.929	C ₂₁ H ₁₄ O ₇	Ph	377.066809	Tephcalostan C	Anastatin A; (2S)-2,3-dihydro-5,7,8-trihydroxy-2-(4-hydroxyphenyl)-4H-benzofuro[3,2-g]-1-benzopyran-4-one
6977	<0.0001	-4.709	C ₁₄ H ₁₂ O ₃	Ph	227.071363	3,4',5-Trihydroxystilbene; resveratrol	Seselin
12602	0.0008	-4.228	C ₂₁ H ₂₄ O ₁₀	Ph	435.129714	Phlorizin; phlorhizin; phloridzin	Epiafzelechin 3-O-β-D-allopyranoside
9554	0.0020	-3.669	C ₁₅ H ₁₈ O ₈	Ph	325.092884	4-O-β-D-Glucosyl-4-hydroxycinnamate	transβ-D-Glucosyl-2-hydroxycinnamate; β-D-glucosyl-2-coumarate
10065	0.0035	-3.436	C ₁₅ H ₁₈ O ₉	Ph	341.087825	Caffeic acid 3-glucoside	1-Caffeoyl-β-D-glucose
7347	0.0005	-3.432	C ₁₄ H ₁₂ O ₄	Ph	243.066281	3,3',4',5-Tetrahydroxystilbene; piceatannol	Wyerone acid
13274	0.0009	-3.431	C ₃₀ H ₄₈ O ₃	Lp	455.353111	Betulinic acid	α-Elemolic acid
15783	0.0021	-3.386	C ₂₇ H ₂₆ O ₁₅	Pp	589.120375	Quercetin 3-(2'',3'',4''-triacylgalactoside)	
10738	0.0010	-3.366	C ₂₁ H ₁₆ O ₆	Ph	363.087543	Justicidin B	Robustone
13297	0.0106	-3.303	C ₂₂ H ₁₈ O ₁₁	Ph	457.077655	Epigallocatechin 3-gallate	8-Demethyltetraacenomycin C
8465	0.0060	-3.271	C ₁₅ H ₁₂ O ₆	Ph	287.056116	Dihydrokaempferol; aromadendrin; (+)-dihydrokaempferol; (+)-aromadendrin	Fustin; 2,3-dihydrofisetin
11475	0.0077	-3.226	C ₂₀ H ₂₂ O ₈	Ph	389.124188	Piceid; polydatin; 3,4,5-trihydroxystilbene-3-β-monoglucoside	Populin
13180	0.0006	-3.216	C ₂₈ H ₂₂ O ₆	Ph	453.134393	Gnetin A	ε-Viniferin
14026	0.0008	-3.159	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone	Gemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(Z)-enyl)-4,2',4'-trihydroxychalcone
11862	0.0044	-3.095	C ₂₀ H ₂₂ O ₉	Ph	405.119108	Astringin	Trichocarpin
14841	0.0129	-3.056	C ₂₄ H ₂₈ O ₁₃	Ph	523.145869	Barbatoflavan	
15613	0.0097	-3.691	C ₂₇ H ₂₈ O ₁₄	Ph	575.140820	Vitexin 6''-(3-hydroxy-3-methylglutarate)	Apigenin 7-[6''-(3-hydroxy-3-methylglutaryl)glucoside]
15406	0.0391	-3.193	C ₂₆ H ₂₄ O ₁₄	Ph	559.109638	Herbacetin 8-(2'',3'',4''-triacylxyloside)	
14261	0.0036	-3.114	C ₂₂ H ₂₂ O ₁₃	Ph	493.098885	Pleurostimin 7-glucoside; 5,6,7,3',4'-Pentahydroxy-8-methoxyflavone 7-glucoside	Laricitrin 3-galactoside
15883	0.0008	-3.104	C ₃₂ H ₃₀ O ₁₂	Ph	605.166662	Skullcapflavone I 2'-(2''-E-cinnamoylglucoside)	Skullcapflavone I 2'-(4''-E-Cinnamoylglucoside)
13584	0.0020	-3.074	C ₂₈ H ₂₂ O ₇	Ph	469.129181	Didymocalyxin B	
13995	0.0011	-3.012	C ₃₀ H ₄₄ O ₅	Lp	483.311656	Propapyriogenin A2	(25S)-3-oxo-12β-acetoxy-cholest-1,4-dien-26-oic acid

^a Selection was made using Perseus applying *t* test (FDR = 0.001 and *S*₀ = 1) and components are ranked according to their *t* test statistic values. If multiple MassTrix annotations for the same formula were obtained, only the two first ones are listed. AC, white wood of asymptomatic vines; SD, brown stripe of diseased vines; ID, component identity; *P*, *t* test associated *P* value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and *m/z*, measured mass value.

discriminant compounds (“top compounds”) were determined in Perseus by strengthening Student’s *t* test parameters such as permutation-based false discovery rate (FDR) and S_0 (two groups threshold fold change). (Supplementary Fig. S2, step f). The resulting significant outliers (FDR and S_0 values detailed on related Tables) were depicted among the identified compounds.

RESULTS

Whole dataset information. The FT-ICR-MS analysis of all provided a total of 6,499 *m/z* that were reduced to 5,842 with validated elemental formulas. These 5,842 formulas covered a high chemical diversity, as shown by the van Krevelen diagram, which represents their distribution on the basis of their H/C and O/C ratios (Supplementary Fig. S3A). Higher proportions of CHO, CHON,

and CHONS species (37, 22, and 15%, respectively) were observed (Supplementary Fig. S3A). These 5,842 formulas were principally equally attributed to phytochemicals and lipids (27 and 28%, respectively), peptides (22%), and then nonmatching (NM) structures (15%) (Supplementary Fig. S3B). For further statistical analyses, data curation based on replicate redundancy was applied on the whole data set, which led to 6,277 *m/z*.

Statistical analysis (ANOVA, *P* value < 0.05) was then performed on the 6,277 *m/z* for [cultivar_wood area] discrimination. This analysis highlighted 3,890 significant *m/z* values. However, the global HCA did not allow a clear discrimination of groups based on cultivars and/or disease (Supplementary Fig. S4). We therefore performed data analysis by two ways. First, the whole data set was split into three subsets according to the cultivar (one by cultivar), in order to get their AC, AD, and SD metabolic fingerprints and

TABLE 4. List of the 33 top *m/z* accumulated in Gewurztraminer SD samples compared with AC samples^a

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	<i>m/z</i>		Annotations
8690	<0.0001	−5.032	C ₁₇ H ₁₂ O ₅	Ph	295.061199	Aspulinone E	Pseudobaptigenin methyl ether; 7-methoxy-3',4'-methylenedioxyisoflavone
8121	<0.0001	−4.548	C ₁₅ H ₁₄ O ₅	Ph	273.076861	Phloretin	α-Pyrufuran
12161	0.0003	−4.167	C ₂₁ H ₂₄ O ₉	Ph	419.134766	Melampodin A	Glycyphyllin; phloretin 2'-O-rhamnoside
11533	<0.0001	−4.104	C ₂₀ H ₂₄ O ₈	Ph	391.139864	Eupatoroxin	10-epi-Eupatoroxin
6697	<0.0001	−4.040	C ₁₄ H ₁₂ O ₂	Ph	211.076478	Benzoin; 2-hydroxy-1,2-diphenylethane; benzoin tincture	Pinosylvin
9308	<0.0001	−4.034	C ₁₇ H ₁₆ O ₆	Ph	315.087404	Cajanol	Homoferreirin
12369	<0.0001	−3.980	C ₂₀ H ₂₅ ClO ₈	Ph	427.116559	Eupachloroxin	
12549	<0.0001	−3.968	C ₂₁ H ₂₂ O ₁₀	Ph	433.114055	Naringenin 7-O-β-D-glucoside; prunin	Phlorizin chalcone; chalcone 2'-O-glucoside
14026	<0.0001	−3.934	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone	Gemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(Z)-enyl)-4,2',4'-trihydroxychalcone
10782	<0.0001	−3.906	C ₁₃ H ₁₉ O ₁₀ P	Ph	365.064466	Salicin 6P	
10675	0.0012	−3.902	C ₁₆ H ₁₅ N ₂ O ₆ P	Ph	361.059764	Fosphenytoin	
9223	<0.0001	−3.898	C ₁₇ H ₁₄ O ₆	Ph	313.071873	Ventinone A	Irisolidone
9783	0.0002	−3.793	C ₁₇ H ₁₆ O ₇	Ph	331.082093	Sulochrin	3'-Deoxydryopteris acid
9272	0.0001	−3.741	C ₁₆ H ₁₂ O ₇	Ph	315.050985	3',4',5,7-Tetrahydroxy-3-methoxyflavone; 3-methoxy-5,7,3',4'-tetrahydroxyflavone; 3-O-methylquercetin	Capillarisin
6004	0.0019	−3.722	C ₆ H ₁₀ O ₅	Cb	161.045569	Lichenin; lichenan	(R)-3,3-Dimethylmalate
15851	0.0001	−3.692	C ₂₇ H ₃₂ O ₁₅	Ph	595.167016	Isobutrin	Butrin; butin 7,3'-di-O-glucoside
10621	0.0001	−3.691	C ₂₂ H ₁₆ O ₅	Ph	359.092546	Pinocembrin 7-O-benzoate	
12189	0.0016	−3.657	C ₂₀ H ₂₂ O ₁₀	Ph	421.114102	Catechin 7-O-β-D-xyloside	Plicatic acid
8017	0.0001	−3.625	C ₁₆ H ₁₄ O ₄	Ph	269.081951	(+)-Medicarpin; 3-hydroxy-9-methoxypterocarpan	Vignafuran
13108	0.0016	−3.614	C ₂₁ H ₂₄ O ₁₁	Ph	451.124596	6-C-Glucopyranosylepicatechin	8-C-Glucopyranosylepicatechin
12556	0.0001	−3.594	C ₂₂ H ₂₆ O ₉	Ph	433.150351	Eleganin	Graminiliatrin
13052	0.0007	−3.570	C ₂₁ H ₂₂ O ₁₁	Ph	449.108960	Neostilbin; (2S,3S)-taxifolin 3-rhamnoside	2',3,4,4',6'-Peptahydroxychalcone 4'-O-glucoside; PHC 4'-O-glucoside
12602	0.0007	−3.569	C ₂₁ H ₂₄ O ₁₀	Ph	435.129714	Phlorizin; phlorhizin; phloridzin	Epiafzelechin 3-O-β-D-allopyranoside
8941	0.0006	−3.558	C ₁₅ H ₁₂ O ₇	Ph	303.051030	Taxifolin; dihydroquercetin; (+)-dihydroquercetin; (+)-taxifolin; trans-dihydroquercetin	Pentahydroxyflavanone; dihydrotricetin
13104	0.0020	−3.546	C ₂₄ H ₂₀ O ₉	Ph	451.103486	Cinchonain 1a	9,10-Dihydro-10-(3,4-dihydroxyphenyl)-pyrano[2,3-h]catechin-8-one
8748	0.0002	−3.510	C ₁₇ H ₁₄ O ₅	Ph	297.076935	Apigenin 7,4'-dimethyl ether; apigenin dimethylether	Afromosin
13497	0.0009	−3.506	C ₂₁ H ₂₂ O ₁₂	Ph	465.103886	Quercetin 7-O-glucoside	Plantagoside
10401	0.0002	−3.483	C ₂₁ H ₁₈ O ₅	Ph	349.108165	Maximaisoflavone B	4'-O-methylalpinumisoflavone
12598	0.0002	−3.482	C ₂₄ H ₂₀ O ₈	Ph	435.108547	9,10-Dihydro-10-(4-hydroxyphenyl)-pyrano[2,3-h]epicatechin-8-one	Epigallocatechin 3-O-cinnamate
10350	0.0002	−3.462	C ₂₁ H ₁₆ O ₅	Ph	347.092536	Calopogonium isoflavone B; 6'',6''-dimethyl-3',4'-methylenedioxyprano[2'',3'':7,8]isoflavone	Tuberostan
6555	0.0002	−3.444	C ₁₃ H ₁₄ O ₂	Ph	201.092122	Tremetone	
8424	0.0002	−3.414	C ₁₃ H ₁₈ O ₇	Ph	285.097974	Salicin; salicoside	Gastrodin; 4-(hydroxymethyl)phenyl-β-D-glucopyranoside
6805	0.0008	−3.252	C ₉ H ₁₇ NO ₅	Lp	218.103393	Pantothenate; pantothenic acid; (R)-pantothenate	Pantothenic acid (see KEGG C00864); 3-[(2R)-2,4-dihydroxy-3,3-dimethylbutanoyl]amino]propanoic acid

