Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Rutin-stevioside and related conjugates for potential control of grapevine trunk diseases

Laura BUZÓN-DURÁN[1], Natalia LANGA-LOMBA[2,3], Vicente GONZÁLEZ-GARCÍA[3,*], José CASANOVA-GASCÓN[2], Eva SÁNCHEZ-HERNÁNDEZ[1], Jesús MARTÍN-GIL[1], Pablo MARTÍN-RAMOS[2]

[1] *Department of Agricultural and Forestry Engineering, ETSIIAA, Universidad de Valladolid, Avda. Madrid 44, 34004 Palencia, Spain*
[2] *Instituto Universitario de Investigación en Ciencias Ambientales de Aragón (IUCA), EPS, Universidad de Zaragoza, Carretera de Cuarte s/n, 22071 Huesca, Spain*
[3] *Plant Protection Unit, Agrifood Research and Technology Centre of Aragón, Instituto Agroalimentario de Aragón—IA2 (CITA-Universidad de Zaragoza), Avda. Montañana 930, 50059 Zaragoza, Spain*
* Corresponding author. E-mail: vgonzalezg@aragon.es

Summary. Flavonoids and phenolic acids play roles in grapevine defence against pathogens causing grapevine trunk diseases (GTDs). Rutin is a major flavonoid in vegetative organs of the grapevines, and this compound, unlike other flavonoids, is non-toxic and non-oxidizable. Rutin was assayed *in vitro* and *in vivo* against two *Botryosphaeriaceae* taxa. The limited bioavailability of this compound was circumvented by conjugation with stevioside, a glycoside obtained from *Stevia rebaudiana*. Clear synergistic effects were observed for the stevioside-rutin adduct, resulting in EC_{50} and EC_{90} values of 306.0 and 714.9 μg·mL^{-1} against *Neofusicoccum parvum* and 241.6 and 457.8 μg·mL^{-1} against *Dothiorella viticola*. In greenhouse experiments, moderate inhibition of *N. parvum* growth and complete inhibition of *D. viticola* were observed. These inhibitory effects were greater than those of ferulic acid, which has been considered the most effective phenolic acid against GTDs. Conjugation with stevioside provided solubility enhancement of rutin, paving the way to the design of glycopesticides based on rutin-rich plant extracts as promising antifungals against GTDs.

Keywords. Antifungal, Botryosphaeria dieback, candyleaf, GTDs, rutoside.

Citation: L. Buzón-Durán, N. Langa-Lomba, V. González-García, J. Casanova-Gascón, E. Sánchez-Hernández, J. Martín-Gil, P. Martín-Ramos (2022) Rutin-stevioside and related conjugates for potential control of grapevine trunk diseases. *Phytopathologia Mediterranea* 61(1): 65-77. doi: 10.36253/phyto-13108

Accepted: November 9, 2021

Published: March 25, 2022

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) cultivars can be severely affected by many pathogens, including fungi, bacteria, oomycetes, and viruses. Grapevine trunk diseases (GTDs) have long been responsible for significant economic losses, with some of these diseases being well-known for more than 100 years. The interest of the viticulture sector in this group of diseases has increased in the last three decades, due to, firstly, increased mortality of young nursery-produced grapevine plants, especially 1–3 years after planting in the field, and secondly, the progressive suspension of the use of chemical

fungicides, which has resulted in progressive increases in the incidence and losses due to these diseases.

Current agricultural policies are provoking interest in the development of alternative, naturally-derived antifungal products for the sustainable management of grapevine diseases. However, there are other factors directly or indirectly involved in the expansion of GTDs, in young and mature vineyards. Some factors are related to changes in cultural practices, such as reduced protection of pruning wounds or reductions in sanitary control measures in certified propagation material (Graniti *et al.*, 2000). It is also commonly accepted that a correlation exists between the increase in the incidence of decay of young grapevine plants and increased demand for new plantings or replacements in the different world production areas. This has led to the advocation of a system where infected propagation material from nurseries is considered the primary source of inoculum causing young vine decline. Numerous studies (see Surico (2001) and Fourie and Halleen (2004)) have correlated the presence of particular fungi causing propagation material decay in nurseries with the dead of grapevine plants in the very first years after vineyard planting.

Natural compounds that have been tested against the three main GTDs (Botryosphaeria dieback, Esca complex, and Eutypa dieback) include chitosan, garlic extract, tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) oil, green coffee extract, lemon peel extract, honey, propolis, seaweed extract, and saponins (Mondello *et al.*, 2018). Nonetheless, few studies have focused on the specific bioactive phytochemicals associated with these natural extracts and compounds, which could provide increased efficacy for products with variable phytochemical composition, resulting from genetic variability, and/or environmental variability influenced by weather or soil fertility.

Phytoalexins, which in grapevines are phenolic compounds including tannins, phenolic acids, flavonoids, and stilbenes, are involved in grapevine defence, increasing host resistance to pathogens (Del Río *et al.*, 2004). The progression of fungal pathogens along grapevine wood is inhibited by polyphenol-rich reaction zones (Fontaine *et al.*, 2016). The possible roles of phenolics in defence against GTD casual agents were studied by Lambert *et al.* (2012a), who analysed the *in vitro* effects of 24 grapevine compounds (eight phenolic acids, three flavan-3-ols, two flavonols, and 11 stilbenoids) on six *Botryosphaeriaceae* taxa. They showed that these pathogens were differentially susceptible to phenolics, and concluded that ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid) was one of the most active compounds, causing an inhibition comparable to that from the stilbenoids ε-viniferin, vitisin A and B, or *trans*-pterostilbene. However, Lambert *et al.* (2012a) detected no inhibitory activity on any wood disease fungi for flavonols (kaempferol and quercitin) and flavan-3-ols [(+)-catechin, (–)-epicatechin, and epicatechin-3-*O*-gallate]. In some cases, these compounds enhanced the growth of some of the assayed fungi. In contrast, other authors have reported that catechin inhibited fungi involved in Petri disease, and other GTDs (caused by *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams, *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai, *Eutypa lata* (Pers.) Tul & C. Tul, and *Stereum hirsutum* (Willd.) Pers.) (Del Río *et al.*, 2004). Furthermore, the antifungal efficacy of flavonoids is well-recognised (Jin, 2019; Al Aboody and Mickymaray, 2020). To gain further insight into these conflicting results, the present study compared the effectiveness of the flavonoid-3-*O*-glycoside rutin (also known as rutoside, phytomelin, or quercetin 3-*O*-rutinoside) with that of ferulic acid. Rutin is one of the most abundant polyphenols (excluding stilbenes) in the vegetative organs of grapevine plants, found at mean concentrations of 257 $mg \cdot kg^{-1}$ (Goufo *et al.*, 2020), and has significant antimicrobial activity (Ganeshpurkar and Saluja, 2017). The use of rutin is advantageous compared with other flavonoids as it is non-toxic and non-oxidizable (Sharma *et al.*, 2013).

Low water-solubility of phenolic acids, e.g., ferulic acid (Shakeel *et al.*, 2017) and flavonoids (Chebil *et al.*, 2007), can limit their bioavailability and applicability (Hussain *et al.*, 2017). This may be circumvented through the formation of inclusion compounds or conjugate complexes with terpene glycosides (Nguyen *et al.*, 2017). Stevioside (the major constituent of *Stevia rebaudiana* (Bertoni) Bertoni extract) may be a suitable option to form conjugate complexes, resulting in enhanced antifungal activity. Clear synergistic effects have been reported from the conjugation of phenolic acids with stevioside against *Fusarium culmorum* (Wm.G.Sm.) Sacc. (Buzón-Durán *et al.*, 2020) and *Phytophthora cinnamomi* Rands (Matei *et al.*, 2018b).

