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A dimeric stilbene extract produced by oxidative coupling of resveratrol active against *Plasmopara viticola* and *Botrytis cinerea* for vine treatments

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ABSTRACT

Aim: Stilbenes are a group of polyphenols that contribute to the defense mechanisms of grapevine under stress conditions. The main active compounds are oligomeric stilbenes. The sourcing of such compounds is limited by plant raw material availability. In order to overcome this limitation, we proceeded to hemisynthesis of oligomeric stilbenes from natural resveratrol by oxidative coupling using metals. The reaction mixture and the obtained pure compounds were tested for their antimicrobial activity *in vitro* against *P. viticola* and *B. cinerea,* and compared with that of resveratrol and grapevine cane extract.

Methods and results: Hemisynthesis was achieved in methanol using silver acetate (AgOAc) as oxidative coupling reagent. Four main compounds were obtained in the reaction mixture: δ-viniferin, parthenostilbenin B, oxistilbenins A and B. Results demonstrate the ability of oxidative coupling reaction to produce potent inhibitors of *P. viticola* and *B. cinerea*. The reaction mixture is enriched in antimicrobial compounds with high potency as it is at least eight times more active than grapevine cane extract against *P. viticola*. In addition, this reaction mixture reveals a high fungitoxicity against *B. cinerea* in contrast to the grapevine extract.

Conclusion: The present findings indicate that metals could be used for producing highly antimicrobial compounds from natural resveratrol.

Significance and impact of the study: In this study, a method to produce active dimeric stilbenes to protect grapevine was developed based on botanical ingredients. The combination of natural extracts, rich in resveratrol, and metals such as copper may be used as alternatives to Bordeaux mixture and chemical fungicides to protect crops.

KEYWORDS

resveratol, stillbene, hemisynthesis, grey mould, mildew

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INTRODUCTION

The use of chemical pesticides to protect crops is increasingly called into question nowadays, due to their damaging impact on human and ecosystems health. This problem is subject to much debate in winegrowing regions, where *Vitis vinifera* grapes and wine production depends on pesticides used to control grapevine microbial diseases. Hence, alternative preventive or curative solutions are necessary in order to achieve the reduction of pesticides use and to ensure the stability of wine production and economy worldwide. Some scientific researches have focused on plant active against grapevine downy mildew (Krzyzaniak *et al.*, 2018), and other on antagonist microbes conferring botrytis bunch rot reduction (Calvo-Garrido *et al.*, 2019), or on the efficacy of benzothiadiazole, a defense stimulator, against *Botrytis cinerea* (Bellée *et al.*, 2018). Among the potential biocontrol strategies, a particular interest has also been in grapevine extracts enriched in stilbenes (Gabaston *et al.*, 2017; Schnee *et al.*, 2013). Stilbenes are a group of polyphenols produced in several plants and especially in grapevine (Pawlus *et al.*, 2012; Rivière *et al.*, 2012). Under stress conditions as pathogen attacks, stilbenes play the role of phytoalexins and thus are involved in the grapevine defense mechanisms (Gabaston *et al.*, 2017; Langcake and Pryce, 1977; Pezet *et al.*, 2003; Richard *et al.*, 2016; Schnee *et al.*, 2013; Vrhovsek *et al.*, 2012). Among stilbenes, oligomers of resveratrol, such as viniferins, are promising antimicrobial compounds (Gabaston *et al.*, 2017). Even if these compounds are present grapevine byproducts (Gabaston *et al.*, 2019), oligomeric stilbenes sourcing could be problematic in case of large-scale use such as crop treatment.

Resveratrol is a highly abundant natural stilbene, especially in canes, but above all it can be obtained in huge amount from other natural sources such as *Polygonum cuspidatum* (Vastano *et al.*, 2000). Moreover, different studies report the production of oligomeric stilbenes from resveratrol hemisynthesis using metals (Keylor *et al.*, 2015; Quideau *et al.*, 2014; Snyder *et al.*, 2011; Takaya *et al.*, 2005). The main purpose of this work is to evaluate the ability of oxidative coupling of natural resveratrol to produce active antimicrobial oligomeric stilbenes as an alternative or complement of natural extracts. Hemisynthesis was achieved in methanol in the presence of silver acetate (AgOAc) as oxidative coupling reagent. Compound identification was confirmed using UPLC-MS and NMR spectroscopy. The efficiency of the extract and purified compounds was evaluated against *P. viticola* and *B. cinerea* using resveratrol and a grapevine canes extract as control.

