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Curse or blessing: Growth- and laccase-modulating properties of polyphenols and their oxidized derivatives on *Botrytis cinerea*

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ABSTRACT

Infection of grapevines with the grey mold pathogen *Botrytis cinerea* results in severe problems for winemakers worldwide. Browning of wine is caused by the laccase-mediated oxidation of polyphenols. In the last decades, *Botrytis* management has become increasingly difficult due to the rising number of resistances and the genetic variety of *Botrytis* strains. During the search for sustainable fungicides, polyphenols showed great potential to inhibit fungal growth. The present study revealed two important aspects regarding the effects of grape-specific polyphenols and their polymerized oxidation products on *Botrytis* wild strains. On the one hand, laccase-mediated oxidized polyphenols, which resemble the products found in infected grapes, showed the same potential for inhibition of growth and laccase activity, but differed from their native forms. On the other hand, the impact of phenolic compounds on mycelial growth is not correlated to the effect on laccase activity. Instead, mycelial growth and relative specific laccase activity appear to be modulated independently. All phenolic compounds showed not only inhibitory but also inductive effects on fungal growth and/or laccase activity, an observation which is reported for the first time. The simultaneous inhibition of growth and laccase activity demonstrated may serve as a basis for the development of a natural botryticide. Yet, the results showed considerable differences between genetically distinguishable strains, impeding the use of a specific phenolic compound against the genetic variety of wild strains. The present findings might have important implications for future understanding of *Botrytis cinerea* infections and sustainable *Botrytis* management including the role of polyphenols.

1. Introduction

Plants synthesize secondary metabolites among other reasons as one of their defense strategies. Most of them act as multitarget agents against various biotic stressors like herbivores and microorganisms. Among these metabolites, phenolic compounds represent one of the most abundant groups with a high structural diversity. Some of them are considered as phytoalexins, which play an important role in the plant's protection system, especially against microbial infections (Dixon, 2001; Wink, 2015). The antimicrobial activity of phenols is related to several growth inhibiting mechanisms. Hydroxycinnamic acids can act as mitochondrial uncouplers (Morales et al., 2017), whereas catechin reduces germ tube length (Tao et al., 2010). Cytological changes such as membrane permeabilization have been observed in fungi in the presence of some phenolic compounds like resveratrol, resulting in growth inhibition of *Botrytis cinerea* and others (Cotoras et al., 2004; Bouarab Chibane et al., 2019).

Botrytis cinerea is a ubiquitous, necrotrophic fungus infecting a wide

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range of plants including the grapevine (*Vitis vinifera* L.) (Williamson et al., 2007). Late infections of ripe grape berries can enhance wine quality due to skin perforation leading to evaporation and concentration of ingredients, which is called noble rot (Ribereau-Gayon et al., 2006; Thibon et al., 2009). On the other side, infections during an early ripening stage result in *Botrytis* bunch rot, also known as grey mold disease. Spoilage is recognizable by characteristic grey spots (Armijo et al., 2016). This disease causes severe problems for winemakers worldwide (Dean et al., 2012; Steel et al., 2013). Quality deteriorations include secondary infections with pathogenic microorganisms, discoloration of the must or formation of off-flavors (La Guerche et al., 2006; Ribereau-Gayon et al., 2006; Ky et al., 2012). Damage can lead to total loss of the harvest (Ribereau-Gayon et al., 2006). Browning is caused by the fungal enzyme laccase, which among other exoenzymes is secreted during the infection process (Gil-ad et al., 2001; Armijo et al., 2016). *B. cinerea* produces laccase for oxidation and thus inactivation of the antimicrobial phytoalexins biosynthesized by the grapevine in response to fungal infestation (Claus et al., 2014).

Laccases (EC 1.10.3.2; benzenediol: oxygen oxidoreductases) are multicopper enzymes. They can oxidize a wide range of mono- and diphenolic compounds with concomitant reduction of molecular oxygen to water. During these reactions, quinones are formed which undergo subsequent polymerization, resulting in the formation of brown pigments (Thurston, 1994; Claus, 2020). Oxidation begins in the infected berry and continues in must and wine because of the stability of the enzyme and its pH optimum in acidic environments, although the ethanol concentration present in wine might suppress enzymatic activity (Mayer & Harel, 1978; Claus, 2017). The resulting wine is of poor visual and organoleptic quality and may even be unsuitable for sale (Mullins et al., 1992; Claus, 2017). Typical enological treatments consist in the addition of bentonite or sulfites but do not result in sufficient inhibition of laccase or remedy of wine faults. To date, only heat treatment of must from *Botrytis* infected grapes shows effective inhibition of the enzyme but poses a high risk of altering the aroma profile. Therefore, the most common prevention of quality losses due to *B. cinerea* still is control of fungal growth by synthetic botryticides (Jacometti et al., 2010; Romanazzi & Feliziani, 2014; Fillinger & Walker, 2016). However, excessive fungicide application leads to resistant *B. cinerea* strains worldwide, with rapidly increasing numbers (Romanazzi & Feliziani, 2014; Avenot et al., 2020; Bertetti et al., 2020; DeLong et al., 2020). This rises attention to sustainable alternatives for synthetic botryticides (Romanazzi & Feliziani, 2014; DeSimone et al., 2020). It is well established that *B. cinerea* growth can be inhibited by polyphenols (Tao et al., 2010; Mendoza et al., 2013; Friedman, 2014; Patzke & Schieber, 2018). With respect to laccase inhibition, enological tannins seem to be a suitable alternative to conventional methods (Vignault et al., 2020). In this respect, it is important to consider that laccase secretion, substrate specificity, and activity differ between *Botrytis* strains (Zouari et al., 1987; Quijada-Morin et al., 2017; Zimdars et al., 2017). Neither the inhibition of laccase activity by polyphenols has been described so far nor the use of polymerized polyphenols, which resemble the products emerging in infected grapes. Such laccase-mediated products have not been tested for *B. cinerea* management at all.

This study aims to increase the knowledge about the relation of phenolic compounds and *B. cinerea* by giving first insights into alterations in laccase secretion and into the effects of polymerized polyphenols. Due to the pronounced complexity (Perna et al., 2018; Weber et al., 2019), the aim of this study does not include structural elucidation of those oxidation products. In view of the dependence of laccase secretion on the *Botrytis* strain (Zimdars et al., 2017), differences in enzymatic activity and mycelial growth between genetically distinguishable strains were further investigated. In this context, the effects of native phenols were compared to those of phenols obtained from laccase-induced oxidation. For this purpose, grape specific polyphenols and their laccase-mediated polymerized polyphenols were tested for their impact on growth and laccase activity of genetically different *B. cinerea* strains isolated from different grape varieties.

