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An emerging problem affecting apple production: *Neofusicoccum parvum*. *Aureobasidium pullulans* L1 and L8 strains as an alternative control strategy

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ABSTRACT

Aureobasidium pullulans strains L1 and L8 were used as an alternative strategy against *Neofusicoccum parvum*, pathogen of apple wood and fruit. Both strains displayed a great ability to reduce pathogen conidia germination of almost 50% and totally stop its sporification in wood of 'Gala' and 'Fuji' apple cultivars. In addition, by *in vitro* tests the antagonistic activity displayed by non-volatile metabolites produced by both yeasts was highlighted by 70% of reduction of the pathogen mycelial growth. On apple fruit, the pathogen severity was reduced by 66% and 50% in 'Gala', and 70% and 60% in 'Fuji' by L1 and L8 respectively. In wood, no statistical differences were observed between L1, L8 and the control, except for 'Fuji' wood, where L8 strain displayed a wood lesion reduction of 23% with respect to the untreated control. Infrared analysis showed how the presence of both strains on woody tissues helped preserving cellulose content, preventing in this way the faster wood degradation by the pathogen. In addition, L1 and L8 simply colonized the wood surface, probably displaying an endophytic attitude, an essential requirement for controlling *N. parvum* in field.

1. Introduction

Neofusicoccum parvum is often associated with numerous woody hosts worldwide (von Arx, 1987) and represents one of the principal species with marked ability to cause diseases (Slippers and Wingfield, 2007; Sakalidis et al., 2013), principally on tree species such as grapevine (Spagnolo et al., 2014; Úrbez-Torres, 2011), apple (Cloete et al., 2011; Rooney-Latham and Soriano, 2016), peach (Thomidis et al., 2011), and kiwifruit (Wu, 2017). The fungus was firstly identified as *Botryosphaeria parva*, belonging to a complex of woody fungi also able to infect fruits, such as apple (Delgado-Cerrone et al., 2016). Moreover, the constant increase of temperatures has probably led to an increase in the occurrence of *N. parvum* infection also in European regions such as Italy (Prodorutti et al., 2012), causing significant market apple fruit losses. In fact, climate changes can influence many ecosystems, including orchards, so increasing the pathogen potential damage (Slippers et al., 2013; Úrbez-Torres, 2011). *Botryosphaeraceae* diseases

control was consistently focused on the use of different synthetic fungicides such as benomyl and tebuconazole (Bester et al., 2007), but different studies showed a decrease in efficiency in time after treatment. For this, the progressive use of fungicides for plant pathogens control requires regular monitoring of pathogen populations for resistance. Alternative strategies such as the use of antagonistic microorganisms offer the potential to provide sustainable and long-term protection of crops (Kotze et al., 2011). The application of biological control agents (BCAs) is considered a safe and environmentally friendly alternative (Zhang et al., 2010) and could represent an alternative method to control *N. parvum* disease. In fact, recent studies showed how BCAs such as *Burkholderia* sp, *Trichoderma harzianum*, and *Bacillus subtilis* (Wu, 2017; Kotze et al., 2011; Alfonzo et al., 2009) were used for increasing resistance in host plants, and for directly compete against the target pathogen since proven to be able to be involved in complex interaction systems between host, pathogen, and environment (Oliveira et al., 2016).

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Among recent studies about BCAs, *Aureobasidium pullulans* molecularly characterized by Di Francesco et al., (2018a) showed a high efficacy principally against fruit postharvest pathogens (Zhang et al., 2010; Di Francesco et al., 2017, 2018a). In the present study, both strains were tested against *N. parvum* of apple wood and fruit as an alternative method to fungicides.