MATERIALS AND METHODS

1. Chemicals and instrumentation

All reagents were of analytical grade and used as received without further purification. The *trans*-Resveratrol (3,4',5-trihydroxystilbene, purity ≥ 99 %) was purchased from Bulk Powders™ (Colchester, United Kingdom). Silver acetate (AgOAc, purity ≥ 99 %) was purchased from Acros organics (Geel, Belgium). Grapevine cane extract (Vineatrol) was kindly provided by Actichem (Montauban, France) and produced using a mixture of Cabernet-Sauvignon and Merlot cultivars grown in Bordeaux region (France). The extract contained mainly 15 % resveratrol, 13 % ε-viniferin, 4 % ampelopsin A, 3 % hopeaphenol, 2 % ω-viniferin, 2 % vitisin A, 2 % vitisin B, 2 % piceatannol and 2 % miyabenol C (Biais *et al.*, 2017; Müller *et al.*, 2009). Resveratrol was extracted and purified from Vineatrol following the method described by Biais *et al.* (Biais *et al.*, 2017). Methanol (CH_3OH, HPLC grade), ethanol (CH_2OH, HPLC grade), acetonitrile (CH_3CN, HPLC and LC-MS grades, purity ≥ 99.9 %) and formic acid (HCOOH, 98/100 %) were purchased from Fisher Scientific (Loughborough, UK). Ultrapure water (8 MΩ.cm) was obtained from an Elga apparatus (High Wycombe, UK). Syringes were purchased from Millipore (Molsheim, France). The preparative HPLC was performed on a Gilson PLC 2050 apparatus (Middleton, WI, USA). Elution was conducted on an Agilent Zorbax SB-C18 column (21.2 mm x 250 mm, 7 µm). The reaction extract powder was solubilized at 50 $mg.mL^{-1}$ in MeOH-H_2O (50/50; v/v). Extract was then eluted with a flow rate of 20 $mL.min^{-1}$ using non-acidified ultrapure water (solvent A) and acetonitrile (solvent B), according the following gradient: 30 % B (0-2 min), 30-40 % B (2-25 min), 40-100 % B (25-26 min) and 100 % B (26-31 min). The eluate was monitored at 280 and 306 nm and the fractions were automatically collected. The solvents were removed under reduced pressure and fractions were lyophilized. Compounds purity was measured using an UHPLC-DAD Agilent 1290 series apparatus (Santa Clara, Canada). The elution was performed on an Agilent SB-C18 (2.1 mm x 100 mm, 1.8 µm) column at a flow rate of 0.4 $mL.min^{-1}$, with acidified water (0.1 % formic acid, solvent A) and acidified acetonitrile (0.1 % formic

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acid, solvent B) according to the following gradient: 10 % B (0.0-1.7 min), 10 20 % B (1.7-3.4 min), 20-30 % B (3.4-5.1 min), 30 % B (5.1-6.8 min), 30-35 % B (6.8-8.5 min), 35-60 % B (8.5-11.9 min), 60-100 % B (11.9-15.3 min), 100 % B (15.3-17.0 min), 100 10 %B (17-17.30 min). Samples were injected at a concentration of 100 µg.mL^{-1} after solubilization in H_2O/MeOH mixture (1/1, v/v). Purity of the isolated compounds was estimated to be ≥ 90 %. The purified compounds structure was determined by ^{1}H-^{13}C NMR, using a Bruker Avance III 600 NMR spectrometer (Rheinstetten, Germany).

2. Synthetic aspects

Based on the protocol described by Sako *et al.* (Sako *et al.*, 2004), regioselective oxidative coupling of resveratrol (2.0 g, 40 mmol, 1.0 equiv.) using AgOAc (2.2 g, 60 mmol, 1.5 equiv.) as a metallic catalyst was conducted in MeOH (100 %, 200 mL). The reaction mixture turned to an ash color as it was heated at 50 °C and stirred for 1 hour. The reaction was then stopped by cooling at 4 °C and centrifuged. The methanol fraction was collected and the solvent was removed under reduced pressure. The fraction residue was lyophilized to afford 2.0 g of a dark yellow powder. Produced compounds were isolated and identified using HPLC and NMR. All the steps were carried out in the dark.

