JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Phenolics and Their Antifungal Role in Grapevine Wood Decay: Focus on the Botryosphaeriaceae Family

Carole Lambert,[†] Jonathan Bisson,[†] Pierre Waffo-Téguo,[†] Yorgos Papastamoulis,[†] Tristan Richard,[†] Marie-France Corio-Costet,[§] Jean-Michel Mérillon,[†] and Stéphanie Cluzet*[,†]

[†]Université de Bordeaux, Groupe d'Etude des Substances Végétales à Activité Biologique (GESVAB), EA 3675, Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte, CS 50008, F-33882 Villenave d'Ornon Cedex, France

[§]INRA, ISVV, UMR Santé et Agroécologie du vignoble (SAVE-1065), B.P. 81, Avenue Edouard Bourleaux, 33883 Villenave d'Ornon, France

ABSTRACT: The interaction between *Vitis vinifera* and trunk disease fungi requires better understanding. We studied the role of phenolics as possible plant defense compounds in this context. The impact of 24 grapevine phenolic compounds was determined on 6 major wood decay fungi by an in vitro agar plate assay. Hydroxystilbenoids, especially oligomers such as miyabenol C, isohopeaphenol, and vitisin A and B, greatly reduced the growth of the fungi, except that of *Phaeoacremonium aleophilum*. A detailed investigation in 10 Botryosphaeriaceae strains revealed that all of the studied members of this family display a common susceptibility to phenolics that is more or less significant. Then we undertook a quantitative analysis of stilbenoid content in grapevine plantlets inoculated with Botryosphaeriaceae to investigate whether in planta these fungi have to counteract the most active phenolics. On the basis of our results, the possible role of phenolics in grapevine defense against trunk disease agents is discussed.

KEYWORDS: *Vitis vinifera, polyphenols, stilbenoids, antifungal activity, wood decay, Botryosphaeriaceae*

INTRODUCTION

Wood afflictions are very destructive grapevine diseases and occur worldwide, causing losses in grape yield and quality and threatening the sustainability of viticulture.[1] They result from the colonization of perennial organs by one or several fungal endophytes, leading eventually to the death of the plant in the more or less long term. Eutypa dieback and Esca syndrome are among the most serious and widespread wood diseases. Eutypiosis is caused by an ascomycete, *Eutypa lata*, whereas Esca syndrome is associated with at least two major ascomycetes, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, and a basidiomycete, *Fomitiporia mediterranea*.[2−4] Botryosphaeriaceae species such as *Neofusicoccum parvum* and *Diplodia seriata* are also associated with a wide range of grapevine decline symptoms including Esca syndrome.[5] The infection process remains unclear, and it is hypothesized that fungi could enter the grapevine through pruning wounds, the graft, the rootstock, and/or the roots.[6] These fungi infect xylem vessels and associated cells[4,7] and produce cankers or rot.[8] Typical external wood decay symptoms such as leaf discoloration become increasingly evident in plants that are 8−10 years or older.[8] Owing to pathogen localization, symptom expression variability, and lack of knowledge about disease onset, no curative treatment is available to control grapevine wood diseases. To date, no resistant Vitaceae species have been identified, although differences in level of tolerance to wood decay exist between grapevine cultivars.[9,10]

The latency time before symptom appearance observed in grapevine trunk diseases suggests that preformed and/or inducible defenses could restrain the development of these pathogens in the wood. Constitutive and induced phenolic compounds are thought to be involved in defense mechanisms of trees against wood decay fungi by forming a chemical barrier that limits pathogen growth.[11,12] In particular, the role of resveratrol derivatives has been emphasized.[13−15]

Several studies suggest the involvement of some phenolics in grapevine response to complex Esca fungi. Phenolic compound levels increase in infected discolored wood,[16] especially those of the hydroxystilbenes, *trans*-resveratrol and ε-viniferin,[17] and those of more complex stilbenoids such as ampelopsins A, B, and H, leachianols F and G, hopeaphenol, isohopeaphenol, and pallidol.[18,19] Inoculation of plants with *P. aleophilum* and *P. chlamydospora* has also demonstrated that phenolics accumulate in xylem vessels and cells adjacent to infected ones or close to infected shoots and roots.[20,21] Again, *trans*-resveratrol and ε-viniferin were shown to increase in colonized tissues, as well as an unidentified resveratrol dimer.[22] Taken together, these data suggest that phenolics, and especially stilbenes, could play a role in limiting the development of fungi in grapevine wood.

In vitro tests have identified numerous simple phenolic acids with antimicrobial activity on fungi involved in grapevine decline and/or with an inhibitory effect on fungal enzymes catalyzing wood degradation.[8,23] Although all stilbenes can play a role against Esca syndrome, only resveratrol has been checked for its antifungal activity on wood disease pathogens,[24−26] though grapevine wood contains many other resveratrol derivatives as mentioned above.

Received: July 30, 2012
Revised: November 12, 2012
Accepted: November 13, 2012
Published: November 13, 2012

