A grapevine by-product extract enriched in oligomerised stilbenes to control downy mildews: focus on its modes of action towards *Plasmopara viticola*

David Taillis, Anthony Pébarthé-Courrouilh, Émilie Lepeltier, Eva Petit, Antonio Palos-Pinto, Julien Gabaston, Jean-Michel Mérellon, Tristan Richard, and Stéphanie Cluzet*

Université de Bordeaux, INRAE, Bordeaux INP, Bordeaux Sciences Agro, OENO, UMR 1366, Équipe Molécules d’Intérêt Biologique (MIB), ISV, F-33140 Villenave d’Ormon, France

ABSTRACT

Natural extracts, and particularly plant by-product extracts, can represent an eco-friendly alternative to synthetic pesticides to control crop diseases. We produced an extract from a mix of grapevine trunk and roots that contain a high amount of complex stilbenes (grapevine phytoalexins), especially ε- and r-viniferins. This extract conferred protection to grapevines against *Plasmopara viticola* (the causal agent of downy mildew) by displaying antimicrobial activities towards zoospore mobility and sporulation and by stimulating plant defences. From the perspective of its application in the field, a formulated preparation of the extract was proposed using polysorbate and sophorolipids. The formulated extract reduced the development of various oomycetes impacting grapevine, potato, tomato and melon in semi-controlled conditions. Thus, such grapevine extract constitutes a promising strategy to protect major cultivated plants against downy mildews.

KEYWORDS: resveratrol oligomers, crop protection, *Vitis vinifera*, antimicrobial activities, defence stimulation

ABBREVIATIONS:

ACT: actin
BM: Bordeaux mixture
CHIT: chitinase
CHS: chalcone synthase
DAD: diode array detector
DPI: days post-inoculation
DPT: days post-treatment
DW: dry weight
EF1: γ-chain of elongation factor 1
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
HPLC: high-performance liquid chromatography
IC50: the concentration of 50 % of downy mildew inhibition
MS: mass spectrometer
NQ: non-quantifiable
PAL: phenylalanine ammonia-lyase
PDS: plant defence stimulator
PR: pathogenesis-related proteins
ROMT: resveratrol-O-methyltransferase
RT-PCR: reverse transcriptase-polymerase chain reaction
STS: stilbene synthase
THIORYLS8: catalytic thioredoxin-like protein 4A
UHPLC: ultra-high-performance liquid chromatography
INTRODUCTION

Oomycetes are responsible for severe diseases affecting major crops. For instance, *Phytophthora infestans* is the causal agent of potato and tomato late blights, and *Pseudoperonospora cubensis* is associated with melon downy mildew. *Plasmopara viticola* affects grapevines, especially European cultivars, and represents one of the most destructive diseases in all vineyards worldwide (Spring et al., 2019). The control of these fungal-like eukaryotes is largely ensured by using synthetic and/or copper-based fungicides (Sharma et al., 2015; Corio-Costet, 2012). However, the intensive use of such pesticides is associated with environmental and health issues, plus the risk of the pathogen developing resistance. Considering copper-based fungicides, their long-term, repeated, and excessive applications result in their accumulation in vineyard soils with negative side effects (such as yield reduction or impacts on soil biota) (Lamichhane et al., 2018). To reduce reliance on chemical fungicides, grape growers need alternative methods for disease management, and, in consequence, the development of eco-friendly control solutions has to be promoted. Amongst these alternatives, the use of microorganisms acting as plant pathogen antagonists, such as *Trichoderma harzianum* T39, has been proposed (Perazzoli et al., 2011). Another promising strategy is based on the use of plant defence stimulators (PDS). For instance, benzothiadiazole has been shown to trigger grapevine protection against *Erysiphe necator* (powdery mildew), *P. viticola* (downy mildew) and *Botrytis cinerea* (grey mould) (Dufourt et al., 2013). This enhanced resistance results from the induction of plant defence reactions such as the accumulation of pathogenesis-related (PR) proteins (e.g., chitinases (CHIT) and β-1,3-glucanase) and the production of phytoalexins (stilbenes). Nevertheless, both biocontrol agents and PDS have been reported to lack constancy and efficacy in fields (Aveline et al., 2019). Additional sustainable strategies have to be offered as the use of natural antimicrobial molecules. Some plant extracts, such as extracts of forest or herbaceous species, displayed oomycidal activities (Krzyzaniak et al., 2018; Mulholland et al., 2017; Thuerig et al., 2011). The capacity of grapevine cane extracts to reduce downy mildew development has been reported both in greenhouse and vineyard conditions (Richard et al., 2016). Stilbenes, the compounds present in high content in cane extracts, were proposed for such protection as they display antimicrobial properties (Alonso-Villaverde et al., 2011). They share a common backbone stilbene structure, exist as monomers and oligomers and differ in the type and position of the substituents (Paulus et al., 2012). Their biosynthesis occurs through the phenylpropanoid pathway with the involvement of two key enzymes, phenylalanine ammonia-lyase (PAL), which ensures the conversion of phenylalanine to cinnamate, and stilbene synthase (STS), which converts cinnamoyl-coenzyme A in stilbenoids (Chong et al., 2009). Resveratrol, the basic unit, shows moderate antimicrobial activity. Besides, its derivatives, such as pterostilbene, a methyalted form, and viniferins, isohopeaphenol and hopeaphenol, oligomerised compounds can be highly effective molecules against downy mildew (Pezet et al., 2004; Gabaston et al., 2017a; Schnee et al., 2013). In addition, stilbenes have been reported to act as PDS (De Bona et al., 2019). Regarding oligomerised stilbenes, they are present in grapevine canes and in higher contents in trunks and roots (Gabaston et al., 2017a; Schnee et al., 2013). These two latter perennial organs represent a huge amount of biomass (e.g., in France, around 0.4 million tons per year) due to the grubbing up of vines (France Agrimer, 2015; Ademe, 2009). This valuable biomass is still today poorly recovered despite its high potential value (Schnee et al., 2013). To benefit from the ability of stilbenes to control diseases, preparations with solvents, emulsifiers, safeners and adjuvants etc., are required to allow these active compounds to counteract adverse climatic conditions (Martinez et al., 1989; Hazra and Purkait, 2019). Eco-friendly formulations have to be developed as the ones based on lipid-related products (e.g., resins or vegetable oils) (Demanèche et al., 2020; Ergodan et al., 2019). Surfactants such as Tween 20 can help encapsulate hydrophobic compounds by forming emulsions with ethanol (Baranaukaite et al., 2019). Amongst adjuvants of natural origin, sophorolipids represent promising candidates (Vaughn et al., 2014; Giessler-Blank et al., 2011).

In this study, we produced an extract enriched in oligomerised stilbenes from a mix of grapevine trunk and roots to study its protective capacities against different oomycetes with a focus on *P. viticola*. A formulated extract was proposed. We evaluate the antimicrobial properties of the oligomerised stilbenes extract (OSE) formulated or not, and in addition, we followed up on its effect on grapevine defence responses. Experiments were done under laboratory and/or semi-controlled conditions.

MATERIALS AND METHODS

1. Chemicals and Standards

For stilbenes extraction and their solubilisation, the solvents (ethyl acetate, methanol and ethanol) were purchased from Fisher Scientific (Loughborough, UK). For analysis, HPLC-grade acetonitrile and methanol, trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO), LC-MS-grade acetonitrile (VWR, Fontenay-Sous-Bois, France) and formic acid (Fisher Scientific (Loughborough, UK). For analysis, HPLC-grade acetonitrile and methanol, trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO), LC-MS-grade acetonitrile (VWR, Fontenay-Sous-Bois, France) and formic acid (Fisher Scientific, Loughborough, UK) were purchased. Water was purified by an Elga water purification system (High Wycombe, UK).