3. Bioassays

3.1. *Plasmopara viticola* sporulation inhibition

Grapevine leaves (*V. vinifera*, Cabernet-Sauvignon cultivar) were collected from the upper part of the shoots of plants produced by wood-cutting propagation and grown in the INRA greenhouse (Villenave-d'Ornon, France), under controlled conditions of temperature (25/20 °C, day/night), with a photoperiod of 15/9h (light/dark), and relative humidity (75 %). The *P. viticola* isolate ANN-01 used in the assay was collected in 2015 from Ugni Blanc grapevine cultivar leaves in a commercial vineyard (Charente, France). To obtain the necessary quantity of pathogen material, an inoculum (sporangia suspension) of *P. viticola* sporulations stored at -20 °C was prepared and grown on the collected Cabernet-Sauvignon leaves for 7 days, before proceeding to the sporulation inhibition assay. Leaf disks are cut from Cabernet-Sauvignon fresh leaves, placed with the abaxial side uppermost on Whatman paper moistened in Petri dishes, and sprayed with the stilbene solutions 1 day before adding sporangia suspension (20 000 sporangia/mL). After 7 days of cultivation, downy mildew development was measured according to the density of mycelium and number of sporulation sites as Corio-Costet *et al.* (Corio-Costet *et al.*, 2011).

3.2. *Botrytis cinerea* mycelium development inhibition

This *in vitro* assay is similar to Adrian *et al.* (Adrian *et al.*, 1997). The culture medium was a mixture of potato dextrose broth (24 g.L^{-1}) and agar (15 g.L^{-1}) (PDA) purchased from Sigma (Saint-Louis, MO, USA), autoclaved at 120 °C for 20 minutes. Ethanol solutions of the evaluated compounds were prepared at the concentrations of 0.5, 1, 2, 5, 10, 20 and 50 mg.mL^{-1}. The compound solutions were then incorporated in the PDA medium (240 µL/12 mL, solution/medium) to obtain final concentrations of 5, 10, 20, 50, 100, 200 and 500 mg.L^{-1}. Six-well plates were used and each mixture was poured in 3 wells, 4 mL/well. A calibrated plug of mycelium of *B. cinerea* (strain CCB16 collected from naturally infected berries in the vineyard of Château Couhins (France) in 2016) was then placed on the surface of culture medium, and the dishes were incubated at 22 °C, with a photoperiod of 12/12h (light/dark). Measurements of the mycelium growth area were performed after 48 and 60h of incubation, leading to the calculation of the inhibition rate according to each concentration of the tested compounds and extracts, and hence determining IC_{50} for each tested compound or extract.

RESULTS AND DISCUSSION

1. Production of oligomeric stilbenes

The oxidative coupling reaction of resveratrol in presence of silver acetate (AcOAg) (Sako *et al.*, 2004). After lyophilization, reaction mixture was characterized through LC-MS analysis, indicating the synthesis of four main compounds and the absence of resveratrol (Figure 1 and Table 1). Preliminary experiments allowed us to note the stirring effect on the oxidative reaction (data not shown). Indeed, conducting the oxidative coupling of resveratrol under stirring condition had a major positive impact on the yield, since the reaction tests without stirring led to the transformation of only 60 % of the resveratrol, whereas under stirring, 100 % of the resveratrol reacted.

Likewise, the total transformation of resveratrol is also due to the molar ratio of resveratrol to

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TABLE 1. Peak number, retention time, compound name, yield, UV data (λ_{max} in nm), and MS data (molecular ion and fragments in negative mode) of the identified compounds.

No	Rt (min)	Compound name	Yield (%)	λ_{max}	$[M-H]^-$	Fragments
1	6.5	parthenostilbenin B	6	280	485	453, 391, 359, 255
2	9.9	oxistilbenin A	21	310	485	453, 257, 227
3	10.0	oxistilbenin B	11	310	485	453, 257, 227
4	10.3	δ-viniferin	57	310	453	435, 411

The chemical structures of the four purified compounds were obtained by NMR and MS analysis. NMR data showed that these four compounds correspond to different resveratrol dimers: parthenostilbenin B **(1)** (Kim *et al.*, 2005), *threo*-5-[1-[4-[(1E)-2-(3,5-dihydroxyphenyl)ethenyl]phenoxy]-2-(4-hydroxyphenyl)-2-methoxyethyl]-1,3-benzenediol, named oxistilbenin A **(2)** (Zhang *et al.*, 2014), *erythro*-5-[1-[4-[(1E)-2-(3,5-dihydroxyphenyl)ethenyl]phenoxy]-2-(4-hydroxyphenyl)-2-methoxyethyl]-1,3-benzenediol, named oxistilbenin B **(3)** (Zhang *et al.*, 2014), and δ-viniferin **(4)** (Pezet *et al.*, 2003). The δ-viniferin is the main compound produced (yield 57 %) whereas parthenostilbenin B was obtained as residual compound (yield 6 %).