dx.doi.org/10.1021/jf303290g | *J. Agric. Food Chem.* 2012, 60, 11859−11868

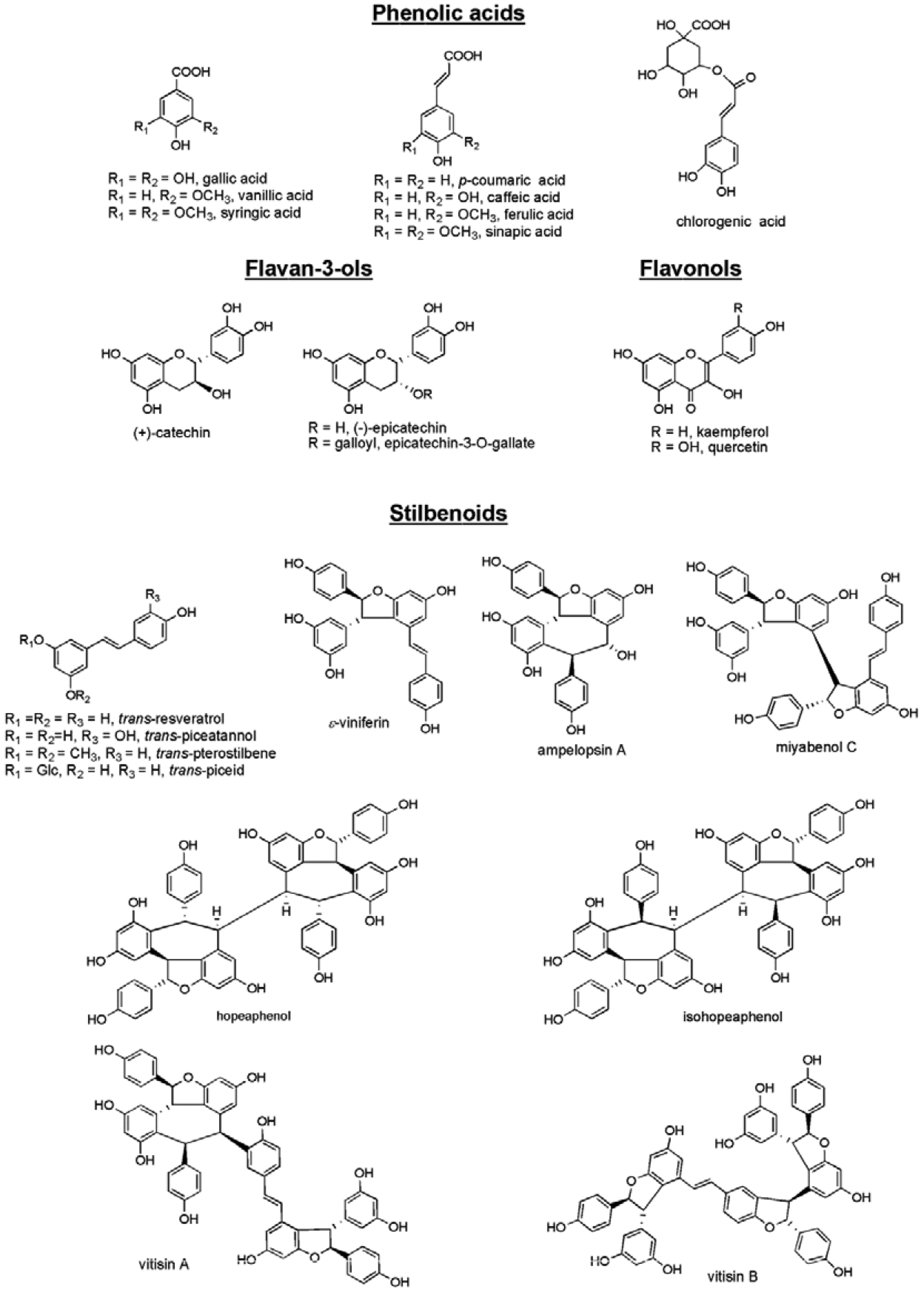

Figure 1. Structures of the 24 phenolic compounds tested.

We sought to improve understanding of the role that stilbenes play in grapevine defense against wood decay. On the basis of the literature, the phenolic content of the wood stem was established and the purification of commercially unavailable phenolics, particularly stilbenoids, was undertaken. Then we triggered an in vitro assay to compare the ability of grapevine stilbenoid compounds to inhibit the growth of six different species of major fungal agents causing wood decay. The synergic effect of some molecules was also evaluated. As stilbenes particularly affect the mycelium development of the two Botryosphaeriaceae species studied, we paid special attention to this fungal family by considering other members. Finally, we performed in planta inoculation to check whether the most active stilbenoid molecules highlighted in our antimicrobial assay were the same as those produced in higher quantities in the woody part of grapevine cuttings inoculated with Botryosphaeriaceae fungi.

MATERIALS AND METHODS

Fungal Strains and Culture Conditions. Fourteen fungal strains from the UMR SAVE (Institute of National Research of Agronomy, Bordeaux, France) monospore collection[27] and from Central Bureau voor Schimmel cultures (Utrecht, The Nederlands) were used: *Phaeomoniella chlamydospora* (SO44), *Phaeoacremonium aleophilum* (SO21), *Fomitiporia mediterranea* (SO35), *Eutypa lata* (BX1-10), four isolates of *Diplodia seriata* (PLU03, LAT28, BoF99-1, BoF98-1), *Botryosphaeria dothidea* (OGE14), two strains of *Neofusicoccum parvum* (Bp0014, PER20), *Lasiodiplodia theobromae* (CBS116460), *Neofusicoccum luteum* (CBS110299), and *Diplodia mutila* (BRA08). The strains were isolated from French *Vitis vinifera* vines except *N. luteum* and *L. theobromae*, which were isolated from *V. vinifera* in Portugal and from *Acacia mangium* in Costa Rica, respectively. The fungi were maintained on sterile potato dextrose agar (PDA) medium except *F. mediterranea* and *P. aleophilum*, which were grown on malt extract agar (MA) at 23 °C in the dark. Media were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Chemicals. Polyphenols (Figure 1) were purchased from Sigma Chemical Co. except epicatechin gallate, ε-viniferin, ampelopsin A, miyabenol C, vitisin A (r-2-viniferin), and B (r-viniferin). We extracted and purified the latter from *V. vinifera* roots and woody stems of cv. Merlot and characterized them by NMR and LC-ESI-MS, as previously described.[28]

Guaiacol, potato dextrose agar, and malt extract were obtained from Sigma Chemical Co.

Antifungal Activity. The antifungal activity of phenolic compounds was adapted from Mazullo et al.[25] and tested using a 24-well plate assay. Phenolics were tested on six fungi involved in grapevine wood decay: *E. lata*, *P. chlamydospora*, *P. aleophilum*, *F. mediterranea*, *N. parvum* Bp0014, and *D. seriata* Bo F99-1. Phenolics were dissolved in ethanol 20% (v/v). For experiments, an appropriate volume of phenolics or ethanol 20% (negative control) was added to an agar medium containing potato broth (12 mg/mL)−malt extract (15 mg/mL). The final concentration of ethanol in each well was 2% (v/v): such an ethanol concentration allows correct growth of fungi and so is often used in antifungal assays, as in the work of Mazullo et al.[25] Inoculum plugs of 4 mm diameter were cut with a cork borer from the margin of fungal growing cultures and then placed at the center of each well containing agar medium ± phenolics. Plates were incubated at 23 °C in the dark. The radial growth of mycelium was determined at various time points between 24 h and 10 days corresponding to each fungal species at 50% of growth in well. Colony diameter was determined at four different points of the mycelium. For each condition (phenolic compound or control), four wells per plate were tested, and each experiment was repeated at least three times. The pH of the medium supplemented or not with phenolic compound was assessed with Litmus paper and a pH-meter (Mettler Toledo MP 225).

The first screening was undertaken with 24 phenolic molecules (Figure 1) at the initial concentration of 500 μM. The concentration inhibiting 50% of fungal growth (IC_{50}) was determined for some compounds by testing various concentrations (0, 50, 100, 250, 500 μM). A potential additive activity was evaluated by combining two molecules of interest (*trans*-resveratrol, *trans*-pterostilbene, *trans*-piceatannol, (+)-catechin, and/or (−)-epicatechin) at 1, 5, 10, 25, 50, or 250 μM each.

Significant results were discriminated by the Student *t* test with $P < 0.05$ or $P < 0.01$. Data on the effect of the 24 compounds on *E. lata*, *P. chlamydospora*, *P. aleophilum*, *F. mediterranea*, *N. parvum* Bp0014, and *D. seriata* Bo F99-1 were organized and visualized using the hierarchical clustering software Cluster version 3.0[29] and Tree View software.[30] The hierarchical cluster analysis of the data obtained with the ten Botryosphaeriaceae in response to five active phenolics at 500 μM was conducted with the same software.

Guaiacol Assay. The ability of fungi to secrete polyphenol oxidase in the culture medium was evaluated by this assay. A fungal plug was placed on plates containing potato dextrose agar supplemented with 0.005% guaiacol and was incubated at 23 °C in the dark. After 7 days of growth, the production of a reddish brown color under and around the fungal colony was considered as a positive reaction resulting from guaiacol oxidation.

Scanning Electron Microscopy. *D. seriata* strain Bo F99-1 was grown on PDA medium supplemented or not (control) with *trans*-pterostilbene at 500 μM final concentration. For the control assay, a PDA medium with 2% ethanol final concentration was used. Mycelium samples from *D. seriata* strain Bo F99-1 were directly examined by environmental scanning electron microscopy with a tungsten electron source (FEI quanta 200, FEI Co., Hillsboro, OR, USA). A gaseous secondary electron detector was used with a chamber pressure at 533 Pa. The acceleration voltages on examination were 7.3−7.6 kV, and the temperature of the Peltier stage was 3 °C. The carrier gas was water. These parameters provided humidity up to 100% on the sample.