The purification of standard stilbenes (E-piceid, E-resveratrol, E-piceatannol, pallidol, amplexopin A, parthenocissin, E-miyabenol C, hopeaphenol, isohopeaphenol, E-ε-, E-ω-, r2- and r- viniferins) from *Vitis vinifera* roots and cane extract was performed by preparative high-performance liquid chromatography (HPLC) as described by Gabaston and co-workers (Gabaston et al., 2017a). Compounds were analysed by ultrahigh-performance liquid chromatography-ultraviolet-diode array detector coupled to a mass spectrometer (UHPLC-UV-DAD-MS) to determine identity and purity.
The purity of compounds was estimated ≥90 %. The percentage of purity of each standard was estimated based on their HPLC peak area regardless of the total mass of powder obtained after their isolation by preparative HPLC.

The Bordeaux mixture (BM) was a commercial preparation (KB Bouillie bordelaise RSR Dispers Jardin, Scotts, France) containing 20 % copper sulphate.

The co-formulants were Tween 20 (polysorbate 20, Radiasurf 7137, Oléon, Compiègne, France), Tween 80 (polysorbate 80, Radiasurf 7157, Oléon, Compiègne, France) and sophorolipids (Rewoferm SL One, Evonik, Essen, Germany).

2. Preparation of the oligomerised stilbene-enriched extracts

Trunk and roots were collected from grapevines of V. vinifera cv. Merlot and Riparia Gloire de Montpellier as rootstock. This vineyard was located in the South-West region of France (Pessac-Léognan, Gironde). The vines were 32 years old and the vineyard was managed in a conventional way. After their collection, trunks and roots were dried at ambient temperature for 8 months until their moisture content was under 10 %. A mix of trunk and roots (70/30, w/w) was crushed to powder of small particles of 4 mm. Two successive extractions with ethyl acetate were performed on grapevine powder at 60 °C for 2 h. Four hundred grams of powder was firstly extracted with 4000 mL of pure ethyl acetate under stirring. After filtration on a cotton layer, the supernatant was collected and a second extraction was done on the plant powder with an ethyl acetate–water mixture (85/15, v/v). This latter filtrate was collected and pooled with the first one. Finally, to enrich the final extract in stilbenes, a water/ethyl acetate (50/50, v/v) liquid–liquid extraction was done. The organic phase was removed with a rotary evaporator at 38 °C and the concentrated extract was evaporated to dryness in a laboratory freeze dryer. The final product, a brown powder, was the oligomerised stilbenes enriched (OSE) extract.

The formulated OSE (F-OSE) extract consisted of a solution containing the OSE extract (300 mg/L) and a mixture of co-formulants (9 g/L). In this latter mixture, the co-formulants and their final concentration were: Tween 20 at 0.8 g/mL, Tween 80 at 0.15 g/mL and sophorolipids at 0.05 g/mL. Tween 20 and Tween 80 act as surfactants and allow a homogeneous distribution of the extract when sprayed on the leaves. Sophorolipids offer a good solvation of the extract.

3. Identification and characterisation of stilbenes by UHPLC-MS analysis

Analyses were carried out on an Agilent 1290 LC system series from Agilent Technologies (Santa Clara, CA, USA). The system is made of a binary pump, a vacuum degasser, an autosampler, a thermostatted column compartment and outfitted with an ultraviolet-visible diode-array detector (UV–vis DAD) (Agilent G4212B). Samples, prepared at 1 mg/mL, were solubilised in the methanol–water mixture (50/50, v/v) by ultrasonication before being filtrated with a PTFE membrane filter (0.2 µm, Millex-LG, Japan). One µL was injected into the system. The mobile phase consisted of water and pure acetonitrile (solvent A and B, respectively), both prepared in formic acid 1 %. The gradient used was the same as described in the work of Gabaston et al. (2017a). Analysis was performed at 30 °C. The mass spectrometer coupled to the UHPLC was an Esquire 6000 ion trap mass spectrometer with an ESI source (Bruker-Daltomics, BillERICA, MA, USA). An Agilent Zorbax SB-C18 (100 mm × 2.1 mm × 1.8 µm) column was used for analysis. Three independent samples were analysed, and, in addition, technical triplicates were performed for each independent sample. Mass analysis conditions were performed as previously mentioned (Gabaston et al., 2017a). Compounds were identified by UV spectrum and retention time from standards. Data were compared with those obtained previously in the laboratory (Gabaston et al., 2017a). Each stilbene was quantified based on its respective standard calibration curve (with standards concentrations from 1, 5, 20, 50 and 100 µg/mL) at its maximum wavelength to not misestimate the quantity.

4. Plant and fungal material

4.1. Grapevine

For bioassays on foliar discs and analyses of defence responses (gene expression and stilbenes), woody cuttings of V. vinifera cv. Cabernet Sauvignon were used. They were provided by UMR SAVE (INRAE, Villenave d’Ornon, France). Canes were potted in sandy soil and developed plants were grown in a greenhouse under controlled conditions at 25/20 °C day/night temperature with 75 % relative humidity and a 15/9 h light/dark photoperiod. V. vinifera cv. Merlot was used for measuring pest incidence and pest severity in semi-controlled conditions (average temperature of 23 °C, relative humidity of 50 % and a 15/9 h light/dark photoperiod). They were produced by Science Agro Atlantique (Saint Germain du Puch, France).

4.2. Other crops

Potato (Solanum tuberosum) variety Bintje, tomato (Solanum lycopersicum) variety Saint-Pierre and melon (Cucumis melo) variety Maltese F1 were cultivated under semi-controlled conditions at 25/20 °C day/night temperature with 75 % relative humidity and a 15/9 h light/dark photoperiod for respectively one month (6 leaves), 40 days (3–4 leaves) and one month (6 leaves) before the application of the first treatments.

4.3. Pathogens

For laboratory conditions, bioassays on grapevine P. viticOLA isolates (ANN-01) were collected in 2015 in a commercial vineyard of V. vinifera cv. Ugni-blanc in Charente (France) were used. The maintenance of P. viticola was done as previously described (Corio-Costet et al., 2011).

For the semi-controlled assays on grapevines, tomato and melon, respective pathogens isolates used were P. viticola, Phytophthora infestans and Pseudoperonospora cubensis; all provided by BIOtransfer (Montreuil, France).
Sporangia were prepared at 48,000 spores/mL for *P. infestans* in the potato assay and 40,000 spores/mL for *P. infestans* and *P. cubensis* in the tomato and melon assays, respectively.

5. Antimicrobial assays

5.1. On *Plasmopara viticola*

To evaluate the direct oomycidal effect of the OSE extract on zoospores, we collected *P. viticola* (ANN-01) sporangia and placed them in sterile distilled water to obtain a solution at 15,000 sporangia/mL. After 1h in the dark, we added the OSE extract at different concentrations (5, 10, 25, 50 and 100 mg/L) to the sporangia solution and immediately performed microscope observation at 400-fold magnification. The inhibition of zoospores mobility was estimated in comparison to the 1 % ethanol control solution (0 % inhibition) after 5 min of treatment. At least 10 representative fields in triplicate were observed and 3 independent experiments were performed.

The ability of the OSE extract to damage the zoospores was also evaluated by an indirect assay. To do that, a solution of sporangia obtained as described before and treated for 5 min with the OSE extract at 5, 10, 25, 50 or 100 mg/L was used to inoculate grapevine foliar discs. The downy mildew development was estimated at six days after inoculation. Details about downy mildew inoculation and assessment of the oomycete development are described below (sub-part 5.2).

5.2. In laboratory conditions

The third and the fourth leaves below the apex of two-months-old grapevine cv. Cabernet Sauvignon cuttings were collected. Foliar discs (25 mm wide) were produced and randomly placed, the abaxial side upwards, into Petri dishes containing humidified Whatman paper. The OSE extract was prepared at different concentrations (from 25 to 300 mg/L) in sterile water with 1 % ethanol (v/v). The control contained only 1 % ethanol in distilled water. After being sprayed with the solutions (86.5 mL/m²) and kept one night in the dark, the foliar discs were inoculated with 3 drops of 15 µL of a *P. viticola* sporangia suspension (15,000 sporangia/mL).