The goal of the present study was to assess the *in vitro* and *in vivo* antifungal activities of rutin against the two most important *Botryosphaeriaceae* taxa that cause GTDs, especially of young grapevines. Rutin was assessed alone and in a conjugate complex with stevioside, and was compared with ferulic acid as a reference substance. This information should be useful for selecting promising plant sources of natural antifungal products for use in organic or integrative viticulture.

MATERIALS AND METHODS

Reagents

Rutin hydrate (CAS 207671-50-9, ≥94%), ferulic acid (CAS 537-98-4, European Pharmacopoeia reference standard), sodium alginate (CAS 9005-38-3), calcium carbonate (CAS 471-34-1, ≥99.0%), and methanol (CAS 67-56-1, UHPLC, suitable for MS) were supplied by Sigma-Aldrich/Merck KGaA (Darmstadt, Germany). Stevioside (CAS 57817-89-7, 99%) was purchased from Wako Chemicals GmbH (Neuss, Germany). Potato dextrose agar (PDA) was supplied by Becton, Dickinson & Company (Franklin Lakes, NJ, USA).

Fungal isolates

Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (code ITACYL_F111; isolate Y-091-03-01c; isolated from 'Verdejo' cultivar grapevines in a nursery in Navarra, Spain, in 2006) and *Dothiorella viticola* A.J.L. Phillips & J. Luque (code ITACYL_F118; isolate Y-103-08-01; isolated from grapevines in Extremadura, Spain, in 2004) were supplied as lyophilized vial cultures (later reconstituted and refreshed as PDA subcultures) by the Agricultural Technological Institute of Castilla and Leon (ITACYL, Valladolid, Spain) (Martin and Cobos, 2007).

Preparation of bioactive formulations

Treatments based on pure stevioside, rutin, or ferulic acid were prepared by dissolving the respective compounds in Milli-Q water (stevioside) or methanol (for rutin and ferulic acid), without further purification.

Ultrasonication-assisted aqueous biphasic system separation was used to prepare the stevioside-polyphenol conjugate complexes in a 1:1 molar ratio. 50 mL of an aqueous solution of stevioside (126 mg, MW = 804.87 g·mol^{-1}, 0.156 mM) were mixed with a 50 mL methanol solution of either ferulic acid (95.2 mg, MW = 610.517 g·mol^{-1}, 0.156 mM) or rutin (75.3 mg, MW = 482.44 g·mol^{-1}, 0.156 mM). The solutions were sonicated with a probe-type UIP1000hdT ultrasonicator (Hielscher, Teltow, Germany; 1000 W, 20 kHz) for 15 min, keeping the temperature below 60°C.

For *in vivo* experiments, the conjugate complexes were dispersed in a calcium alginate matrix, in the form of hydrogel beads. The beads were prepared as follows: each bioactive product was added to a 3% sodium alginate solution in a 2:8 ratio (20 mL bioactive product:80 mL sodium alginate), and this solution was then dispensed drop by drop onto a 3% calcium carbonate solution to spherify (polymerize) the solution. Beads of ⌀ = 0.4–0.6 cm containing the different treatments were obtained.

In vitro *tests of mycelium growth inhibition*

The biological activity of the different treatments was determined using the agar dilution method, incorporating aliquots of stock solutions into PDA medium to provide final concentrations of 62.5, 93.75, 125, 187.5, 250, 375, 500, 750, 1000, and 1500 μg·mL^{-1}. Mycelium plugs (⌀ = 5 mm) of each pathogen from the edges of 7-day-old cultures were transferred to plates filled with amended media (three plates per treatment and concentration). Plates containing only PDA without amendment were used as experimental controls. The experiment was carried out twice.

Radial mycelium growth was determined by calculating the average of two perpendicular colony diameters for each replicate. Mycelium growth inhibition, after 7 days of incubation at 25°C in the dark for each treatment and concentration, was calculated according to the formula: $((d_c\text{-}d_t)/d_c) \times 100$, where d_c is the average fungal colony diameter in the experimental control and d_t is the average colony diameter treated with composite.

Fitting the radial growth inhibition values (%) with a DoseResp function, using an orthogonal distance regression (ODR) algorithm, allowed expression of the results as 50% (EC_{50}) and 90% (EC_{90}) effective concentrations.

For treatment interactions, synergy factors (SF) were estimated using Wadley's method (Levy *et al.*, 1986). This method assumes that one component of a mixture can substitute at a constant proportion for the other component. The expected effectiveness of the mixture is then directly predictable from the effectiveness of the constituents if the relative proportions are known (as in this case). The synergy factor (SF) was estimated as:

$$SF = \frac{ED(exp)}{ED(obs)} = \frac{\left(\frac{a+b}{\frac{a}{ED_A}+\frac{b}{ED_B}}\right)}{ED(obs)}$$

where a and b are the proportions of the products A and B in the mixture and $a + b = 1$, ED_A and ED_B are their equally effective doses, *ED(exp)* is the expected equally effective dose and *ED(obs)* is the equally effective dose observed in the experiment.

If SF = 1, the hypothesis of similar joint action (i.e., additivity) can be accepted; if SF > 1, there is synergistic action; and if SF < 1, there is antagonistic action between the two fungicide products.

Greenhouse bioassays on grafted plants

The protective capabilities of the most promising formulations and dosages, as indicated from the *in vitro* mycelium growth inhibition experiments, were further assayed in grafted plants against the two selected *Botryosphaeriaceae*, using the method described by Buzón-Durán *et al.* (2021). Briefly, 68 plants were used, half of which were 2-year-old 'Tempranillo' vines (CL. 32 clone) grafted on 775P rootstock, and the rest were 1-year-old 'Garnacha' vines (VCR3 clone) grafted on 110R rootstock. The two cultivars were tested to assess potential differences in their sensitivity to the pathogens under study. The cultivar choice was guided by the relevance of 'Tempranillo' and 'Garnacha' in the protected designations of origin in Aragón, Spain.

Plants were grown on 3.5 L capacity plastic pots containing a mixed substrate of moss peat and sterilized natural soil (75:25), to which a slow-release fertilizer was incorporated when needed. The plants were kept in a greenhouse with drip irrigation and anti-weed ground cover for 6 months (from June to December 2020).

One week after placing in the greenhouse, the grapevine plants were artificially inoculated with the two pathogens (*N. parvum* and *D. viticola*) and simultaneously treated with either the stevioside–rutin or the stevioside–ferulic acid treatment. Inoculations of both pathogens and bioactive products were carried out directly on the trunks of the living plants at two sites on each plant, at least 5 cm apart from each other, and below the grafting point (and not reaching the root crown). For the pathogens, agar plugs from 5-day-old fresh PDA cultures of each species were used as the fungal inoculum. At the two inoculation points, slits (⌀ ≈ 15 mm, 5 mm deep) were made with a scalpel. Agar plugs (⌀ = 5 mm) were then placed so that the mycelium was in contact with the stem vascular tissue. The beads containing the bioactive product were then placed at both sides of the agar plug, and the agar plug and beads were covered with cotton soaked in sterile double distilled water and sealed with Parafilm™ tape.