^a Selection was made using Perseus applying *t* test (FDR = 0.0001 and S_0 = 2) and components are ranked according to their *t* test statistic values. If multiple MassTrix annotations for the same formula were obtained, only the two first ones are listed. AC, white wood of asymptomatic vines; SD, brown stripe of diseased vines; ID, component identity; *P*, *t* test associated *P* value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and *m/z*, measured mass value.

TABLE 5. List of the 50 top *m/z* differentially accumulated in Mourvèdre AC and SD samples^a

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	<i>m/z</i>	Annotations	
7586	0.0007	−3.173	C ₁₆ H ₁₆ O ₃	Ph	255.102666	Xenognosin A	Broussin; 7-hydroxy-4'-methoxyflavan
10621	0.0017	−2.948	C ₂₂ H ₁₆ O ₅	Ph	359.092546	Pinocembrin 7-O-benzoate	
15613	0.0084	−2.829	C ₂₇ H ₂₈ O ₁₄	Ph	575.14082	Vitexin 6''-(3-hydroxy-3-methylglutarate)	Apigenin 7-[6''-(3-hydroxy-3-methylglutaryl)glucoside]
5635	0.0147	−2.792	C ₅ H ₁₀ N ₂ O ₃	NM	145.061919	L-Glutamine; L-2-aminoglutaric acid	Glutamine; 2-aminoglutaric acid
8065	0.0032	−2.779	C ₁₆ H ₁₆ O ₄	Ph	271.097597	1,2-Bis(4-hydroxy-3-methoxyphenyl)ethylene	5-O-methylallopataeroxylin
6461	0.0284	−2.763	C ₉ H ₈ O ₅	Ph	195.029896	3-(3,4-Dihydroxyphenyl)pyruvate; 3,4-dihydroxyphenylpyruvate	
10401	0.0031	−2.757	C ₂₁ H ₁₈ O ₅	Ph	349.108165	Maximaisoflavone B	4'-O-methylalpinumisoflavone
13547	0.0033	−2.742	C ₂₉ H ₂₄ O ₆	Ph	467.149957	Dichamanetin	
14026	0.0032	−2.728	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone	Gemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(Z)-enyl)-4,2',4'-trihydroxychalcone
13180	0.0036	−2.717	C ₂₈ H ₂₂ O ₆	Ph	453.134393	Gnetin A	ε-Viniferin
16444	0.0044	−2.650	C ₃₅ H ₃₂ O ₁₃	Ph	659.177046	Isophylloflavanine	Phylloflavanine
13104	0.0092	−2.629	C ₂₄ H ₂₀ O ₉	Ph	451.103486	Cinchonain 1a	9,10-Dihydro-10-(3,4-dihydroxyphenyl)-pyrano[2,3-h]catechin-8-one
8715	0.0054	−2.598	C ₁₈ H ₃₂ O ₃	Lp	295.227864	(9Z,12Z)-(8S)-Hydroxyoctadeca-9,12-dienoic acid; laetisarinic acid; 8-hydroxylinoleic acid	(9Z)-(12S,13R)-12,13-Epoxyoctadecenoic acid; vernolic acid; cis-12,13-epoxy-cis-9-octadecenoic acid
12598	0.0065	−2.584	C ₂₄ H ₂₀ O ₈	Ph	435.108547	9,10-Dihydro-10-(4-hydroxyphenyl)-pyrano[2,3-h]epicatechin-8-one	Epigallocatechin 3-O-cinnamate
13971	0.0056	−2.579	C ₂₀ H ₂₀ O ₁₄	Ph	483.078086	1-O,6-O-Digalloyl-β-D-glucose	
7485	0.0091	−2.543	C ₁₂ H ₁₃ NO ₅	Ph	250.072108	N-Feruloylglycine	
16669	0.0058	−2.540	C ₄₂ H ₃₂ O ₉	Lp	679.197769	Canaliculatol	Copalliferol B
11962	0.0065	−2.530	C ₂₂ H ₁₈ O ₈	Ph	409.092984	Aklanonic acid methyl ester	Aklaviketone
7299	0.0146	−2.530	C ₁₅ H ₁₄ O ₃	Ph	241.087001	Fenoprofen	7,4'-Dihydroxyflavan
8690	0.0049	−2.508	C ₁₇ H ₁₂ O ₅	Ph	295.061199	Aspulinone E	Pseudobaptigenin methyl ether; 7-methoxy-3',4'-methylenedioxyisoflavone
9593	0.0089	−2.500	C ₁₈ H ₃₀ O ₅	Lp	325.202058	2,3-Dinor-8-iso prostaglandin F2α; 2,3-Dinor-8-iso PGF2α	2R-Hydroperoxy-9Z,12Z,15Z-octadecatrienoic acid
10302	0.0073	−2.495	C ₂₂ H ₁₈ O ₄	Ph	345.11328	Ovalifolin	
17789	0.0117	−2.483	C ₄₂ H ₇₇ O ₁₃ P	Lp	819.503489	PI(13:0/20:2(11Z,14Z)); 1-tridecanoyl-2-(11Z,14Z-eicosadienoyl)-glycero-3P-(1'-myo-inositol)	PI(14:1(9Z)/19:1(9Z)); 1-(9Z-tetradecenoyl)-2-(9Z-nonadecenoyl)-glycero-3P-(1'-myo-inositol)
13584	0.0097	−2.470	C ₂₈ H ₂₂ O ₇	Ph	469.129181	Didymocalyxin B	
14603	0.0090	−2.450	C ₃₂ H ₄₈ O ₅	Lp	511.342965	Acetoxolone; acetylglucyrrhethinic acid	ZK 159222; (5Z,7E,22E)-(1S,3R,24R)-25-carbobutoxy-26,26-cyclo-9,10-seco-5,7,10(19),22-cholestatetraene-1,3,24-triol
13995	0.0106	−2.444	C ₃₀ H ₄₄ O ₅	Lp	483.311656	Propapyriogenin A2	(25S)-3-oxo-12β-acetoxy-cholest-1,4-dien-26-oic acid
8658	0.0108	−2.442	C ₁₈ H ₃₀ O ₃	Lp	293.212217	(9Z)-(13S)-12,13-Epoxyoctadeca-9,11-dienoic acid; (9Z)-(13S)-12,13-Epoxyoctadeca-9,11-dienoate; 13(S)-EOD	8-[(1R,2R)-3-Oxo-2-{(Z)-pent-2-enyl}cyclopentyl]octanoate; OPC-8:0
9859	0.0090	−2.428	C ₁₇ H ₁₈ O ₇	Ph	333.098004	Byakangelicin	Mesquitol-4β-ol 3,8-dimethyl ether
15952	0.0103	−2.418	C ₃₂ H ₃₀ O ₁₂	Ph	605.166662	Skullcapflavone I 2'-(2''-E-cinnamoylglucoside)	Skullcapflavone I 2'-(4''-E-Cinnamoylglucoside)
6805	0.0155	−2.375	C ₉ H ₁₇ NO ₅	Lp	218.103393	Pantothenate; pantothenic acid; (R)-pantothenate	Pantothenic acid (see KEGG C00864); 3-[[[(2R)-2,4-dihydroxy-3,3-dimethylbutanoyl]amino]propanoic acid
13413	0.0106	−2.375	C ₂₃ H ₂₆ O ₁₀	Ph	461.145196	Methylnissolin 3-O-glucoside	Agehouston A
13805	0.0119	−2.374	C ₂₂ H ₂₂ O ₁₂	Ph	477.104045	5,2',4',5'-Tetrahydroxy-7-methoxy-4-phenylcoumarin 5-O-glucoside	Isorhamnetin 3-glucoside
7635	0.0343	−2.365	C ₁₄ H ₁₀ O ₅	Ph	257.04554	Gentisin; 1,7-dihydroxy-3-methoxyxanthone	Isogentisin; 1,3-dihydroxy-7-methoxyxanthone
10350	0.0114	−2.353	C ₂₁ H ₁₆ O ₅	Ph	347.092536	Calopogoniumisoflavone B; 6'',6''-dimethyl-3',4'-methylenedioxyprano[2'',3'':7,8]isoflavone	Tuberostan
14261	0.0219	−2.351	C ₂₂ H ₂₂ O ₁₃	Ph	493.098885	Pleurostimin 7-glucoside; 5,6,7,3',4'-Pentahydroxy-8-methoxyflavone 7-glucoside	Laricitrin 3-galactoside
11575	0.0116	−2.348	C ₂₃ H ₂₂ O ₆	Ph	393.134354	Rotenone	Macluraxanthone
6187	0.0117	−2.346	C ₈ H ₁₅ NO ₃	Lp	172.097901	N-Acetyl-L-leucine	Swainsonine
15851	0.0120	−2.346	C ₂₇ H ₃₂ O ₁₅	Ph	595.167016	Isobutrin	Butrin; butin 7,3'-di-O-glucoside
9064	0.0117	−2.344	C ₁₅ H ₁₆ O ₇	Ph	307.082253	Allamandin	
11486	0.0120	−2.337	C ₂₁ H ₂₇ ClN ₂ O ₃	Lp	389.164013	Compound II(R/S); 5-(7-(6-chloro-4-(5-hydro-4-methyl-2-oxazolyl)phenoxy)heptyl)-3-methylisoxazole	
13581	0.0131	−2.309	C ₂₄ H ₂₂ O ₁₀	Ph	469.114305	Pongamoside B	Pongamoside C