Then they were incubated at 22 °C with a photoperiod of 16/8 h day/night for 7 days. Six foliar discs per dish, three dishes per condition and three independent experiments were performed. The density of sporulation was estimated by visual scoring to assess disease development. The control was set at 100 % sporulation and the values obtained for extracts conditions were compared to this control and estimated in the percentage of inhibition (Corio-Costet et al., 2011). A dose–response curve was established and the concentration of 50 % of downy mildew inhibition (IC₅₀) was calculated as described by Corio-Costet et al. (2011).

To estimate the level of protection conferred by the OSE extract that resulted from defence stimulating properties, the following experiments were conducted. In the greenhouse, all leaves of the grapevine cuttings were treated by a spray of the OSE extract at 300 mg/L. The third and the fourth leaves were collected 1, 2, 3 and 6 days post-treatment (dpt) and washed carefully with distilled water. Foliar discs were taken and the remaining part of the leaves was freeze-dried for further phytoalexin analysis. *P. viticola* inoculation was performed on foliar discs as described above. Seven days after, disease development was estimated. Five independent biological replicates were used for each timing sample and condition and two independent experiments were done.

5.3. In semi-controlled conditions

For *P. viticola* assays, solutions of OSE extract at 300 mg/L, formulated or not, were tested as described above. BM was applied at 4.2 g/L. An untreated condition was done. Three independent experiments were done and the schedule of the first experiment was provided as an example in Figure 1. The modalities were evenly distributed by blocks in the greenhouse with 4 blocks of 4 plants by modality. The design of the experiments is presented in the supplementary Figure S1. In brief, the protocol was as follows. Every 8–10 days, the solutions were sprayed on all grapevine leaves for a total of three treatments (T₁, T₂, T₃). Three inoculations were performed: a first and artificial inoculation (primary infection, PI) and two secondary inoculations (SI₁ and SI₂), mimicking natural infections by misting water. To carry out the natural inoculations, a blowing fog was generated and dispersed into the air, thus allowing homogeneous distribution of the zoospores in the greenhouse. PI was carried out one day after T₁ and SI₁ was done as soon as the first symptoms appeared (2–3 days after T₁). Then SI₂ 9–10 days after T₁. Three assessments (A₁, A₂, A₃) were performed at least 7 days after PI, SI₁ and SI₂, respectively. The disease development was evaluated by visual observation. It was estimated as pest incidence and pest severity that represent the number of infected leaves and the leaf area covered by sporulation, respectively, and expressed in the percentage of inhibition compared to the untreated control.

![Figure 1](image-url)
For oomycetes affecting tomato (*Solanum lycopersicum*), melon (*Cucumis melo*) and potato (*Solanum tuberosum*), greenhouse trials were conducted with the formulated OSE extract at 1 g/L in the presence of co-formulants at 9 g/L. An untreated condition was done. The treatment was performed on 7-leaf stage plants for potato and 3-leaf stage plants for tomato and melon. The experimental designs for all pathosystems were similar to the one used for the *P. viticola* assay (Supplemental Figure S1). Potato and melon were artificially inoculated with *Phytophthora infestans* and *Pseudomonas cubensis*, respectively, 1 day after treatment, and tomato was inoculated with *P. infestans* 2 days after treatment. Pest incidence and severity were estimated as described before. Two assessments were performed at 7 and 14 days post-inoculation (dpi) for *P. infestans*/potato, 4 and 7 dpi for *P. infestans*/tomato and 6 and 12 dpi for *P. cubensis*/melon.

6. Real-time quantitative PCR analysis

For gene expression analysis, two conditions were considered: control (1 % ethanol) and OSE extract (300 mg/L). The solutions were sprayed on all leaves of Cabernet Sauvignon cuttings. The third and fourth leaves from the apex were collected at 0, 1, 2, 3 and 6 dpi. Five plants were used for each time of sampling and condition. Three independent experiments were done. After harvest, leaves were immediately frozen in liquid nitrogen and kept at –80°C until use. RNAs were obtained from 150 mg of grounded leaves with Spectrum™ Plant Total RNA kit (Merck, Germany) and treated with RNase-free DNase I kit (Promega Corp., USA). Reverse transcription was conducted on 2 µg of RNA using the GoScript™ Reverse Transcriptase (Promega Corp., USA). Real-time quantitative PCR analysis (qPCR) was carried out to determine the mRNA copy number of genes of interest. The selected genes and corresponding primer sets used were presented in Table 1. PCR reactions were performed in triplicates with the GoTaq® qPCR Master Mix (Promega Corp., USA) in 96-well plates (20 µL per well), according to supplier instructions. Melting curves were done to verify primer specificity. *THIORYLS8*, coding a catalytic thioredoxin-like protein 4A, was chosen as a housekeeping gene as it was the most stable according to BestKeeper© (Pfaffl et al., 2004) software out of four housekeeping genes tested (glyceraldehyde-3-phosphate dehydrogenase, γ-chain of elongation factor 1, THIORYLS8 and actin) (Dufour et al., 2016). PCR efficiency amplification was evaluated. The slope of the linear regression of the log-transformed cDNA concentration was plotted against the respective CTs and according to the following formula E = 10^(−slope). As efficiencies for each primer pair were not equal to 2, the ratio of expression was calculated according to the following formula (Pfaffl et al., 2004).

7. Stilbenes extraction from grapevine leaves and analysis

We quantified the stilbene content of leaves treated with the OSE extract from the leaves that were also used for antimicrobial assays in laboratory conditions (Section 5.1). After the production of foliar discs for antimicrobial assays, the remaining part of the leaves was washed with tap water, dried gently with paper and immediately freeze-dried and put in powder. Then, two successive methanol (100 %) extractions followed by a third with a methanol-water mixture (90/10, v/v) were carried out on 60 mg of leaf powder, each for 1h30 under stirring and at room temperature. All the organic phases were pooled and evaporated, and the remaining extract was resuspended in a methanol–water mixture (30/70, v/v). Purification on a Supelclean® LC18 column (Supelco®, Bellefonte, USA) was performed. The resulting extract was resuspended in a methanol-water mixture (50/50, v/v) and analysed by UHPLC-MS as described above.

8. Statistical analyses

Statistical analyses were performed using R software. For variance homogeneity, a one-way analysis of variance (ANOVA) was applied (p < 0.05) and the mean values were separated by the Tukey post-hoc statistic.

**RESULTS**

1. Chemical characterisation of the oligomerised stilbene-enriched extract

The stilbenes present in the OSE extract that was produced from the grapevine trunk and roots (70/30, w/w) were characterised by UHPLC-MS. Twelve molecules were identified and quantified: 3 monomers (*E*-piceid, *E*-piceatannol and *E*-resveratrol), 5 dimers (ampelopsin A, pallidol, parthenocissin, *E*-viniferin and *E*-o-viniferin) and 4 tetramers (hopeaphenol, isohopeaphenol, r- and r2-viniferins) (Table 2).

**TABLE 1.** Defence-related genes and corresponding primer sets used for qPCR.

| Names | GenBank accession number | Forward primer (5′−3′) | Reverse primer (5′−3′) |
|-------|--------------------------|------------------------|------------------------|
| THIORYLS8 | XM_002283586 | TCCCAATCGTGCCGGAACCG | TCACCTCAGGATGGGCGGTTCG |
| PAL | X75967 | ACAACAATGGAGCTCCATCA | CACTTCAGCATGGTGGTGG |
| CHS | X75969 | CCACCAATGCTGTCAGTGGC | CTCGGTCTAGTGGCTACTGT |
| STS | X74892 | ATGGAAAGATCACCCACCTTG | GTAGGGGGTTCGAGAGCA |
| ROMT | FM178870 | TGAGTCTCCCACTGACACCCAGA | CGCATGAGACCGTACACCAGT |
| PR3 | VVU97521 | TACCATGGGCTGCGTCCA | TGAATCCAAATGGTCTT |
| PR10 | AJ291705 | GCTCAAAAGTGCTGGTTTTC | CTCTACACATCGCCTTTC |

Names, Genbank accession number, forward and reverse primers.
The limits of detection and quantification, the accuracy and the linearity were determined. Another stilbene, the \( E \)-miyabenol C trimer, was identified, but it was under the limit of quantification.