The objectives of this study were to evaluate the efficacy of L1 and L8 *A. pullulans* strains against *N. parvum* through *in vitro* assays for verifying the effect of volatile and non-volatile metabolites produced by the two strains on the target pathogen growth and the antagonistic effect on pathogen conidia germination, and through *in vivo* assays detecting the efficacy of both antagonistic strains against the pathogen in apple wood and fruit. In particular, the effects of fungal attack on the main chemical components of apple wood (i.e. cellulose, hemicellulose and lignin) were evaluated by using infrared (IR) spectroscopy analysis, a technique that has already proved to be useful to monitor analogous transformations (Pandey and Nagveni, 2007; Ozgenc et al., 2018; Beck et al., 2018; Lucejko et al., 2018). Moreover, the same spectroscopic technique has been applied in order to distinguish the different yeasts glucans in polysaccharide matrices of various origin (Synytsya and Novak, 2014). As a matter of fact, *A. pullulans* produces both pullulan: α -glucan that may act as a protective agent on cell membranes and β -glucans that can elicit cells' response to fungal attack (Bozoudi and Tsaltas, 2018). This paper describes the first application of IR spectroscopy to monitor the antagonistic effect on fungal wood decay in fruit trees.

2. Material and method

2.1. Antagonists

Aureobasidium pullulans strains L1 and L8 molecularly characterized by Di Francesco et al., (2018a) were maintained on nutrient yeast dextrose agar (NYDA) consisting of nutrient broth: 8 g; yeast extract: 5 g; dextrose: 10 g and technical agar: 15 g (Oxoid, Basingstoke, UK) in 1 L of distilled water and stored at 4 °C until use. Two days before the experiments, each antagonist was grown on NYDA at 25 °C, and yeast cells were collected in sterile distilled water containing 0.05% (v/v) of Tween80 (Sigma-Aldrich, St. Louis, MO, USA) then adjusted to a final concentration of 10^8 cell mL⁻¹.

2.2. Pathogen

Neofusicoccum parvum (strain NP1) was isolated from cankers of internal wood necrosis of 'Gala' apple tree located in the experimental orchards of the State University of Mid-Western of Paraná (Brazil) and molecularly identified by sequencing of ribosomal DNA ITS regions analysis.

For *in vitro* experiments, *N. parvum* was grown on Malt Extract Agar (MEA, 50 g per 1000 mL of distilled water) (Sigma, St. Louis, MO, USA) in Petri dishes incubated at 25 °C $\pm$ 1 °C with 12-h dark: 12-h light cycles for 8 d and maintained at 4 °C until use. For the *in vivo* experiments, conidial suspensions were prepared by washing *N. parvum* pycnidia grown on 'Gala' twigs for 12 d. Conidia were collected in sterile distilled water containing 0.05% (v/v) of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) then adjusted to a final concentration of 10^5 cell mL⁻¹ with hemocytometer.

2.3. Plant material: apple twigs and fruits

Twigs (50 cm long) were collected from 'Fuji' and 'Gala' apple trees of an experimental orchard located in Cadriano (Bologna, Italy), stored at 5 °C, and used within 5 d of collection. 'Fuji' and 'Gala' apple fruits (*Malus domestica* L. Borkh) were obtained from the same above-mentioned experimental orchards. Harvested fruits, homogeneous in size, were stored at 0 °C and used for experiments within 5 d after harvest.

2.4. Effect of L1 and L8 strains on *Neofusicoccum parvum* conidia germination

The antifungal activity assay was performed on a microscope slide; aliquots of 25 μ L of *N. parvum* 10^5 conidia mL⁻¹ were added to the same aliquots of yeasts (10^8 cells mL⁻¹) suspensions. Slides were introduced in a sterile Petri dish and the dish was immediately sealed with a layer of Parafilm (Pechiney Plastic Packaging, USA) to maintain high humidity. After 9 h of incubation at 25 °C, the slide was observed through a microscope (Nikon Eclipse TE2000-E) and the conidia germination (30 conidia per microscopic field) of each sample were determined. An aliquot of 25 μ L of distilled water was considered as the control. For each treatment, three microscope slides (replicates) were observed. The experiment was repeated twice.

2.5. Antagonistic effect of volatile and non-volatile metabolites

The effects of L1 and L8 strains volatile and non-volatile metabolites on *N. parvum* mycelial growth were investigated by *in vitro* experiments.