(Continued on next page)

^a *m/z* are either accumulated in AC (positive *t* statistic value) or in SD (negative *t* statistic value). Selection was made using Perseus applying *t* test (FDR = 0.04 and *S*₀ = 2) and components are ranked according to their *t* test statistic values. If multiple MassTrix annotations for the same formula were obtained, only the two first ones are listed. AC, white wood of asymptomatic vines; SD, brown stripe of diseased vines; ID, component identity; *P*, *t* test associated *P* value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and *m/z*, measured mass value.

TABLE 5. (Continued from previous page)

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	<i>m/z</i>	Annotations	
14573	0.0227	−2.300	C ₃₂ H ₄₆ O ₅	Lp	509.32727	11-(3-acetoxy-1-propynyl)-1 α ,25-dihydroxy-9,11-didehydrovitamin D3 / 11-(3-acetoxy-1-propynyl)-1 α ,25-dihydroxy-9,11-didehydrocholecalciferol; (5Z,7E)-(1S,3R)-11-(3-acetoxy-1-propynyl)-9,10-seco-5,7,9(11),10(19)-cholestatetraene-1,3,25-triol	ZK 168281; (5Z,7E,22E)-(1S,3R,24R)-25-(carboethoxy-methylene)-26,26-cyclo-9,10-seco-5,7,10(19),22-cholestatetraene-1,3,24-triol
10793	0.0139	−2.289	C ₂₁ H ₁₈ O ₆	Ph	365.103108	Glycyrol; neoglycyrol	Derrubone; 5,7-Dihydroxy-3',4'-methylenedioxy-6-prenylisoflavone
10476	0.0155	−2.254	C ₂₀ H ₁₈ O ₆	Ph	353.103165	Cyclokievitone	Licoisoflavone A
15158	0.0157	−2.250	C ₂₇ H ₂₈ O ₁₂	Ph	543.151233	Premithramycin A1	
11571	0.0157	−2.250	C ₂₂ H ₁₈ O ₇	Ph	393.098044	Justicidin A	12a-Hydroxyisomillettone
13537	0.0164	−2.236	C ₂₄ H ₂₀ O ₁₀	Ph	467.0984	Epigallocatechin 3-O-cafeate	Apocynin A
15570	0.0167	−2.229	C ₂₅ H ₄₉ O ₁₂ P	Lp	571.288944	PI(16:0/0:0); 1-hexadecanoyl-sn-glycero-3P-(1'-myo-inositol)	
17113	0.0251	+2.183	C ₃₃ H ₃₈ O ₁₈	Ph	721.199197	6'''-(3-Hydroxy-3-methylglutaryl)isoviolanthin	
13904	0.0172	+2.392	C ₃₀ H ₂₄ O ₆	Lp	479.150345	Blestriarene B	

compare them. Secondly, *m/z* values common to the three cultivars were used to identify metabolic fingerprints of AC, AD, and SD samples, compare them, and find possible markers.

Cultivar-dependent metabolic fingerprints. Data subsets obtained for Chardonnay, Gewurztraminer, and Mourvèdre were composed of a similar number of *m/z* values among with 3,914, 4,048, and 3,896, respectively, were assigned with a validated formula (Fig. 1). For each cultivar, lipids and phytochemicals were the two main categories observed at around 30% (Fig. 1), as observed for the analysis of the global data set (Supplementary Fig. S3B). When compared, the three cultivars shared 2,612 formulas, which were obtained from 2,910 common *m/z* (Fig. 2, top panel). Conversely, 580, 344, and 640 ones were specifically observed in Chardonnay, Gewurztraminer, and Mourvèdre, respectively (Fig. 2, top panel). The 2,612 common formulas were mainly distributed among lipids (32%) and phytochemicals (25%) (Fig. 2, bottom panel).

Using ANOVA statistical analysis ($P < 0.05$), significant *m/z* lists were obtained with a similar number of *m/z* for Chardonnay and Gewurztraminer (2,171 and 2,140 *m/z*, respectively) and a lower one for Mourvèdre (only 656 *m/z*) (Supplementary File S1). For each cultivar, the data subset was then *t* test pair-compared (FDR < 0.05) on the basis of sample type (AC, AD, and SD). Significant *m/z* having a reliable elemental formula (determined by VK criteria) were submitted to MassTrix identification. Among them, only annotated *m/z* were finally considered to select the discriminating ones (i.e., the most discriminant putative metabolites) by adjusting both FDR values and S_0 in Perseus software, in order to get the lowest number of putative discriminant candidates.

For AC and AD sample comparison, the greatest number of significant *m/z* differently accumulated was observed for Chardonnay (1,723 *m/z*), then Gewurztraminer (229 *m/z*), whereas only one could be found for Mourvèdre (Fig. 3). Among them, 23 top *m/z* were listed for Chardonnay (all accumulated in AD), 39 for Gewurztraminer (38/39 accumulated in AD), and none for Mourvèdre (the single discriminant *m/z* was not annotated) (Tables 1 and 2). In both Chardonnay and Gewurztraminer cultivars, all/most of the top metabolites were more accumulated in AD, and were mostly belonging to lipids, mainly fatty acids for Chardonnay and phospholipids (phosphatidic acids, phosphatidyl glycerol, and phosphatidyl inositol) for Gewurztraminer (Tables 1 and 2). Phytochemicals were also accumulated, such as hiosloppin, bichananine, and sinapyl alcohol in Chardonnay; diphyllin and 1-caffeoyl-4-deoxyquinic acid in Gewurztraminer, but to a lesser extent. Peptides (such as Tryptophan for Chardonnay) and amino sugars (such as N-acetyl-L-glutamate for Gewurztraminer) were scarcely listed.

A similar comparison was made between AC and SD samples. The highest number of *m/z* differently accumulated was observed for Gewurztraminer (1,701 *m/z*), then Mourvèdre (540 *m/z*), and the lowest one for Chardonnay (64 *m/z*) (Fig. 3). Among them, most were accumulated in SD, 24 top *m/z* were identified for Chardonnay, 33 for Gewurztraminer, and 50 (48/50 accumulated in SD) for Mourvèdre (Tables 3, 4, and 5). For the three cultivars, the metabolites more accumulated in SD samples were mainly phytochemicals (over 87%). The grapevine stilbene phytoalexins resveratrol, piceid, astringin, and ϵ -viniferin were among the most accumulated in Chardonnay, while only ϵ -viniferin was listed in Mourvèdre top ones, and none of them for Gewurztraminer (Tables 3, 4, and 5). Among other phytochemicals accumulated in Gewurztraminer AD samples were phenolic compounds, mainly flavonoids. As examples, one could find quercetin 7-O-glucoside, dihydroquercetin, or naringenin 7-O-beta-D-glucoside, as putative structures (Table 4).

A last discrimination was made between AD and SD samples. The highest number of *m/z* differently accumulated was observed for Chardonnay (1,628 *m/z*), then Gewurztraminer (1,382 *m/z*), whereas none was found for Mourvèdre (Fig. 3). Among them, 28 top *m/z* were identified for Chardonnay (2/28 accumulated in SD, 26/28 accumulated in AD), and 20 for Gewurztraminer (all accumulated in SD) (Tables 6 and 7). For Chardonnay, only the two phytoalexins resveratrol and ϵ -viniferin were more accumulated in SD areas compared with AD ones, whereas mostly lipids (fatty acids and phospholipids) were accumulated in AD (57%) (Table 6). For Gewurztraminer, the top metabolites were mostly phytochemicals among which the two phytoalexins piceid and astringin, flavonoids (i.e., naringenin 7-O-beta-D-glucoside), and other ones of various chemical families (Table 7).

Altogether, these three comparisons pointed out that in diseased vines, phytochemicals largely contribute to the fingerprint of the brown stripe area (SD), whereas lipids contribute to that of the adjacent white area (AD).

Sample type metabolic fingerprints. Data analysis thus allowed a discrimination of the different sample types (AC, AD, and SD) for each cultivar. Further investigation was next conducted in order to highlight the *m/z* characteristic of the three trunk areas, regardless of the cultivar, i.e., putative markers of the three sample types. The 2,910 *m/z* common to the three cultivars (see above and Supplementary File 1) were selected and ANOVA statistical process ($P < 0.05$) was performed to compare sample types.

AC versus AD comparison revealed 139 common *m/z* values (122 ones with assigned formulas, 68 annotations) between Chardonnay and Gewurztraminer, and none in common with Mourvèdre (Fig. 3, left side). Among the 122 common formulas, 55% belong to lipids (Fig. 4A). One hundred and fifteen were more abundant in AD (57%

Lipids) for both cultivars, whereas seven were inversely accumulated in AC and AD samples, depending on the cultivar (Fig. 4A, noted “conflict”). Among these 115 AD-accumulated m/z , 68 could also be annotated by MassTriX (including 18 isotopologues) (Fig. 3, Table 8). Most of them were lipids including phosphatidic acids (PA), phosphatidylglycerols (PG), and phosphatidyl inositols (PI). A lower number of phytochemicals were accumulated, such as the flavonoid hesloppin. Besides, the three metabolites L-tryptophan, O-propanoylcarnitine, and gratiogenin were inversely accumulated between both cultivars, which renders them ineligible as AD area markers.