Five repetitions were arranged for each pathogen/bioactive product and plant (cultivar/rootstock) combination. Four positive controls/(pathogen*cultivar) and three negative controls (only the bioactive product) for each treatment were used (Table 1).

During the assay period, cuprous oxide (75%) was applied in mid-July to control downy mildew outbreaks, together with a first sprouting (followed by periodic sprouting). *Amblyseius* (*Typhlodromips*) *swirskii* Athias-Henriot was used for the biological control of whitefly, thrips, and spider mite; *Encarsia formosa* Gahan/*Eretmocerus eremicus* Rose & Zolnerowich for whitefly; and *Aphelinus abdominalis* Dalman for aphids at the end of July (Biobest Group NV, Almería, Spain). The grapevine plants were visually examined each week throughout the assay period for the presence of foliar symptoms (including interveinal and veinal necroses).

At the end of the experiment, plants were removed and two transversal sections of each inoculated stem, between the grafting point and the root crown, were prepared and sectioned longitudinally. The effects of the inoculated fungi were evaluated by measuring the lengths of longitudinal vascular necroses in each direction from the inoculation point.

Samples from the assayed plants were further processed to re-isolate the previously inoculated fungi, and to fulfill Koch's postulates. Wood chips (length = 5 mm) exhibiting vascular necroses (1–2 cm around the wounds) were washed, surface sterilized, and placed in PDA plates amended with streptomycin sulphate (to avoid bacterial contamination). The plates were incubated at 26°C in the dark for 2–3 days in a culture chamber. Emerging colonies were identified based on their morphological characters. A selection of the isolates recovered from vascular lesions was identified by comparing ribosomal ITS sequences with those from the inoculated isolates.

Table 1. Details of plant/treatment combinations for the greenhouse bioassay. Each grafted plant was inoculated at two sites below the grafting point.

Plant	Treatment	Pathogen	Number of replicates
'Tempranillo' (CL. 32 clone) on 775P rootstock	Stevioside-ferulic acid	*N. parvum*	5
		D. viticola	5
		Nil (negative control)	3
	Stevioside-rutin	*N. parvum*	5
		D. viticola	5
		Nil (negative control)	3
	Nil (positive control)	*N. parvum*	4
		D. viticola	4
'Garnacha' (VCR3 clone) on 110R rootstock	Stevioside-ferulic acid	*N. parvum*	5
		D. viticola	5
		Nil (negative control)	3
	Stevioside-rutin	*N. parvum*	5
		D. viticola	5
		Nil (negative control)	3
	Nil (positive control)	*N. parvum*	4
		D. viticola	4

Statistical analyses

Given that the homogeneity and homoscedasticity requirements were satisfied, according to Shapiro–Wilk and Levene tests, the results of the *in vitro* mycelium growth inhibition experiments were statistically analyzed using one-way analysis of variance (ANOVA), followed by *post hoc* comparison of means through Tukey's test at $P < 0.05$. For the greenhouse assays, since normality and homoscedasticity requirements were not met, Kruskal-Wallis non-parametric test was used, with Conover-Iman test for *post hoc* multiple pairwise comparisons. R statistical software was used for all the statistical analyses (R Core Team, 2020).

RESULTS

In vitro *tests of mycelium growth inhibition*

From *in vitro* tests (Figure 1 and Figure S1), greater antifungal activity was recorded from stevioside or rutin alone than for ferulic acid against both *Botryosphaeriaceae* taxa, especially against *N. parvum* (Table S1). Statistically significant increases in antifungal activity were observed for the stevioside-rutin and stevioside-ferulic acid conjugate complexes. For stevioside-rutin, almost complete inhibition of *N. parvum* occurred at 1000 µg·mL^{-1}, and for *D. viticola* complete inhibition was observed at 750 µg·mL^{-1}, compared with 1500 µg·mL^{-1} for the non-conjugated compounds against both fungi. For the ferulic acid adduct, efficacy was also slightly lower than that of the rutin adduct: concentrations of 1500 and 1000 µg·mL^{-1} were required for complete inhibition of *N. parvum* and *D. viticola*, respectively. Inhibition of 64% for *N. parvum* and 74% for *D. viticola* resulted from the 1500 µg·mL^{-1} ferulic acid treatment.

Comparison of EC_{50}s and EC_{90}s (Table 2) for colony diameter measurements corroborated the lower EC values for rutin than for ferulic acid, especially against *D. viticola*. On the basis of the EC_{90} values, synergism was observed for the two complexes, with SF values close to 1.45 for *N. parvum*, and ranging from 1.6 to 2.2 for *D. viticola*.

Greenhouse bioassays with grafted plants

After removing, cutting, and measuring vascular necroses present in the different treated plants, no statistically significant differences were detected between cultivar/rootstock combinations, as shown in Table S2.

Statistically significant differences were detected for mean lengths of vascular necroses between treated and non-treated plants for both pathogens. Against *N. parvum* (Figure 2), the two assayed formulations gave similar results (Table 3). Against *D. viticola*, the stevioside-rutin treatments gave a greater reduction in lesion lengths than the stevioside-ferulic acid treatment, as it occurred in the *in vitro* tests (Table 4). Mean lengths of necroses for the treated plants were not significantly different from those of the negative controls (i.e., those from plants treated with conjugate complexes without pathogens), indicating strong inhibition of *D. viticola*. Lesions from the two negative controls were similar.

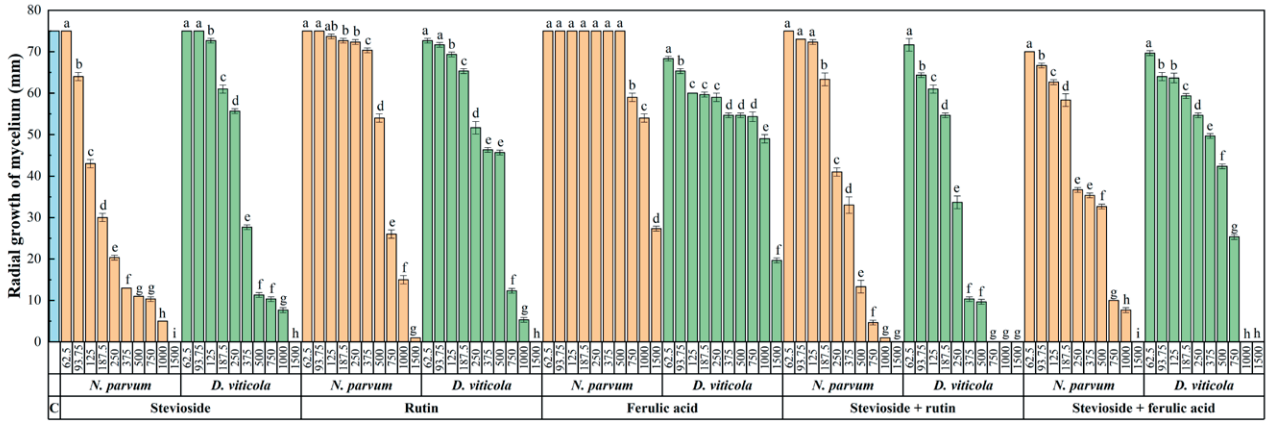

Figure 1. Mean colony radial diameters for *Neofusicoccum parvum* and *Dothiorella viticola* strains when cultured in PDA plates containing the different treatments) at concentrations ranging from 62.5 to 1500 µg·mL^{-1}. Means accompanied by the same letters are not significantly different ($P < 0.05$), and bars indicate standard deviations.