The effect of volatile metabolites on pathogen colony growth was determined as described by Di Francesco et al., (2015). For this purpose, NYDA plates were inoculated by spreading 100 μ L of *A. pullulans* cell suspension (10^8 cell mL⁻¹) of L1 and L8, respectively. The lid of the plate was replaced, after 48 h of incubation at 25 °C, by a base plate of MEA inoculated with 5 mm pathogen mycelial plug. The two base plates were sealed immediately with a double layer of Parafilm and incubated at 25 °C for 5 d. Plates inoculated with the pathogen only represented the control. Ten plates for each strain and control were used.

The action of L1 and L8 non-volatile metabolites on pathogen colony growth was determined according to Lundberg and Unestam (1980), and Dennis and Webster (1971) methods. Plugs (5 mm diameter) of each yeast strain cultured for 48 h in NYDA were grown on a sterile cellophane disc (Courtauld Films: 90 mm thick) laying on MEA Petri dishes for 48 h at 25 °C; the cellophane with the colony was then removed and, in the same position where the BCAs were grown, a mycelial plug 5 mm diameter of *N. parvum* was inoculated. A NYDA plug without antagonist positioned on cellophane represented the control. Ten plates for each antagonist and control were used. Each experiment was repeated twice.

2.6. In vivo experiments: apple wood and fruit

Apple twigs, disinfected with 70% alcohol and air-dried were cut using a sterile blade on intermediate internodes. Superficial wounds were inoculated with 50 μ L of *A. pullulans* L1 and L8 (10^8 cell mL⁻¹) suspensions and subsequently air-dried for 24 h. A mycelium agar plug (4 mm) from 8 d-old fungal colony was placed in the wound and wrapped with sterile cotton wetted by 5 mL of sterile water and suddenly covered with Parafilm. Twigs were soaked in 200 mL of sterile water and incubated at room temperature. Negative control was constituted by twigs treated with sterile water and after inoculated with pathogen mycelium plug. Fifteen twigs for each antagonist and control of both cultivars were used. The lesions were evaluated after 12 d of inoculation and measured by the software ImageJ 1.x (Wisconsin, USA). Apple fruits were sterilized in sodium hypochlorite solution (1%), washed with tap water, air-dried and inoculated through artificial wounds by a sterile nail (3 $\times$ 3 $\times$ 3 mm) on the equator (one wound per fruit) with 20 μ L of antagonists suspensions (10^8 cell mL⁻¹ respectively for L1 and L8). After 1 h, 20 μ L of *N. parvum* suspension (10^5 conidia mL⁻¹) were inoculated in treated fruit wound. The control consisted of fruits inoculated with sterile water. The apples were kept at 20 °C and 90% of relative humidity (RH). The lesion diameters were measured after 5 d from the inoculation. Fifteen apples for each antagonist and control of both cultivars were used. Each experiment was repeated twice.

2.7. Wood IR/ATR spectroscopy

‘Fuji’ and ‘Gala’ twigs were inoculated with 50 μ L of *A. pullulans* L1 and L8 (10^8 cell mL^{-1}) suspensions and subsequently air-dried for 24 h. A mycelium agar plug (4 mm) from an 8 d-old fungal colony was placed in the wound and wrapped with sterile cotton wetted by 5 mL of sterile water and suddenly covered with Parafilm. Twigs were soaked in 200 mL of sterile water and incubated at room temperature. Negative control was constituted by twigs treated with sterile water and after inoculated with pathogen mycelium plug. After 12 d, portions of decay tissues from fifteen twigs for each antagonist and control of both cultivars were examined by IR spectroscopy. IR spectra were recorded with a Bruker ALPHA series FT-IR spectrophotometer (Bruker, Ettlingen, Germany) equipped with a crystal diamond cell for attenuated total reflectance (ATR). The ATR configuration was chosen in order to preserve samples integrity and to focus the analysis on the surface of apple wood; in fact, the penetration depth of the IR radiation in the ATR mode in cellulosic materials was estimated to be in the range 2–3 μ m (Rampazzo and Di Foggia, 2018). The spectra were collected from 4000 to 400 cm^{-1} and averaged over 100 scans (resolution = 4 cm^{-1} on five different wood samples for each yeast strain).