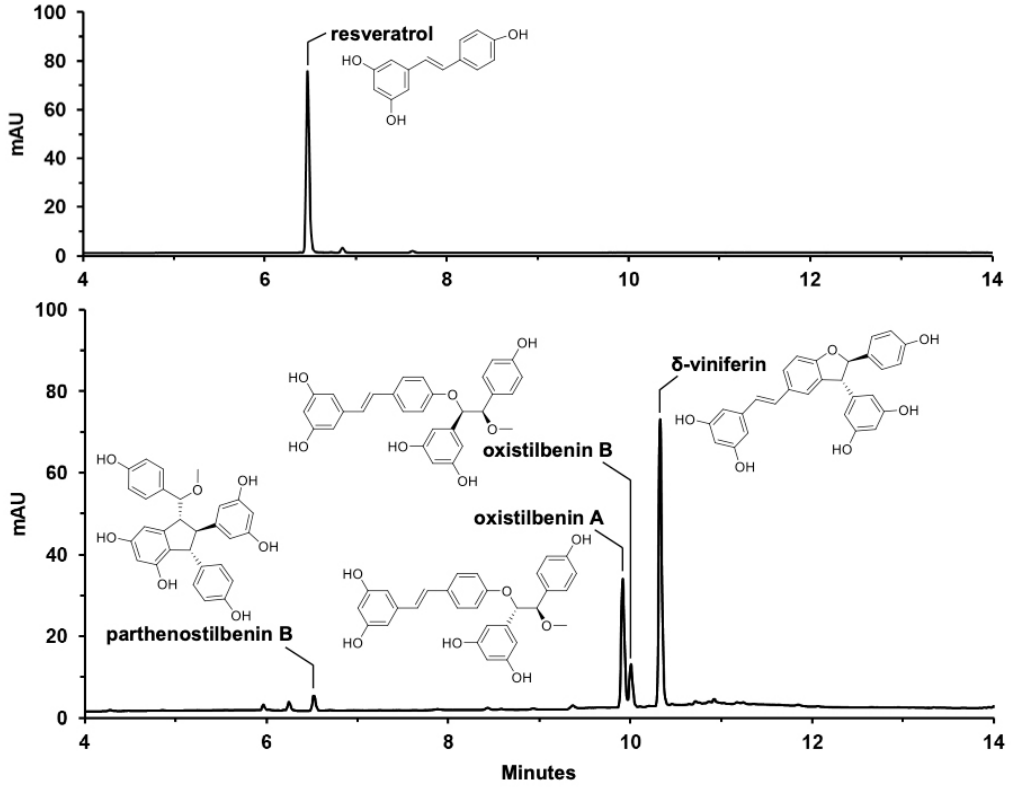

FIGURE 1. UHPLC-DAD chromatograms of resveratrol (A) and oxidative coupling reaction result (B) at 280 nm.

AgOAc 1:1.5 used in the reaction. In preliminary experiments, using reduced molar proportions of AgOAc resulted in a partial transformation of resveratrol.

The produced compounds presented in this work differ from the previous work of Sako and coauthors (Sako *et al.*, 2004). They used a resveratrol:AgOAc 1:1 molar ratio and observed a complete transformation of resveratrol in δ-viniferin, with no mention of other dimers. This high regioselectivity of the dimerization reaction is not observed in the present work, since three other dimers were formed (Table 1). Moreover, it is noteworthy that the resveratrol dimerization was solvent controlled. Indeed, three dimers present an O-methylation provided by the methanol used in the reaction mixture. This observation aligns with those of Velu *et al.* who studied the impact of the solvent used during the reaction on the product distribution and structure (Velu *et al.*, 2008). Another example of the solvent influence is found in Takaya *et al.* work on biomimic synthesis of resveratrol dimers in ethanol using soybean peroxidase (Takaya *et al.*, 2005). Using ethanol as solvent, they obtained quadrangularin B and