Table 2. Mean EC_{50}s and EC_{90}s ($\mu g \cdot mL^{-1}$; ± standard errors), and calculated synergy factors (S.F.).

Pathogen	Effective Concentration	Stevioside	Rutin	Ferulic acid	Stevioside–rutin	S.F.	Stevioside–ferulic acid	S.F.
N. parvum	EC_{50}	154.9±13.5	656.9±25.4	1394.4±63.0	306.0±23.6	0.82	435.6±66.8	0.64
	EC_{90}	923.8±56.7	1156.5±72.2	4121.3±313.5	714.9±31.9	1.44	1032.2±43.1	1.46
D. viticola	EC_{50}	309.6±16.6	575.1±34.9	1287.2±51.3	241.6±12.8	1.67	574.4±46.3	0.87
	EC_{90}	1007.1±66.0	981.1±58.6	2948.6±168.0	457.8±21.7	2.17	921.8±72.5	1.63

Figure 2. Foliar symptoms and vascular necroses observed in grapevine plants artificially inoculated with *Neofusicoccum parvum* and treated with two conjugate complexes of natural products. Top row, left to right; general aspect of plants treated with *N. parvum* (positive control), stevioside-ferulic acid, stevioside-rutin, *N. parvum* + stevioside-ferulic acid and *N. parvum* + stevioside-rutin. Bottom row; vascular lesions after sectioning the stems of the grapevine plants in the top row.

Table 3. Mean lengths of the vascular necroses in grapevine plants after inoculations with *Neofusicoccum parvum*. Mean of rank values accompanied by the same letters are not significantly different ($P < 0.05$; Kruskal-Wallis test, and multiple pairwise comparisons using the Conover-Iman procedure).

Treatment	Mean of ranks	Groups		
Stevioside-rutin negative control	42.573	A		
Stevioside-ferulic acid negative control	48.188	A		
Stevioside-rutin	145.656		B	
Stevioside-ferulic acid	155.638		B	
Positive control	183.313			C

Table 4. Mean lengths of vascular necroses in grapevine plants after inoculations with *Dothiorella viticola*. Mean of rank values accompanied by the same letters are not significantly different ($P < 0.05$; Kruskal-Wallis test, and multiple pairwise comparisons using the Conover-Iman procedure).

Treatment	Mean of ranks	Groups			
Stevioside-rutin	90.472	A			
Stevioside-rutin negative control	110.813	A	B		
Stevioside-ferulic acid negative control	123.713		B	C	
Stevioside-ferulic acid	145.632			C	
Positive control	260.766				D

DISCUSSION

Comparison with reported antifungal efficacies for bioactive substances

When comparing results of sensitivity of fungal pathogens to exposure to fungicidal compounds, susceptibility profiles in these microorganisms are usually species, and isolate-dependent, so comparisons of effective concentrations discussed below should be taken with caution.

Previous research has advocated ferulic acid as the phenolic acid having the strongest anti-fungal activity (Lambert *et al.*, 2012a; Sabel *et al.*, 2017; Zabka and Pavela, 2013). In assessments of the efficacy of ferulic acid against GTDs, Lambert *et al.* (2012b) assayed a concentration of 500 µM (97 µg·mL^{-1}), and measured *in vitro* growth inhibitions of 23% against *N. parvum* strain PER20 and 35% against *N. parvum* strain Bp0014. However, these authors did not report MIC or EC values for ferulic acid against these fungi. Gómez *et al.* (2016) reported EC_{50} values of 3530 µg·mL^{-1} for ferulic acid against several *Botryosphaeriaceae* species and 4740 µg·mL^{-1} against *Phaeoacremonium minimum* (Tul. & C. Tul.) Gramaje, L. Mostert & Crous. Dekker *et al.* (2002) reported 62% inhibition for ferulic acid against *Botryosphaeria* Ces. & de Not. species at 25 mM (4855 µg·mL^{-1}), with an EC_{50} value of 15 mM (2913 µg·mL^{-1}). In general terms, EC_{50}s in these reports were 2 to 3 times greater than those recorded in the present study (1340 and 1454 µg·mL^{-1}). Srivastava *et al.* (2013) tested ten naturally occurring phenolic compounds from plants against isolates from different *Botryosphaeriaceae* genera (viz. *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *B. obtusa* (Schwein.) Shoemaker, and *Neofusicoccum ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips)). They reported that ferulic acid at 25 mM (4855 µg·mL^{-1}) gave ca. 80% mycelium growth inhibition of *L. theobromae* and ca. 70% inhibition of *N. ribis*, while 100% inhibition was attained at 20 mM (3885 µg·mL^{-1}) for *B. obtusa*. These concentrations are similar to the EC_{90} values obtained in the present study against *N. parvum* (3230 µg·mL^{-1}) and *D. viticola* (3921 µg·mL^{-1}).

Zabka and Pavela (2013) assessed the efficacy of 21 phenolic components of essential oils and plant substances against several toxicogenic filamentous fungi. They reported MIC values >1000 µg·mL^{-1} for ferulic acid against *Fusarium oxysporum* Schltdl., *F. verticillioides* (Sacc.) Nirenberg, *Penicillium brevicompactum* Dierckx, *P. expansum* Link, *Aspergillus flavus* Link, and *A. fumigatus* Fresen. EC_{50} values ranged from 411 (*P. expansum*) to 895 µg·mL^{-1} (*A. flavus*). Wu *et al.* (2010) found that ferulic acid inhibited conidium germination of the watermelon soil-borne pathogen *F. oxysporum* f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hansen at concentrations of 800 µg·mL^{-1}. Asiegbu *et al.* (1996) reported that ferulic acid at 5000 µg·mL^{-1} severely repressed growth of the lignocellulolytic fungi *Trichoderma harzianum* Rifai, *Chaetomium cellulolyticum* Chahal & D. Hawksw., *Phanerochaete chrysosporium* Burds., *Trametes versicolor* (L.) Lloyd and *Pleurotus sajor-caju* (Fr.) Singer. Ferulic acid or ferulic acid-rich extracts have also been suggested as natural alternatives for reducing post-harvest fruit losses. Hernández *et al.* (2021) reported almost 100% inhibition of *Monilinia fructicola* (G. Winter) Honey at a dose of 2 mM (390 µg·mL^{-1}), and 90% inhibition of *Alternaria alternata* (Fr.) Keissl. at 3 mM (583 µg·mL^{-1}), and of *Botrytis cinerea* Pers. at 7.5 mM (1457 µg·mL^{-1}).

There have been no previous reports of the antifungal activity of rutin against GTDs. To date, more than 70 plant species have been shown to contain rutin (Gullón *et al.*, 2017), and different plant extracts with high contents of this compound have shown inhibitory effects on the growth of fungi. Devi *et al.* (2007) found significant growth inhibition from extracts of *Eupatorium birmanicum* DC [*Eupatorium cannabinum* subsp. *cannabinum*]

against *F. oxysporum* (at 1000 ppm), *Curvularia lunata* Boedijn (at 500 ppm), and *Trichoderma viride* Pers. (at 100 ppm). Salvador *et al.* (2004) showed that ethanolic extracts from *Alternanthera maritima* (Mart.) St. Hil gave moderate inhibition of *Candida albicans* (C.P. Robin) Berkhout, *C. tropicalis* (Castell.) Berkhout, *C. glabrata* (H.W. Anderson) S.A. Mey. & Yarrow, *C. parapsilosis* (Ashford) Langeron & Talice, *Trichophyton mentagrophytes* C.P. Robin) R. Blanch. and *T. rubrum* (Castell.) Sabour. Pure rutin against the same fungi gave MIC values of 500 $\mu g \cdot mL^{-1}$.