2.8. Statistical analysis

All experiments were carried out in a completely randomized design. Obtained data were analyzed by one-way ANOVA ($P < 0.05$). Separation of means was performed using the least significant difference (LSD) test, at $P < 0.05$. All analyses were performed with the software Statgraphic Plus Version 2.1 (Statistical Graphics Corp., USA 1996).

3. Results

3.1. Effect of L1 and L8 strains on *Neofusicoccum parvum* conidia germination and mycelial growth

The antagonistic activity of both yeast strains was tested on *N. parvum* conidia and their efficacy for germination inhibition evaluated, showing that L1 and L8 *A. pullulans* strains reduced *N. parvum* conidia germination of 48.5% and 52.9% respectively (Table 1).

The activity of both volatile and non-volatile metabolites produced by L1 and L8 on *N. parvum* mycelial growth was then analyzed through double Petri dishes assay. With respect to volatile metabolites both L1 and L8 strain did not significantly reduce the pathogen colony growth compared to control. Conversely, in non-volatile metabolites interaction experiment, both L1 and L8 strains significantly reduced the pathogen colony growth (69.2%) with respect to the control without a substantial difference between two antagonists (Table 1).

Table 1

Effect of *Aureobasidium pullulans* L1 and L8 strains on *Neofusicoccum parvum* conidia germination (%) after 9 h of co-culturing and on pathogen colony growth (mm) exposed to antagonists volatile and non-volatile metabolites for 5 days. Data are the means of three independent experiments $\pm$ standard deviation, each consisting of three replicates per treatment. Different letters within the same column indicate significant differences, LSD test ($P < 0.05$).

	Experiments		
	Conidia germination (%)	Volatile metabolites	Non-volatile metabolites
Control	68 $\pm$ 5.89b	60 $\pm$ 5.20a	65 $\pm$ 8.21b
L1	35 $\pm$ 3.49a	59 $\pm$ 9.54a	20 $\pm$ 4.80a
L8	32 $\pm$ 2.89a	61 $\pm$ 9.28a	23 $\pm$ 1.90a

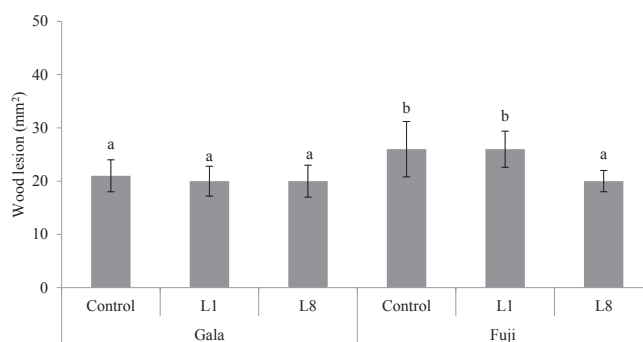


Fig. 1. Wood area lesions (mm^2) measured on ‘Gala’ and ‘Fuji’ twigs after 12 days after antagonists (L1 and L8) and *Neofusicoccum parvum* inoculation. Data are the means of two independent experiments $\pm$ standard deviation, each consisting of fifteen replicates per treatment. Different letters within the same cultivar indicate significant differences, LSD test ($P < 0.05$).

3.2. In vivo experiments

The antagonistic effect of *A. pullulans* strains was assayed against *N. parvum* on ‘Gala’ and ‘Fuji’ wood and fruit. At 12 d from the pathogen inoculation in ‘Gala’ apple wood, no statistical differences were observed between wood treated with either strain or the control wood (Fig. 1). On the contrary, in ‘Fuji’ wood, L8 strain was capable of inhibitory activity against *N. parvum*, displaying a wood lesion reduction of 23% with respect to the untreated control, whereas no inhibitory activity was detected in L1-treated wood compared to control (Fig. 1). Despite this, after 12 d from the fungal inoculation, both strains prevented pathogen sporification on apple wood of both cvs (data not shown).

The susceptibility of ‘Gala’ and ‘Fuji’ apple fruit to *N. parvum*, measured as lesion diameter (mm), was significantly different, with ‘Gala’ as the most susceptible fruit (Fig. 2). Here, both L1 and L8 strains inhibited the pathogen growth by 66% and 50% in ‘Gala’ apples, and by 70% and 60% in ‘Fuji’, respectively (Fig. 2).