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C and other resveratrol dimers with ethyl moiety as substituent. These dimers present the same chemical structure as parthenostilbenin B, except that the ethyl moiety is replaced by a methyl group, since the oxidative coupling was conducted in methanol. Hence, while the chemoselective aspect of resveratrol dimers hemisynthesis can hardly be controlled, the solvent choice could be an option to promote the production of specific compounds among others. It is recalled that resveratrol dimers synthesis is based on oxidatively obtained phenyl radicals coupling, thus involving intermolecular and intramolecular cyclization. Depending on the regioisomeric positions involved in the radical coupling, various groups of dimers can be produced. In this study, the major regioisomeric mode was the connectivity 3-8', which generated δ-viniferin, and oxistilbenins, while the regioisomeric mode 8-8' was allowed the synthesis of the parthenostilbenin B. In comparison with oxistilbenins formation, δ-viniferin required a further intramolecular cyclization following the 3-8' intermolecular radical coupling, whereas oxistilbenins cyclization was prevented by the nucleophilic addition of a MeOH moiety on the double bond 7'-1'.

2. Antimicrobial activity on *Plasmopara viticola*

In order to evaluate the preventive antimicrobial activity of the reaction mixture and the purified stilbenes against *P. viticola*, *in vitro* assays were conducted on grapevine leaves. Moreover, as controls and to allow comparison, resveratrol and grapevine cane extract (Vineatrol) were also tested. The growth measures led to the calculation of the 50 % inhibitory concentration (IC_{50}), which corresponds to the concentration that inhibits 50 % of the *P. viticola* sporulation. The results were presented in Table 2 and Figure 2A.

The reaction mixture totally inhibited the development of *P. viticola* from 200 $mg.L^{-1}$, as did grapevine extract at 500 $mg.L^{-1}$ (Figure 2A). The results for the grapevine cane extract are in agreement with Gabaston et coauthors (Gabaston *et al.*, 2017). Grapevine extract effectivity against downy mildew has been demonstrated *in vitro* (Schnee *et al.*, 2013), in greenhouse and vineyard (Richard *et al.*, 2016). The reaction mixture was eight times more potent than the cane extract, with IC_{50} of 23 and 197 $mg.L^{-1}$, respectively (Table 2).

This result confirms that some metals could be used to potentialize stilbene natural extracts rich in resveratrol.

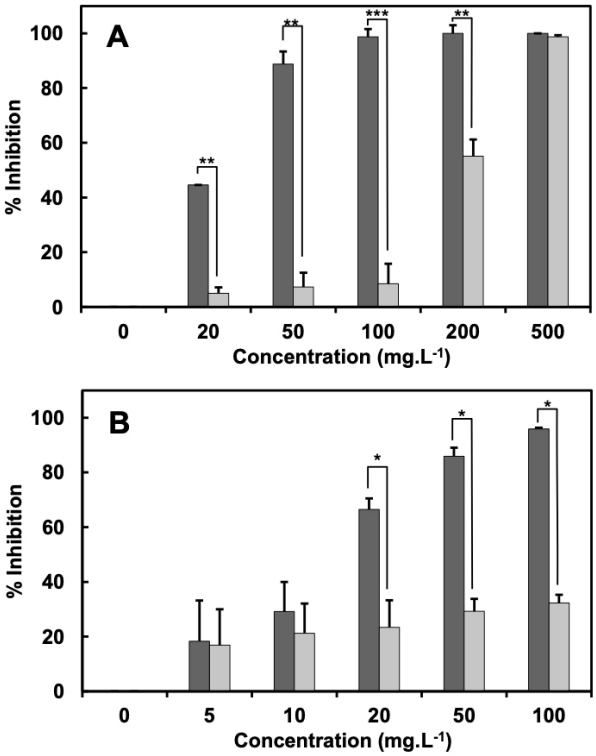

FIGURE 2. Effect of oxidative coupling reaction mixture and cane grapevine extract on *P. viticola* sporulation (A) and *B. cinerea* mycelial growth (B).
Results are expressed as means ± SEM. Significant difference between each extract was set at ***p<0.001, **p<0.01, and *p<0.1. Dark grey bar = oxidative coupling reaction mixture, and light grey bar = cane grapevine extract.

TABLE 2. Concentration ($mg.L^{-1}$) of different compounds and extracts causing 50 % inhibition of mildew development monitored by *P. viticola* sporulation.