2. Materials and methods

2.1. Chemicals, reagents, and fungal strains

Malt extract-peptone agar was prepared using 10 $g*L^{-1}$ agar (Merck, Darmstadt, Germany), 30 $g*L^{-1}$ malt extract (Carl Roth, Karlsruhe, Germany and Extrakt Chemie Dr. Bruno Stellmach GmbH & Co. KG, Stadthagen, Germany) and 5 $g*L^{-1}$ soy peptone (Carl Roth, Karlsruhe, Germany). The phenolic compounds used were ferulic acid (purity $\geq$ 99 %, Thermo Scientific, Kandel, Germany), caffeic acid (purity $\geq$ 95 %, Merck, Darmstadt, Germany), *p*-coumaric acid (purity $\geq$ 98 %, Thermo Scientific, Kandel, Germany), (+)-catechin hydrate (purity $\geq$ 98 %, Sigma-Aldrich, Saint Louis, MO, USA), and resveratrol (purity 99.94 %, Apollo Scientific, Bredburry, UK). Sodium acetate buffer (46 mM, pH 5) contained sodium acetate (purity $\geq$ 98.5 %, Carl Roth, Karlsruhe, Germany) and glacial acetic acid (purity 99.8–100.5 %, VWR Chemicals, Radnor, PA, USA). *Botrytis cinerea* strains isolated from white and red grape varieties in the Palatinate region of Germany (Riesling, Scheurebe, Roter Riesling and one unknown red variety) as well as the reference strain DSM877 from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) were tested. All chemicals and materials used for experiments with *B. cinerea* were sterilized. Wild *B. cinerea* isolates used originated from the Palatinate Region and the following grape varieties: Strain A from Scheurebe, Strain B from Riesling, Strain C from Red Riesling, and Strain D from an unknown red variety.

2.2. PCR analysis of B. cinerea

Botrytis cinerea DNA was extracted following the RED Extract Plant PCR Kit protocol (Merck KGaA, Darmstadt, Germany). Mycelium was scratched from the agar plates using sterile pincers and transferred to a 2 mL capped reaction tube. Extraction solution (100 µL) was added to the tube and incubated in a heat block at 95 °C for 10 min at 300 rpm. After incubation, 100 µL of dilution solution was added and vortexed to mix. The sample was centrifuged at 16,200 *g* for 10 min and the supernatant was stored in the freezer until used later. The *B. cinerea* DNA obtained was then analyzed using PCR. Eight different simple sequence repeat (SSR) markers described by Fournier et al., 2002 were used. The following protocol was conducted according to Backmann et al., 2024: The SSR markers were paired in different combinations as shown in **Table A1**, which is displayed in Appendix A: Supplementary Data. PCR was performed using the adapted primer temperatures: 10 µL of RED Extract (Sigma-Aldrich), 4 µL MilliQ water, 1 µL forward/reverse primer each, and 4 µL sample DNA were added to the reaction tubes. PCR program: 94 °C for 3 min (initial denaturation), 94 °C for 30 s (denaturation), primer dependent temperature (50 °C, 60 °C) for 30 s (annealing), 72 °C for 30 s (elongation) for a total of 36 cycles, 72 °C for 10 min (final elongation). The PCR was performed on an Eppendorf MasterCycler personal 5332 (Eppendorf SE, Hamburg, Germany). PCR samples were stored at 4 °C or at –20 °C for later use.

For agarose gel electrophoresis, a 3.1 % agarose gel was used following the method by Backmann et al., 2024. For this purpose, 3.1 g Agarose (AppliChem GmbH, Darmstadt, Germany) was combined with 100 ml of 10X TAE buffer (AppliChem GmbH, Darmstadt, Germany) and microwaved at 600 W for 3 to 4 min. The mixture was cooled to 50 °C and 10 µL GelRed (GeneON, Ludwigshafen, Germany) was added. Aliquots of 5 µL of the PCR probes and 2 µL ladder (Thermo Fisher Scientific, Waltham, MA, USA), respectively, were added to the gel and incubated for 2:45 h at a current of 90 V. The resulting gel bands were analyzed using a UV-camera lens and the ImageJ software tool (Schneider et al., 2012) and compared to the ladder to obtain fragment lengths of the different primer pairs.

2.3. Spore suspensions

All strains were cultivated for two weeks at 25 °C in the dark on malt extract-peptone agar plates (diameter 8.4 cm, filled with 15–20 mL MPA). Afterwards, the plates were covered with approximately 10 mL of sterile Tween® 20 solution (0.1 % v/v, Carl Roth, Karlsruhe, Germany). The spores were harvested using a Drigalski spatula and the suspension filtered through sterile cheesecloth. For spore counting, a Fuchs–Rosenthal chamber was used. The spore suspensions contained at least 10^5 mL^{-1} and were stored at −20 °C.

2.4. Laccase-mediated oxidation of phenolic compounds

Oxidation of the five grape specific phenolic compounds (ferulic acid, caffeic acid, *p*-coumaric acid, (+)-catechin, and resveratrol) was performed with laccase from *Trametes versicolor* (Sigma-Aldrich, St. Louis, MO, USA) in a sodium acetate buffer (46 mM, pH 5) containing 10 % (v/v) acetone (min. 99.8 %, CHEMSOLUTE, Renningen, Germany) in case of resveratrol for enhanced solubility. Based on preliminary experiments, substrate-enzyme ratios were chosen as followed to obtain maximum yields: 100 U laccase from *T. versicolor* was used for the oxidation of either 29.8 mmol ferulic acid, 20.8 mmol caffeic acid, 14.9 mmol (+)-catechin, 7.44 mmol *p*-coumaric acid, or 11.9 mmol resveratrol. The reaction was conducted at 35 °C in an incubator for 24 h and was stopped by heat treatment at 70 °C for 5 min. Polymerization proceeded at 4 °C for 3 days and was followed by lyophilization (Beta 2–8 LSCbasic, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The yield of oxidation products was calculated gravimetrically. To determine potential residues of non-oxidized monomers in the reaction solution after incubation, an aliquot of the solution was analyzed using UHPLC-ESI-MS/MS (Appendix A). Residues were neither detectable nor quantifiable using external calibration.