Mass values of each stilbene were in accordance with those obtained previously in the laboratory (Table S1) (Gabaston et al., 2017a). The compounds present in a relatively high amount in the OSE extract (in mg/g of DW extract) were \( E \)-resveratrol (95.41), \( E \)-\( \varepsilon \)-viniferin (94.02), \( r \)-viniferin (73.74), isohopeaphenol (63.67) and hopeaphenol (47.07) (Table 2). Stilbenes present in minor quantity were ampelopsin A (23.08), \( r^2 \)-viniferin (21.77), pallidol (18.52), \( E \)-\( \omega \)-viniferin (12.75), \( E \)-piceatannol (11.07) and parthenocissin A (6.47). The presence of \( E \)-piceid was also noted (2.38).

2. Protection assays in vitro against grapevine downy mildew
2.1. Antimicrobial assays on zoospores
First, we evaluated the potential of the OSE extract at different concentrations (5 to 100 mg/L OSE) to reduce \( P. \ viticola \) zoospore mobility. The mobility of zoospores (Figure 2) was not impaired by the extract at 5 mg/L. At 10 mg/L, we noted a reduction of zoospores mobility by 50 % after 5 min of treatment. From 25 mg/L, during the first 2 min after stilbenes addition, the spores were unaffected (Figure 3). Then, the zoospores were unable to move, seemed to vibrate and finally burst after 3-4 min.

Second, we performed an inoculation assay to confirm the ability of the OSE extract to damage the zoospores. For that, \( P. \ viticola \) spores that were treated for 5 min with different concentrations of the OSE extract were used to inoculate grapevine foliar discs. After 7 days, the downy mildew development was estimated by assessing the sporulation level. Regarding foliar discs inoculated by spores pre-treated with the OSE extract at 5 mg/L, no difference was noted compared to the control (non-treated spores) (Figure 2). For the foliar discs inoculated by spores that have been placed in contact with the OSE extract at 10 mg/L, a 24 % inhibition of \( P. \ viticola \) growth was observed and a total inhibition was noted for the 25 mg/L OSE extract condition.

2.2. Protection assays on grapevine foliar discs
The capacity of the OSE extract to reduce the development of \( P. \ viticola \) was evaluated in laboratory conditions using a foliar disc assay. For that, the OSE extract was sprayed at different concentrations (25 to 300 mg/L) on grapevine discs 24 h prior to their inoculation. Control (1 % ethanol) did not inhibit sporulation (Figure 4A). Regarding the OSE extract, for the lowest concentration (25 mg/L), the level of sporulation was similar to the control. From 50 to 300 mg/L, concentration-dependent inhibition of the sporulation was noted: at 50 mg/L, a 30 % sporulation reduction was achieved, at 200 mg/L, a strong inhibition (88 %) was obtained and, at 300 mg/L, the extract totally prevented the \( P. \ viticola \) development. The determined IC\(_{50}\) was equal to 70 mg/L.

| Compound            | LOD | LOQ  | \( R^2 \) | Content (mg/g OSE extract) | Content (%) |
|---------------------|-----|------|----------|---------------------------|-------------|
| \( E \)-piceid       | 0.6 | 1.8  | 1        | 2.38 ± 0.07               | 0.2         |
| \( E \)-piceatannol  | 2.6 | 7.9  | 0.997    | 11.07 ± 0.24              | 1.1         |
| \( E \)-resveratrol  | 1.4 | 4.2  | 0.999    | 95.41 ± 1.68              | 9.5         |
| Total monomers      |     |      |          | 108.86 ± 10.18            | 10.9        |
| ampelopsin A        | 0.6 | 1.7  | 1        | 23.08 ± 0.52              | 2.3         |
| pallidol            | 1.4 | 4.2  | 0.999    | 18.52 ± 0.24              | 1.9         |
| parthenocissin A    | 2.1 | 6.3  | 0.999    | 6.47 ± 0.29               | 0.6         |
| \( E \)-\( \varepsilon \)-viniferin | 0.8 | 2.6  | 1        | 94.02 ± 1.48              | 9.4         |
| \( E \)-\( \omega \)-viniferin | 2.7 | 8.0  | 0.997    | 12.75 ± 0.75              | 1.3         |
| Total dimers        |     |      |          | 154.83 ± 17.05            | 15.5        |
| \( E \)-miyabenol C | 2.9 | 8.7  | 0.999    | NQ (8.69 ± 1.21)          | NQ          |
| Total trimer        |     |      |          | NQ                        | NQ          |
| hopeaphenol         | 1.5 | 4.7  | 1        | 47.07 ± 0.32              | 4.7         |
| isohopeaphenol      | 1.8 | 5.5  | 1        | 63.67 ± 0.43              | 6.4         |
| \( r \)-viniferin    | 1.6 | 4.9  | 0.999    | 73.74 ± 1.51              | 7.4         |
| \( r^2 \)-viniferin  | 1.7 | 5.2  | 0.999    | 21.77 ± 0.40              | 2.2         |
| Total tetraters     |     |      |          | 206.26 ± 23.53            | 20.6        |
| Total               |     |      |          | 469.95 ± 46.83            | 47.0        |

TABLE 2. Stilbenes content in the OSE extract. Limits of Detection (LOD in μg/mL), Limits of Quantification (LOQ in μg/mL) and Calibration Curve Coefficients (\( R^2 \)) of standard stilbenes. Stilbene content is expressed in mg/g DW as the means ± S.D. (in italics) of three independent samples analysed in triplicate and the percentage of presence in the total extract. NQ means detected but not quantified because of low levels.
FIGURE 2. Effect of the OSE extract on Plasmopara viticola zoospores.

Control corresponds to 1 % ethanol condition. The percentage of inhibition of zoospores mobility (bars) and the percentage of inhibition of sporulation (curve) were indicated. The significant difference between each treatment was set at p < 0.05, letters represent significance (straight and italic letters apply to the mobility and sporulation assessment).

FIGURE 3. Microscopic observation of Plasmopara viticola zoospores in the presence of OSE extract.

The mobility of zoospores was followed during 3.5 min post-OSE extract treatment at 25 mg/L: 0–2 min (A), 3 min after treatment (B) and after 3.5 min (C). Degraded zoospores are indicated by dark arrows. Pictures are taken using a 400× optical microscope.

FIGURE 4. Protection level on grapevine treated by the OSE extract against Plasmopara viticola evaluated by foliar disc assay.

Sporulation inhibition was evaluated post-inoculation on foliar discs treated by 1 % ethanol (Control), OSE extract at different concentrations (A), and on foliar discs obtained from leaves of greenhouse plants pretreated by 1 % ethanol (Control), OSE extract at 300 mg/L (light grey) or Bordeaux Mixture (BM, black) at 1, 2, 3 and 6 dpt (days post-treatment) (B). Results are expressed as means ± SEM of a percentage of P. viticola sporulation inhibition compared to the control (100 % infected, 0 % inhibition). The significant difference between each condition was set at p < 0.05, the letters represent significance.
To estimate the duration of the protective effect of the OSE extract, experiments were performed on grapevine cuttings in a greenhouse. The OSE extract was sprayed at its IC$_{100}$ (300 mg/L) on the leaves and the Bordeaux mixture (BM at 4.2 g/L) was applied as a positive control.

At 1, 2, 3 and 6 dpt, leaves were collected, leaf disks generated and inoculated with $P$. viticola spores. Whatever the day of sampling, the OSE extract triggered a similar level of protection (Figure 4 B). The protection was relatively high, up to 72% at 3 dpt, and it tended to decrease over time (46% of protection at 6 dpt). Leaves treated by BM presented no disease development.

3. Protection assays in planta against downy mildews

3.1. On grapevine cuttings

We carried out assays in semi-controlled conditions to estimate the protective effect of the OSE extract in planta. Moreover, to be as close as possible to the final mode of application of the OSE extract in the field, we tested a formulated OSE extract. It consisted of the OSE extract with 9 g/L of a mixture of 80% Tween 20, 15% Tween 80 and 5% sophorolipids. Thus, four modalities were considered: non-formulated OSE extract (OSE) at 300 mg/L, formulated OSE (F-OSE) extract at 300 mg/L, copper sulphate (BM for “Bordeaux Mixture” at 4.2 g/L) and untreated condition (NT). Whatever the treatment, no phytotoxicity was observed for the duration of the experiment.