Inoculation of Plants by *D. seriata* or *N. parvum*. Grapevine plants (*V. vinifera* L. cv. Merlot) were propagated from wood cuttings in a greenhouse (INRA, Villenave d'Ornon, France). After 3 weeks, rooted cuttings were potted in sandy soil and were grown under controlled conditions at 25/20 °C day/night temperature with 75% relative humidity and a 16 h photoperiod (350 $\mu mol/m^2/s$) with weekly fertilization (2 g L^{-1}, N−P−K 20% with trace elements). Two-month-old plants with 10−12 leaves were used for the experiments. *D. seriata* and *N. parvum* mycelia were maintained on malt agar medium (15 g L^{-1} malt and 20 g L^{-1} agar) at 22 °C (± 1 °C) in darkness. The inoculation was done by drilling a hole in the wood cuttings to the pith at 2 cm below the upper bud. Pieces of 5 mm diameter young mycelium (3 days of culture) were collected by punching the surface of the malt agar culture medium. In each hole, a piece of mycelium or agar medium (control plants) was placed into the hole of the cutting, and the inoculation site was immediately covered with paraffin wax. For each condition, 20 plants were used. Cuttings were maintained in the greenhouse (same conditions as previously) for 2 months. Then, the inoculated parts of the plants were collected, the size of necrosis caused by fungi was measured, and the samples were stored at −80 °C.

Quantification of Stilbenoids in Plants Inoculated by *D. seriata* or *N. parvum*. About 500 mg of inoculated part was cut into small pieces of <1 mm. Stilbenoids were extracted from this material overnight with 10 mL of acetone/water solution (6:4, v/v) at 4 °C. After centrifugation (2500 rpm, 5 min), 5 mL of the supernatant was evaporated to dryness. Dry extract was then recovered in water/methanol solution (8:2, v/v) and extracted twice with 2 mL of hexane and then three times with 2 mL of ethyl acetate. Ethyl acetate extracts that contained the compounds of interest were evaporated to dryness, dissolved in methanol 50% (v/v) (1 mL), and filtered through 0.45 μm PTFE membrane filters (Fioroni SA, France). Analysis of stilbenoids was performed by HPLC on a 250 × 4 mm Prontosil reverse-phase C18 column (5 μm, Bischoff Chromatography, Leonberg, Germany) protected by a guard column of the same material. Separation was performed at a flow rate of 1 mL/min with a mobile phase composed of (A) H_2O/TFA 1% (97.5/2.5, v/v) and (B)

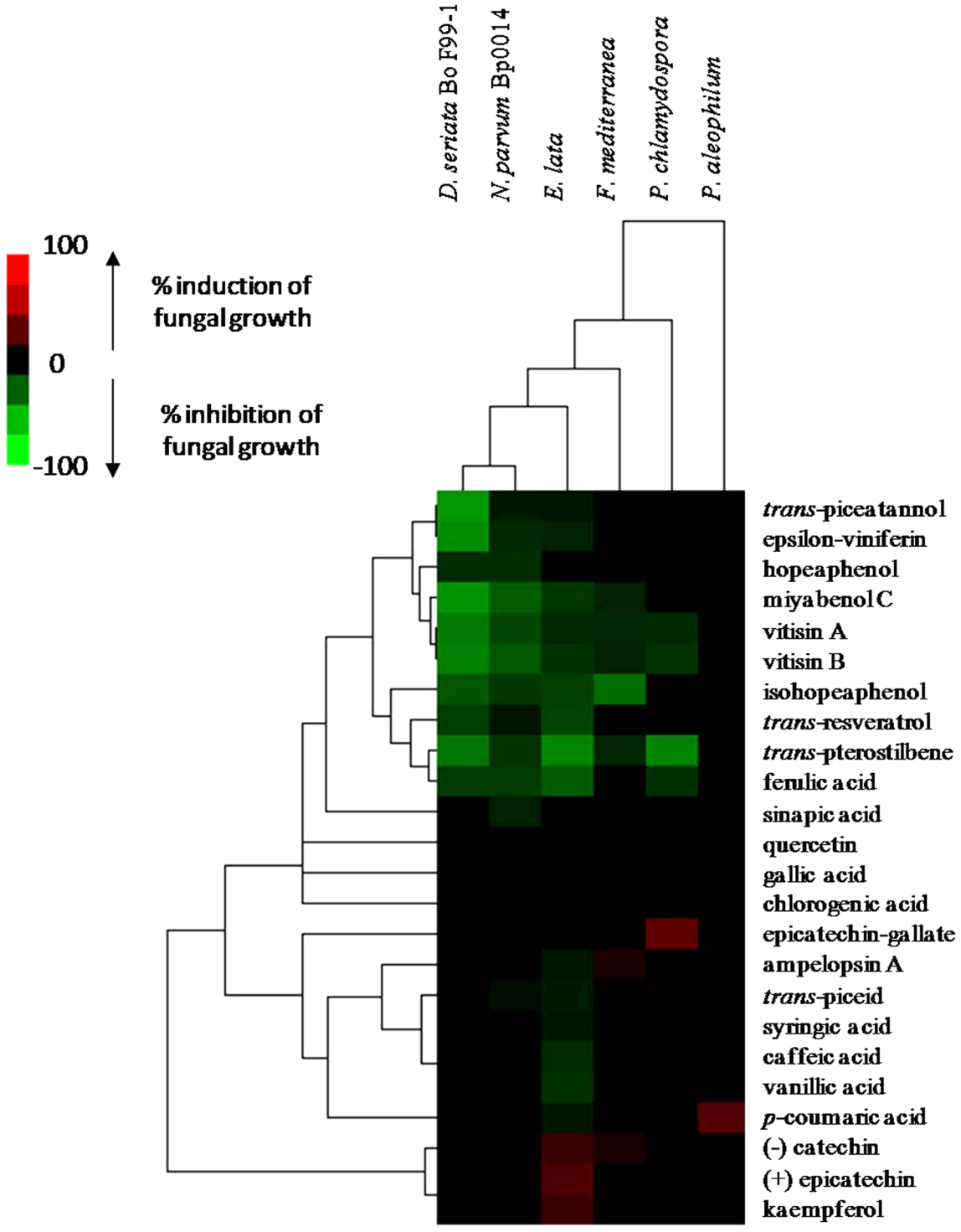

Figure 2. Percentage of growth inhibition or induction of fungi in the presence of one of the 24 grapevine phenolic compounds tested. Results are presented by hierarchical clustering. Green represents an inhibitory compound, black a compound with no effect, and red a stimulating one. Color is proportional to the effect of the molecule.

ACN/TFA 1%. A volume of 20 μL was injected in the column. The run was as follows: 0−5 min, 17% B; 5−25 min, from 17 to 30% B; 25−35 min, from 30 to 38% B; 35−45 min, from 38 to 100% B; 45−55 min, 100% B; 55−56 min, from 100 to 17% B; 56−70 min, 17% B. UV detection was performed at 280 and 306 nm. Stilbenoids were identified by comparing the retention time of pure standards and by LC-ESI-MS analysis. Absolute contents of *trans*-piceatannol, *trans*-resveratrol, ε-viniferin, miyabenol C, hopeaphenol, and isohopeaphenol were estimated from calibration curves prepared with pure standards. Stilbenoid contents significantly different from the control were discriminated by Kruskal−Wallis one-way ANOVA using α = 0.05 and performed with Statistica 10 software.

RESULTS AND DISCUSSION

Antifungal Activity of Phenolics. *Responses of Six Wood Decay Fungi to 24 Grapevine Phenolics.* Twenty-four grapevine phenolic compounds were selected on the basis of their reported presence in grapevine wood:[17,22,23,31] 8 phenolic

acids (gallic, vanillic, syringic, *p*-coumaric, caffeic, ferulic, sinapic, chlorogenic acids), 3 flavan-3-ols ((+)-catechin, (−)-epicatechin, epicatechin-3-*O*-gallate), 2 flavonols (kaempferol, quercetin), and 11 stilbenoids (*trans*-resveratrol, *trans*-piceatannol, *trans*-pterostilbene, *trans*-piceid, ε-viniferin, ampelopsin A, miyabenol C, hopeaphenol, isohopeaphenol, vitisins A and B). We also included pterostilbene as it is well-known for its antifungal activity against fungi such as *Plasmopara viticola*[32] and *Botrytis cinerea*,[33] which are responsible for downy mildew and gray mold, respectively. This set of phenolics on major grapevine trunk disease agents (*P. chlamydospora*, *P. aleophilum*, *F. mediterranea*, *E. lata*, *D. seriata* strain Bo F99-1, and *N. parvum* strain Bp0014) was screened by evaluating the growth of target organisms on solid media in the presence of each phenolic compound at 500 μM. We selected this concentration on the basis of resveratrol and ε-viniferin contents in grapevine wood[31,34] and previously used phenolic concentrations in in vitro antifungal tests.[8,23,25,34] Agar plate tests were used to bioassay very small quantities of phenolics that are not easily purified in the laboratory and which were particularly useful for this comparative study.