2.5. Application of the compounds on agar plates with B. cinerea

For increased solubility of the structurally diverse compounds tested, dimethyl sulfoxide (1 % v/v, min. 99.9 %, CHEMSOLUTE, Renningen, Germany) or acetone (10 % v/v) were added to the aqueous solutions. The solvents did not show any impact on fungal growth or laccase activity at the concentrations used. All experiments were carried out in triplicate. A positive control was included, for which a commercially available botryticide was used in the recommended concentration. The compounds were applied in four different concentrations between 50 and 500 $mg*L^{-1}$ by spreading 2 mL solution on MPA plates. Inhibitory effects were calculated with respect to the negative control, which consisted of treatment with the same amount of sterile water.

2.6. Growth inhibitory effects

Agar plates (diameter 5.5 cm, filled with 10 mL MPA with a Dispensette® S, BRAND GMBH+CO KG, Wertheim, Germany) which had been treated as described in 2.5 were inoculated with 2500 spores of the respective *B. cinerea* strain. After incubation for 3 days at 25 °C in the dark, the plates were lyophilized and homogenized using liquid nitrogen. DNA was extracted using a REDExtract-N-Amp™ Plant PCR extraction kit according to the manufacturer's instructions. Subsequently, real-time PCR analysis (qPCR) was carried out in a MyGo Pro ESR cycler (IT-IS International Ltd., Stokesley, Middlesbrough, UK). For DNA amplification, specific *B. cinerea* primers targeting the ribosomal region between 28S and 18S genes (intergenic spacer) based on primers reported by Suarez et al. (2005) and Diguta et al. (2010) were used with slight modifications. Bc10nt_3R: 5'-GGA GCA ACA ATT CGC ATT TCA AAC ATG CTG −3' and Bc10nt_3F: 5'- GCT GTA ATT TCA ATG TGC AGA ATC CTG TCC CCG GT-3'. PowerTrack SYBR Green Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania) was used according to manufacturer's instructions with slight changes described in Appendix A: Supplementary Data. Sterile water served as no template control (NTC). Furthermore, ROX was used as a passive reference dye, which is included in the SYBR Green Master Mix. DNA was quantified using a calibration curve obtained from 10-fold template dilution of a 10^5 or 10^6 mL^{-1} spore suspension of the respective strain. The curve was generated by plotting the logarithm of the spore concentration against the C_t value determined by qPCR. The resulting $spores*ml^{-1}$ were converted into amount of DNA (m_{DNA}) by evaluating the average amount of DNA in 10^5 spores with an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA). Growth inhibitory effects were calculated through the following equation using the average of the triplicate ($\overline{m}_{DNA}$): $\text{Growth Inhibition}\,[\%] = \left(\overline{m}_{DNA(control)} - \overline{m}_{DNA(sample)}\right) * \overline{m}_{DNA(control)}{}^{-1} * 100\,\%$. Negative values represent growth enhancement.

2.7. Suppression of laccase activity

To induce production of external laccase, liquid cultivation in the presence of gallic acid (purity 98 %, Alfa Aesar, Ward Hill, MA, USA) was necessary (Marbach et al., 1983). For this purpose, 100 mL of medium containing 30 $g*L^{-1}$ malt extract, 5 $g*L^{-1}$ soy peptone, and 1 $g*L^{-1}$ gallic acid was filled in a 500 mL Erlenmeyer flask in duplicate for each strain. The flasks were inoculated with 10^5 spores and incubated at 25 °C for 3 days in the dark under continuous shaking. After the mycelium was homogenized using a T 25 digital Ultra-Turrax® (IKA-Werke GmbH & CO. KG, Staufen, Germany), 100 µL were transferred to the treated MPA plates in triplicate (diameter 8.4 cm, filled with 20 mL MPA with a Dispensette® S). The plates were incubated for another 11 days under the same conditions. A defined number of pieces (diameter 0.5 cm) was punched out and extracted with sodium acetate buffer (150 rpm at 35 °C for 90 min). Laccase activity was determined using the syringaldazine assay (Grassin & Dubourdieu, 1989), where the absorption was measured with a FLUOstar Omega microplate reader spectrophotometer (BMG Labtech, Ortenberg, Germany) at 595 nm as described by Zimdars et al. (2017). For quantification, a standard curve with laccase from *T. versicolor* was established. Determination of the biomass was carried out *via* qPCR, for which the plates were lyophilized, homogenized using liquid nitrogen and analyzed as described above (see 2.6). One unit (U_{Lacc}) was defined as the amount of enzyme oxidizing 1 µmol syringaldazine per minute. Correction of laccase activity by the corresponding biomass was conducted to obtain relative specific laccase activity [$U_{Lacc} * m_{DNA}^{-1}$]. The percentage of activity related to the water treatment was used as an indicator of inhibition. Negative values indicated induction of enzymatic activity.

2.8. Statistical analysis

For statistical analysis, XLSTAT software (Version 2019.1.1, Addinsoft Technologies, Paris, France) was used. An ANOVA (analysis of variance) with a defined significance level of $p < 0.05$ was performed in the case of variance homogeneity. A Dunnett's mean test was used to compare the samples with their respective control. Pearson correlation coefficients with different significance levels were calculated to determine correlations between variables.

3. Results and discussion

3.1. Laccase-mediated polymerization of phenolic compounds

We hypothesized that the effects of phenolic compounds on mycelial growth and laccase activity differ between genetically distinguishable *B. cinerea* strains. To assess the differences after application of native or oxidized polyphenols, laccase-oxidized grape-specific polyphenols were prepared in a semi-synthesis first. Oxidation of the selected five polyphenols was verified by the absence of educts in the reaction mix after

24 h analyzed *via* UHPLC-ESI-MS/MS. Further evidence was given by the formation of resulting laccase-specific dimeric compounds and a brown precipitate. Sufficient product yields of 74.5 – 100.0 % were obtained (data not shown). Residues of precipitated buffer and enzyme in the oxidation products were calculated and tested negative for any impact on *B. cinerea*. Potential residues of non-oxidized monomers in the precipitate were determined *via* UHPLC-ESI-MS/MS, which showed no antifungal effects and may thus be neglected.

3.2. Selection of B. cinerea strains

The selection of strains for the present study is based on the differentiation shown in Backmann et al., 2024. The agarose gel electrophoresis was able to distinguish all tested strains using the eight microsatellite markers described by Fournier et al., 2002. Each strain was assigned with an individual "fingerprint" resulting from the eight tested primer pairs, with different strains showing different fragment lengths between the primer pairs, which was demonstrated by Backmann et al., 2024. Even strains from geographical regions close to each other could be distinguished. Strain properties might differ between grape varieties, regions, and vintages due to adaption of the fungus to specific resilience or climate factors such as phenolic composition of the grape variety, leading to altered laccase expression.