At the date of the first assessment ($A_1$, after the primary inoculation (PI)), the pest incidence was similar regardless of the treatment done with no reduction (Figure 5A). Concerning pest severity, the OSE extract cannot trigger protection against this artificial infection (Figure 5B).

However, the F-OSE extract displayed a high protection efficiency (70% pest severity reduction), higher than BM (51% reduction).

At $A_2$ (after the secondary inoculations (SI)), the protection level related to pest incidence for the OSE extract was 6% and was not significantly different from the control. F-OSE extract and BM inhibited pest incidence with a respective reduction of 23 and 63%. Based on the third assessment ($A_3$), we noticed that both F-OSE and OSE extracts could not reduce the pest incidence and only BM significantly moderated it (30% of reduction). Regarding pest severity at $A_2$ and $A_3$ (the two “natural” inoculations (SI)), the OSE extracts, formulated or not, significantly diminished it by approximately 35 to 50% (Figure 5B). BM, the fungicide reference, strongly limited the level of pest severity (83%).

3.2. On tomato, potato and melon

All these experiments were carried out in a greenhouse. To proceed as close to its final way of use as possible, only the F-OSE extract was considered. We determined its activity towards oomycetes that are responsible for late blight on popular fresh market vegetable crops by using the following pathosystems: $P$. infestans/potato, $P$. infestans/tomato and $P$. cubensis/melon. As grapevine leaves were not fully protected against $P$. viticola by F-OSE when used at 300 mg/L in greenhouse assays (Figure 4) and we lacked hindsight on the effectiveness of such extract against other oomycetes, a higher concentration of F-OSE extract of 1 g/L was used for potato, tomato and melon.

Regarding potato late blight, we observed a strong inhibition of both pest incidence (68%) and pest severity (approx. 90%) at $A_1$ and $A_2$ (Table 3). For tomato late blight, F-OSE did not significantly reduce pest incidence (approx. 15%);
however, it strongly limited pest severity (approx. 75 % inhibition). Similar results regarding pest severity were obtained for *P. cubensis* at A₁; nevertheless, the reduction was slight at A₂ (from 75 to 34 % at A₁ and A₂, respectively). The inhibition of pest incidence for melon late blight was 6 % at A₁ and null at A₂.

### 4. Grapevine defence responses and resistance induced against downy mildew by the OSE extract

To investigate the capacity of the OSE extract to modulate defence responses in grapevine leaves, we used the stilbenes extract at 300 mg/L, not formulated. F-OSE was not selected to avoid the potential co-formulants effect. The control consisted of 1 % ethanol. Treated leaves were harvested at 1, 2, 3 and 6 dpt.

#### 4.1. Defence gene expression

We studied the expression of six defence-related genes: two genes encoding pathogenesis-related (PR) proteins (a chitinase (*PR3*) and a *PR10* protein) and four genes encoding enzymes involved in polyphenol biosynthesis (phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*), stilbene synthase (*STS*) and resveratrol-O-methyl transferase (*ROMT*)).

One day after OSE application, five genes out of the six were slightly but significantly modulated: *PAL*, *ROMT*, *CHS* and *PR3* were up-regulated, whereas *STS* was inhibited (Figure 6). Two days post-treatment, only *PR3* was still significantly induced. Three days after treatment, no genes were modulated. At 6 dpt, the expression of *PR3* was highly induced, while strong repression of *STS* was observed. *PR10* expression was not modified throughout the time of the study.

#### 4.2. Phytoalexin production

To determine the effect of the OSE extract on stilbenes production, leaves were collected at 0, 1, 2, 3 and 6 dpt, and, after their harvest, they were washed thoroughly with water to remove sprayed solutions. Whatever the time-point, no stilbenes from the OSE extract can be detected in the leaves (data not shown). Only *trans*-piceid, the glycosylated form of resveratrol, was detected in a sufficient amount to be quantified (Figure 7). No differences in its content were observed after OSE extract treatment (approx. 1.5 µg/mg DW), except at 1 dpt with a significant accumulation of this stilbene (2.3 µg/mg DW) in the leaves, with a value 1.5 times higher than that of the control.

### Table 3. Pest incidence and pest severity inhibition of the formulated OSE extract (F-OSE) extract in different pathosystems.

The experiments were performed in a greenhouse. Two assessments (A₁ and A₂) were carried out. Results are expressed in percentage of inhibition as means ± SEM compared to the untreated control. The significant difference between each condition and untreated control was set at *p < 0.05.*

| Pathosystem          | Pest incidence inhibition (%) | Pest severity inhibition (%) |
|----------------------|-------------------------------|------------------------------|
|                      | A₁               | A₂               | A₁               | A₂               |
| *P. infestans*/potato| 68.02 ± 14.51* | 68.51 ± 7.52*   | 86.85 ± 5.62*   | 88.28 ± 6.85*   |
| *P. infestans*/tomato| 15.0 ± 8.40      | 13.35 ± 12.18   | 77.29 ± 4.17*   | 70.59 ± 14.27*  |
| *P. cubensis*/melon  | 6.25 ± 4.79       | 0                | 75.63 ± 11.66*  | 33.64 ± 9.24*   |

The experiments were performed in a greenhouse. Two assessments (A₁ and A₂) were carried out. Results are expressed in percentage of inhibition as means ± SEM compared to the untreated control. The significant difference between each condition and untreated control was set at *p < 0.05.*

**FIGURE 6.** The pattern of relative expression of defence genes in grapevine leaves treated by OSE extract (300 mg/L).

Columns represent the sampling date in days post-treatment (dpt) and line the genes. A three-coloured scale was used to show log-2 transformed fold induction of each gene. Upregulated genes appear in shades of red, with expression levels higher than 2 in bright red. Downregulated genes appear in shades of green, with intensity lower than −2 in bright green. Numbers in boxes represent the significant changes in gene expression (*p ≤ 0.05*) in treated leaves compared to untreated ones (control). Values are means of triplicate data of one representative experiment out of three.
4.3. Evaluation of the protection conferred by the OSE extract

To consider the conferred protection of OSE extract against *P. viticola* due to its defence stimulating activities and not to its antimicrobial capacity, leaves were treated with the OSE not formulated at 300 mg/L or ethanol 1 % (Control) and collected at 1, 2, 3 and 6 dpt (days post-treatment). After harvesting, leaves were washed and foliar discs were generated and inoculated.

We noted that the percentage of sporulation was reduced at 1 to 3 dpt in the OSE extract condition (Figure 8). At 1 dpt, the protection level conferred by OSE was 36 %. The highest inhibition was observed 2 dpt with values reaching 59 %. Then the protection decreased at 3 dpt (31 %) until it was no longer effective at 6 dpt.

**DISCUSSION**

In this study, we evaluated the protective effect of a grapevine extract obtained from a mix of grapevine trunk and roots which are vineyard co-products. This extract, named the OSE extract, was particularly enriched in stilbenes as it contains 470 mg/g DW of stilbenes. This value is in accordance and even higher than the ones of grapevine canes and wood and root extracts, which presented a stilbene content of 340, 351 and 224 mg/g, respectively (Gabaston *et al.*, 2017a). Furthermore, the OSE extract displayed a higher stilbene content in comparison to other extracts of plant species containing stilbenes as *Pinus pinaster* (knot) and *Picea abies* (bark) that presented 5 and 18.5 mg/g of stilbenes, respectively (Gabaston *et al.*, 2017b; Gabaston *et al.*, 2017c).
This discrepancy can be relative to the plant species, the plant part and/or the extraction methods. Concerning individual stilbenes, the OSE extract contained resveratrol, dimers and oligomerised stilbenes in higher quantity than other grapevine extracts (Gabaston et al., 2017a). For instance, it was richer in hopeaphenol and isohopeaphenol than a grapevine root extract and richer in hopeaphenol in comparison to a wood extract (Gabaston et al., 2017a). The quantity in total and individual stilbenes that we have extracted reflected their content in the plant organs. Indeed, it is in accordance with the mean values of stilbenes quantified in wood and roots of different grapevine cultivars that were presented in several studies and reviewed by Goufo and his colleagues (Goufo et al., 2020).