For AC versus SD comparison, 33 m/z values were common to the three cultivars (30 ones with assigned formula, 12 annotations) (Fig. 3, top of the middle part). Among the 30 common formulas, 70% belong to phytochemicals. All were more abundant in SD, mainly

phytochemicals (70%) (Fig. 4B). Only 12 m/z could be annotated by MassTriX, among which two isotopologues (Fig. 3, Table 9), including the grapevine phytoalexins resveratrol, ϵ -viniferin (isomer of gnetin A), and piceatannol.

A last comparison was made between AD and SD samples (Fig. 3, right side); 235 m/z (213 with assigned formulas, 63 annotations) were common for Chardonnay and Gewurztraminer but not for Mourvèdre. Among the 213 common formulas, 36% correspond to phytochemicals and 24% to lipids (Fig. 4C). Moreover, from them, 94 were more abundant in SD samples, 96 more abundant in AD samples, and 23 showed variable abundance among sample types, depending on the cultivar. Among the 190 formulas similarly accumulated in both cultivars, 57 could be annotated, among which 18 isotopologues (Table 10). When looking at structural categories (Fig. 4C), mainly phytochemicals (67%) were accumulated in SD

TABLE 6. List of the 28 top m/z differentially accumulated in Chardonnay AD and SD samples^a

ID	<i>P</i>	<i>t</i> test	Formula	Cat.	m/z	Annotations
6977	<0.0001	−4.120	C ₁₄ H ₁₂ O ₃	Ph	227.071363	3,4',5-Trihydroxystilbene; resveratrol
13180	0.0006	−3.138	C ₂₈ H ₂₂ O ₆	Ph	453.134393	Gnetin A
8552	0.0029	+2.979	C ₁₀ H ₁₈ N ₄ O ₆	Pp	289.115364	N-(L-arginino)succinate; 2-(nomega-L-arginino)succinate; L-argininosuccinate; L-argininosuccinic acid
13140	0.0028	+3.025	C ₂₁ H ₄₄ NO ₇ P	Lp	452.278290	LysoPE(0:0/16:0); 1-hydroxy-2-hexadecanoyl-sn-glycero-3-phosphoethanolamine
6674	0.0008	+3.040	C ₁₁ H ₁₄ O ₄	Ph	209.081940	Sinapyl alcohol; sinapoyl alcohol
9257	0.0028	+3.100	C ₁₃ H ₂₁ N ₃ O ₆	Pp	314.135789	Clavamycin D
12659	0.0014	+3.104	C ₂₁ H ₄₃ O ₇ P	Lp	437.267420	LPA(0:0/18:0) (see KEGG C00416); 2-octadecanoyl-glycero-3P
9037	0.0053	+3.110	C ₁₀ H ₁₇ N ₃ O ₆ S	Pp	306.076520	Glutathione; 5-L-glutamyl-L-cysteinylglycine; N-(N-γ-L-glutamyl-L-cysteinyl)glycine; γ-L-glutamyl-L-cysteinyl-glycine; GSH; reduced glutathione
12020	0.0048	+3.184	C ₂₅ H ₄₈ O ₄	Lp	411.348011	MG(0:0/22:1(13Z)/0:0); 2-(13Z-docosenoyl)-glycerol
10154	0.0019	+3.184	C ₂₀ H ₃₈ O ₄	Lp	341.269697	Eicosanedioic acid
7059	0.0004	+3.244	C ₉ H ₁₄ N ₂ O ₅	Pp	229.082987	Aspartyl-L-proline; (2S)-1-[(2S)-2-amino-3-carboxy-propanoyl]pyrrolidine-2-carboxylic acid
13611	0.0006	+3.319	C ₁₇ H ₂₈ NO ₁₄ R	Lp	470.151575	R replaced by H in α-N-acetylneuraminyl-2,3-β-D-galactosyl-R
8394	0.0009	+3.321	C ₁₂ H ₁₉ N ₃ O ₅	Pp	284.125188	Glycylprolylhydroxyproline
10356	0.0022	+3.337	C ₁₈ H ₂₁ ClN ₂ O ₃	Lp	347.116987	Compound VII; 5-(5-(6-chloro-4-(4,5-dihydro-2-oxazolyl)phenoxy)pentyl)-3-methylisoxazole
11306	0.0026	+3.425	C ₂₃ H ₄₄ O ₄	Lp	383.316655	MG(0:0/20:1(11Z)/0:0); 2-(11-eicosenoyl)-glycerol
6556	0.0025	+3.439	C ₁₀ H ₁₈ O ₄	Lp	201.113231	Sebacic acid; decanedioic acid
8030	0.0002	+3.468	C ₁₇ H ₃₄ O ₂	Lp	269.248606	Methyl palmitate
9202	0.0024	+3.715	C ₁₄ H ₁₉ NO ₇	Lp	312.108877	Tyramine glucuronide (see KEGG C03033); (2S,3S,4S,5R,6S)-6-[4-(2-aminoethyl)phenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid
7319	0.0037	+3.735	C ₁₃ H ₂₅ NO ₃	Lp	242.176162	N-Undecanoylglycine
8849	0.0011	+3.789	C ₁₇ H ₃₂ O ₄	Lp	299.222777	MG(0:0/14:1(9Z)/0:0); 2-(9Z-tetradecenoyl)-glycerol
11529	0.0010	+3.838	C ₁₆ H ₂₄ O ₁₁	NM	391.124610	Shanzhiside
6527	0.0012	+3.884	C ₁₀ H ₁₆ O ₄	Lp	199.097596	cis-4-Decenedioic acid; (4Z)-dec-4-enedioic acid
8390	< 0.0001	+3.942	C ₁₂ H ₁₅ NO ₇	Ph	284.077572	Buchananine
10808	0.0001	+4.006	C ₂₄ H ₄₆ O ₂	Lp	365.342494	(15Z)-Tetracosenoic acid; nervonic acid; (Z)-15-tetracosenoic acid
10006	< 0.0001	+4.148	C ₂₀ H ₃₆ O ₄	Lp	339.254064	15S-HpEDE; 15S-hydroperoxy-11Z,13E-eicosadienoic acid
11754	0.0001	+4.171	C ₂₀ H ₃₄ O ₈	Lp	401.218068	5S-HETE di-endoperoxide; 5S,15S-dihydroxy-9S,11R,8S,12S-diperoxy-6E,13E-eicosadienoic acid
6580	< 0.0001	+4.522	C ₁₁ H ₁₂ N ₂ O ₂	Pp	203.082615	L-Tryptophan; tryptophan; (S)-α-amino-β-(3-indolyl)-propionic acid
10010	0.0015	+4.592	C ₁₇ H ₁₁ NO ₇	Ph	340.046275	Aristolochic acid; aristolochic acid I

^a m/z are either accumulated in AD (positive *t* statistic value) or in SD (negative *t* statistic value). Selection was made using Perseus applying *t* test (FDR = 0.01 and *S*₀ = 1) and components are ranked according to their *t* test statistic values. If multiple MassTriX annotations for the same formula were obtained, only the two first ones are listed. AD, white wood of diseased vines; SD, brown stripe of diseased vines; ID, component identity; *P*, *t* test associated *P* value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

samples, whereas it was lipids (34%) and peptides (21%) in AD samples. Phytochemicals accumulated in SD included the phytoalexins resveratrol, ϵ -viniferin, piceatannol, and astringin. In AD samples, most accumulated lipids were phospholipids. As no toxin known to be produced by Botryosphaeriaceae was listed among discriminant compounds provided by statistical analysis, their presence was investigated in the global data file. Interestingly, putative terremutin hydrate ($m/z = 173.0455679$ [M-H]⁻), mellein ($m/z = 177.0557238$ [M-H]⁻), hydroxymellein ($m/z = 193.0506336$ [M-H]⁻), and succinate terremutin hydrate ($m/z = 273.061045$ [M-H]⁻) could be detected (Fig. 5). Their distribution was sample type- and cultivar-dependent. Chardonnay seemed to contain all these toxins in AD and, to a lesser extent, in SD area. Conversely, all toxins (except mellein) were more likely localized in SD for Gewurztraminer and Mourvèdre, while they were not detected (hydroxymellein for both cultivars; terremutin hydrate for Mourvèdre) or less accumulated in AD (succinate terremutin hydrate for both cultivars, terremutin hydrate for Gewurztraminer). Succinate terremutin hydrate was detected in all samples, even in AC of Mourvèdre. Similar investigation of resveratrol and ϵ -viniferin indicated that for the three cultivars, they were mostly accumulated in SD samples.

DISCUSSION

Spagnolo et al. (2014) used proteomic and targeted gene expression approaches to compare the impact of Botryosphaeria dieback infection on cultivars Chardonnay, Gewurztraminer (white cultivars), and Mourvèdre (red cultivar). They observed cultivar-dependent differences in the nature and abundance of proteins in the brown striped area (SD) and adjacent area (AD) of the trunk of diseased vines. In the present study, the same samples were analyzed by FT-ICR-MS to compare their metabolic fingerprints to deepen the characterization of this typical brown stripe associated to the Botryosphaeriaceae infection and adjacent asymptomatic area. Samples were collected in July, i.e., during cambial functioning and before phellogen one. They exclusively consisted of wood tissue, i.e., mainly vessels surrounded by perivascular parenchyma and wood parenchyma (fibers). This tissue is characterized by a

high degree of lignification of the cell walls. Vessels are dead cells but we cannot exclude that raw sap was partly collected during sampling. Biological isolation showed that cultivable Botryosphaeriaceae species were present in the wood area sampled,

Fig. 4. Relative abundance (%) of the structural categories corresponding to formula obtained from the m/z differently accumulated between sample types. The structural categories were predicted by multidimensional stoichiometric constraints classification on the filtered m/z common to the three cultivars and resulting from **A**, AC versus AD, **B**, AC versus SD, and **C**, AD versus SD comparisons. Conflict means reverse accumulation between sample types, depending on the cultivar. AC, white wood of asymptomatic vines; AD, white wood of diseased vines; SD, brown stripe of diseased vines; CH, Chardonnay; GW, Gewurztraminer; MV, Mourvèdre; and NM, nonmatching structures.