3.3. Impact of phenolic compounds on B. cinerea growth

Alteration of fungal growth and relative specific laccase activity might be based on different mechanisms because laccase activity was corrected by the respective biomass and should therefore be considered separately. **Table A4**, included in Appendix A: Supplementary Data, displays the results of *B. cinerea* growth modulation. All compounds showed the ability to reduce total biomass of at least one strain, which confirms the antifungal properties of polyphenols. However, considerable differences between the strains were observed. Apart from strain D, the results for resveratrol are in complete agreement with existing studies on its antifungal properties. Strain D showed significant growth induction in the presence of resveratrol, which is somehow conflicting with the role of resveratrol as a phytoalexin (Adrian & Jeandet, 2012). Ferulic acid led to the inhibition of all strains except strain B, which was inhibited only after treatment with (+)–catechin. Inhibitory effects on the reference strain were more frequent than on the wild strains. It apparently possesses a higher sensitivity to the phenolic compounds in terms of mycelial growth. This behavior can be attributed to the well-known formation of resistances formed by *B. cinerea* wild strains against synthetic fungicides and natural compounds that are involved in the plants defense system (Nakajima & Akutsu, 2014; Fillinger & Walker, 2016). The strains tested not only show resistances against phenolic compounds but even growth stimulation, which is dependent on the fungal strain and polyphenol applied. Such growth promoting effects were also reported for oak-associated fungi with species-related differences in sensitivity regarding different phenolic compounds (Nickerson et al., 2023).

The results obtained for polymerized polyphenols confirm their ability to inhibit fungal growth (Claus et al., 2014) and demonstrate antifungal effects on *B. cinerea* specifically for the first time. Direct comparison between native and oxidized forms reveals differences. Polymerized caffeic acid showed almost exclusively growth inducing effects, whereas native caffeic acid exhibited great potential for growth inhibition. In contrast, polymerized catechin as well as polymerized coumaric acid demonstrated the same, or an even higher potential for inhibition than their native forms. Polymerized resveratrol was comparable to native resveratrol with respect to growth inhibitory effects, which is consistent with a previously reported mechanism of self-intoxication. Resveratrol acts as a natural profungicide and is converted by laccase into products of higher fungistatic activity (Schouten et al., 2002). No correlations between the growth modulation of native and oxidized polyphenols were found apart from one strain. Due to the lack of correlations, the corresponding data from the statistical evaluation are not shown. This finding supports the hypothesis that laccase-mediated oxidation products possess activities different from those of their non-oxidized precursors. Hence, polymerization of phenolic compounds can lead to increased inhibitory effects as well as inductive effects depending on the fungal strain and substrate. Structural differences in the monomeric substrates like the number and position of oxidizable groups can lead to heterogeneous polymerization products. These will have distinct molecular structures with different accessible functional groups, which influences interactions of the polymerized compounds with fungal cell walls and enzymes. In this context, hydrophobicity should be taken into account since the lipophilic character of the compounds represents a crucial factor regarding the mechanism of antimicrobial activities (Bouarab Chibane et al., 2019). The lack of knowledge about the chemical structures of the polymers precludes the proposition of inhibitory mechanisms and explanations concerning the different outcomes compared to their non-oxidized forms.

3.4. Impact of phenolic compounds on laccase activity

Phenolic compounds can induce laccase production since it is based on a stress-regulated gene expression (Marbach et al., 1984). Our findings additionally reveal that also laccase suppression up to complete inhibition may take place. The decrease in enzyme activity might be reasoned by several mechanisms. Suppression of the biomass-corrected laccase activity may result from lowered enzyme secretion. The expression of laccase-coding genes might be downregulated in the presence of phenolic compounds, leading to an attenuated production of the enzyme (Buddhika et al., 2020). Otherwise, the decreased activity may also be a result of direct inhibition by phenolic compounds interacting with the protein through van der Waals forces, hydrogen binding, hydrophobic binding, or electrostatic forces (Martinez-Gonzalez et al., 2017). For example, it was reported that enological tannins act as uncompetitive inhibitors of *Botrytis* laccase (Vignault et al., 2020). The hypothesis that secretion of laccase and the resulting enzyme activity is highly dependent on the *Botrytis* strain (Zimdars et al., 2017) is supported by the results displayed in **Table A5**. Broad differences in biomass-corrected laccase activity between the tested strains are obvious. The reference strain substantially differs in its enzymatic modulation compared to the wild strains. Strain D either showed strong induction or almost complete inhibition of laccase activity. It exhibits a high sensitivity against (+)-catechin and resveratrol and their respective polymers. In contrast, strain A was strikingly resistant regarding its laccase activity against all compounds applied. This fungal strain seems to display strong resistances in terms of laccase inhibition in general. Differences between fungal strains regarding their laccase-associated sensitivity might be linked to their genetic differences discussed in 3.2, which is corroborated by the results of recent studies showing that the secretion of laccase is strain-dependent (Park et al., 2015). However, the authors investigated the fungal response to external copper, which may not be comparable to the presence of polyphenols.

Like their non-oxidized precursors, polymerized phenolic compounds demonstrate inhibitory and inductive properties. Most likely, the underlying mechanism is different, given the substantially different molecular structure. Oxidation may lead to enhanced inhibitory effects, e.g., in case of (+)–catechin and resveratrol, or result in laccase induction, as observed for caffeic acid. Yet, these effects are highly dependent on the *Botrytis* strain and polymers applied. Enzyme–inhibiting properties of polymerized phenolic compounds have been described for polyphenol oxidase (Le Bourvellec et al., 2004; Uyama & Kobayashi, 2006), but enzyme-inducing effects of these compounds have not been discussed so far. Regarding the interactions between polymers and polyphenol oxidases, there is evidence that the degree of polymerization plays a role in inhibitory properties (Le Bourvellec et al., 2004).

3.5. Comparison of fungal growth and laccase activity

Investigation of biomass-corrected laccase activity allowed for a separate consideration of the influence on growth and enzyme activity. For an integrated interpretation, Fig. 1 shows inhibitory and inductive effects of the phenolic compounds applied on the five tested *B. cinerea* strains, where percentage of growth inhibition is plotted against percentage laccase activity. Samples are distributed over all quadrants of the scatter plots of all fungal strains. This distribution is very important as it underlines the fungus' ability to adapt mycelial growth and laccase activity independently. The hypothesis of independent regulatory mechanisms of growth and enzymatic activity is further supported by the lack of statistical correlations between the two inhibition parameters. The corresponding statistical results may not be shown because of the extent of the tables.