	IC_{50}
cane extract	197 ± 3
reaction mixture	23 ± 4
parthenostilbenin B (**1**)	not determined
oxistilbenin A (**2**)	63 ± 12 (130 [a])
oxistilbenin B (**3**)	20 ± 3 (41 [a])
δ-viniferin **(4)**	18 ± 2 (39 [a])
Resveratrol	99 ± 10 (434 [a])

[a] concentration expressed in μM

The four main resveratrol oligomers formed into the reaction mixture were purified as described in Materials and Methods section. Oligomers along with their precursor compound, resveratrol, were tested at different concentrations. Data for IC_{50} were expressed both in $mg.L^{-1}$ and in μM (Table 2). The lowest IC_{50} values were shown by δ-viniferin (39 μM) and

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oxistilbenin B (41 µM) followed by oxistilbenin A (130 µM). The parthenostilbenin B had nearly no inhibition effect on *P. viticola* sporulation. For resveratrol, IC_{50} was high, reaching 434 µM, which came in agreement with Gabaston *et al.* works (Gabaston *et al.*, 2017), and confirmed again the low antimicrobial potency of resveratrol and its role as a precursor for the synthesis of effective stilbene oligomers. Our data concerning δ-viniferin are in contrast with those of Pezet and coauthors that reported a higher IC_{50} value (14.7 µM) for downy mildew development (Pezet *et al.*, 2004). In this study, they observed that δ-viniferin exhibited a significative inhibition on the mobility of zoospore. The variation in IC_{50} could be related to differences in the antimicrobial bioassays, such as the *P. viticola* strain used (Gabaston *et al.*, 2017). Nevertheless, as in our results, δ-viniferin is one of the most potent toxic stilbenes against downy mildew development. In addition to δ-viniferin, this work led to identify oxistilbenin B as a new potent antimicrobial stilbene. Finally, no evident synergic effects were observed between the constituents of the reaction mixture.

3. Antifungal activity against *Botrytis cinerea*

B. cinerea growth areas were measured leading to the calculation of the inhibition rate according to each concentration of the tested compounds and extracts, and hence determining IC_{50}. The results were presented in Table 3 and Figure 2B.

Efficacy of reaction mixture was compared to that of grapevine cane extract (Figure 2B). Interestingly, the reaction mixture is more active than the grape vine extract. The reaction mixture totally inhibited the development of *B. cinerea* from 100 $mg.L^{-1}$. Whereas grapevine extract presents a moderate efficacy as previously reported (Schnee *et al.*, 2013). The reaction mixture had an IC_{50} of 16 $mg.L^{-1}$. The reduced toxicity of the grapevine extract against *B. cinerea* could be due to the presence of laccases in this fungus (Pezet *et al.*, 1991).

TABLE 3. Inhibitory concentration 50 % ($mg.L^{-1}$) of different compounds and extracts against *B. cinerea* mycelium development.

	IC_{50}
cane extract	not determined
reaction mixture	16 ± 2
parthenostilbenin B **(1)**	not determined
oxistilbenin A **(2)**	62 ± 3 (127 [a])
oxistilbenin B **(3)**	26 ± 3 (53 [a])
δ-viniferin **(4)**	6 ± 2 (13 [a])
resveratrol	98 ± 10 (430 [a])

[a] concentration expressed in µM

In addition, fungitoxicity of the four main constituents of oxidative coupling reaction was evaluated using resveratrol as control. Among the four main compounds of the reaction mixture, δ-viniferin presented the lowest IC_{50} value (13 µM), followed by oxistilbenin B and A (53 and 127 µM, respectively). Whereas resveratrol and parthenostilbenin B were the less active compounds. It should be noted that δ-viniferin is one of the most abundant stilbenes synthesized by stressed grapevine leaves after inoculation with *B. cinerea* (Timperio *et al*., 2012).

CONCLUSION

To summarize, these results demonstrate the ability of oxidative coupling reactions of resveratrol to generate dimeric stilbenes. The reaction mixture could be a promising solution as it is at least eight times more than the native grapevine cane extract. These increased properties of the oxidized extract against microbes can be relative to the compounds present in each extract. Indeed, the grapevine cane extract contains mainly resveratrol and ε-viniferin, which exhibited lowest antimicrobial effects, while the reaction mixture is mainly constituted of δ-viniferin and 2 other dimers, strongly active, especially against *P. viticola* development. This approach could be a promising solution to add value to compounds from grapevine by-products. Furthermore, as for all future biocontrol products, the reuse of all compounds of the reaction has to be improved and by this way the used metals can be easily removed from the extracts and recycled for another round of oxidized stilbene production. Such natural antimicrobial products can represent a sustainable wine-growing concept to control vineyard diseases. Further studies are needed to experiment and validate their efficiency in environmental conditions.