Parvu *et al.* (2015) found rutin contents of 130 $\mu g \cdot mL^{-1}$ in flower extracts of *Hedera helix* L. (ivy), and 170 $\mu g \cdot mL^{-1}$ in fruit extracts of the same plant, and assayed these against *Aspergillus niger* Tiegh., *B. cinerea, F. oxysporum* f. sp. *tulipae* Apt., *Penicillium gladioli* L. McCulloch & Thom, and *Sclerotinia sclerotiorum* (Lib.) Korf & Dumont. Full inhibition of mycelium growth of these fungi was attained at concentrations of 8 to 12% for flower extracts, and of 10 to 14% for fruit extracts.

Elansary *et al.* (2020a) assayed the stem extracts of six *Ferocactus* species (*F. gracilis, F. pottsii, F. herrerae, F. horridus, F. glaucescens,* and *F. emoryi*), with rutin contents of up to 108 mg per 100 g DW, against several bacteria and fungi. They found strong antifungal effects against *A. flavus, A. ochraceus, A. niger, C. albicans, Penicillium funiculosum,* and *P. ochrochloron* (with MICs from 100 to 730 $\mu g \cdot mL^{-1}$). They found slightly greater rutin concentrations (139 mg/100 g DW) in *Ocimum basilicum* L. (basil), giving MIC values from 290 to 560 $\mu g \cdot mL^{-1}$ against these fungal pathogens (Elansary *et al.*, 2020d). Much greater rutin concentrations (1533 and 1010 mg/100 g DW) were found in leaves of *Acacia saligna* L. and *Ruta graveolens* L. leaves by Elansary *et al.* (2020b; 2020c). In the associated *in vitro* assays, conducted for the leaf methanolic extracts and several pure bioactive compounds detected by high-performance liquid chromatography–diode array detection (HPLC-DAD), these authors found MIC values against the different fungi ranging from 180 to 300 $\mu g \cdot mL^{-1}$ for pure rutin, from 300 to 580 $\mu g \cdot mL^{-1}$ for *A. saligna* extract, and from 330 to 780 $\mu g \cdot mL^{-1}$ for *R. graveolens* extract.

Concerning the antifungal activity of stevioside (*Stevia rebaudiana* extracts), Ghosh *et al.* (2008) and Abou-Arab and Abu-Salem (2010) reported growth inhibition effects against *A. solani, Helminthosporium solani, A. niger, A. ochraceus* K. Wilh., *A. parasiticus* Speare, *A. flavus*, and *Penicillium chrysogenum* Thom, but MIC values were not determined in these studies. Arya *et al.* (2012) also demonstrated the antifungal activity of stevioside (at 50000 $\mu g \cdot mL^{-1}$) against *A. flavus, A. fumigatus, A. niger,* and *Fusarium oxysporum*. Abdel-Fatt *et al.* (2018) measured MIC values of 250 to 300 $\mu g \cdot mL^{-1}$ for stevioside against *A. flavus, A. ochraceus, A. niger,* and *Fusarium moniliforme* Sheldon. Shukla *et al.* (2013) reported stevioside MIC values of 3 $mg \cdot mL^{-1}$ against *B. cinerea* and 2 $mg \cdot mL^{-1}$ against *F. oxysporum*, and Guerra Ramírez *et al.* (2020) found that the hexane extract of stevioside at 833 ppm inhibited mycelium growth of *F. oxysporum* by up to 50%.

No data are available on the antifungal activity of stevioside-polyphenol conjugate complexes against fungi associated with GTDs. Buzón-Durán *et al.* (2020) reported an EC_{50} of 123 $\mu g \cdot mL^{-1}$ and an EC_{90} of 160 $\mu g \cdot mL^{-1}$ against *F. culmorum* for conjugate complexes based on a 1:1 mixture of stevioside with polyphenols present in milk thistle seeds (*Silybum marianum* (L.) Gaertn). Composites based on stevioside:ferulic acid inclusion compounds (in a 5:1 molar ratio), combined with chitosan oligomers in hydroalcoholic solution or in choline chloride:urea deep eutectic solvent media, were assayed against *F. culmorum* by Matei *et al.* (2018a), obtaining EC_{50}s ranging from 175 to 292 $\mu g \cdot mL^{-1}$ and EC_{90}s in the 377 to 713 $\mu g \cdot mL^{-1}$ interval, depending on the dispersion medium. Inclusion compounds from stevioside and ferulic acid in 6:1 ratio, dispersed in a hydroalcoholic solution of chitosan oligomers, were also assayed against *P. cinnamomi* by Matei *et al.* (2018b) and Matei *et al.* (2020), obtaining EC_{50}s of 171 to 229 $\mu g \cdot mL^{-1}$ and EC_{90}s of 446 to 450 $\mu g \cdot mL^{-1}$, depending on the presence or absence of silver nanoparticles.

Mechanisms of action

The antimicrobial activity of ferulic acid can involve different modes of action, mainly related to the destabilization and permeabilization of cytoplasmatic membranes and to enzyme inhibition by the oxidized products (Borges *et al.*, 2013). Phenolic acids also affect the physicochemical surface properties of microbial cells, given that these compounds are electrophilic and change hydrophobicity. Due to their partially lipophilic character, it is assumed that ferulic acid crosses cell membrane by passive diffusion in undissociated form, disturbing cell membrane structure by localized hyperacidification, and possibly acidifying the cytoplasm and causing protein denaturation. The alteration of cell membrane potential makes it more permeable and causes leakage of cell constituents, including proteins and nucleic acids. Additionally, ferulic acid (like p-coumaric acid and caffeic acid) affects the cell membrane structure and rigidity, and alters phospholipid chain dynamics (Ota *et al.*, 2011). Shi *et al.* (2016) also noted that ferulic acid causes changes in intracellular ATP concentrations.

The antifungal mechanism of action of flavonoids was comprehensively reviewed by Al Aboody and Mickymaray (2020). Flavonoids inhibit fungal growth via various underlying mechanisms, including disruption of plasma membranes, induction of mitochondrial dysfunction, and inhibition of efflux mediated pumping, cell division, cell wall formation, and protein and RNA syntheses. For rutin, the mechanisms of pharmacological action were summarized by Koval'skii *et al.* (2014), who also noted that this compound can interact with various structures at molecular levels (including free radicals, protein systems, and enzymes).

In addition to direct effects, which cause reductions of fungal growth by altering hyphal morphology, grapevine phenolic compounds also exert their actions against GTD fungi through indirect effects, via inhibition of the extracellular fungal manganese peroxidase (MnP) involved in lignin degradation (Gómez *et al*., 2016). A decrease of laccase production and pectinase activity of *Botryosphaeria* isolates resulting from phenolic compounds was also reported by Srivastava *et al.* (2013).

Khan *et al.* (2017) suggested that the antimycotic activity of phytoglycosides is mediated through different and multiple targets that are not fully understood. However, there is a consensus that the main antimycotic mechanism is related to their ability to complex with sterols of fungal membranes. This produces spore-like structures that cause pore formations in membranes, losses in membrane integrity, and even membrane rupture, leading to fungal cell death.