The scatterplots emphasize the differences between fungal strains in their overall response to the applications as discussed in chapters 3.3 and 3.4. Strains A and C show robustness in terms of laccase activity. In contrast, strain D was highly sensitive in its laccase activity and more robust in its mycelial growth. The reference strain and strain B showed high sensitivity in general, since a certain number of samples can be found in the first quadrant. This quadrant comprises applications that were able to inhibit growth and laccase activity at the same time. The differences observed between strains might result from the genetic varieties discussed in 3.2. Research on population genetics showed many structuring factors leading to genetic diversity, with the host crop being the most important factor (Walker, 2016). In the case of grapevine as a host, secondary metabolites like polyphenols represent a crucial part, leading to the assumption of a link between the strain-dependent differences observed here and the polyphenol profile of the original grape variety. However, the assertion that resistance formation is a function of grapevine secondary metabolites is limited by the natural variations of the polyphenol profile due to abiotic parameters such as temperature, water availability, and many others (Nadal, 2010).

Fig. 2 focuses on the first quadrants of the scatterplots shown in Fig. 1 including information on the concentrations applied. Growth suppressing effects can already be obtained at concentrations around 0.05 $g*L^{-1}$, which is comparable to previously reported minimal inhibitory concentrations (MIC) of polyphenols or even lower, but MICs are highly dependent on the *Botrytis* strain (Adrian & Jeandet, 2012; Xu et al., 2018). Concentrations of 0.05 $g*L^{-1}$ can suppress laccase activity as well, depending on the compound and *Botrytis* strain. Recent studies discovered significant effects in laccase reduction by application of at least 0.5 $g*L^{-1}$ tannins, which exceeds the concentrations used in the present study (Vignault et al., 2020). It should be noted that the application of tannins was conducted in botrytized must, whereas this study focuses on the effect on laccase secreted into malt extract peptone agar. Dunnett's test showed significant reductions in biomass and laccase activity compared to the respective controls for all strains apart from DSM877. The only compounds capable of reducing fungal biomass and laccase activity of all tested strains were resveratrol and its polymers. These results reveal a potential of phenolic compounds to inhibit *Botrytis* wild strains not only in terms of fungal growth but also laccase activity.

4. Conclusions

Modulation of growth and laccase activity of *B. cinerea* in response to application of phenolic compounds appears to follow independent regulatory mechanisms. The modes of actions are yet to be elucidated. The lack of knowledge about the structure of laccase-oxidized polyphenols hinders a conclusion about their interactions with fungal cell walls or enzymes. The insufficient knowledge about the biological basis for the regulation of laccase genes compromises any conclusion of the role of (polymerized) polyphenols in this mechanism.

Due to the vast number of *B. cinerea* resistances, there is an urgent need to develop new viticultural strategies against this pathogen. The results of the research presented in this study demonstrate significant inducing effects, indicating that treatment of *B. cinerea* with phenolic compounds poses the risk of accelerating the infection or laccase-associated quality losses. These effects are highly dependent on the specific fungal strain. Host adaption, apart from fungicide resistances, constitute the most crucial factors in genetic diversity and might be related to the significant differences between the genetically distinguishable strains tested here. This also indicates that the results obtained for laboratory strains need to be interpreted with caution because wild strains might show a different behavior. Future investigations into the

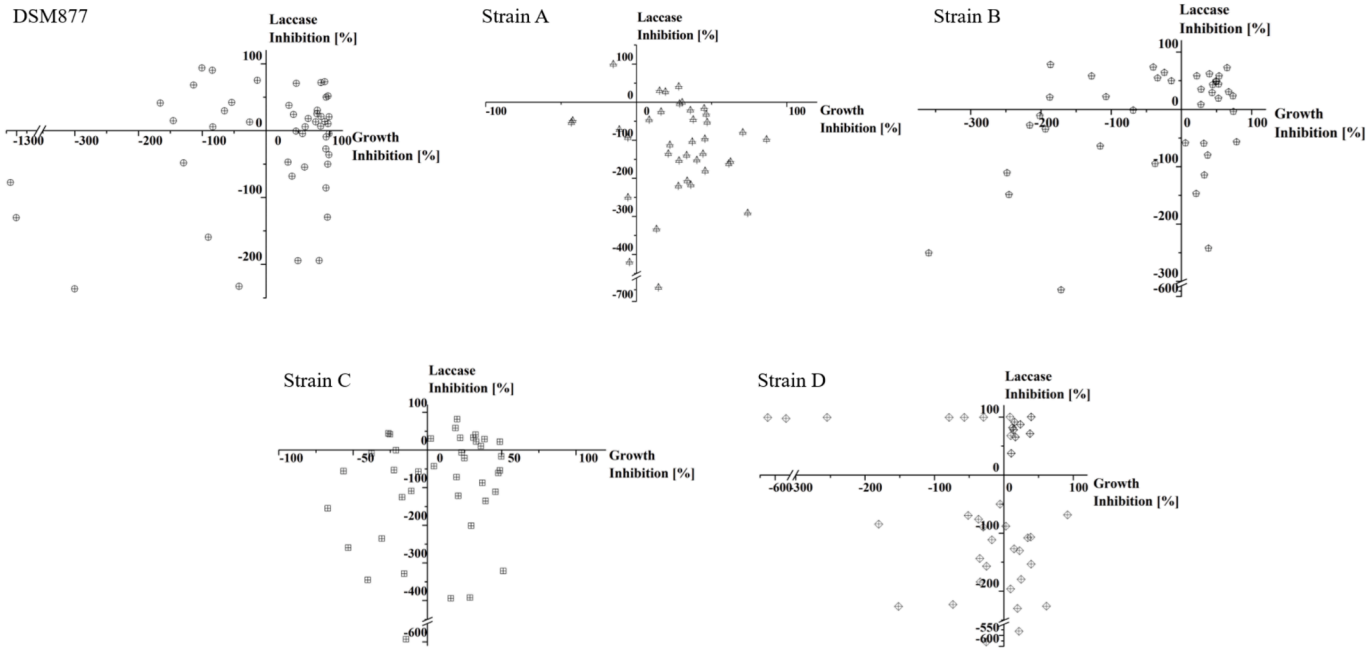

Fig. 1. Simultaneous effects of phenolic compounds on *B. cinerea* growth and laccase activity. Growth inhibition in percentage is displayed against laccase inhibition in percentage. Negative values indicate inductive effects.