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REFERENCES

Adrian M., Jeandet P., Veneau J., A. Weston L. and Bessis R., 1997. Biological activity of resveratrol, a

stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *Journal of Chemical Ecology* 23, 1689-1702. doi:10.1023/B:JOEC.0000006444.79951.75

Bellée A., Cluzet S., Dufour M.C., Mérillon J.M. and Corio-Costet M.F., 2018. Comparison of the Impact of Two Molecules on Plant Defense and on Efficacy against *Botrytis cinerea* in the Vineyard: A Plant Defense Inducer (Benzothiadiazole) and a Fungicide (Pyrimethanil). *Journal of Agricultural and Food Chemistry* 66(13), 3338-3350. doi:10.1021/acs.jafc.7b05725

Biais B., Krisa S., Cluzet S., Da Costa G., Waffo-Teguo P., Mérillon J.M. and Richard T., 2017. Antioxidant and cytoprotective activities of grapevine stilbenes. *Journal of Agricultural and Food Chemistry* 65(24), 4952-4960. doi:10.1021/acs.jafc.7b01254

Calvo-Garrido C., Roudet J., Aveline N., Davidou L., Dupin S. and Fermaud M., 2019. Microbial antagonism toward botrytis bunch rot of grapes in multiple field tests using one bacillus ginsengihumi strain and formulated biological control products. *Frontiers in Plant Science* 10. doi:10.3389/fpls.2019.00105

Corio-Costet M.F., Dufour M.C., Cigna J., Abadie P. and Chen W.J., 2011. Diversity and fitness of *Plasmopara viticola* isolates resistant to QoI fungicides. *European Journal of Plant Pathology* 129(2), 315-329. doi:10.1007/s10658-010-9711-0

Gabaston J., Cantos-Villar E., Biais B., Waffo-Teguo P., Renouf E., Corio-Costet M.F., Richard T. and Mérillon J.M., 2017. Stilbenes from *Vitis vinifera* L. waste: a sustainable tool for controlling *Plasmopara viticola*. *Journal of Agricultural and Food Chemistry* 65(13), 2711-2718. doi:10.1021/acs.jafc.7b00241

Gabaston J., Leborgne C., Waffo-Teguo P., Valls J., Palos Pinto A., Richard T., Cluzet S. and Mérillon J.M., 2019. Wood and roots of major grapevine cultivars and rootstocks: A comparative analysis of stilbenes by UHPLC-DAD-MS/MS and NMR. *Phytochemical Analysis*. doi:10.1002/pca.2815

Keylor M.H., Matsuura B.S. and Stephenson C.R.J., 2015. Chemistry and biology of resveratrol-derived natural products. *Chemical Reviews* 115(17), 8976-9027. doi:10.1021/cr500689b

Kim H.J., Saleem M., Seo S.H., Jin C. and Lee Y.S., 2005. Two new antioxidant stilbene dimers, parthenostilbenins A and B from *Parthenocissus tricuspidata*. Planta Medica 71(10), 973-976. doi:10.1055/s-2005-871229

Krzyzaniak Y., Trouvelot S., Negrel J., Cluzet S., Valls J., Richard T., Bougaud A., Jacquens L., Klinguer A., Chiltz A., Adrian M. and Héloir M.C., 2018. A plant extract acts both as a resistance inducer and an oomycide against grapevine downy mildew. *Frontiers in Plant Science* 9. doi:10.3389/fpls.2018.01085

Langcake P. and Pryce R.J., 1977. A new class of phytoalexins from grapevines. *Experientia* 33(2), 151-152.

Müller C., Ullmann K., Wilkens A., Winterhalter P., Toyokuni S. and Steinberg P., 2009. Potent antioxidative activity of vineatrol®30 grapevine-shoot extract. Bioscience, *Biotechnology, and Biochemistry* 73(8), 1831-1836. doi:10.1007/BF02124034

Pawlus A.D., Waffo-Téguo P., Shaver J. and Mérillon J.M., 2012. Stilbenoid chemistry from wine and the genus *Vitis*, a review. *Journal International des Sciences de la Vigne et du Vin* 46(2), 57-111. doi:10.20870/oeno-one.2012.46.2.1512

Pezet R., Gindro K., Viret O. and Richter H., 2004. Effects of resveratrol, viniferins and pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. *Vitis* 43.