TABLE 7. List of the 20 top m/z accumulated in Gewurztraminer SD samples compared with AD samples^a

ID	P	t test	Formula	Cat.	m/z	Annotations
8690	<0.0001	-5.032	C ₁₇ H ₁₂ O ₅	Ph	295.061199	Aspulinone E
8121	<0.0001	-4.186	C ₁₅ H ₁₄ O ₅	Ph	273.076861	Pseudobaptigenin methyl ether; 7-methoxy-3', 4'-methylenedioxyisoflavone
10782	<0.0001	-4.055	C ₁₃ H ₁₉ O ₁₀ P	Ph	365.064466	α -Pyruvuran
9308	<0.0001	-4.044	C ₁₇ H ₁₆ O ₆	Ph	315.087404	Homoferreirin
6697	<0.0001	-4.040	C ₁₄ H ₁₂ O ₂	Ph	211.076478	Pinosylvin
11533	<0.0001	-4.008	C ₂₀ H ₂₄ O ₈	Ph	391.139864	Benzoin; 2-hydroxy-1,2-diphenylethanone; benzoin tincture
12369	<0.0001	-3.980	C ₂₀ H ₂₅ ClO ₈	Ph	427.116559	Eupatoroxin
14026	<0.0001	-3.945	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Eupachloroxin
9223	<0.0001	-3.898	C ₁₇ H ₁₄ O ₆	Ph	313.071873	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone
13648	0.0005	-3.830	C ₃₀ H ₄₈ O ₄	Lp	471.348021	Ventinone A
13274	0.0002	-3.797	C ₃₀ H ₄₈ O ₃	Lp	455.353111	Gratiogenin
15851	0.0001	-3.692	C ₂₇ H ₃₂ O ₁₅	Ph	595.167016	Betulic acid
10621	0.0001	-3.691	C ₂₂ H ₁₆ O ₅	Ph	359.092546	Isobutrin
12549	<0.0001	-3.665	C ₂₁ H ₂₂ O ₁₀	Ph	433.114055	Pinocembrin 7-O-benzoate
8424	0.0002	-3.649	C ₁₃ H ₁₈ O ₇	Ph	285.097974	Naringenin 7-O- β -D-glucoside; prunin
8017	0.0001	-3.625	C ₁₆ H ₁₄ O ₄	Ph	269.081951	Salicin; salicoside
11475	0.0003	-3.605	C ₂₀ H ₂₂ O ₈	Ph	389.124188	(+)-Medicarpin; 3-hydroxy-9-methoxypterocarpan
12556	0.0001	-3.594	C ₂₂ H ₂₆ O ₉	Ph	433.150351	Piceid; polydatin; 3,4,5-trihydroxystilbene-3- β -monoglucoside
11862	0.0003	-3.566	C ₂₀ H ₂₂ O ₉	Ph	405.119108	Eleganin
						Astringin

^a Selection was made using Perseus applying t test (FDR = 0.005 and $S_0 = 1$). Components are ranked according to their t test statistic values. If multiple MassTrix annotations for the same formula were obtained, only the two first ones are listed. AD, white wood of diseased vines; SD, brown stripe of diseased vines; ID, component identity; P , t test associated P value; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

TABLE 8. List of the common annotated m/z differently accumulated in AC and AD, resulting from AC versus AD comparison (t test FDR < 0.05)^a

ID	CH <i>t</i> test	GW <i>t</i> test	Formula	Cat	<i>m/z</i>	Annotations	
11523	−3.938	−2.617	C ₂₂ H ₁₆ O ₇	Ph	391.082331	Hosloppin	8-C-p-Hydroxybenzylkaempferol
7693	−3.803	−2.524	C ₁₁ H ₂₀ N ₂ O ₅	Pp	259.129924	L-γ-Glutamyl-L-isoleucine	L-γ-Glutamyl-L-leucine
9942	−3.292	−2.909	C ₁₆ H ₁₈ O ₈	Ph	337.092840	1-Caffeoyl-4-deoxyquinic acid	4-p-Coumaroylquinic acid
13981	−3.121	−2.855	C ₂₁ H ₂₄ O ₁₃	Ph	483.114397	Diospyrin	
8172	−3.072	−2.460	C ₁₁ H ₁₆ O ₈	Ph	275.077231	Ranunculin	
7433	−3.072	−2.500	C ₁₀ H ₁₇ NO ₆	Lp	246.098303	Linamarin	Malonylcarnitine; (3S)-3-(3-hydroxy-3-oxopropanoyl)oxy-4-trimethylazaniumylbutanoate
17171	−3.006	−2.692	C ₃₉ H ₆₉ O ₁₀ P	Lp	727.455621	PG(13:0/20:4(5Z,8Z,11Z,14Z)); 1-tridecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-glycero-3-phospho-(1'-sn-glycerol)	PG(15:0/18:4(6Z,9Z,12Z,15Z)); 1-pentadecanoyl-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phospho-(1'-sn-glycerol)
11978	−3.005	−2.626	C ₁₉ H ₃₉ O ₇ P	Lp	409.236062	1-Palmitoylglycerol 3P; 1-hexadecanoyl-sn-glycero-3P	LPA(0:0/16:0) (see KEGG C00416); 2-hexadecanoyl-glycero-3P
8145	−2.982	−2.499	C ₁₀ H ₁₃ NO ₈	Ph	274.057019	3-β-D-Glucopyranuronosyloxy-5-methylisoxazole	
12506	−2.894	−2.901	C ₂₁ H ₃₇ O ₇ P	Lp	431.220429	PA(18:3(6Z,9Z,12Z)/0:0); 1-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3P	PA(18:3(9Z,12Z,15Z)/0:0); 1-(9Z,12Z,15Z-octadecatrienoyl)-glycero-3P
12745	−2.848	−2.464	C ₂₀ H ₂₄ O ₉ S	Ph	439.106483	Hallactone B	R replaced by H in 3,5,7,9,11,13,15,17-Octa-oxo-eicosanoyl-[acp]
12566	−2.838	−2.683	C ₂₁ H ₃₉ O ₇ P	Lp	433.236085	Oleoylglycerone phosphate	LPA(0:0/18:2(9Z,12Z)) (see KEGG C00416); 2-(9Z,12Z-octadecadienoyl)-glycero-3P
12192	−2.783	−2.910	C ₁₇ H ₂₆ O ₁₂	Cb	421.135131	Lamiide	10-Hydroxymorroniside
6371	−2.768	−2.951	C ₇ H ₁₁ NO ₅	As	188.056451	N-Acetyl-L-glutamate; N-acetyl-L-glutamic acid	L-2-Amino-6-oxoheptanedioate; L-2-Amino-6-oxopimelate
17357	−2.747	−2.710	C ₄₃ H ₇₉ O ₈ P	Lp	753.544006	PA(18:1(9Z)/22:2(13Z,16Z)); 1-(9Z-octadecenoyl)-2-(13Z,16Z-docosadienoyl)-glycero-3P	PA(18:2(9Z,12Z)/22:1(11Z)); 1-(9Z,12Z-octadecadienoyl)-2-(11Z-docosenoyl)-glycero-3P
8347	−2.696	−2.698	C ₁₂ H ₁₇ NO ₇	Lp	286.093239	Volkenin	
11190	−2.662	−2.624	C ₂₁ H ₁₆ O ₇	Ph	379.082660	Diphyllin	Tetracenomycin F1 methylester
16981	−2.624	−2.625	C ₃₇ H ₇₁ O ₁₀ P	Lp	705.471462	PG(12:0/19:1(9Z)); 1-dodecanoyl-2-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-sn-glycerol)	PG(13:0/18:1(9Z)); 1-tridecanoyl-2-(9Z-octadecenoyl)-glycero-3-phospho-(1'-sn-glycerol)
13994	−2.570	−2.744	C ₂₂ H ₄₅ O ₉ P	Lp	483.272898	PG(16:0/0:0); 1-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	
17882	−2.535	−2.520	C ₄₃ H ₇₉ O ₁₃ P	Lp	833.519168	PI(16:0/18:2(9Z,12Z)) (see KEGG C00626); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphoinositol	PI(16:1(9Z)/18:1(11Z)) (see KEGG C00626); 1-(9Z-hexadecenoyl)-2-(11Z-octadecenoyl)-sn-glycero-3-phosphoinositol
17172	−2.533	−2.470	C ₄₁ H ₇₇ O ₈ P	Lp	727.528590	PA(16:0/22:2(13Z,16Z)); 1-hexadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3P	PA(16:1(9Z)/22:1(11Z)); 1-(9Z-hexadecenoyl)-2-(11Z-docosenoyl)-glycero-3P
6804	−2.513	−2.503	C ₈ H ₁₃ NO ₆	As	218.067023	O-Succinyl-L-homoserine	N-Acetyl-D-mannosaminolactone
16418	−2.479	−2.714	C ₃₆ H ₆₅ O ₈ P	Lp	655.434640	PA(13:0/20:3(8Z,11Z,14Z)); 1-tridecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3P	PA(15:0/18:3(6Z,9Z,12Z)); 1-pentadecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3P
17860	−2.477	−2.668	C ₄₃ H ₇₇ O ₁₃ P	Lp	831.503409	PI(14:0/20:3(8Z,11Z,14Z)); 1-tetradecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phospho-(1'-myo-inositol)	PI(14:1(9Z)/20:2(11Z,14Z)); 1-(9Z-tetradecenoyl)-2-(11Z,14Z-eicosadienoyl)-glycero-3-phospho-(1'-myo-inositol)
17364	−2.476	−2.681	C ₄₃ H ₈₁ O ₈ P	Lp	755.560009	PA(18:0/22:2(13Z,16Z)); 1-octadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3P	PA(18:1(9Z)/22:1(11Z)); 1-(9Z-octadecenoyl)-2-(11Z-docosenoyl)-glycero-3P
14298	−2.469	−2.650	C ₂₁ H ₂₀ O ₁₄	Ph	495.078067	Hibiscetin 3-glucoside	
16924	−2.464	−2.506	C ₃₉ H ₇₃ O ₈ P	Lp	699.497295	PA(18:0/18:2(9Z,12Z)) (see KEGG C00416); 1-octadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3P	PA(18:1(11Z)/18:1(11Z)) (see KEGG C00416); 1,2-di(11Z-octadecenoyl)-rac-glycero-3P
16562	−2.456	−2.574	C ₃₇ H ₆₉ O ₈ P	Lp	671.466004	PA(16:0/18:2(9Z,12Z)) (see KEGG C00416); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3P	PA(16:0/18:2(9Z,12Z)); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3P
16538	−2.433	−2.618	C ₃₇ H ₆₇ O ₈ P	Lp	669.450309	PA(14:0/20:3(8Z,11Z,14Z)); 1-tetradecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3P	PA(14:1(9Z)/20:2(11Z,14Z)); 1-(9Z-tetradecenoyl)-2-(11Z,14Z-eicosadienoyl)-glycero-3P
16184	−2.406	−2.481	C ₃₄ H ₆₅ O ₈ P	Lp	631.434712	PA(12:0/19:1(9Z)); 1-dodecanoyl-2-(9Z-nonadecenoyl)-glycero-3P	PA(13:0/18:1(9Z)); 1-tridecanoyl-2-(9Z-octadecenoyl)-glycero-3P
16871	−2.389	−2.523	C ₃₉ H ₆₉ O ₈ P	Lp	695.466015	PA(16:0/20:4(5Z,8Z,11Z,14Z)); 1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3P	PA(14:0/22:4(7Z,10Z,13Z,16Z)); 1-tetradecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P
16853	−2.370	−2.536	C ₃₉ H ₆₇ O ₈ P	Lp	693.450342	PA(14:1(9Z)/22:4(7Z,10Z,13Z,16Z)); 1-(9Z-tetradecenoyl)-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P	PA(18:1(9Z)/18:4(6Z,9Z,12Z,15Z)); 1-(9Z-octadecenoyl)-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3P