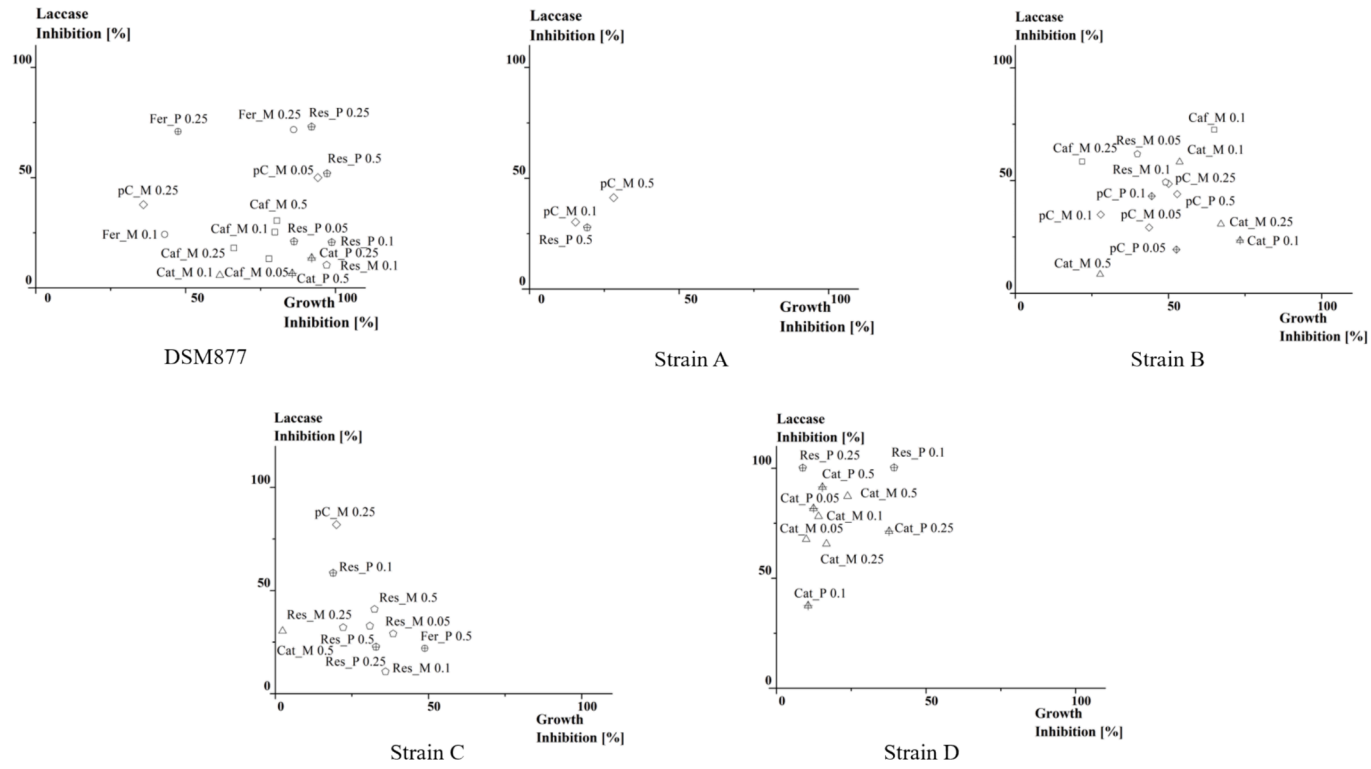

Fig. 2. Simultaneous inhibition of mycelial growth and laccase activity of *B. cinerea* strains after treatment with phenolic compounds. Fer, ferulic acid; caf, caffeic acid; cat, (+)-catechin; pC, *p*-coumaric acid; res, resveratrol; m, monomer; p, polymer. Numbers indicate the applied concentration in $g*L^{-1}$.

correlations between genetic characteristics of *B. cinerea* strains and growth- and enzyme-modulating patterns may support the development of strategies for sustainable *Botrytis* management and a diagnostic methodology for an early detection in the vineyard. Resveratrol and its laccase-mediated polymers possess great potential for *B. cinerea* management in general and with respect to the uprising challenges coming along with the high genetic differences between strains.

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CRediT authorship contribution statement

Kim Marie Umberath: Writing – original draft, Investigation, Data curation. **Anna Mischke:** Investigation. **Rita Caspers-Weiffenbach:** Investigation. **Louis Backmann:** Investigation. **Maren Scharfenberger-Schmeer:** Writing – review & editing, Funding acquisition. **Pascal Wegmann-Herr:** Funding acquisition, Conceptualization. **Andreas Schieber:** Writing – review & editing, Funding acquisition. **Fabian Weber:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kim Marie Umberath reports financial support was provided by German Ministry of Economics and Technology. Louis Backmann reports financial support was provided by German Ministry of Economics and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114782.

References

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

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C., & Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. *Frontiers in Plant Science, 7*, 382. https://doi.org/10.3389/fpls.2016.00382

Avenot, H. F., Morgan, D. P., Quattrini, J., & Michailides, T. J. (2020). Phenotypic and molecular characterization of fenhexamid resistance in *Botrytis cinerea* isolates collected from pistachio orchards and grape vineyards in California. *Crop Protection, 133*, Article 105133. https://doi.org/10.1016/j.cropro.2020.105133

Backmann, L., Schmidtmann, K., Wegmann-Herr, P., Jürgens, A., & Scharfenberger-Schmeer, M. (2024). Molecular biological methods to assess different *Botrytis cinerea* strains on grapes. *Microbiol Res, 15*(2), 567–581. https://doi.org/10.3390/microbiolres15020037

Bertetti, D., Monchiero, M., Garibaldi, A., & Gullino, M. L. (2020). Monitoring activities on fungicide resistance in *Botrytis cinerea* carried out in vineyards in North-West Italy in 2018. *Journal of Plant Diseases and Protection, 127*, 123–127. https://doi.org/10.1007/s41348-019-00263-3

Bouarab Chibane, L., Degraeve, P., Ferhout, H., Bouajila, J., & Oulahal, N. (2019). Plant antimicrobial polyphenols as potential natural food preservatives. *Journal of the Science of Food and Agriculture, 99*, 1457–1474. https://doi.org/10.1002/jsfa.9357

Buddhika, U. V. A., Savocchia, S., & Steel, C. C. (2020). Copper induces transcription of BcLCC2 laccase gene in phytopathogenic fungus, *Botrytis cinerea*. *Mycology, 12*, 48–57. https://doi.org/10.1080/21501203.2020.1725677

Claus, H., Sabel, A., & König, H. (2014). Wine phenols and laccase: An ambivalent relationship. In Y. El Rayess (Ed.), *Wine: Phenolic composition, classification and health benefits* (pp. 155–185). USA: New York.