Solubility and synergistic behaviour

A major disadvantage associated with rutin is its poor bioavailability, mainly caused by its low aqueous solubility and poor stability. Gullón *et al.* (2017) indicated that this hinders the *in vivo* biological effects of rutin, although the compound may have detectable bioactivity in different *in vitro* systems. Common approaches used to enhance rutin bioavailability include particle diminution to the submicron range and complex formation with cyclodextrins and various metals. Also, various carrier systems have been proposed for rutin delivery, including micro- and nano-emulsions, nanocrystals and nanosuspensions (Sharma *et al*., 2013).

An alternative approach is to use steviol glycosides as natural solubilizers, an approach that has been successfully tested for several natural phenols, such as curcumin (a diarylheptanoid) (Zhang *et al*., 2011; Nguyen *et al*., 2017), liquiritin (the 4'-*O*-glucoside of the flavanone liquiritigenin) (Nguyen *et al*., 2014), and betulinic acid (a pentacyclic triterpenoid) (Zhang *et al*., 2016). For rutin, Ko *et al.* (2016) optimized its solubility by the Box-Behnken design with the aid of microwave treatment (instead of ultrasonic treatment, as in the present study). Nguyen *et al.* (2015) attained similar results for quercetin, by complexation with rubusoside and rebaudioside, finding that as the glycoside concentration increased, the solubility of quercetin in water increased, without reducing its biological functions. Solubility optimization could be responsible for synergistic effects of conjugates of the two glycodrugs against GTD pathogens. The same rationale may be applied to tentatively explain the synergy observed for ferulic acid (although this was weaker than that attained for rutin).

Opportunities for future GTDs treatments

Levels of phenolic compounds have been reported to increase in the discoloured wood of Esca-affected grapevines (Agrelli *et al*., 2009; Amalfitano *et al*., 2011), while phenolic compounds have also been shown to limit fungal development in grapevine vascular tissues (Lambert *et al*., 2012a; Lima *et al*., 2011). Spagnolo *et al.* (2014) found the greatest levels of total phenolics in the brown striped wood of three grapevine cultivars infected with *N. parvum* and *Diplodia seriata* De Not. Martin *et al.* (2009) showed that stilbene polyphenols such as resveratrol and ε-viniferin increased in the wood of vines artificially inoculated with *Phaeomoniella chlamydospora*. Quercetin-3-*O*-glucoside and *trans*-caffeoyltartaric acid (analogous to rutin and ferulic acid studied here) were associated with resistance to *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni in grapevine leaves (Ali *et al*., 2012), and increases in quercetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside have been found in asymptomatic leaves of plants infected with the Bois noir phytoplasma (Rusjan *et al*., 2012).

The approach presented here, based on mimicking the grapevine response via intrinsic phenolic compounds, along with solubility and bioavailability enhancements, is a "natural" and effective way to control the development of particular GTD pathogens. Although pure reagents were assayed in this study, the results attained indicate that selection of natural antifungal compounds could show promise. In the case of rutin, extracts from *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli, *Sambucus nigra* L., *Drimys winteri* J.R.Forst. or *Taraxacum officinale* Weber ex Wiggins (Meinhart *et al*., 2020) may deserve further attention for large-scale field experiments. If plants rich in rutin and ferulic were preferred, the activity of the extracts from sea buckthorn (*Hippophae Rhamnoides* L.) (Criste *et al*., 2020), *Rhinacanthus nasutus* (L.) Kurz (Huang *et al*., 2015), *Artemisia absinthium* L., *Achil-*

lea millefolium L., *Sambucus nigra* L. or *Salvia officinalis* L. (Bljajić *et al*., 2021) could be explored.

CONCLUSIONS

In an attempt to mimic grapevine defence responses against GTDs, the anti-fungal efficacy of rutin, either alone or in conjugate complexes with stevioside, was assayed against two *Botryosphaeriaceae* taxa. While the *in vitro* performance of the pure flavonoid-3-*O*-glycoside was moderate, with EC_{90}s of 1157 μg·mL^{-1} against *N. parvum* and 981 μg·mL^{-1} against *D. viticola*, considerable increases in activity (EC_{90}s of 715 and 458 μg·mL^{-1}, respectively) were attained for stevioside-rutin. The synergistic behaviour (with SFs of 1.44 and 2.17) may be due to solubility and bioavailability optimization. Testing of the formulations in greenhouse *in vivo* conditions showed moderate inhibition of *N. parvum* and full inhibition against *D. viticola* for the stevioside-rutin treatments. These EC_{90}s and *in vivo* results were consistently better than those found for ferulic acid and stevioside-ferulic acid, used as references. The promising results attained with this approach provide guidance for the selection of new plant extracts that could be utilized as antifungal agents in organic viticulture.

ACKNOWLEDGMENTS

V.G.-G thanks C. Julián (Plant Protection Unit, CITA) for technical assistance. The financial support for this study was provided by Junta de Castilla y León under project VA258P18, with FEDER co-funding; by Cátedra Agrobank under the "IV Convocatoria de Ayudas de la Cátedra AgroBank para la transferencia del conocimiento al sector agroalimentario" programme, and by Fundación Ibercaja-Universidad de Zaragoza under "Convocatoria Fundación Ibercaja-Universidad de Zaragoza de proyectos de investigación, desarrollo e innovación para jóvenes investigadores".

LITERATURE CITED

Abdel-Fatt, S. M., Badr, A. N., Abu Seif, F. A.-H., Mohamed Al, S., and Ahmed Hass, R., 2018. Anti-fungal and anti-mycotoxigenic impact of eco-friendly extracts of wild Stevia. *Journal of Biological Sciences* 18: 488–499.

Abou-Arab, E., and Abu-Salem, F., 2010. Evaluation of bioactive compounds of *Stevia rebaudiana* leaves and callus. *Journal of Food and Dairy Sciences* 1: 209–224.

Agrelli, D., Amalfitano, C., Conte, P., and Mugnai, L., 2009. Chemical and spectroscopic characteristics of the wood of *Vitis vinifera* cv. Sangiovese affected by esca disease. *Journal of Agricultural and Food Chemistry* 57: 11469–11475.

Al Aboody, M. S., and Mickymaray, S., 2020. Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics* 9: 45.

Ali, K., Maltese, F., Figueiredo, A., Rex, M., Fortes, A. M., Zyprian, E., … Choi, Y. H., 2012. Alterations in grapevine leaf metabolism upon inoculation with *Plasmopara viticola* in different time-points. *Plant Science* 191: 100–107.

Amalfitano, C., Agrelli, D., Arrigo, A., Mugnai, L., Surico, G., and Evidente, A., 2011. Stilbene polyphenols in the brown red wood of *Vitis vinifera* cv. Sangiovese affected by "esca proper". *Phytopathologia Mediterranea* 50: S224-S235.

Arya, A., Kumar, S., and Kasana, M., 2012. In vitro regeneration of Stevia and evaluation of antimicrobial and antiprotozoal properties of regenerated calli and plants. *Electronic Journal of Plant Breeding* 3: 916–924.

Asiegbu, F. O., Paterson, A., and Smith, J. E., 1996. Inhibition of cellulose saccharification and glycolignin-attacking enzymes of five lignocellulose-degrading fungi by ferulic acid. *World Journal of Microbiology and Biotechnology* 12: 16–21.

Bljajić, K., Brajković, A., Čačić, A., Vujić, L., Jablan, J., Saraiva de Carvalho, I., and Zovko Končić, M., 2021. Chemical composition, antioxidant, and α-glucosidase-inhibiting activity of aqueous and hydroethanolic extracts of traditional antidiabetics from Croatian ethnomedicine. *Horticulturae* 7: 15.