(Continued on next page)

^a Isotopologues forms were removed leading to 50 metabolites. m/z accumulated either in AC (positive t test statistic) or AD (negative t test statistic) samples. Only MassTrix two first annotations are mentioned. AC, white wood of asymptomatic vines; AD, white wood of diseased vines; ID, component identity; Chardonnay (CH); Gewurztraminer (GW); Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

abundantly in SD samples and in lesser extent in AD samples (Spagnolo et al. 2014). AD areas were also colonized by other cultivable fungal species, whereas no-cultivable fungal species could be isolated from AC samples (Spagnolo et al. 2014). Overall, these results indicate that metabolites detected and identified in both SD and AD samples may be of plant or microbial origin.

FT-ICR-MS analysis allowed the detection of 6,499 raw mass values, which seems low compared with what could be detected in grapevine leaves of Chardonnay (Magnin-Robert et al. 2017). HCA of all samples (Supplementary Fig. S4) did not allow a distinction of cultivar or sample type (AC, AD, and SD) groups, making it necessary to dissociate sample sets for analysis.

The three cultivars were first considered separately for comparison of sample types. They were characterized by a similar number of m/z (about 4,500) and structural category proportions (as defined by MSCC), with a majority of phytochemicals and lipids. Phytochemicals category, which corresponds to secondary metabolites, was

expected since woody tissues are rich in lignin related metabolites (especially monolignols), tannins, and other organic components such as phenolic compounds and terpenes (Belgacem and Pizzi 2016). Although cellulose is an abundant wood component, the lipid category was more represented than the carbohydrate one. It can be explained by the fact that lipids ionize very well in MS analysis compared with carbohydrates (Boutegrabet et al. 2012).

Comparison of AC and AD samples highlighted important differences in the number of m/z differently accumulated in the three cultivars studied: high for Chardonnay (1,723), low for Gewurztraminer (229), and only one for Mourvèdre. These results suggest that the asymptomatic area close to the brown stripe is not impacted by infection in a similar manner for the three cultivars. The metabolome of AD area could be differently impacted for several reasons: either by different defense responses, or by an asymptomatic stage of fungal infection (hypothetical situation in Chardonnay and Gewurztraminer); or no plant response and no

TABLE 8. (Continued from previous page)

ID	CH <i>t</i> test	GW <i>t</i> test	Formula	Cat	<i>m/z</i>	Annotations	
16337	-2.354	-2.516	C ₃₅ H ₆₉ O ₈ P	Lp	647.465942	PA(16:0/16:0) (see KEGG C00416); 1,2-dihexadecanoyl-rac-glycero-3P	PA(16:0/16:0)[rac]; 1,2-dihexadecanoyl-sn-glycero-3P
18022	-2.328	-2.573	C ₄₅ H ₇₉ O ₁₃ P	Lp	857.519267	PI(16:0/20:4(5Z,8Z,11Z,14Z)) (see KEGG C00626); 1-hexadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphoinositol	PI(16:0/20:4(8Z,11Z,14Z,17Z)) (see KEGG C00626); 1-hexadecanoyl-2-(8Z,11Z,14Z,17Z-eicosapentaenoyl)-sn-glycero-3-phosphoinositol
6859	-2.305	-2.709	C ₁₃ H ₁₈ O ₃	Lp	221.118327	Dehydrovomifoliol; (6R)-6-hydroxy-3-oxo- α -ionone	(+/-)-6-Hydroxy-3-oxo- α -ionone
16702	-2.301	-2.572	C ₃₈ H ₆₇ O ₈ P	Lp	681.450280	PA(13:0/22:4(7Z,10Z,13Z,16Z)); 1-tridecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P	PA(15:0/20:4(5Z,8Z,11Z,14Z)); 1-pentadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-glycero-3P
6414	-2.300	-2.768	C ₇ H ₁₂ O ₆	Cb	191.056114	Quinate; quinic acid; kinic acid; chinic acid; L-quinic acid; L-quinate; (-)-quinic acid	2D-5-O-Methyl-2,3,5/4,6-pentahydroxycyclohexanone; 5D-5-O-methyl-2,3,5/4,6-pentahydroxycyclohexanone; D-5-O-methyl-2,3,5/4,6-pentahydroxycyclohexanone
16436	-2.294	-2.533	C ₃₆ H ₆₇ O ₈ P	Lp	657.450319	PA(13:0/20:2(11Z,14Z)); 1-tridecanoyl-2-(11Z,14Z-eicosadienoyl)-glycero-3P	PA(14:1(9Z)/19:1(9Z)); 1-(9Z-tetradecenoyl)-2-(9Z-nonadecenoyl)-glycero-3P
17118	-2.234	-2.615	C ₃₈ H ₇₅ O ₁₀ P	Lp	721.502885	PG(16:0/16:0); 1,2-dihexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	PG(18:0/14:0); 1-octadecanoyl-2-tetradecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
17801	-2.188	-2.733	C ₄₂ H ₇₉ O ₁₃ P	Lp	821.518938	PI(13:0/20:1(11Z)); 1-tridecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-myo-inositol)	PI(14:0/19:1(9Z)); 1-tetradecanoyl-2-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-myo-inositol)
17340	-2.160	-2.618	C ₄₀ H ₇₉ O ₁₀ P	Lp	749.534147	PG(16:0/18:0); 1-hexadecanoyl-2-octadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	PG(18:0/16:0); 1-octadecanoyl-2-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
17699	-2.144	-2.621	C ₄₁ H ₇₇ O ₁₃ P	Lp	807.503428	PI(16:0/16:1(9Z)) (see KEGG C00626); 1-hexadecanoyl-2-(9Z-hexadecenoyl)-sn-glycero-3-phosphoinositol	PI(16:1(9Z)/16:0) (see KEGG C00626); 1-(9Z-hexadecenoyl)-2-hexadecanoyl-sn-glycero-3-phosphoinositol
18042	-2.139	-2.815	C ₄₅ H ₈₃ O ₁₃ P	Lp	861.550160	PI(16:0/20:2(11Z,14Z)) (see KEGG C00626); 1-hexadecanoyl-2-(11Z,14Z-eicosadienoyl)-sn-glycero-3-phosphoinositol	PI(18:0/18:2(9Z,12Z)) (see KEGG C00626); 1-octadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphoinositol
17301	-2.100	-2.652	C ₄₀ H ₇₅ O ₁₀ P	Lp	745.502826	PG(16:0/18:2(9Z,12Z)); 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	PG(16:1(9Z)/18:1(11Z)); 1-(9Z-hexadecenoyl)-2-(11Z-octadecenoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)
17284	-2.056	-2.663	C ₄₀ H ₇₃ O ₁₀ P	Lp	743.487156	PG(16:0/18:3(6Z,9Z,12Z)); 1-hexadecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)	PG(16:0/18:3(9Z,12Z,15Z)); 1-hexadecanoyl-2-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycero-3-phospho-(1'-sn-glycerol)
15855	-2.013	-2.459	C ₂₇ H ₄₉ O ₁₂ P	Lp	595.289019	PI(18:2(9Z,12Z)/0:0); 1-(9Z,12Z-octadecadienoyl)-glycero-3-phospho-(1'-myo-inositol)	PG(13:0/18:0); 1-tridecanoyl-2-octadecanoyl-glycero-3-phospho-(1'-sn-glycerol)
16999	-2.012	-2.680	C ₃₇ H ₇₃ O ₁₀ P	Lp	707.487030	PG(12:0/19:0); 1-dodecanoyl-2-nonadecanoyl-glycero-3-phospho-(1'-sn-glycerol)	D-Tryptophan
6580	-4.254	+2.506	C ₁₁ H ₁₂ N ₂ O ₂	Pp	203.082615	L-Tryptophan; tryptophan; (S)- α -amino- β -(3-indolyl)-propionic acid	Propionylcarnitine (see KEGG C03017); 3-propanoyloxy-4-trimethylammonio-butanate
6773	-2.991	+3.849	C ₁₀ H ₁₉ NO ₄	Lp	216.124136	O-Propanoylcarnitine; O-propionylcarnitine	Echinocystic acid
13648	-1.984	+2.730	C ₃₀ H ₄₈ O ₄	Lp	471.348021	Gratiogenin	