The data reported in the presented work showed that the OSE extract could impair P. viticola development thanks to different modes of action. This is in accordance with the publication of De Bona et al., who reported a dual mode of action for a grapevine cane extract (De Bona et al., 2019). Firstly, we showed that the OSE extract displayed a direct antimicrobial action. Indeed, it strongly impaired P. viticola zoospores: it reduced their mobility, a basic function to initiate infection (Spring et al., 2019) and impacted their viability as zoospores burst in its presence. Such OSE pre-treated zoospores, when inoculated on grapevine foliar discs, had probably a reduced ability in their infection process as the oomycete growth was diminished. We also noticed that the OSE extract, if sprayed on foliar discs prior to their inoculation, resulted in a protective effect against P. viticola with an IC$_{50}$ of 70 mg/L in accordance with the IC$_{50}$ previously reported for wood and roots extracts (60 and 120 mg/L, respectively) (Gabaston et al., 2017a). The IC$_{100}$ of the OSE extract was determined at 300 mg/L based on foliar disc assays. However, if sprayed at this concentration on whole leaves of plants placed in a greenhouse, the protection was incomplete (average of 64 %). This discrepancy can result from differences in the repartition of the OSE extract at the leaf surface and/or in the environmental conditions. The direct antimicrobial capacity of the OSE extract probably resulted from its high content in stilbenes representing 47 % of the extract and particularly in its richness in complex forms (Gabaston et al., 2017a; Schnee et al., 2013). Indeed, stilbenes, especially oligomerised forms (hopeaphenol, ε- and t-viniferins) and trans-resveratrol, have been shown to affect spore mobility and display toxicity on them (Gabaston et al., 2017a; De Bona et al., 2019). This lethal activity can result partly from the inhibition of cellular respiration and/or from the degradation of plasma membranes (Fröbel and Zyprian, 2019; Adrian and Jeandet, 2012; Koh et al., 2016). Considering the environmental conditions that could impact the OSE extract activity when applied in the greenhouse, it has been reported, for instance, that UV triggers isomerisation of stilbenes (Martinez et al., 1989), thus potentially modifying their oomycidal activity.

Secondly, in addition to its direct antimicrobial mode of action, the OSE extract can confer grapevine resistance toward P. viticola by activating plant defence mechanisms. Indeed, even after its removal from the leaves (by washing) prior to inoculation, the OSE extract treatment triggered sporulation inhibition up to 60 % at 2 dpt. This conferred resistance resulted in its ability to modulate the expression of some defence-related genes and the production of polyphenols. For instance, PR3 was induced at 6 dpt. PR3 could be involved in oomycete resistance as several oomycetes possess chitin-synthesising enzymes and their timing of expression suggests their role in the prevention of host colonisation (Hinkel and Ospina-Giraldo, 2017; Yan et al., 2017). To our knowledge, the expression of this gene was never assessed after treatment with an extract enriched in stilbenes. Nevertheless, a maple leaf extract containing a high content of polyphenols was shown to trigger the overexpression of PR3 in tobacco and a grape marc extract enhanced chitinase activity in grapevine (Peghaire et al., 2020; Filippi et al., 2019). PR10 was not affected by the OSE extract treatment. De Bona and its colleagues mentioned that the expression of this gene was unaffected after a grapevine cane extract treatment, whereas they reported a strong induction of PR5 and PR6 and repression of PR1 and PR14 (De Bona et al., 2019). The OSE extract also modulated, even very slightly, at 1 dpt, four genes involved in polyphenol synthesis: PAL, ROMT, CHS and STS. The study done by De Bona indicated that the application of a cane stilbene extract inhibits the expression of STS (De Bona et al., 2019). Our data were quite consistent with this result as STS was repressed after the OSE application at 6 dpt. This effect could be attributed to the presence of resveratrol in these extracts as this latter was reported to negatively regulate VmMYB14, a transcription factor of STS (Jeandet et al., 2019; Gindro et al., 2017). Moreover, ROMT, a gene encoding a protein involved in the biosynthesis of pterostilbene, a highly active compound against P. viticola, tended to be repressed at 6 dpt. Regarding PAL, the key enzyme in the phenol synthesis pathway, its gene was slightly up-regulated at 1 dpt. Nevertheless, none of the analysed antimicrobial stilbenes, like resveratrol and pterostilbene, seemed to be newly synthesised. However, piceid accumulated, which is the glycosylated form of resveratrol that does not exhibit any antimicrobial capacity. The production of this compound may indicate the occurrence of a general plant defence response (Figura et al., 2012; Lambert et al., 2012; Jeandet et al., 2002). In addition to PAL induction at 1 dpt, CHS was up-regulated. Thus, the assumption of an up-regulation of the flavonoid pathway can be proposed. Flavonoids have been previously shown to play a part in resistance to P. viticola (Agati et al., 2008) and it will be relevant to perform further experiments to unveil the role of these compounds in the tolerance conferred by the OSE extract treatment.

The protective effect of the OSE extract treatment resulting from its different modes of action was noted on grapevine cuttings in semi-controlled conditions. Indeed, treatment with the OSE extract at 300 mg/L reduced the pest severity by up to 40 to 50 % due to P. viticola in the context of assessments done after secondary infections (the ones mimicking a “natural infection”).
These results were in accordance with the reduction of attack frequency from 59 to 69 % and disease reduction from 83 to 88 % obtained after a cane extract treatment (Richard et al., 2016). However, this latter cane extract was applied at 5 g/L, a relatively high concentration, and no protective effect was obtained if used at 1 g/L. We remarked that the OSE extract treatment, and also BM, cannot inhibit neither the pest incidence nor the pest severity after the first inoculation (the “artificial” one). This probably resulted in the too high level of disease pressure of this type of inoculation.

In this study, we have also performed the treatment with a formulated OSE extract (F-OSE) to evaluate its protective effect under end-use conditions. Eco-friendly co-formulants (Tween® 20, Tween® 80 and sophorolipids) were used to optimise the long-term solubility of the OSE extract compounds, particularly the one of stilbenes, and to obtain a good wettability of the target surface and a resistance to leaching to develop its use in the vineyard. All the assays were performed in semi-controlled conditions and with various crops (grapevine, melon, tomato and potato).

Considering grapevine, the F-OSE treatment at 300 mg/L resulted in a protection efficiency of 40 to 50 % against P. viticola regarding pest severity, a level of protection similar to the one obtained for the OSE extract after secondary infections (“natural infection”). In addition, F-OSE inhibited pest severity after a primary inoculation and in a strong way (approx. 70 %). The presence of co-formulants may result in this enhanced protection, compared to OSE alone, thanks to better solubilisation of the OSE extract active compounds and/or their effective spread at the leaf surface. The co-formulants may have also formed a protective layer at the leaf surface, thus avoiding penetration of P. viticola zoospores. In addition, they can directly inhibit the pathogen as they possess antimicrobial activities (Figura et al., 2012; Haque et al., 2019; Sen et al., 2017). Regarding P. infestans on potato and tomato and P. cubensis on melon, the F-OSE extract (at 1 g/L) effectively reduced pest incidence (6 to 68 %) and pest severity (33 to 88 %) of these other oomycetes. The level of reduction mainly differed according to the pathosystem considered, with the highest protection obtained for potato and the lowest for melon. These discrepancies might result from the ability of each downy mildew species to bypass the toxicity of stilbenes and/or other compounds present in the F-OSE extract. Other plant extracts have been reported to reduce the development of mildew species and late blight (Goufo et al., 2010; Forrer et al., 2017). For instance, an extract of rhubarb, a stilbene-producing plant, was effective against the potato late blight (Jeandet et al., 2019). The oomycidal properties of pure stilbene were also reported; as for pterostilbene, the methylated form of resveratrol, which inhibited P. infestans (Ward et al., 1975). To our knowledge, this is the first time that a stilbene extract obtained from grapevine by-products was reported for its action against different mildews, especially in greenhouse conditions.