The pH of the medium displayed a value of 5.0 and was not modified by addition of either ethanol or phenolic. The data are presented with a hierarchical clustering of growth percentage of each fungus in the presence of phenolics at 500 μM compared to the control condition set at 100% (Figure 2).

The 24 phenolic compounds that we tested at 500 μM differently affected fungal growth, depending on the molecule studied and the fungus of interest. Sensitivity toward phenolic compounds differed among the fungi. On the one hand, the compounds could display an antifungal activity, exhibit no activity, and even enhance the growth of some of the pathogens. On the other hand, *P. aleophilum* was not susceptible to any of the phenolics, *P. chlamydospora* was susceptible to 4 molecules, *F. mediterranea* to 5, *D. seriata* to 10, *N. parvum* to 11, and *E. lata* to 15. Botryosphaeriaceae strains and *E. lata* were very susceptible to phenolics, especially stilbenes.

Fifteen molecules had an inhibitory activity on fungal growth, four stimulated it, three showed no activity, and two triggered both an inhibition and a stimulation.

In the hierarchical cluster, ten molecules were grouped together. These molecules represented the most active ones: nine were stilbenoids and the tenth was ferulic acid. The highly antifungal phenolic was pterostilbene, which reduced the growth of five target fungi about 3.8−5.5-fold. It has never been reported to be present in the wood, and it was used as a control in our experiments.

Two tetramers of resveratrol, vitisins A and B, also had an intense activity on five fungi, and the trimer miyabenol C and tetramer isohopeaphenol exhibited interesting antifungal properties on four trunk disease agents. These compounds were identified in recent decades in *V. vinifera*,[30,35] and little is known about their biological activity or their quantities in grapevine woody tissues.[36] To our knowledge, this is the first time that their antimicrobial activity is reported against wood diseases and even against other micro-organisms. Nevertheless, miyabenol C was recently identified in leaves of *V. vinifera* hybrids challenged with *P. viticola*, the causal agent of downy mildew.[37] These results suggest that owing to their high in vitro antifungal activity, these resveratrol oligomers may be key compounds in grapevine defense against trunk disease pathogens. Piceatannol, ε-viniferin, resveratrol, and hopeaphenol had growth inhibitory effects with very different levels on at least two of the fungi. Resveratrol, a major stilbenoid of grapevine wood, inhibited the growth of *D. seriata* (1.6-fold), *N. parvum* (1.1-fold), and *E. lata* (1.7-fold). Coutos-Thévenot et al.[26] also demonstrated that resveratrol at 500 μM inhibited *E. lata* growth about 1.7-fold, whereas Mazullo et al.[25] found that it had no effect on the anamorph of *E. lata*, *Libertella blepharis*, even at 2200 or 4400 μM. In our hands, *F. mediterranea* was not susceptible to resveratrol, although Bruno et al.[34] showed that a 220 μM concentration inhibits its growth. A guaiacol assay allowed us to notice the ability of *F. mediterranea* to secrete polyphenol oxidase in the culture medium. Such a capacity could explain its ability to grow in the presence of resveratrol in our experiment, so resveratrol was certainly oxidized. However, the guaiacol assay failed to demonstrate the presence of polyphenol oxidase in the medium of *P. chlamydospora* and *P. aleophilum*. In the case of *P. chlamydospora*, this is consistent with the results indicated by Mazullo et al.[25] and Bruno and Sparapano,[38] who showed that a concentration around 500 μM (exactly under 440 μM) seems to stimulate *P. chlamydospora* growth. Other studies demonstrated no effect or an inhibition, but they were conducted at higher concentrations (2200 and 876 μM, respectively).[25,39] Bruno and Sparapano[38] found *P. aleophilum* growth was inhibited at 220 μM of resveratrol, although Mazullo et al.[25] found that at concentrations between 44 and 440 μM fungal growth was stimulated, but was inhibited at 2200 μM resveratrol. These contradictions could be due to the use of different experimental conditions: different media (pH, composition that may influence the molecule effect) and different fungal isolates able or not to metabolize phenolics. The *P. chlamydospora* and *P. aleophilum* isolates that we studied were not found to display this ability (guaiacol assay). Besides, some authors have reported that such fungi exhibit limited phenol oxidase activity in vitro.[8,39]

Nearly all stilbenoids strongly inhibited the growth of the two tested Botryosphaeriaceae, *D. seriata* and *N. parvum*. To our knowledge, the susceptibility of Botryosphaeriaceae to phenolic compounds has received little attention. Djoukeng et al.[40] performed a disk diffusion antifungal assay and reported that stilbenoids (resveratrol, ε- and δ-viniferin) had no effect on *D. seriata*. This result was contrary to what we obtained and could be due to a different experimental method.

Piceatannol, which was tested for the first time on these fungi, displayed an inhibitory profile that was rather similar to that obtained with resveratrol. However, it almost completely inhibited the growth of *D. seriata*, whereas its ability to reduce the growth of *E. lata* was 1.5-fold lower than that of resveratrol. Because each fungus had a specific growth response to a given molecule, no general structure/activity relationship can be extrapolated. Piceid and ampelopsin A did not cluster with the other stilbenoids and displayed a slight inhibitory effect. Piceid is a glycosylated derivative of resveratrol. The effect of glycosylation increases the stability and the solubility of the molecule[41] and generally reduces its antifungal activity,[42] which might explain its only slight activity on trunk disease fungi. With regard to the antimicrobial impact of ampelopsin A, the only study to our knowledge is that of Yim et al.[43] Focusing on oral bacteria causing human dental caries, it evidenced a moderate inhibitory activity at 220 μM against *Streptococcus mutans* and *Streptococcus sanguis*. Moreover, they found that other phenolic compounds such as resveratrol, ε-viniferin, and pterostilbene act at a lower concentration (around 55 μM) than that used for ampelopsin A. This could explain why ampelopsin