Borges, A., Ferreira, C., Saavedra, M. J., and Simões, M., 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance* 19: 256–265.

Buzón-Durán, L., Martín-Gil, J., Ramos-Sánchez, M. d. C., Pérez-Lebeña, E., Marcos-Robles, J. L., Fombellida-Villafruela, Á., and Martín-Ramos, P., 2020. Anti-fungal activity against *Fusarium culmorum* of stevioside, *Silybum marianum* seed extracts, and their conjugate complexes. *Antibiotics* 9(8): 440.

Buzón-Durán, L., Langa-Lomba, N., González-García, V., Casanova-Gascón, J., Martín-Gil, J., Pérez-Lebeña, E., and Martín-Ramos, P., 2021. On the applicability of chitosan oligomers-amino acid conjugate complexes as eco-friendly fungicides against grapevine trunk pathogens. *Agronomy* 11(2): 324.

Chebil, L., Humeau, C., Anthoni, J., Dehez, F., Engasser, J.-M., and Ghoul, M., 2007. Solubility of flavonoids in

organic solvents. *Journal of Chemical & Engineering Data* 52: 1552–1556.

Criste, A., Urcan, A. C., Bunea, A., Pripon Furtuna, F. R., Olah, N. K., Madden, R. H., and Corcionivoschi, N., 2020. Phytochemical composition and biological activity of berries and leaves from four Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Molecules* 25: 1170.

Dekker, R. F. H., Barbosa, A. M., and Sargent, K., 2002. The effect of lignin-related compounds on the growth and production of laccases by the ascomycete, *Botryosphaeria* sp. *Enzyme and Microbial Technology* 30: 374–380.

Del Río, J. A., Gómez, P., Báidez, A., Fuster, M. D., Ortuño, A., and Frías, V., 2004. Phenolic compounds have a role in the defence mechanism protecting grapevine against the fungi involved in Petri disease. *Phytopathologia Mediterranea* 43: 87–94.

Devi, L. R., Singh, T. S., and Laitonjam, W. S., 2007. Antifungal and phytochemical studies of *Eupatorium birmanicum* DC. *Indian Journal of Chemistry Section B-Organic Chemistry Including Medicinal Chemistry* 46: 1868–1872.

Elansary, H. O., Szopa, A., Klimek-Szczykutowicz, M., Ekiert, H., Barakat, A. A., and Al-Mana, F. A., 2020a. Antiproliferative, antimicrobial, and antifungal activities of polyphenol extracts from *Ferocactus* species. *Processes* 8: 138.

Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Al-Mana, F. A., and Al-Yafrsi, M. A., 2020b. Antioxidant and biological activities of *Acacia saligna* and *Lawsonia inermis* natural populations. *Plants* 9: 908.

Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., El-Ansary, D. O., Al-Mana, F. A., and Mahmoud, E. A., 2020c. Polyphenol content and biological activities of *Ruta graveolens* L. and *Artemisia abrotanum* L. in Northern Saudi Arabia. *Processes* 8: 531.

Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., El-Ansary, D. O., Al-Mana, F. A., and Mahmoud, E. A., 2020d. Saudi *Rosmarinus officinalis* and *Ocimum basilicum* L. polyphenols and biological activities. *Processes* 8: 446.

Fontaine, F., Pinto, C., Vallet, J., Clement, C., Gomes, A. C., and Spagnolo, A., 2016. The effects of grapevine trunk diseases (GTDs) on vine physiology. *European Journal of Plant Pathology* 144: 707–721.

Fourie, P. H., and Halleen, F., 2004. Proactive Control of Petri Disease of Grapevine Through Treatment of Propagation Material. *Plant Disease* 88: 1241–1245.

Ganeshpurkar, A., and Saluja, A. K., 2017. The pharmacological potential of rutin. *Saudi Pharmaceutical Journal* 25: 149–164.

Ghosh, S., Subudhi, E., and Nayak, S., 2008. Antimicrobial assay of *Stevia rebaudiana* Bertoni leaf extracts against 10 pathogens. *International Journal of Integrative Biology* 2: 27–31.

Gómez, P., Báidez, A. G., Ortuño, A., and Del Río, J. A., 2016. Grapevine xylem response to fungi involved in trunk diseases. *Annals of Applied Biology* 169: 116–124.

Goufo, P., Singh, R. K., and 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.

Graniti, A., Surico, G., and Mugnai, L., 2000. Esca of grapevine a disease complex or a complex of diseases? *Phytopathologia Mediterranea* 39: 16–20.

Guerra Ramírez, P., Guerra Ramírez, D., Zavaleta Mejía, E., Aranda Ocampo, S., Nava Díaz, C., and Rojas Martínez, R. I., 2020. Extracts of *Stevia rebaudiana* against *Fusarium oxysporum* associated with tomato cultivation. *Scientia Horticulturae* 259: 108683.

Gullón, B., Lú-Chau, T. A., Moreira, M. T., Lema, J. M., and Eibes, G., 2017. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology* 67: 220–235.

Hernández, A., Ruiz-Moyano, S., Galván, A. I., Merchán, A. V., Pérez Nevado, F., Aranda, E., … Martín, A., 2021. Anti-fungal activity of phenolic sweet orange peel extract for controlling fungi responsible for post-harvest fruit decay. *Fungal Biology* 125: 143–152.

Huang, R. T., Lu, Y. F., Inbaraj, B. S., and Chen, B. H., 2015. Determination of phenolic acids and flavonoids in *Rhinacanthus nasutus* (L.) Kurz by high-performance-liquid-chromatography with photodiode-array detection and tandem mass spectrometry. *Journal of Functional Foods* 12: 498–508.

Hussain, Z., Thu, H. E., Amjad, M. W., Hussain, F., Ahmed, T. A., and Khan, S., 2017. Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: A review of new trends and future perspectives. *Materials Science and Engineering C* 77: 1316–1326.

Jin, Y. S., 2019. Recent advances in natural antifungal flavonoids and their derivatives. *Bioorganic & Medicinal Chemistry Letters* 29: 126589.

Khan, H., Khan, Z., Amin, S., Mabkhot, Y. N., Mubarak, M. S., Hadda, T. B., and Maione, F., 2017. Plant bioactive molecules bearing glycosides as lead compounds for the treatment of fungal infection: A review. *Biomedicine & Pharmacotherapy* 93: 498–509.

Ko, J.-A., Nam, S.-H., and Kim, Y., 2016. Optimization of soluble rutin using stevioside as a natural solubilizer. *Journal of Chitin and Chitosan* 21: 181–187.

Koval'skii, I. V., Krasnyuk, I. I., Krasnyuk, I. I., Nikulina, O. I., Belyatskaya, A. V., Kharitonov, Y. Y., … Lutsenko, S. V., 2014. Mechanisms of rutin pharmacological action. *Pharmaceutical Chemistry Journal* 48: 73–76.

Lambert, C., Bisson, J., Waffo-Téguo, P., Papastamoulis, Y., Richard, T., Corio-Costet, M.-F., … Cluzet, S., 2012a. Phenolics and their antifungal role in grapevine wood decay: Focus on the Botryosphaeriaceae family. *Journal of Agricultural and Food Chemistry* 60: 11859–11868.

Lambert, C., Papastamoulis, Y., Waffo-Téguo, P., Mérillon, J., and Cluzet, S., 2012b. Effects of phenolic compounds towards grapevine wood decay fungi. In *Oeno2011: Actes de colloques du 9e symposium international d'oenologie de Bordeaux*, Dunod, Bordeaux, France, 90–94.