asymptomatic stage of the fungal infection (hypothetical case in Mourvèdre). For Chardonnay and Gewurztraminer, the metabolites more accumulated in AD than in AC samples were mainly lipids, especially fatty acids for Chardonnay and the phospholipids phosphatidic acids (PA), phosphatidyl glycerol (PG), and phosphatidyl inositol (PI) for Gewurztraminer. Fatty acids are constituents of glycerolipids. They are now considered as signal molecules, especially jasmonic acid and traumatic acid (Farmer 1994), and play a key role in plant–pathogen interactions (Siebers et al. 2016; Walley et al. 2013). Jasmonic acid and traumatin were not listed among top m/z but in the common 2,612 m/z shared by the three cultivars (Supplementary File S1). The other fatty acids identified could be released from glycerolipids by lipases (Lee and Park 2019), but we cannot conclude if they are just a result of degradation or if they play a role in defense signaling. Salvatore et al. (2018) also detected fatty acids in the culture medium of *N. parvum* and *N. vitifusiforme*, suggesting their production and secretion. They only found linoleic, elaidic, and stearic acids for both fungi, whereas palmitoleic and palmitic acids were specifically found for *N. vitifusiforme*. Among them, only palmitic acid was listed among significant compounds in the present study. Phospholipids are major constituents in the biological membrane of organisms among which plants and fungi. They can also be found in lesser extent in fungi cell walls. Their higher abundance in AD samples suggests a fungal colonization of the wood, despite the absence of visible symptoms, which is consistent with the presence of fungi detected in this area. They may result, at least partially, from hydrolysis of membrane phospholipids by phospholipase D (PLD). PLD and its hydrolysis product phosphatidic acid are known for their role in plant immunity (for review, see Li and Wang 2019; Siebers et al. 2016; Zhang and Xiao 2015; Zhao 2015). In plant–pathogen interactions, the plant PLD is indeed activated after MAMP (microbe associated molecular pattern) or effector recognition and the released phosphatidic acid contributes to signaling of defense response (Hong et al. 2016). We can therefore hypothesize that Botryosphaeriaceae are perceived by grapevine and that defense responses in xylem parenchyma cells are in progress in AD area. Spagnolo et al. (2014) did not report PLD as overexpressed protein; the peak of PLD activity might have occurred prior to sampling time, making it nondiscriminant in their statistical analysis. Another possibility is that the identified lipid compounds could be the results of degradation by fungal action on wood composition. As reported

by Esteves et al. (2014), fungi involved in Botryosphaeria dieback produced extracellular enzymes with lipase activity. Phytochemicals were also accumulated to a lesser extent, with for instance sinapyl alcohol, b Buchananine, hosloppin, and diphyllin. Sinapyl alcohol is a monolignol that can be incorporated into plant cell wall lignin polymer after oxidation by peroxidase and laccases (Cesarino et al. 2012). Its presence might contribute to cell wall strengthening, a defense response that is likely to limit infection (Sattler and Funnell-Harris 2013). It could result from plant peroxidase activity or from fungal laccase activity as it was reported that Botryosphaeriaceae fungi produce such enzymes (Esteves et al. 2014; Stempien et al. 2017). Buchananine is a pyridine alkaloid first identified in the stems of the climbing tree *Cryptolepis buchanani* (Dutta et al. 1978). Hosloppin is a pyrone-substituted flavonoid first identified in *Hoslundia opposita* (Ngadjui et al. 1995). Diphyllin was extracted from leaves of *Cleistanthus collinus* and its antimicrobial activity was demonstrated against *Candida* species (Suman et al. 2018). Since the occurrence of these four compounds in grapevine has never been reported, further specific studies would merit to be performed to definitely confirm their presence and study their role. Among them, it would be interesting to evaluate the antimicrobial activity of diphyllin against Botryosphaeriaceae.

Comparison between AC and SD samples also highlighted differences in the number of m/z differently accumulated in the three cultivars studied: higher for Gewurztraminer (1,701), intermediate for Mourvèdre (540), and lower for Chardonnay (64). Botryosphaeria dieback thus clearly impacts the metabolome of symptomatic area, regardless of the cultivar, and even for Mourvèdre for which no obvious significant difference could be highlighted for AC versus AD comparison. Most of m/z values, which were more accumulated in SD samples compared with AC samples were predicted to be phytochemicals. Among them were the stilbenes resveratrol and ϵ -viniferin for Chardonnay and Mourvèdre, and also piceatannol, astringin, and piceid for Chardonnay. These stilbenic derivatives are grapevine phytoalexins, among which ϵ -viniferin, which has the highest antimicrobial activity (Adrian and Jeandet 2012; Adrian et al. 2012; Chong et al. 2009; Jeandet et al. 2002; Lambert et al. 2012). It was previously shown that metabolites secreted by *N. parvum* and *D. seriata* induce phytoalexin production and other defense responses in grapevine cell suspensions (Abou-Mansour et al. 2015; Stempien et al. 2018) and in plantlets (Trotel-Aziz et al.

TABLE 9. List of the common annotated m/z differently accumulated in SD samples and resulting from AC versus SD comparison (t test FDR < 0.05)^a

ID	CH t test	GW t test	MV t test	Formula	Cat.	m/z	Annotations
6977	-4.709	-2.679	-1.932	C ₁₄ H ₁₂ O ₃	Ph	227.071363	3,4',5'-Trihydroxystilbene; resveratrol
7347	-3.432	-2.390	-1.949	C ₁₄ H ₁₂ O ₄	Ph	243.066281	3,3',4',5'-Tetrahydroxystilbene; piceatannol
7635	-7.962	-1.961	-2.365	C ₁₄ H ₁₀ O ₅	Ph	257.045540	Gentisin; 1,7-dihydroxy-3-methoxyxanthone
10738	-3.366	-3.108	-2.285	C ₂₁ H ₁₆ O ₆	Ph	363.087543	Justicidin B
13180	-3.216	-3.184	-2.717	C ₂₈ H ₂₂ O ₆	Ph	453.134393	Gnetin A
13584	-3.074	-3.290	-2.470	C ₂₈ H ₂₂ O ₇	Ph	469.129181	Didymocalyxin B
13995	-3.012	-2.920	-2.444	C ₃₀ H ₄₄ O ₅	Lp	483.311656	Propapyriogenin A2
14026	-3.159	-3.934	-2.728	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone
14261	-3.114	-2.935	-2.351	C ₂₂ H ₂₂ O ₁₃	Ph	493.098885	Pleurostemin 7-glucoside; 5,6,7,3',4'-pentahydroxy-8-methoxyflavone 7-glucoside
15952	-3.116	-2.233	-2.417	C ₃₂ H ₃₀ O ₁₂	Ph	605.166662	Skullcapflavone I 2'-(2''-E-cinnamoylglucoside)

^a Isotopologues forms were removed leading to 10 metabolites. Only MassTriX two first annotations are mentioned. AC, white wood of asymptomatic vines; SD, brown wood of diseased vines; ID, component identity; CH, Chardonnay; GW, Gewurztraminer; MV, Mourvèdre; Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

2019). Stilbene accumulation was also reported in the woody tissues of GTD-affected vines (Amalfitano et al. 2011; Amalfitano et al. 2000; Fontaine et al. 2016; Magnin-Robert et al. 2016). As far as Botryosphaeriaceae are concerned, Massonnet et al. (2018) detected stilbenes in the wood of Cabernet Sauvignon cuttings

infected by *N. parvum* and Reis et al. (2016) in the green stems of Tempranillo cuttings infected by *N. parvum* or *D. seriata*. Spagnolo et al. (2014) also reported the presence of stilbene phytoalexins, together with overexpression of defense proteins, mainly PR-proteins, in the brown striped area. Altogether, these results suggest

TABLE 10. List of the common annotated m/z differently accumulated in AD and SD, resulting from AD versus SD comparison (t test FDR < 0.05)^a

ID	CH t test	GW t test	Formula	Cat.	m/z	Annotations
6977	-4.120	-2.709	C ₁₄ H ₁₂ O ₃	Ph	227.071363	3,4',5-Trihydroxystilbene; resveratrol
13180	-3.139	-3.190	C ₂₈ H ₂₂ O ₆	Ph	453.134393	Gnetin A
7347	-3.070	-2.429	C ₁₄ H ₁₂ O ₄	Ph	243.066281	3,3',4',5'-Tetrahydroxystilbene; piceatannol
14026	-3.064	-3.945	C ₂₉ H ₂₆ O ₇	Ph	485.160643	Isogemichalcone B; 3'-(4-coumaroyloxy-3-methylbutyl-2(E)-enyl)-4,2',4'-trihydroxychalcone
13995	-3.012	-2.920	C ₃₀ H ₄₄ O ₅	Lp	483.311656	Propapyriogenin A2
10738	-2.890	-3.089	C ₂₁ H ₁₆ O ₆	Ph	363.087543	Justicidin B
16669	-2.804	-3.244	C ₄₂ H ₃₂ O ₉	Lp	679.197769	Canaliculatol
13274	-2.784	-3.797	C ₃₀ H ₄₈ O ₃	Lp	455.353111	Betulinic acid
15952	-2.640	-2.234	C ₃₂ H ₃₀ O ₁₂	Ph	605.166662	Skullcapflavone I 2'-(2"-E-cinnamoylglucoside)
13584	-2.631	-3.219	C ₂₈ H ₂₂ O ₇	Ph	469.129181	Didymocalyxin B
13905	-2.560	-2.001	C ₂₃ H ₂₈ O ₁₁	Ph	479.156055	Bruceine B
14261	-2.365	-2.779	C ₂₂ H ₂₂ O ₁₃	Ph	493.098885	Pleurostemin 7-glucoside; 5,6,7,3',4'-pentahydroxy-8-methoxyflavone 7-glucoside
14603	-2.345	-2.888	C ₃₂ H ₄₈ O ₅	Lp	511.342965	Acetoxolone; acetylglucyrrhettinic acid
13971	-2.298	-2.477	C ₂₀ H ₂₀ O ₁₄	Ph	483.078086	1-O,6-O-Digalloyl-β-D-glucose
13805	-2.178	-2.842	C ₂₂ H ₂₂ O ₁₂	Ph	477.104045	5,2',4',5'-Tetrahydroxy-7-methoxy-4-phenylcoumarin 5-O-glucoside
9308	-2.168	-4.044	C ₁₇ H ₁₆ O ₆	Ph	315.087404	Cajanol
16464	-2.159	-2.544	C ₃₇ H ₅₈ O ₁₀	Lp	661.396011	Cimicifetiside A; 15α,25-dihydroxy-16β,23R:16α,24S-diepoxo-9β,19-cyclolanostan-3β-yl 2-O-acetyl-α-L-arabinopyranoside
10621	-2.120	-3.691	C ₂₂ H ₁₆ O ₅	Ph	359.092546	Pinocembrin 7-O-benzoate
12602	-2.063	-3.065	C ₂₁ H ₂₄ O ₁₀	Ph	435.129714	Phlorizin; phlorhizin; phloridzin
11862	-2.056	-3.566	C ₂₀ H ₂₂ O ₉	Ph	405.119108	Astringin
7635	-2.015	-2.756	C ₁₄ H ₁₀ O ₅	Ph	257.045540	Gentisin; 1,7-dihydroxy-3-methoxyxanthone
15851	-2.002	-3.692	C ₂₇ H ₃₂ O ₁₅	Ph	595.167016	Isobutrin
15976	-1.992	-2.471	C ₂₇ H ₂₈ O ₁₆	Ph	607.130772	Luteolin 7-glucuronide-4'-rhamnoside
12259	+2.053	+2.298	C ₂₀ H ₄₁ O ₇ P	Lp	423.251744	PA(17:0/0:0); 1-heptadecanoyl-glycero-3P
15463	+2.258	+2.015	C ₂₈ H ₅₅ O ₉ P	Lp	563.335503	PG(22:2(13Z,16Z)/0:0); 1-(13Z,16Z-docosadienoyl)-glycero-3-phospho-(1'-sn-glycerol)
13904	+2.321	+2.381	C ₃₀ H ₂₄ O ₆	Lp	479.150345	Blestriarene B
13072	+2.399	+2.293	C ₂₂ H ₄₃ O ₇ P	Lp	449.267381	PA(19:1(9Z)/0:0); 1-(9Z-nonadecenoyl)-glycero-3P
6414	+2.412	+2.094	C ₇ H ₁₂ O ₆	Cb	191.056114	Quinate; quinic acid; kinic acid; chinic acid; L-quinic acid; L-quinate; (-)-quinic acid
9942	+2.702	+1.938	C ₁₆ H ₁₈ O ₈	Ph	337.092840	1-Caffeoyl-4-deoxyquinic acid
16184	+2.704	+2.331	C ₃₄ H ₆₅ O ₈ P	Lp	631.434712	PA(12:0/19:1(9Z)); 1-dodecanoyl-2-(9Z-nonadecenoyl)-glycero-3P
8447	+2.809	+1.989	C ₁₂ H ₁₇ NO ₇	Lp	286.093239	Volkenin
15031	+2.817	+2.211	C ₁₈ H ₃₅ O ₁₈	Cb	535.151681	1,4-Beta-D-glucan (see KEGG C00760)
16612	+2.863	+2.242	C ₃₆ H ₇₀ NO ₈ P	Lp	674.476862	PC(14:0/14:1(9Z)) (see KEGG C00157); 1-tetradecanoyl-2-(9Z-tetradecenoyl)-sn-glycero-3-phosphocholine
16702	+2.881	+2.276	C ₃₈ H ₆₇ O ₈ P	Lp	681.450280	PA(13:0/22:4(7Z,10Z,13Z,16Z)); 1-tridecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3P
12094	+2.991	+1.967	C ₂₁ H ₃₇ O ₆ P	Lp	415.225543	CPA(18:2(9Z,12Z)/0:0); 1-(9Z,12Z-octadecadienoyl)-glycero-3-cyclophosphate
13994	+3.021	+2.031	C ₂₂ H ₄₅ O ₉ P	Lp	483.272898	PG(16:0/0:0); 1-hexadecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
12659	+3.105	+1.990	C ₂₁ H ₄₃ O ₇ P	Lp	437.267420	LPA(0:0/18:0) (see KEGG C00416); 2-octadecanoyl-glycero-3P
9037	+3.111	+1.945	C ₁₀ H ₁₇ N ₃ O ₆ S	Pp	306.076520	Glutathione; 5-L-glutamyl-L-cysteinylglycine; N-(N-γ-L-glutamyl-L-cysteinyl)glycine; γ-L-glutamyl-L-cysteinyl-glycine; GSH; reduced glutathione
11190	+3.271	+2.652	C ₂₁ H ₁₆ O ₇	Ph	379.082660	Diphyllin
						Tetracenomycin F1 methylester