Claus, H. (2017). Laccases of *Botrytis cinerea*. In H. König, G. Unden, & J. Fröhlich (Eds.), *Biology of microorganisms on grapes, in must and in wine* (pp. 339–356). Berlin/Heidelberg: Springer International Publishing.

Claus, H. (2020). How to deal with uninvited guests in wine: Copper and copper-containing oxidases. *Fermentation, 6*, 38. https://doi.org/10.3390/fermentation6010038

Cotoras, M., Folch, C., & Mendoza, L. (2004). Characterization of the antifungal activity on *Botrytis cinerea* of the natural diterpenoids kaurenoic acid and 3beta-hydroxy-kaurenoic acid. *Journal of Agricultural and Food Chemistry, 52*, 2821–2826. https://doi.org/10.1021/jf030672j

Dean, R., van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular plant pathology, 13*, 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

DeLong, J. A., Saito, S., Xiao, C.-L., & Naegele, R. P. (2020). Population genetics and fungicide resistance of *Botrytis cinerea* on *Vitis* and *Prunus* spp. in California. *Phytopathology, 110*, 694–702. https://doi.org/10.1094/PHYTO-09-19-0362-R

DeSimone, N., Pace, B., Grieco, F., Chimienti, M., Tyibilika, V., Santoro, V., Capozzi, V., Colelli, G., Spano, G., & Russo, P. (2020). *Botrytis cinerea* and table grapes: A review of the main physical, chemical, and bio-based control treatments in post-harvest. *Foods, 9*. https://doi.org/10.3390/foods9091138

Diguta, C. F., Rousseaux, S., Weidmann, S., Bretin, N., Vincent, B., Guilloux-Benatier, M., & Alexandre, H. (2010). Development of a qPCR assay for specific quantification of *Botrytis cinerea* on grapes. *FEMS microbiology letters, 313*, 81–87. https://doi.org/10.1111/j.1574-6968.2010.02127.x

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature, 411*, 843–847. https://doi.org/10.1038/35081178

Fillinger, S., & Walker, A.-S. (2016). Chemical control and resistance management of *Botrytis* diseases. In S. Fillinger, & Y. Elad (Eds.), *Botrytis – the fungus, the pathogen and its management in agricultural systems* (pp. 189–216). Cham, Heidelberg, New York: Springer.

Fournier, E., Giraud, T., Loiseau, A., Vautrin, D., Estoup, A., Solignac, M., Cornuet, J. M., & Brygoo, Y. (2002). Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). *Molecular Ecology Notes, 2*, 253–255. https://doi.org/10.1046/j.1471-8286.2002.00207.x

Friedman, M. (2014). Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *Journal of Agricultural and Food Chemistry, 62*, 6025–6042. https://doi.org/10.1021/jf501266s

Gil-ad, N. L., Bar-Nun, N., & Mayer, A. M. (2001). The possible function of the glucan sheath of *Botrytis cinerea*: Effects on the distribution of enzyme activities. *FEMS microbiology letters, 199*, 109–113. https://doi.org/10.1111/j.1574-6968.2001.tb10659.x

Grassin, C., & Dubourdieu, D. (1989). Quantitative determination of *Botrytis* laccase in musts and wines by the syringaldazine test. *Journal of the Science of Food and Agriculture, 48*, 369–376. https://doi.org/10.1002/jsfa.2740480312

Jacometti, M. A., Wratten, S. D., & Walter, M. (2010). Review: Alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. *Australian Journal of Grape and Wine Research, 16*, 154–172. https://doi.org/10.1111/j.1755-0238.2009.0067.x

Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Gény, L., Rey, P., Doneche, B., & Teissedre, P.-L. (2012). Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. *Australian Journal of Grape and Wine Research, 18*, 215–226. https://doi.org/10.1111/j.1755-0238.2012.00191.x

La Guerche, S., Dauphin, B., Pons, M., Blancard, D., & Darriet, P. (2006). Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. *Journal of Agricultural and Food Chemistry, 54*, 9193–9200. https://doi.org/10.1021/jf0615294

Le Bourvellec, C., Le Quéré, J.-M., Sanoner, P., Drilleau, J.-F., & Guyot, S. (2004). Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. *Journal of Agricultural and Food Chemistry, 52*, 122–130. https://doi.org/10.1021/jf034461q

Marbach, I., Harel, E., & Mayer, A. M. (1983). Inducer and culture medium dependent properties of extracellular laccase from *Botrytis cinerea*. *Phytochemistry, 22*, 1535–1538. https://doi.org/10.1016/0031-9422(83)80083-1

Marbach, I., Harel, E., & Mayer, A. M. (1984). Molecular properties of extracellular *Botrytis cinerea* laccase. *Phytochemistry, 23*, 2713–2717. https://doi.org/10.1016/0031-9422(84)83001-0

Martinez-Gonzalez, A. I., Díaz-Sánchez, Á. G., La Rosa, L. A.d., Vargas-Requena, C. L., Bustos-Jaimes, I., & Alvarez-Parrilla, A. E. (2017). Polyphenolic compounds and digestive enzymes: *In vitro* non-covalent interactions. *Molecules, 22*, 669. https://doi.org/10.3390/molecules22040669

Mayer, A. M., & Harel, E. (1978). Polyphenol oxidases in plants. *Phytochemistry, 193–215*. https://doi.org/10.1016/0031-9422(79)80057-6

Mendoza, L., Yañez, K., Vivanco, M., Melo, R., & Cotoras, M. (2013). Characterization of extracts from winery by-products with antifungal activity against *Botrytis cinerea*. *Industrial Crops and Products, 43*, 360–364. https://doi.org/10.1016/j.indcrop.2012.07.048

Morales, J., Mendoza, L., & Cotoras, M. (2017). Alteration of oxidative phosphorylation as a possible mechanism of the antifungal action of *p*-coumaric acid against *Botrytis cinerea*. *Journal of Applied Microbiology, 123*, 969–976. https://doi.org/10.1111/jam.13540

Mullins, M. G., Bouquet, A., & Williams, L. E. (1992). *Biology of the grapevine. Biology of horticultural crops*. Cambridge: Cambridge University Press.

Nadal, M. (2010). Phenolic maturity in red grapes. In S. Delrot, H. Medrano, E. Or, L. Bavaresco, & S. Grando (Eds.), *Methodologies and Results in Grapevine Research* (pp. 389–409). Dordrecht: Springer, Netherlands.