3.3. IR/ATR spectroscopy

The spectroscopic measurements by means of IR/ATR technique were carried out to determine the extent of structural changes in the spectra of apple wood specimens exposed to fungal attack in comparison to the spectra of specimens where the antagonistic effect of *A.*

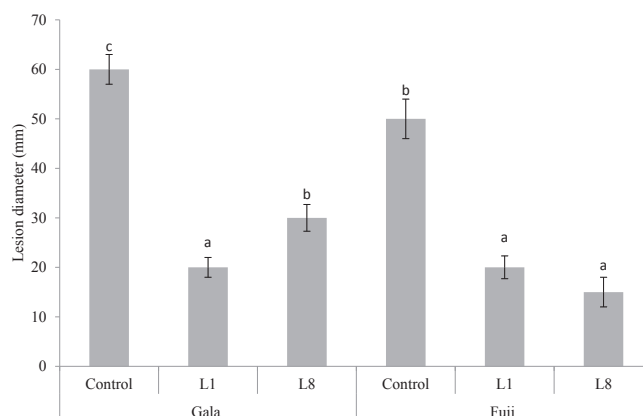


Fig. 2. Fruit diameter lesions (mm) measured on ‘Gala’ and ‘Fuji’ apple fruit after 5 days, previously wounded and treated with antagonists L1 and L8 strains (20 μ L) and *Neofusicoccum parvum* (20 μ L). Data are the means of two independent experiments $\pm$ standard deviation, each consisting of fifteen replicates per treatment. Different letters within the same cultivar indicate significant differences, LSD test ($P < 0.05$).

Table 2

Main IR bands investigated to assess the effects of fungal attack on apple wood.

IR band/cm ⁻¹	Attribution (Tomak et al., 2013)	IR intensity ratio	Attribution
2900	ν C–H (cellulose + hemicellulose)	I_{1370}/I_{2900}	Crystallinity index of cellulose
1730	ν C=O (hemicellulose)	I_{897}/I_{1730}	Cellulose/hemicellulose ratio
1510	ν C=C (lignin)	I_{1510}/I_{1370}	Lignin/(cellulose + hemicellulose) ratio
1430	δ C–H ₂ (cellulose)	I_{1510}/I_{1430}	Lignin/cellulose ratio
1370	δ C–H (cellulose I and II + hemicellulose)	I_{1510}/I_{1730}	Lignin/hemicellulose ratio
897	δ C–H + ν C–C (cellulose)	I_{160}/I_{1023}	β -glucans/ α -glucans ratio

Table 3Diagnostic IR intensity ratios. Different letters indicates a significant difference between treatments using the LDS statistical test ($P < 0.05$, $n = 5$).

	I_{1370}/I_{2900}	I_{897}/I_{1730}	I_{1510}/I_{1370}	I_{1510}/I_{1430}	I_{1510}/I_{1730}	I_{160}/I_{1023}
Fuji control	1.44 ± 0.15 ns	2.46 ± 0.18 ns	0.93 ± 0.04a	0.93 ± 0.05a	2.00 ± 0.18 ns	0.153 ± 0.005b
Fuji L1	1.47 ± 0.13 ns	2.56 ± 0.10 ns	0.90 ± 0.03a	0.93 ± 0.03a	2.09 ± 0.16 ns	0.169 ± 0.015ab
Fuji L8	1.38 ± 0.10 ns	2.65 ± 0.19 ns	0.83 ± 0.03b	0.81 ± 0.05b	1.78 ± 0.18 ns	0.183 ± 0.014a
Gala control	1.55 ± 0.09 ns	2.24 ± 0.15b	0.96 ± 0.05a	1.00 ± 0.07a	2.13 ± 0.14a	0.149 ± 0.016 ns
Gala L1	1.62 ± 0.14 ns	2.68 ± 0.15a	0.85 ± 0.03b	0.90 ± 0.03b	1.81 ± 0.16b	0.154 ± 0.022 ns
Gala L8	1.47 ± 0.09 ns	2.41 ± 0.11b	0.86 ± 0.03b	0.89 ± 0.03b	1.69 ± 0.20b	0.162 ± 0.011 ns

ns = not statistically significant.