^a Isotopologues forms were removed leading to 39 metabolites. m/z are either accumulated in AD (positive t test statistic) or SD (negative t test statistic) samples. Only MassTrix two first annotations are mentioned. AD, white wood of diseased vines; SD, brown wood of diseased vines; ID, component identity; Chardonnay (CH); Gewurztraminer (GW); Cat., multidimensional stoichiometric constraints classification (MSCC) predicted structural category; amino sugars (As); phytochemicals (Ph); peptides (Pp); lipids (Lp); carbohydrates (Cb); and m/z , measured mass value.

that cells of infected xylem parenchyma respond to the pathogens by immune responses.

Comparison between AD and SD also revealed specificity for Mourvèdre. For Chardonnay and Gewurztraminer, it confirmed a higher accumulation of lipids (mostly phospholipids) in AD samples and of phytochemicals (encompassing phytoalexins) in SD ones. In both AC/SD and AD/SD comparisons, resveratrol and its dimer ϵ -viniferin were listed as top m/z for Chardonnay, but not for other cultivars. As Chardonnay is known to be less susceptible to GTDs than the two other cultivars analyzed (Bruez et al. 2013; Grosman and Doublet 2012; Murolo and Romanazzi 2014), this suggests that ϵ -viniferin contribute to this lower susceptibility. One can also not exclude the possible role of fatty acids that were found as Chardonnay significant top m/z in diseased wood. Curiously, few phenolic compounds were listed among phytochemicals, although they have been reported to be accumulated in leaves of vines affected by GTDs (Lima et al. 2010, 2017; Moret et al. 2018), and in the wood of *Botryosphaeria* affected vines.

Some of them have antifungal activities and can cause alteration of hyphal morphology and inhibition of fungal ligninolytic activities (Gómez et al. 2016; Spagnolo et al. 2014). We can hypothesize that they are effectively present in samples, but not in high-enough concentrations for their selection as top m/z by statistical analysis.

Amazingly, no difference was found between AD and SD samples for Mourvèdre, despite visually clear differences, at least in color. It is probably due to the huge sample heterogeneity between replicates of this cultivar, as revealed by HCA analysis (Supplementary Fig. S4). Hence, half (4/9) of the Mourvèdre SD samples demonstrated an over-accumulation of m/z while the other half looked like AD ones. In the same manner, replicates of AC samples were for half of them (4/8) different in pattern. Altogether, only few compounds could be highlighted by statistical analysis on the Mourvèdre m/z subset (Supplementary Fig. S2, step b). Further experimentation would merit to be conducted in order to check if this heterogeneity is cultivar dependent.

Fig. 5. Abundance of putative fungal toxins and vine phytoalexins resveratrol and ϵ -viniferin in the different sample types (AC, AD, and SD) for the three cultivars Chardonnay, Gewurztraminer, and Mourvèdre (CH, GW, and MV, respectively). Values correspond to signal intensity. AC, white wood of asymptomatic vines; AD, white wood of diseased vines; SD, brown stripe of diseased vines; resveratrol (Resv); viniferin (Vinif); mellein (Mln); hydroxy-mellein (OHMln); terremin hydrate (TrmH); and succinate terremin (STrm).

It was demonstrated that the fungi associated to *Botryosphaeria dieback* secretes phytotoxins (Andolfi et al. 2011; Bellée et al. 2017; Benard-Gellon et al. 2015; Blanco-Ulate et al. 2013; Massonnet et al. 2017, 2018; Morales-Cruz et al. 2015). Investigation of the occurrence in the global data file edited in this study allowed us to find putative compounds of this family, including terremin hydrate, mellein, hydroxymellein, and succinate terremin hydrate. In the literature, attention is especially paid on these metabolites since their production could be associated to the fungal aggressiveness (Abou-Mansour et al. 2015; Benard-Gellon et al. 2015; Reveglia et al. 2019; Trotel-Aziz et al. 2019). Abou-Mansour et al. (2015) reported the occurrence of several toxins encompassing (–)-terremin, (+)-terremin hydrate, 4-hydroxysuccinate-terremin hydrate, (R)-(–)-mellein, and hydroxymellein. Curiously, we found putative terremin and terremin succinate that were previously reported as produced by *N. parvum* but not by *D. seriata*; despite only *D. seriata* was isolated from our samples (Spagnolo et al. 2014). As microbiological methods were used, the identification of *D. seriata* does not exclude the possibility that other *Botryosphaeriaceae* were present. Bruez et al. (2014) and Larignon et al. (2015) indeed showed that *N. parvum* and *D. seriata*, and in lesser extent *B. dothidea*, were frequently identified in French vineyards. In this study, the presence of the toxins was cultivar and sample type dependent. Succinate terremin hydrate was detected in all samples, even in AC of Mourvèdre. This last observation would suggest that Mourvèdre control vines were probably also infected, despite the absence of visible symptoms. For succinate terremin hydrate, it is the first report suggesting its presence in vine tissue since Abou-Mansour et al. (2015) only described this compound in in vitro cultures of *N. parvum*. However, these authors used 0.2 to 3 g of wood powder for analysis, and up to 0.5 g for Magnin-Robert et al. (2016), whereas only 0.15 g was used herein. Despite the high resolutive power of FT-ICR-MS, the amounts of the putative toxins might be under the level of detection in some samples, making it difficult to draw conclusions. It would be interesting to start a detailed study to specifically analyze *Botryosphaeriaceae* phytotoxins in AD and SD wood areas to have a better estimation of their abundance and spatial distribution. Interestingly, for Chardonnay, toxins were mostly accumulated in AD samples, whereas phytoalexins were mostly accumulated in SD samples, where cultivable *Botryosphaeriaceae* species were richly detected. This result suggests that the presence of phytoalexin is correlated with the presence of pathogens, and not with that of toxins. It also indicates that toxins are not accumulated in the brown stripe where there is maximal cultivable *Botryosphaeriaceae* species but in the adjacent asymptomatic area. Conversely, for Mourvèdre and Gewurztraminer, there is a colocalization of pathogens, toxins and stilbenes. This cultivar-dependent accumulation of toxins would merit deeper investigation. These results also indicate that phytoalexins are not sufficient to stop the biosynthesis of toxins by pathogens.

In a second time, the sample types AC, AD, and SD were compared and only the discriminant *m/z* common to cultivars were selected for study. According to what was observed for the cultivar-dependent analysis, Mourvèdre was different, with neither AC versus AD nor AD versus SD discriminant *m/z* common to Chardonnay and Gewurztraminer. As reported for the first comparison, lipids were more accumulated in AD samples whereas phytochemicals were more accumulated in SD samples, confirming that such evolution is not cultivar specific. Although specific markers of AC, AD, and SD areas could not be identified, our findings serve as a basis to draw attention to metabolic fingerprints of these areas.

Conclusion. Using a proteomic approach, Spagnolo et al. (2014) reported differential responses to *Botryosphaeria dieback* of Chardonnay, Gewurztraminer, and Mourvèdre. The present study shows that such differential responses are also expressed at the metabolome level. Phytochemicals and lipids were highlighted.

These results are original for lipids as this is the first time that a study reports the impact of *Botryosphaeria dieback* on wood phospholipid profiles.

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