Nakajima, M., & Akutsu, K. (2014). Virulence factors of *Botrytis cinerea*. *Journal of General Plant Pathology, 80*, 15–23. https://doi.org/10.1007/s10327-013-0492-0

Nickerson, M. N., Moore, L. P., & U'Ren, J. M. (2023). The impact of polyphenolic compounds on the *in vitro* growth of oak-associated foliar endophytic and saprotrophic fungi. *Fungal Ecology, 62*, Article 101226. https://doi.org/10.1016/j.funeco.2023.101226

Park, J.-W., Kang, H.-W., Ha, B.-S., Kim, S.-I., Kim, S., & Ro, H.-S. (2015). Strain-dependent response to Cu(2+) in the expression of laccase in *Pycnoporus coccineus*. *Archives of Microbiology, 197*, 589–596. https://doi.org/10.1007/s00203-015-1090-7

Patzke, H., & Schieber, A. (2018). Growth-inhibitory activity of phenolic compounds applied in an emulsifiable concentrate - ferulic acid as a natural pesticide against *Botrytis cinerea*. *Food Research International, 113*, 18–23. https://doi.org/10.1016/j.foodres.2018.06.062

Perna, V., Agger, J. W., Holck, J., & Meyer, A. S. (2018). Multiple reaction monitoring for quantitative laccase kinetics by LC-MS. *Scientific Reports, 8*, 8114. https://doi.org/10.1038/s41598-018-26523-0

Quijada-Morin, N., Garcia, F., Lambert, K., Walker, A.-S., Tiers, L., Viaud, M., Sauvage, F.-X., Hirtz, C., & Saucier, C. (2017). Strain effect on extracellular laccase activities from *Botrytis cinerea*. *Australian Journal of Grape and Wine Research, 24*, 241–251. https://doi.org/10.1111/ajgw.12322

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., & Lonvaud, A. (2006). *Handbook of enology: Vol. I: The microbiology of wine and vinifications*. Chichester, United Kingdom: John Wiley & Sons Ltd.

Romanazzi, G., & Feliziani, E. (2014). *Botrytis cinerea* (Gray Mold). In S. Bautista-Banos (Ed.), *Postharvest Decay: Control Strategies* (pp. 131–146). Elsevier.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schouten, A., Wagemakers, L., Stefanato, F. L., van der Kaaij, R. M., & van Kan, J. A. L. (2002). Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. *Molecular Microbiology, 43*, 883–894. https://doi.org/10.1046/j.1365-2958.2002.02801.x

Steel, C. C., Blackman, J. W., & Schmidtke, L. M. (2013). Grapevine bunch rots: Impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry, 61*, 5189–5206. https://doi.org/10.1021/jf400641r

Suarez, M. B., Walsh, K., Boonham, N., O'Neill, T., Pearson, S., & Barker, I. (2005). Development of real-time PCR (TaqMan) assays for the detection and quantification of *Botrytis cinerea* in planta. *Plant physiology and biochemistry PPB, 43*, 890–899. https://doi.org/10.1016/j.plaphy.2005.07.003

Tao, S., Zhang, S., Tsao, R., Charles, M. T., Yang, R., & Khanizadeh, S. (2010). *In vitro* antifungal activity and mode of action of selected polyphenolic antioxidants on *Botrytis cinerea*. *Archives Of Phytopathology and Plant Protection, 43*, 1564–1578. https://doi.org/10.1080/03235400802583834

Thibon, C., Dubourdieu, D., Darriet, P., & Tominaga, T. (2009). Impact of noble rot on the aroma precursor of 3-sulfanylhexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food chemistry, 114*, 1359–1364. https://doi.org/10.1016/j.foodchem.2008.11.016

Thurston, C. F. (1994). The structure and function of fungal laccases. *Microbiology, 19–26*. https://doi.org/10.1099/13500872-140-1-19

Uyama, H., & Kobayashi, S. (2006). Enzymatic synthesis and properties of polymers from polyphenols. In S. Kobayashi, H. Ritter, & D. Kaplan (Eds.), *Enzyme-Catalyzed Synthesis of Polymers* (pp. 51–67). Berlin/Heidelberg: Springer-Verlag.

Vignault, A., Gombau, J., Jourdes, M., Moine, V., Canals, J. M., Fermaud, M., Roudet, J., Zamora, F., & Teissedre, P.-L. (2020). Oenological tannins to prevent *Botrytis cinerea* damage in grapes and musts: Kinetics and electrophoresis characterization of laccase. *Food Chemistry, 126334*. https://doi.org/10.1016/j.foodchem.2020.126334

Walker, A.-S. (2016). Diversity within and between species of *Botrytis*. In S. Fillinger, & Y. Elad (Eds.), *Botrytis: The fungus, the pathogen and its management in agricultural systems* (pp. 91–125). Cham, Heidelberg, New York, Dordrecht, London: Springer.

Weber, F., Engelke, G. H., & Schieber, A. (2019). Structure elucidation and tentative formation pathway of a red colored oxidation product of caffeic acid. *Food Chemistry, 297*, Article 124932. https://doi.org/10.1016/j.foodchem.2019.05.206

Williamson, B., Tudzynski, B., Tudzynski, P., & van Kan, J. A. L. (2007). *Botrytis cinerea*: The cause of grey mould disease. *Molecular Plant Pathology, 8*, 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x

Wink, M. (2015). Sekundärstoffe - die Geheimwaffen der Pflanzen. *Biologie in unserer Zeit, 45*, 225–235. https://doi.org/10.1002/biuz.201510569

Xu, D., Yu, G., Xi, P., Kong, X., Wang, Q., Gao, L., & Jiang, Z. (2018). Synergistic effects of resveratrol and pyrimethanil against *Botrytis cinerea* on grape. *Molecules, 23*, 1455. https://doi.org/10.3390/molecules23061455

Zimdars, S., Hitschler, J., Schieber, A., & Weber, F. (2017). Oxidation of wine polyphenols by secretomes of wild *Botrytis cinerea* strains from white and red grape varieties and determination of their specific laccase activity. *Journal of Agricultural and Food Chemistry, 65*, 10582–10590. https://doi.org/10.1021/acs.jafc.7b04375

Zouari, N., Romette, J.-L., & Thomas, D. (1987). Purification and properties of two laccase isoenzymes produced by *Botrytis cinerea*. *Applied Biochemistry and Biotechnology, 15*, 213–225. https://doi.org/10.1007/bf02798450