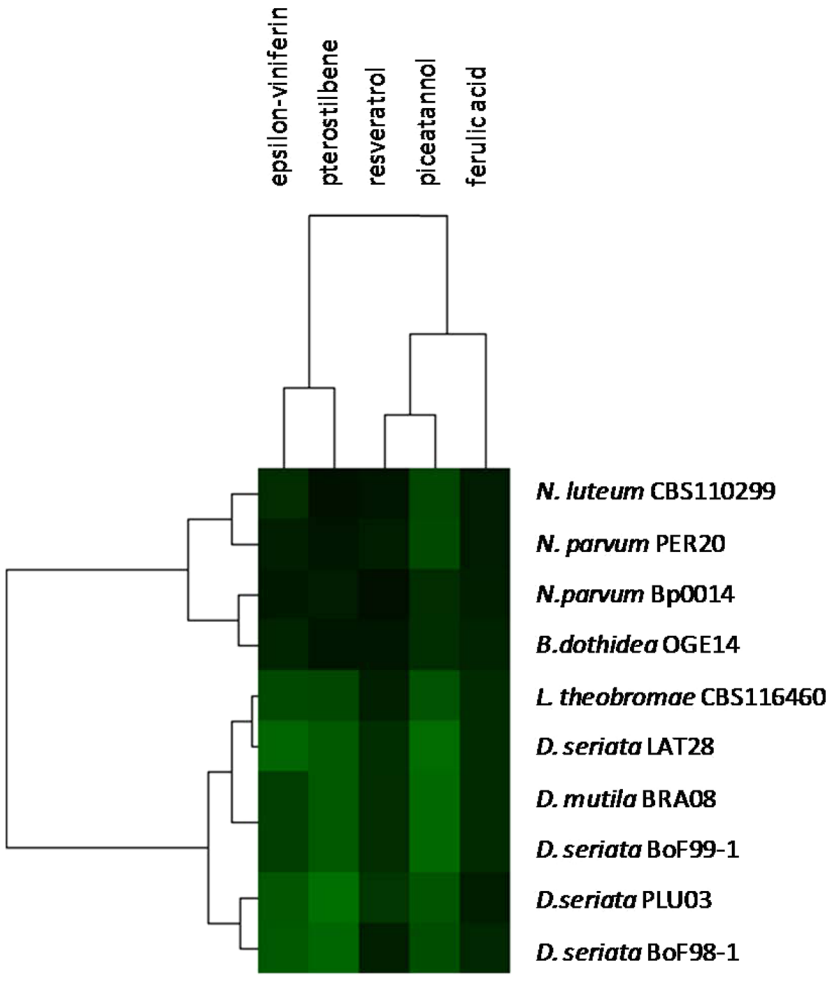

Figure 3. Tree image representing the effect of 5 phenolic compounds on 10 fungi from the Botryosphariaceae family. Hierarchical clustering presents the results expressed as percentage of growth inhibition compared to control (100%).

A at 500 μM was not active in our hands against wood decay fungi.

Except for chlorogenic and gallic acid, phenolic acids exhibited antifungal activity, particularly against *E. lata*. Ferulic acid, which is clustered with stilbenoids, was remarkable for its inhibitory activity: it reduced the growth of *D. seriata*, *N. parvum*, *E. lata*, and *P. chlamydospora* by about 1.6-, 1.5-, 2.2-, and 1.4-fold, respectively. This is the first time that the antifungal activity of ferulic acid has been demonstrated against these pathogens, although its antifungal activity has already been reported against *Pythium* sp. at 50 μM[44] and against *Fusarium oxysporum* at 1030 μM.[45] Some authors have already shown that caffeic, gallic, coumaric, syringic, and vanillic acid exhibit growth inhibition of grapevine wood decay fungi but at concentrations 2−10-fold higher than those used in our study.[8,23] Vanillic, syringic, *p*-coumaric, and caffeic acid affected *E. lata* growth only between 1.1- and 1.3-fold. Sinapic acid inhibited only *N. parvum*. *p*-Coumaric acid was the only phenolic acid displaying two conflicting activities: a negative effect against *E. lata* and a positive one on the growth of *P. aleophilum*.

Among the flavonols, kaempferol stimulated the growth of *E. lata*, whereas quercetin had no effect. Flavan-3-ols had no inhibitory activity on any wood disease fungi but enhanced the growth of some target fungi. Witzell and Martin[46] reported that catechin when tested in antimicrobial agar plate tests failed to demonstrate any inhibitory activity, but similar assays with agar well diffusion showed such an activity on bacteria infecting humans.[47] In our case, even if we could not evidence an antimicrobial role for flavan-3-ols, it should be noted that these molecules could play a role by creating chemical barriers in wood.[48] Nevertheless, epicatechin gallate induced the growth of *P. chlamydospora*, catechin and epicatechin induced the growth of *E. lata*, and catechin also stimulated the growth of *F. mediterranea*.

IC_{50} of Active Compounds and Additive Activity of Polyphenols. To assess their absolute effectiveness, we determined the IC_{50} of three polyphenols: pterostilbene, piceatannol, and ε-viniferin. These molecules were chosen because they were highly effective against the tested fungi at 500 μM and were available. Pterostilbene IC_{50} was evaluated at 163, 251, and 250 μM toward *D. seriata*, *E. lata*, and *N. parvum*, respectively. Piceatannol and ε-viniferin exhibited IC_{50} values of 299 and 260 μM on *D. seriata*, respectively. The IC_{50} of these two compounds was not assessed against *E. lata* and *P. chlamydospora* owing to a slight or nonsignificant inhibitory activity at 500 μM. To our knowledge, this is the first time that the IC_{50} has been investigated with these molecules and pathogens.

As grapevine wood contains various phenolic compounds, trunk disease pathogens might encounter and deal with these molecules in different ways. On this basis, we carried out assays

not only with one phenolic molecule but with two. We expected to see a greater inhibitory effect with these mixtures compared to when the compounds were tested separately. The additive activity of the following combination of inhibitory polyphenolic compounds was investigated: resveratrol and/or pterostilbene and/or piceatannol at 250 μM each against all six fungi. The same assay was carried out with lower concentrations (1, 5, 10, 25, 50, 100 μM) of both resveratrol and pterostilbene against *E. lata* and *D. seriata*, the two fungi most sensitive to these molecules. No additive inhibitory activity was noted. Because some authors[49] reported that some flavan-3-ol compounds such as catechin have been identified as potent stilbene oxidase inhibitors, we sought a potential additive effect of a combination of catechin and/or resveratrol and/or pterostilbene and/or piceatannol at 250 μM final concentration for each molecule and against all fungi. Again, no additive activity was observed, even in the case of *F. mediterranea*, which produced polyphenol oxidase.

Phenolic Effects on 10 Strains of the Botryosphariaceae Family. As shown in the first screening carried out with six trunk disease fungi at 500 μM (Figure 2), *D. seriata* and *N. parvum*, the only two fungi belonging to Botryopshaeriaceae, were highly susceptible to stilbenes and to one phenolic acid, ferulic acid. To date, the Botryosphaeriaceae family has been recognized to contain 21 species pathogenic on grapes.[50] To search for a potential common behavior of fungi belonging to Botryosphaeriaceae, representatives of this family were selected. We chose six species, sometimes with several strains in the same species. Besides *D. seriata* strain BoF99-1 and *N. parvum* strain Bp0014, we studied three other *D. seriata* isolates BoF98-1, LAT28, and PLU03, *B. dothidea*, one other *N. parvum* strain, PER20, *L. theobromae*, *N. luteum*, and *D. mutila*. Their potential growth alteration was also checked in the presence of the active and available phenolic compounds at 500 μM. The growth of almost all Botryosphaeriaceae was more or less affected by the molecules tested (Figure 3). The least affected fungi were *B. dothidea*, *N. parvum* Bp0014 and PER20, and *N. luteum*. Their growth was either not modified or only slightly reduced from 14 to 62%. *D. mutila* and *L. theobromae* and the four *D. seriata* (LAT28, BoF99-1, BoF98-1, PLU03) were more severely inhibited by phenolics, with a growth reduction ranging from 24 to 100%. *D. seriata* strains were particularly susceptible to piceatannol, pterostilbene, and ε-viniferin. Our study carried out with these 10 Botryosphaeriaceae strains revealed for the first time that susceptibility to phenolics is a common feature to the different genera and species of this family. Moreover, the tree concerning the fungi groups suggests that the fungi react to these phenolics according to their genus, because they were clearly separated into two groups, the first one grouping *Neofusicoccum* (*N. luteum*, *N. parvum* PER20 and Bp0014) and *Fusicoccum* (*B. dothidea*) genera and the second grouping *Lasidiplodia*/*Diplodia* genera. This observation is the result of a difference in susceptibility: *Diplodia* species seem to be more susceptible to phenolics than *Neofusicoccum*. This would be in accordance with the fact that *Diplodia* are less virulent than *Neofusicoccum*, as demonstrated elsewhere.[51,52] It was interesting to note a parallel between the tree concerning the behavior of the fungi toward phenolics that we obtained and phylogenetic trees based on ITS analysis or elongation factor 1-α sequence comparison.[52,53]