CONCLUSION

Taken together, the present findings showed that a grapevine extract obtained from trunks and roots and enriched in oligomerised stilbenes displayed a protective effect on various mildews affecting different crops (grapevine, melon, tomato and potato). This ability to protect plants against oomycetes was conferred by direct inhibition properties and by the stimulation of some plant defences (stilbenes and pathogenesis-related proteins expression). These results can help the future development of active extracts based on stilbenoids for crop protection and, more particularly, for vineyard disease control.

ACKNOWLEDGEMENTS

The authors gratefully thank StILNov Laboratory (ANR LabCom Project ANR-14-LAB5-0005-01) and Château Rochemorin (Martillac, France) for providing grapevine material and for the purification of stilbene standards. We are also grateful to M-F. Corio-Costet and S. Gambier for providing the plants and the grapevine downy mildew strain (INRAE, UMR SAVE).

Notes

The authors declare no competing financial interest.

Funding

This work was supported by the Comité Interprofessionnel des Vins de Bordeaux (CIVB). We are thankful to the Bordeaux Metabolome Facility and MetaboHUB (ANR-11-INBS-0010 project) for their financial support.

REFERENCES

Ademe (2009). Biomasse forestière, populicole et bocagère disponible pour l’énergie à l’horizon 2020.

Adrian, M., & Jeandet, P. (2012). Effects of resveratrol on the ultrastructure of Botrytis cinerea conidia and biological significance in plant/pathogen interactions. Fitoterapia, 83, 1345–1350. https://doi.org/10.1016/j.fitote.2012.04.004

Agati, G., Ceronie, Z. G., Marta, A. D., Di Stefano, V., Pinelli, P., Traversi, M. L., & Orlandini, S. (2008). Optically-assessed preformed flavonoids and susceptibility of grapevine to Plasmopara viticola under different light regimes. Functional Plant Biology, 35, 77–84. https://doi.org/10.1071/FP07178

Alonso-Villaverde, V., Voinesco, F., Viret, O., Spring, J. L., & Gindro, K. (2011). The effectiveness of stilbenes in resistant Vitaceae: Ultrastructural and biochemical events during Plasmopara viticola infection process. Plant Physiology and Biochemistry., 49, 265–274. https://doi.org/10.1016/j.plaphy.2010.12.010

Aveline, N., Bidaut, F., & Daire, X. (2019). Les stimulateurs de défense en viticulture. Phytoma la Défense des Végétaux, 723, 16-18.

Banauskaite, J., Kopustinskiene, D. M., & Bernatoniene, J. (2019). Impact of gelatin supplemented with gum arabic, tween 20, and β-cyclodextrin on the microencapsulation of Turkish oregano extract. Molecules, 24, 1–16. https://doi.org/10.3390/molecules24010176

Baranauskaitė, J., Kopustinskiene, D. M., & Bernatoniene, J. (2019). Impact of gelatin supplemented with gum arabic, tween 20, and β-cyclodextrin on the microencapsulation of Turkish oregano extract. Molecules, 24, 1–16. https://doi.org/10.3390/molecules24010176

David Taillis et al.
Chong, J., Poutaraud, A., & Hugueney, P. (2009). Metabolism and roles of stilbenes in plants. *Plant Science*, 177, 143–155. https://doi.org/10.1016/j.plantsci.2009.05.012

Corio-Costet, M. F. (2012). Fungicide resistance in *Plasmopara viticola* in France and anti-resistance measures. In *Fungicide Resistance in Crop Protection: Risk and Management*, Third, T. S., Ed., 157–171. https://doi.org/10.1007/978-1845939052-0157

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

De Bona, G. S., Adrian, M., Negrel, J., Chiltz, A., Héloïc, M.-C., Poinssot, B., Angelini, E., Vincenzi, S., & Bertazonn, N. (2019). Dual mode of action of grape cane extracts against *Botrytis cinerea*. *Journal of Agricultural and Food Chemistry*, 67, 5512–5520. https://doi.org/10.1021/acs.jafc.8b00708

Demaneché, S., Mirabel, L., Abbe, O., Eberst, J. B., & Souche, J. L. (2020). A new active substance derived from lyzed *Willaria muore* c2c maky cells to fight grapevine downy mildew. *Plants*, 9, 1–19. https://doi.org/10.3390/plants901013

Dufour, M. C., Lambert, C., Bouscaut, J., Merillon, J. M., & Corio-Costet, M. F. (2013). Benzothiadiazole-primed defence responses and enhanced differential expression of defence genes in *Vitis vinifera* infected with biotrophic pathogens *Erysiphe necator* and *Plasmopara viticola*. *Plant Pathology*, 62, 370–382. https://doi.org/10.1111/j.1365-3059.2012.02628.x

Dufour, M. C., Magnin, N., Dumas, B., Vergnes, S., & Corio-Costet, M. F. (2016) High-throughput gene-expression quantification of grapevine defense responses in the field using microfluidic dynamic arrays. *BMC Genomics*, 17, 957. https://doi.org/10.1186/s12864-016-3304-z

Erdogan, P., Esin Kilinc, G., Aksu, P., Kahyaoglu, M., & Babaroglu, N. E. (2019) Investigation of formulation preparation of two plant extracts and determination of the effectiveness on *Tetranychus urticae* koch. *Arachnida tetranychidae*. *Horticulture International Journal*, 3, 160–164. https://doi.org/10.15406/hij.2019.03.00124

Figura, N., Marcolongo, R., Cavallo, G., Santucci, A., Collodel, G., Sprefaci, A., & Moretti, E. (2012). Polysorbate 80 and *Helicobacter pylori*: A microbiological and ultrastructural study. *BMC Microbiology*, 12, 1-10. https://doi.org/10.1186/1471-2180-12-217

Filippi, A., Petruzza, E., Boscuiti, F., Vuerich, M., Vrhovsek, U., Rabiei, Z., & Braidot, E. (2019). Bioactive polyphenols modulate enzymes involved in grapevine pathogenesis and chitinase activity at increasing complexity levels. *International Journal of Molecular Sciences*, 20. https://doi.org/10.3390/jms20246357

Forrer, H. R., Vogelsgang, S., & Musa, T. (2017). Botanicals and phosphate show potential to replace copper for control of potato late blight. *Journal of Fungi*, 3. https://doi.org/10.3390/jof3040065

France Agrimer (2015). *L’observatoire national des ressources en biomasse*. Évaluation des ressources. 181.

Fröbel, S., & Zyprian, E. (2019). Colonization of different grapevine tissues by *Plasmopara viticola* - A histological study. *Frontiers in Plant Science*, 10, 1–13. https://doi.org/10.3389/fpls.2019.00951

Gabaston, J., Richard, T., Cluzet, S., Palos Pinto, A., Dufour, M. C., Corio-Costet, M. F., & Merillon, J. M. (2017b). *Pinus pinaster* knot: A source of polyphenols against *Plasmopara viticola*. *Journal of Agricultural and Food Chemistry*, 65, 8884–8891. https://doi.org/10.1021/acs.jafc.7b04129

Gabaston, J., Richard, T., Biais, B., Waffo-Teguo, P., Pedrot, E., Jourdes, M., Corio-Costet, M. F., & Merillon, J. M. (2017c). Stilbenes from common spruce *Picea abies* bark as natural antifungal agent against downy mildew *Plasmopara viticola*. *Industrial Crops and Products*, 103, 267–273. https://doi.org/10.1016/j.indcrop.2017.04.009

Giessler-Blank, S., Schilling, M., Thum, O., & Sieverding, E. (2011). Use of sophorolipids and derivatives thereof in combination with pesticides as adjuvant/additive for plant protection and the industrial non-crop field, US Patent.