Levy, Y., Benderly, M., Cohen, Y., Gisi, U., and Bassand, D., 1986. The joint action of fungicides in mixtures: comparison of two methods for synergy calculation. *EPPO Bulletin* 16: 651–657.

Lima, M. R. M., Ferreres, F., and Dias, A. C. P., 2011. Response of *Vitis vinifera* cell cultures to *Phaeomoniella chlamydospora*: changes in phenolic production, oxidative state and expression of defence-related genes. *European Journal of Plant Pathology* 132: 133–146.

Martin, M. T., and Cobos, R., 2007. Identification of fungi associated with grapevine decline in Castilla y León (Spain). *Phytopathologia Mediterranea* 46: 18–25.

Martin, N., Vesentini, D., Rego, C., Monteiro, S., Oliveira, H., and Ferreira, R. B., 2009. *Phaeomoniella chlamydospora* infection induces changes in phenolic compounds content in *Vitis vinifera*. *Phytopathologia Mediterranea* 48: 101–116.

Matei, P., Iacomi, B., Martín-Gil, J., Pérez-Lebeña, E., Ramos-Sánchez, M., Barrio-Arredondo, M., and Martín-Ramos, P., 2018a. *In vitro* antifungal activity of composites of AgNPs and polyphenol inclusion compounds against *Fusarium culmorum* in different dispersion media. *Agronomy* 8(11): 239.

Matei, P., Martín-Gil, J., Michaela Iacomi, B., Pérez-Lebeña, E., Barrio-Arredondo, M., and Martín-Ramos, P., 2018b. Silver nanoparticles and polyphenol inclusion compounds composites for *Phytophthora cinnamomi* mycelial growth inhibition. *Antibiotics* 7(3): 76.

Matei, P. M., Buzón-Durán, L., Pérez-Lebeña, E., Martín-Gil, J., Iacomi, B. M., Ramos-Sánchez, M. C., and Martín-Ramos, P., 2020. *In vitro* antifungal activity of chitosan-polyphenol conjugates against *Phytophthora cinnamomi*. *AgriEngineering* 2(1): 72–77.

Meinhart, A. D., Damin, F. M., CaldeirÃO, L., Teixeira-Filho, J., and Godoy, H. T., 2020. Rutin in herbs and infusions: screening of new sources and consumption estimation. *Food Science and Technology* 40: 113–120.

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., … Fontaine, F., 2018. Grapevine trunk diseases: A review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease* 102: 1189–1217.

Nguyen, T. T. H., Jung, S.-J., Kang, H.-K., Kim, Y.-M., Moon, Y.-H., Kim, M., and Kim, D., 2014. Production of rubusoside from stevioside by using a thermostable lactase from *Thermus thermophilus* and solubility enhancement of liquiritin and teniposide. *Enzyme and Microbial Technology* 64–65: 38–43.

Nguyen, T. T. H., Yu, S.-H., Kim, J., An, E., Hwang, K., Park, J.-S., and Kim, D., 2015. Enhancement of quercetin water solubility with steviol glucosides and the studies of biological properties. *Functional Foods in Health and Disease* 5: 437.

Nguyen, T. T. H., Si, J., Kang, C., Chung, B., Chung, D., and Kim, D., 2017. Facile preparation of water soluble curcuminoids extracted from turmeric (*Curcuma longa* L.) powder by using steviol glucosides. *Food Chemistry* 214: 366–373.

Ota, A., Abramovič, H., Abram, V., and Poklar Ulrih, N., 2011. Interactions of p-coumaric, caffeic and ferulic acids and their styrenes with model lipid membranes. *Food Chemistry* 125: 1256–1261.

Parvu, M., Vlase, L., Parvu, A. E., Rosca-Casian, O., Gheldiu, A. M., and Parvu, O., 2015. Phenolic compounds and antifungal activity of *Hedera helix* L. (ivy) flowers and fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 43: 53–58.

R Core Team, 2020. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Rusjan, D., Halbwirth, H., Stich, K., Mikulič-Petkovšek, M., and Veberič, R., 2012. Biochemical response of grapevine variety 'Chardonnay' (*Vitis vinifera* L.) to infection with grapevine yellows (Bois noir). *European Journal of Plant Pathology* 134: 231–237.

Sabel, A., Bredefeld, S., Schlander, M., and Claus, H., 2017. Wine phenolic compounds: antimicrobial properties against yeasts, lactic acid and acetic acid bacteria. *Beverages* 3: 29.

Salvador, M. J., Zucchi, O., Candido, R. C., Ito, I. Y., and Dias, D. A., 2004. *In vitro* antimicrobial activity of crude extracts and isolated constituents of *Alternanthera maritima*. *Pharmaceutical Biology* 42: 138–148.

Shakeel, F., Salem-Bekhit, M. M., Haq, N., and Siddiqui, N. A., 2017. Solubility and thermodynamics of ferulic acid in different neat solvents: Measurement, correla-

tion and molecular interactions. *Journal of Molecular Liquids* 236: 144–150.

Sharma, S., Ali, A., Ali, J., Sahni, J. K., and Baboota, S., 2013. Rutin: therapeutic potential and recent advances in drug delivery. *Expert Opinion on Investigational Drugs* 22: 1063–1079.

Shi, C., Zhang, X., Sun, Y., Yang, M., Song, K., Zheng, Z., … Xia, X., 2016. Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action. *Foodborne Pathogens and Disease* 13: 196–204.

Shukla, S., Mehta, A., and Bajpai, V. K., 2013. Phytochemical screening and anthelmintic and antifungal activities of leaf extracts of *Stevia rebaudiana*. *Journal of Biologically Active Products from Nature* 3: 56–63.

Spagnolo, A., Larignon, P., Magnin-Robert, M., Hovasse, A., Cilindre, C., Van Dorsselaer, A., … Fontaine, F., 2014. Flowering as the most highly sensitive period of grapevine (*Vitis vinifera* L. cv Mourvèdre) to the Botryosphaeria dieback agents *Neofusicoccum parvum* and *Diplodia seriata* infection. *International Journal of Molecular Sciences* 15: 9644–9669.

Srivastava, P., Andersen, P. C., Marois, J. J., Wright, D. L., Srivastava, M., and Harmon, P. F., 2013. Effect of phenolic compounds on growth and ligninolytic enzyme production in *Botryosphaeria* isolates. *Crop Protection* 43: 146–156.

Surico, G., 2001. Towards commonly agreed answers to some basic questions on esca. *Phytopathologia Mediterranea* 40: S487-S490.

Wu, H.-s., Luo, J., Raza, W., Liu, Y.-x., Gu, M., Chen, G., … Shen, Q.-r., 2010. Effect of exogenously added ferulic acid on in vitro *Fusarium oxysporum* f. sp. *niveum*. *Scientia Horticulturae* 124: 448–453.

Zabka, M., and Pavela, R., 2013. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. *Chemosphere* 93: 1051–1056.

Zhang, F., Koh, G. Y., Jeansonne, D. P., Hollingsworth, J., Russo, P. S., Vicente, G., … Liu, Z., 2011. A novel solubility-enhanced curcumin formulation showing stability and maintenance of anticancer activity. *Journal of Pharmaceutical Sciences* 100: 2778–2789.

Zhang, J., Chou, G., Liu, Z., and Liu, M., 2016. Employing rubusoside to improve the solubility and permeability of antitumor compound betulonic acid. *Nanomedicine* 11: 2829–2844.