Microscopic Study of the Impact of Pterostilbene on D. seriata. All of our previous results suggested that the Botryosphaeriaceae species are particularly susceptible to stilbenes. To better characterize how these active compounds affect mycelial growth, we investigated the impact of pterostilbene at 500 μM on the morphology of *D. seriata* BoF99-1 mycelium by environmental scanning electron microscopy (ESEM). Figure 4 shows the mycelium of *D. seriata* grown in a medium supplemented or not with pterostilbene at macroscopic (A, C) and microscopic (B, D) levels. At the macroscopic level, in control condition (A), *D. seriata* exhibited hyphae growing on the whole surface of the substrate. In the pterostilbene condition (C), the mycelium seemed to be denser around the initial plug and was colonizing the medium. At the microscopic level, the mycelium was observed at two growth stages corresponding to opposite sides of the plug taken at the margin of the colony. No difference was noted between control and treated fungal samples at the level of the older part of the mycelium, hyphae forming an intricate network in both cases (data not shown). However, in the younger part of the mycelium, even though no modification of hyphal morphology was observed, the mycelial spread was very different and in accordance with macroscopic observations. In the control condition (B), the fungal development looked like a spider's web with the mycelium stretching out to noncolonized medium, whereas in the pterostilbene condition (D), the mycelium seemed to be halted by a virtual barrier that blocked the passage of hyphae. These data suggest that the compound studied has a more fungistatic effect than a fungitoxic one. In the future, it could be of great interest to investigate eventual mycelia modifications of cell organization at the margin of the colony as observed by Pezet and Pont.[54]

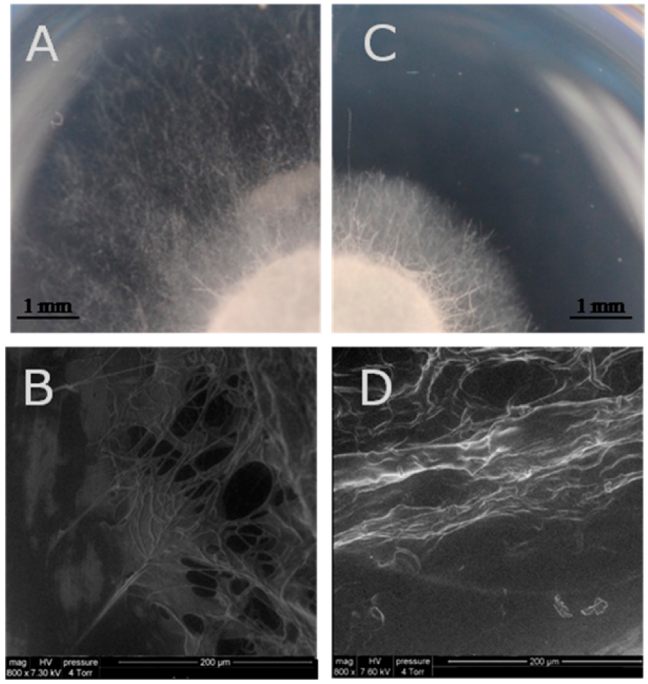

Figure 4. Observation of the effect of *trans*-pterostilbene on *D. seriata* mycelium. Images show mycelium after 2 days of culture in control medium and observed at the macroscopic level (A) and at the colony margin by environmental scanning electron microscopy (B) or grown in medium supplemented with pterostilbene and observed at a macroscopic level (C) and in the same microscopy conditions (D).

Stilbenoid Content in Plants Inoculated by Botryosphaeriaceae Fungi. In the previous parts of this work, it was

Table 1. Means of Stilbenoid Content in Plants Inoculated by *Diplodia seriata* or *Neofusicoccum parvum* Compared to Noninoculated Control Plants and Means of the Extent of Necrosis Caused by Fungi ± Standard Deviation (SD)

		stilbenoid content[a] (μg/g FW)							
	length of necrosis (cm)	*trans*-piceid	*trans*-piceatannol	*trans*-resveratrol	ampelopsin A	hopeaphenol	isohopeaphenol	miyabenol C	ε-viniferin
control	0.03	39	64	104	64	1147	876	202	649
SD	0.08	10	35	49	43	460	387	81	233
N. parvum	5.99	35	66	69	151*	1887*	3197*	487*	1208*
SD	1.17	4	14	19	34	330	693	106	337
D. seriata	0.42	50	38	94	89	1252	1080	276	772
SD	0.35	28	18	44	41	557	557	138	305

[a]An asterisk (*) indicates stilbenoid content significantly different from control according to Kruskal–Wallis one-way ANOVA using $\alpha = 0.05$.

demonstrated that stilbenoids display an antifungal activity in vitro against grapevine wood decay fungi. We demonstrated that Botryosphaeriaceae, *D. seriata* and *N. parvum*, were the most sensitive fungi. As these two fungi display differential aggressiveness,[51,52] we wanted to see whether stilbenoids were produced to a greater or lesser extent qualitatively and/or quantitatively after inoculation of grapevine plants by one type of wood decay fungus and, if so, if these stilbenoid contents are able to prevent fungal development in planta.

The woody parts of foliar cuttings were infected with *D. seriata* (BoF98-1) and *N. parvum* (Per20). The fungal development in the plant was assessed by measuring the length of the necrosis that they caused. Necrosis was negligible in the control. In plants infected by *D. seriata*, the extent of the necrosis was not significantly different from that of the control, suggesting that it did not grow. On the contrary, in plants infected by *N. parvum*, necrosis measured 5.99 cm, suggesting that *N. parvum* grew easily in the cuttings. This is in accordance with previous data showing *D. seriata* to be less aggressive than *N. parvum*.[51,52] Stilbenoids were extracted from approximately 500 mg of fresh woody material that correspond to the part 2.5 cm above and below the site of inoculation. In our conditions, we were able to detect eight stilbenes (retention time in minutes) in the wood: piceid (10), piceatannol (17.5), resveratrol (24.4), ε-viniferin (34.5), and miyabenol C (36.2) detected at 306 nm and ampelopsin A (16.5), hopeaphenol (30), and isohopeaphenol (31.2) detected at 280 nm. Quantitation of resveratrol oligomers that are not easy to purify is an innovative part of our work (Table 1).

Quantitation of stilbenoids showed that the resveratrol, piceid, and piceatannol monomers did not vary significantly between the three conditions (around 90, 45, and 55 μg g^{-1} FW of wood, respectively). With regard to the oligomers, ε-viniferin, miyabenol C, hopeaphenol, and isohopeaphenol, they were at least 6-fold more present than the monomers. Moreover, oligomer content was significantly greater in plants infected with *N. parvum* than in control or *D. seriata*-infected plants (1.3–3-fold more depending on the molecule). Amalfitano et al.[19] compared the profile of resveratrol oligomers in asymptomatic wood (AW) and brown red wood (BRW) of a 'Sangiovese' grapevine naturally infected by *P. chlamydospora*, *P. aleophilum*, and *F. mediterranea*. They detected 14 molecules: *cis*- and *trans*-resveratrol, pallidol, *cis*- and *trans*-ε-viniferin, ampelopsins A and B, leachianols G and F, α-viniferin, *cis*-miyabenol C, hopeaphenol, isohopeaphenol, and ampelopsin H. Six of them (*trans*-resveratrol, ampelopsin A, hopeaphenol, isohopeaphenol, miyabenol C, and ε-viniferin) were common to their Esca-infected plants and our plants inoculated by Botryosphaeriaceae. They also reported that the total concentration of stilbene polyphenols was higher in symptomatic wood (3.7% in BRW vs 1.2% in AW), particularly ε-viniferin and resveratrol.