Pezet R., Perret C., Jean-Denis J.B., Tabacchi R., Gindro K. and Viret O., 2003. δ-viniferin, a resveratrol dehydrodimer: one of the major stilbenes synthesized by stressed grapevine leaves. *Journal of Agricultural and Food Chemistry* 51(18), 5488-5492. doi:10.1021/jf030227o

Pezet R., Pont V. and Hoang-Van K., 1991. Evidence for oxidative detoxication of pterostilbene and resveratrol by a laccase-like stilbene oxidase produced by *Botrytis cinerea*. *Physiological and Molecular Plant Pathology* 39(6), 441-450. doi:10.1016/0885-5765(91)90010-F

Quideau S., Deffieux D. and Pouységu, L., 2014. Oxidative coupling of phenols and phenol ethers, *Comprehensive Organic Synthesis: Second Edition*, pp. 656-740. doi:10.1016/B978-0-08-097742-3.00318-9

Richard T., Abdelli-Belhad A., Vitrac X., Waffo Teguo P. and Mérillon J.-M., 2016. *Vitis vinifera* canes, a source of stilbenoids against downy mildew. *OENO One* 50(3), 137-143. doi:10.20870/oeno-one.2016.50.4.1178

Rivière C., Pawlus A.D. and Mérillon J.M., 2012. Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Natural Product Reports* 29(11), 1317-1333. doi:10.1039/c2np20049j

Sako M., Hosokawa H., Ito T. and Iinuma M., 2004. Regioselective oxidative coupling of 4-hydroxystilbenes: synthesis of resveratrol and ε-viniferin (E)-dehydrodimers. *Journal of Organic Chemistry* 69(7), 2598-2600. doi:10.1021/jo035791c

Schnee S., Queiroz E.F., Voinesco F., Marcourt L., Dubuis P.H., Wolfender J.L. and Gindro K., 2013. *Vitis vinifera* canes, a new source of antifungal compounds against *Plasmopara viticola*, *Erysiphe necator*, and *Botrytis cinerea*. *Journal of Agricultural and Food Chemistry* 61(23), 5459-5467. doi:10.1021/jf4010252

Snyder S.A., Gollner A. and Chiriac M.I., 2011. Regioselective reactions for programmable resveratrol oligomer synthesis. *Nature* 474, 461. doi:10.1038/nature10197

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Takaya Y., Terashima K., Ito J., He Y.H., Tateoka M., Yamaguchi N. and Niwa M., 2005. Biomimic transformation of resveratrol. *Tetrahedron* 61(43), 10285-10290. doi:10.1016/j.tet.2005.08.023

Timperio A.M., D'Alessandro A., Fagioni M., Magro P. and Zolla L., 2012. Production of the phytoalexins *trans*-resveratrol and delta-viniferin in two economy-relevant grape cultivars upon infection with *Botrytis cinerea* in field conditions. *Plant Physiology and Biochemistry* 50, 65-71. doi:10.1016/j.plaphy.2011.07.008

Vastano B.C., Chen Y., Zhu N., Ho C.T., Zhou Z., Rosen R.T., 2000. Isolation and Identification of Stilbenes in Two Varieties of *Polygonum cuspidatum*. *Journal of Agricultural and Food Chemistry* 48(2), 253-256. doi:10.1021/jf9909196

Velu S.S., Buniyamin I., Ching L.K., Feroz F., Noorbatcha I., Gee L.C., Awang K., Wahab I.A. and Weber J.F.F., 2008. Regio- and stereoselective biomimetic synthesis of oligostilbenoid dimers from resveratrol analogues: influence of the solvent, oxidant, and substitution. *Chemistry-A European Journal* 14(36), 11376-11384. doi:10.1002/chem.200801575

Vrhovsek U., Malacarne G., Masuero D., Zulini L., Guella G., Stefanini M., Velasco R. and Mattivi F., 2012. Profiling and accurate quantification of trans-resveratrol, trans-piceid, trans-pterostilbene and 11 viniferins induced by *Plasmopara viticola* in partially resistant grapevine leaves. *Australian Journal of Grape and Wine Research* 18(1), 11-19. doi:10.1111/j.1755-0238.2011.00163.x

Zhang Y., Xianfen W., Hou Q., Lin M., Yao C.-S. and Shi J., 2014. Preparation of active resveratrol dimeric derivatives by oxidative coupling reaction using AgOAc as oxidant. *Chinese Journal of Organic Chemistry* 34(5), 886-892. doi:10.6023/cjoc201402006

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