Gindro, K., Schnee, S., Righi, D., Marcourt, L., Nejad Ebrahimi, S., Codina, J. M., Voinesco, F., Michellod, E., Wolfender, J. L., & Queiroz, E. F. (2017). Generation of antifungal stilbenes using the enzymatic secretome of *Botrytis cinerea*. *Journal of Natural Products*, 80, 887–898. https://doi.org/10.1021/acs.jnatprod.6b00760

Goufo, P., Fontem, D. A., & Ngnokam, D. (2010). Evaluation of plant extracts for tomato late blight control in Cameroon. *New Zealand Journal of Crop and Horticultural Science*, 38, 171–176. https://doi.org/10.1080/01140671.2010.495374

Goufo, P., Singh, R. K., Cortez, I. (2020). A reference list of phenolic compounds (including stilbenes) in grapevine (*Vitis vinifera* L.) roots, woods, canes, stems, and leaves. *Antioxidants*, 9, 398. https://doi.org/10.3390/antiox9050398

Haque, F., Verma, N.K., Alfatah, M., Bibiani, S., & Bhattacharyya, M. S. (2019). Sophorolipid exhibits antifungal activity against ROS mediated endoplasmic reticulum stress and mitochondrial dysfunction pathways in: *Candida albicans*. *RSC Advances*, 9, 41639–41648. https://doi.org/10.1039/C9RA07599B

Hazra, D.K., & Purkait, A. (2019). Role of pesticide formulations for sustainable crop protection and environment management: A review. *Journal of Pharmacognosy and Phytochemistry*, 8, 686–693.

Hinkel, L., & Ospina-Giraldo, M.D. (2017). Structural characterization of a putative chitin synthase gene in *Phytophthora spp.* and analysis of its transcriptional activity during pathogenesis on potato and soybean plants. *Current Genetics*, 63, 909–921. https://doi.org/10.1007/s00294-017-0687-6

Jeandet, P., Douillet-Breuil, A. C., Bessis, R., Debord, S., Shaghi, M., & Adrian, M. (2002). Phytalexins from the vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *Journal of Agricultural and Food Chemistry*, 50, 2731–2741. https://doi.org/10.1021/jf011429s

Jeandet, P., Clément, C., & Cordelier, S. (2019). Regulation of resveratrol biosynthesis in grapevine: new approaches for disease resistance? *Journal of Experimental Botany*, 70, 375–378. https://doi.org/10.1093/jxb/ery446

Koh, J. C. O., Barbulescu, D. M., Salisbury, P. A., & Slater, A. T. (2016). Pertosilibene is a potential candidate for control of blackleg in canola. *PLoS One*, 11, 1–17. https://doi.org/10.1371/journal.pone.0156186

Krzyzanika, Y., Trouvelot, S., Negrel, J., Cluzet, S., Valls, J., Richard, T., Bouguad, A., Jacquens, L., Klinguer, A., Chiltz, A.M., & Héloïr, M.-C. (2018). A plant extract acts both as a resistance inducer and an oomycide against grapevine downy mildew. *Frontiers in Plant Science*, 9, 1–14. https://doi.org/10.3389/fpls.2018.01085
Lambert, C., Bisson, J., Waffo-Teguo, P., Papastamoulis, Y., Richard, T., Corio-Costet, M. F., Mérillon, J. M., & Cluzet, S. (2012). Phenolics and their antifungal role in grapevine wood decay: Focus on the Botryosphaeriaceae family. *Journal of Agricultural and Food Chemistry*, 60, 11859–11868. https://doi.org/10.1021/jf303290g

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J. N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. *Agronomy for Sustainable Development*, 38, 1–18. https://doi.org/10.1007/s13593-018-0503-9

Martinez, R., Sastre, R., & Catalina, F. (1989). Photosensitized *trans-cis* isomerization of stilbene in a pure polystyrene matrix. *Journal of Photochemistry and Photobiology A: Chemistry*, 46, 113–119. https://doi.org/10.1016/1010-6030(89)87037-6

Mulolland, D. A., Thuerig, B., Langat, M. K., Tam, I., Nawrot, D. A., James, E. E., Quyyum, M., Shen, D., Ennis, K., Jones, A., et al. (2017). Efficacy of extracts from eight economically important forestry species against grapevine downy mildew *Plasmopara viticola* and identification of active constituents. *Crop Protection*, 102, 104–109. https://doi.org/10.1016/j.cropro.2017.08.018

Pawlus, A. D., Waffo-Teguo, P., Shaver, J., & 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, 57–111. https://doi.org/10.20870/oeno-one.2012.46.2.1512

Peghaire, E., Hamdache, S., Galien, A., Sleiman, M., ter Halle, A., El Alaoui, H., Kocer, A., Richard, C., & Goupil, P. (2020). Inducing plant defense reactions in Tobacco plants with phenolic-rich extracts from red maple leaves: A characterization of main active ingredients. *Forests*, 11, 1–14. https://doi.org/10.3390/f11060705

Perazzolli, M., Roatti, B., Bozza, E., & Pertot, I. (2011). *Trichoderma harzianum* T39 induces resistance against downy mildew by priming for defense without costs for grapevine. *Biological Control*, 58, 74–82. https://doi.org/10.1016/j.biocontrol.2011.04.006

Pezet, R., Gindro, K., Viret, O., & Richter, H. (2004). Effects of resveratrol, viniferins and pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. *Vitis*, 43, 145–148.

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. (2004). Determination of most stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper. *Biotechnology Letters*, 26, 509–515. https://doi.org/10.1023/B:BILE.0000019559.84305.47

Richard, T., Abdelli-Belhad, A., Vitrac, X., Waffo-Teguo, P., & Mérillon, J. M. (2016). *Vitis vinifera* canes, a source of stilbenoids against downy mildew. *Oeno One*, 50, 137–143. https://doi.org/10.20870/oeno-one.2016.50.3.1178

Schnee, S., Queiroz, E. F., Voinescu, F., Marcourt, L., Dubuis, P. H., Wolfender, J. L., & 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, 5459–5467. https://doi.org/10.1021/jf4010252

Sen, S., Borah, S. N., Bora, A., & Deka, S. (2017). Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. *Microbial Cell Factories*, 16, 1–14. https://doi.org/10.1186/s12934-017-0711-z

Sharma, P., Saika, M. K., & Nath, B. C. (2015). A review on management of late blight of potato through host resistance: a review on management of late blight of potato through host resistance and chemical fungicides. *Trends in Biosciences*, 8, 4459–4466.

Spring, O., Gomez-Zeledon, J., Hadziabdic, D., Trigiano, R. N., Thines, M., & Lebeda, A. (2019). Biological characteristics and assessment of virulence diversity in pathosystems of economically important biotrophic oomycetes. *Critical Reviews in Plant Sciences*, 37, 439–495. https://doi.org/10.1080/07352689.2018.1530848

Thuerig, B., Ramseyer, J., Hamburger, M., Oberhänsli, T., Potterat, O., Schärer, H. J., & Tam, L. (2016). Efficacy of a *Juncus effusus* extract on grapevine and apple plants against *Plasmopara viticola* and *Venturia inaequalis*, and identification of the major active constituent. *Pest Management Science*. https://doi.org/10.1002/ps.4199

Vaughn, S. F., Behle, R. W., Skory, C. D., Kurtzman, C. P., & Price, N. P. J. (2014). Utilization of sophorolipids as biosurfactants for postemergence herbicides. *Crop Protection*, 59, 29–34. https://doi.org/10.1016/j.cropro.2014.01.014

Ward, E. W. B., Unwin, C. H., & Stoesel, A. (1975). Postinfectional inhibitors from plants. XV. Antifungal activity of the phytoalexin orchinol and related phenanthrenes and stilbenes. *Canadian Journal of Botany*, 964-971. https://doi.org/10.1139/b75-115

Yan, H., Zhong, Y., Jiang, B., Zhou, B., Wu, B., & Zhong, G. (2017). Guanggan (*Citrus reticulata*) shows strong resistance to *Phytophthora nicotianae*. *Sciencia Horticulturae*, 225, 141-149. https://doi.org/10.1016/j.scienta.2017.06.068