The synthesis of stilbenoid oligomers and at a high level seems to be specifically induced by *N. parvum*, although its invasion in woody tissues is not inhibited. On the contrary, *D. seriata* did not spread in grapevine, although the stilbenoid content in *D. seriata*-inoculated plants did not vary from that of the noninoculated control. This result seems surprising but is in accordance with our observations on agar plate tests. Indeed, *D. seriata* proved to be more susceptible than *N. parvum* to ε-viniferin, miyabenol C, and isohopeaphenol. This could explain the differences observed in cuttings infected by the fungi. Nevertheless, stilbenoid concentrations in cuttings infected by *N. parvum* were 2.4–7.5-fold higher than those found in agar plate tests (in μg of compound par g of medium) and only up to 3.7-fold higher in cuttings infected by *D. seriata*. With an attack by *N. parvum*, the grapevine tries to block the invader by increasing the production of stilbenes. However, *N. parvum* bypasses the obstacle, even though like all Botryosphaeriaceae, it was sensitive to polyphenolics in the in vitro assay. Nevertheless, the polyphenols displayed only a fungistatic activity and not a fungicidal one, so the fungi tolerate these molecules.

Taken together, these findings indicate that polyphenols can participate in plant reactions to trunk disease pathogens. However, their antimicrobial activity depends on the pathogen involved.

AUTHOR INFORMATION

Corresponding Author

*Phone: +33 (0)5 57 57 59 54. Fax: +33 (0)5 57 57 59 52. E-mail: stephanie.cluzet@u-bordeaux2.fr.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Isabelle Svahn and Etienne Gontier (Bordeaux Imaging Center, France) for the scanning electron microscopy study and Gwenaelle Comont (INRA Villenave d'Ornon, France) for providing the fungal material. We are grateful to Dr. Ray Cooke for revising the English manuscript.

REFERENCES

(1) Bertsch, C.; Larignon, P.; Farine, S.; Clément, C.; Fontaine, F. The spread of grapevine trunk disease. *Science* **2009**, *8*, 721.

(2) Larignon, P.; Fontaine, F.; Farine, S.; Clément, C.; Bertsch, C. Esca et black dead arm: deux acteurs majeurs des maladies du bois chez la vigne. *C. R. Biol.* **2009**, *332*, 765−783.

(3) Larignon, P.; Dubos, B. Fungi associated with esca disease in grapevine. *Eur. J. Plant Pathol.* **1997**, *103*, 147−157.

(4) Moller, W.; Kasimatis, A. Dieback of grapevine caused by *Eutypa armeniacae*. *Plant Dis. Rep.* **1978**, *62*, 254−258.

(5) Van Niekerk, J.; Fourie, P.; Halleen, F.; Crous, P. *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathol. Mediterr.* **2006**, *45*, 43−54.

(6) Gramaje, D.; Armengol, J. Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Dis.* **2011**, *95*, 1040−1055.

(7) Feliciano, A. J.; Gubler, W. D. Histological investigations on infection of grape roots and shoots by *Phaeoacremonium* spp. *Phytopathol. Mediterr.* **2001**, *40*, 387−393.

(8) Mugnai, L.; Graniti, A.; Surico, G. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Dis.* **1999**, *83*, 404−418.

(9) Dubos, B. Fungal antagonism in aerial biocenoses. In *Innovative Approaches to Plant Disease Control*; Chet, I., Ed.; Wiley: New York, 1987; pp 107−135.

(10) Surico, G.; Marchi, G.; Braccini, P.; Mugnai, L. Epidemiology of esca in some vineyards in Tuscany (Italy). *Phytopathol. Mediterr.* **2000**, *39*, 190−205.

(11) Shain, L. Dynamic responses of differentiated sapwood to injury and infection. *Phytopathology* **1979**, *69*, 1143.

(12) Shigo, A. L.; Marx, H. G. Compartmentalization of decay in trees. *Agric. Inf. Bull.* **1977**, *405*, 73.

(13) Hart, J. H.; Shrimpton, D. Role of stilbenes in resistance of wood to decay. *Phytopathology* **1979**, *69*, 1138−1143.

(14) Schultz, T. P.; Hubbard, T. F.; LeHong, J.; Fischer, T.; Nicholas, D. D. Role of stilbenes in the natural durability of wood: fungicidal structure-activity relationships. *Phytochemistry* **1990**, *29*, 1501−1507.

(15) Venäläinen, M.; Harju, A. M.; Saranpaa, P.; Kainulainen, P.; Tiitta, M.; Velling, P. The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood. *Wood Sci. Technol.* **2004**, *38*, 109−118.

(16) Agrelli, D.; Amalfitano, C.; Conte, P.; Mugnai, L. Chemical and spectroscopic characteristics of the wood of *Vitis vinifera* cv. Sangiovese affected by esca disease. *J. Agric. Food Chem.* **2009**, *57*, 11469−11475.

(17) Amalfitano, C.; Evidente, A.; Surico, G.; Tegli, S.; Bertelli, E.; Mugnai, L. Phenols and stilbene polyphenols in the wood of esca-diseased grapevines. *Phytopathol. Mediterr.* **2000**, *39*, 178−183.

(18) Amalfitano, C.; Peduto, F.; Mugnai, L.; Evidente, A.; Surico, G. Chemical characterisation of stilbenic polyphenols from esca-diseased wood and their role in defense mechanisms. 4th International Workshop on Grapevine Trunk Diseases. *Phytopathol. Mediterr.* **2005**, *44*, 99.

(19) Amalfitano, C.; Agrelli, D.; Arrigo, A.; Mugnai, L.; Surico, G.; Evidente, A. Stilbene polyphenols in the brown red wood of *Vitis vinifera* cv. Sangiovese affected by esca proper. *Phytopathol. Mediterr.* **2011**, *50* (Suppl.).

(20) Del Rio, J.; Gonzalez, P.; Fuster, M. D.; Botia, J. M.; Gomez, P.; Frias, V.; Ortuño, A. Tylose formation and changes in phenolic compounds of grape roots infected with *Phaeomoniella chlamydospora* and *Phaeoacremonium* species. *Phytopathol. Medit.* **2001**, *40*, 394−399.

(21) Troccoli, L.; Calamassi, R.; Mori, B.; Mugnai, L.; Surico, G. *Phaeomoniella chlamydospora*-grapevine interaction: histochemical reactions to fungal infection. *Phytopathol. Mediterr.* **2001**, *40*, 400−406.

(22) Martin, N.; Vesentini, D.; Rego, C.; Monteiro, S.; Oliveira, H.; Boavida Ferreira, R. *Phaeomoniella chlamydospora* infection induces changes in phenolic compounds content in *Vitis vinifera*. *Phytopathol. Mediterr.* **2009**, *48*, 101−116.

(23) Del Río, J. A.; Gomez, P.; Baidez, A.; Fuster, M. D.; Ortuño, A.; Frias, V. Phenolic compounds have a role in the defence mechanism protecting grapevine against the fungi involved in Petri disease. *Phytopathol. Mediterr.* **2004**, *43*, 87−94.

(24) Bruno, G.; Sparapano, L. Effects of three esca-associated fungi on *Vitis vinifera* L.: V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. *Physiol. Mol. Plant Pathol.* **2007**, *71*, 210−229.

(25) Mazullo, A.; Di Marco, S.; Osti, F.; Cesari, A. Bioassays on the activity of resveratrol, pterostilbene and phosphorous acid towards fungi associated with esca of grapevine. *Phytopathol. Mediterr.* **2000**, *39*, 357−365.

(26) Coutos-Thévenot, P.; Poinssot, B.; Bonomelli, A.; Yean, H.; Breda, C.; Buffard, D.; Esnault, R.; Hain, R.; Boulay, M. *In vitro* tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase *Vst1* gene under the control of a pathogen-inducible PR 10 promoter. *J. Exp. Bot.* **2001**, *52*, 901.

(27) Comont, G.; Corio-Costet, M. F.; Larignon, P.; Delmotte, F. AFLP markers reveal two genetic groups in French population of the grapevine fungal pathogen *Phaeomoniella chlamydospora*. *Eur. J. Plant Pathol.* **2010**, *127*, 451−464.

(28) Zga, N.; Papastamoulis, Y.; Toribio, A.; Richard, T.; Delaunay, J. C.; Jeandet, P.; Renault, J. H.; Monti, J. P.; Mérillon, J. M.; Waffo-Téguo, P. Preparative purification of antiamyloidogenic stilbenoids from *Vitis vinifera* (Chardonnay) stems by centrifugal partition chromatography. *J. Chromatogr., B* **2009**, *877*, 1000−1004.

(29) De Hoon, M. J. L.; Imoto, S.; Nolan, J.; Miyano, S. Open source clustering software. *Bioinformatics* **2004**, *20*, 1453−1454.

(30) Page, R. D. M. TreeView: an application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **1996**, *12*, 357−358.

(31) Püssa, T.; Floren, J.; Kuldkepp, P.; Raal, A. Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 7488−7494.

(32) Pezet, R.; Gindro, K.; Viret, O.; Spring, J. L. Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. *Physiol. Mol. Plant Pathol.* **2004**, *65*, 297−303.

(33) Pezet, R.; Pont, V. Mise en évidence de ptérostilbène dans les grappes de *Vitis vinifera*. *Plant Physiol. Biochem.* **1988**, *26*, 603−607.

(34) Bruno, G.; Sparapano, L. Effects of three esca-associated fungi on *Vitis vinifera* L.: III. Enzymes produced by the pathogens and their role in fungus-to-plant or in fungus-to-fungus interactions. *Physiol. Mol. Plant Pathol.* **2006**, *69*, 182−194.

(35) Yan, K. X.; Terashima, K.; Takaya, Y.; Niwa, M. A novel oligostilbene named (+)-viniferol A from the stem of *Vitis vinifera* "Kyohou". *Tetrahedron* **2001**, *57*, 2711−2715.

(36) Pawlus, A.; Waffo-Téguo, P.; Mérillon, J. M. Stilbenoid chemistry from wine and the genus *Vitis*, a review. *J. Int. Sci. Vigne Vin* **2012**, *46*, 57−111.

(37) Mattivi, F.; Vrhorsek, U.; Malacarne, G.; Masuero, D.; Zulini, L.; Stefanini, M.; Mose, C.; Velasco, R.; Guella, G. Profiling of resveratrol oligomers, important stress metabolites accumulating in the leaves of hybrid *V. vinifera* (Merzling × Teroldego) genotypes infected with *Plasmopara viticola*. *J. Agric. Food Chem.* **2011**, *59*, 5364−5375.

(38) Bruno, G.; Sparapano, L. Effects of three esca-associated fungi on *Vitis vinifera* L.: I. Characterization of secondary metabolites in culture media and host responses to the pathogens in calli. *Physiol. Mol. Plant Pathol.* **2006**, *69*, 209−223.

(39) Santos, C.; Fragoeiri, S.; Valentim, H.; Phillips, A. Phenotypic characterization of *Phaeoacremonium* and *Phaeomoniella* strains isolated from grapevines: enzyme production and virulence of extra-cellular filtrate on grapevine calluses. *Sci. Hortic.* **2006**, *107*, 123−130.

(40) Djoukeng, J. D.; Polli, S.; Larignon, P.; Abou-Mansour, E. Identification of phytotoxins from *Botryosphaeria obtusa*, a pathogen of black dead arm disease of grapevine. *Eur. J. Plant Pathol.* **2009**, *124*, 303−308.

(41) Morales, M.; Bru, R.; Garcia-Carmona, F.; RosBarcelo, A.; Pedreno, M. A. Effect of dimethyl-beta-cyclodextrins on resveratrol

metabolism in Gamay grapevine cell cultures before and after inoculation with *Xylophilus ampelinus*. *Plant Cell Tiss. Org.* **1998**, *53*, 179−187.
(42) Báidez, A. G.; Gómez, P.; Del Río, J. A.; Ortuño, A. Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahlia* Kleb. Role of phenolic compounds in plant defense mechanism. *J. Agric. Food Chem.* **2007**, *55*, 3373−3377.
(43) Yim, N. H.; Ha, D. T.; Trung, T. H.; Kim, J. P.; Lee, S. M.; Na, M. K.; Jung, H. J.; Kim, H. S.; Kim, Y. H.; Bae, K. H. The antimicrobial activity of compounds from the leaf and stem of *Vitis amurensis* against two oral pathogens. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1165−1168.
(44) Tawata, S.; Taira, S.; Kobamoto, N.; Zhu, J.; Ishihara, M.; Toyama, S. Synthesis and antifungal activity of cinnamic acid esters. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 909.
(45) Wu, H.-S.; Luo, J.; Raza, W.; Liu, Y.-X.; Gu, M.; Chen, G.; Hua, X.-F.; Wanga, J.-H.; Mao, Z.-S.; Shen, Q.-R. Effect of exogenously added ferulic acid on *in vitro Fusarium oxysporum* f. sp *niveum*. *Sci. Hortic.* **2010**, *124*, 448−453.
(46) Witzell, J. W. J.; Martín, J. A. Phenolic metabolites in the resistance of northern forest trees to pathogens-past experiences and future prospects. *Can. J. For. Res.* **2008**, *38*, 2711−2727.
(47) Maria, J. K. M.; Mandal, A. K. A.; Rajesh, J.; Sampath, N. Antioxidant and antimicrobial activity of individual catechin molecules: a comparative study between gallated and epimerized catechin molecules. *Res. J. Biotechnol.* **2012**, *7*, 5−8.
(48) Mace, M. E.; Howell, C. R. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. *Rev. Can. Bot.* **1974**, *52*, 2423−2426.
(49) Goetz, G.; Fkyerat, A.; Métais, N.; Kunz, M.; Tabacchi, R.; Pezet, R.; Pont, V. Resistance factors to grey mould in grape berries: identification of some phenolics inhibitors of *Botrytis cinerea* stilbene oxidase. *Phytochemistry* **1999**, *52*, 759−767.
(50) Urbez-Torres, J. R. The status of *Botryosphaeriaceae* species infecting grapevines. *Phytopathol. Mediterr.* **2011**, *18*, 5−45.
(51) Laveau, C.; Letouze, A.; Louvet, G.; Bastien, S.; Guerin-Dubrana, L. Differential aggressiveness of fungi implicated in esca and associated diseases of grapevine in France. *Phytopathol. Mediterr.* **2009**, *48*, 32−46.
(52) Urbez-Torres, J. R.; Adams, P.; Kamas, J.; Gubler, W. D. Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *Am. J. Enol. Vitic.* **2009**, *60*, 497−507.
(53) Pitt, W.; Huang, R.; Steel, C. C.; Savocchia, S. Identification, distribution and current taxonomy of *Botryosphaeriaceae* species associated with grapevine decline in New South Wales and South Australia. *Aust. J. Grape Wine Res.* **2010**, *16*, 258−271.
(54) Pezet, R.; Pont, V. Mode of action of Vitaceae stilbenes on fungal cells. In *Handbook of Phytoalexin Metabolism and Action*; Daniel, M., Purkayastha, R. E., Eds.; Dekker: New York, 1995; pp 317−331.