pullulans could show variations on the main components of apple wood, i.e. cellulose, hemicellulose and lignin (Supplemental Fig. S1). Moreover, spectra were used to estimate the relative content of branched (1 → 3)(1 → 6)- β -D-glucan and linear (1 → 3)- α -D-glucan, produced by *A. pullulans* (Synytsya and Novak, 2014). The assignment of the different IR peaks is described in Table 2 together with the IR intensity ratios that were used in order to quantitatively estimate the above-mentioned effects: the results are summarized in Table 3. The IR peak attributed to the aromatic skeletal vibration (ν C=C) of lignin (I_{1510}) was chosen as an internal standard since the lignin content is the least affected by fungal degradation (Ozgenc et al., 2018).

In ‘Fuji’ apple wood the effects of L8 strain were statistically different from those induced by L1 strain (and from control samples) for the IR ratios involving lignin and cellulose (I_{1510}/I_{1430} and I_{1510}/I_{1370}): in both cases, the L8 strain induced a decrease in the ratio indicating a preserving effect on the cellulose component, while L1 was found not to be different from the control samples. Both strains showed to be less effective on the hemicellulose fraction, since both the I_{1510}/I_{1730} and the I_{897}/I_{1730} ratios were not significantly affected by the presence of L1 or L8 strain. Moreover, both strains were found to increase the relative content of β -glucans secreted by *A. pullulans* (Table 3).

In ‘Gala’ apple wood, the effects of L1 and L8 strain on the degradation of the different wood component were statistically different from the control for almost all the calculated ratios (Table 3). In particular, all the ratios between lignin and the carbohydrates component (I_{1510}/I_{1730} : lignin/hemicellulose; I_{1510}/I_{1430} : lignin/cellulose; I_{1510}/I_{1370} : lignin/carbohydrates) decreased, indicating a high activity on preventing cellulose and hemicellulose degradation. Moreover, the ratio between cellulose and hemicellulose (I_{897}/I_{1730}) was found to be higher than in control samples (and statistically significant for the L1 strain), indicating an inhibition in cellulose degradation induced by the presence of yeasts. Despite the above-mentioned activity of both strains in preserving the cellulosic component from degradation, no clear effects were detected on cellulose crystallinity (I_{1370}/I_{2900} ratio). Contrarily to what observed in ‘Fuji’ wood, no statistically significant effect was detected by ATR/IR spectroscopy on the α/β glucans ratio.

4. Discussion

Usually, control of *Botryosphaeraceae* diseases relies on the use of different synthetic fungicides such as benomyl and tebuconazole (Bester et al., 2007). The present study shows the potential of *A. pullulans* as BCA of the apple wood and fruit disease pathogen *N. parvum*.

Beside *N. parvum*, this family includes other emerging pathogens of fruit crops, as for example *Botryosphaeria* spp., recently reported to cause severe losses for apple production in the Italian region of Trentino Alto Adige, Italy (Prodorutti et al., 2012). These pathogens are probably going to increase their virulence under predicted climate change events, and represent a real risk of new epidemics in several geographical regions. Previously, L1 and L8 strains were tested against apple fungal pathogens, mainly associated to postharvest phase, such as *Botrytis cinerea*, *Colletotrichum acutatum*, and *Penicillium expansum* (Di Francesco et al., 2015), showing a great ability to control fungal diseases. Here, we show that both strains have the ability to exert the epiphyte and endophyte attitude (Scheda et al., 1999), considering the antagonistic effect exerted on fruit and wood and the *N. parvum* pathogenic attitude.

Also, our *in vitro* assay showed the capability of *A. pullulans* L1 and L8 strains to reduce the fungal conidia germination probably due to the mechanism of action of competition for nutrients, as previously demonstrated against *M. laxa* and *B. cinerea*, where both strains competed against the target pathogens for the main fruits amino acids displaying a great ability to reduce fungal conidia/mycelia growth (Di Francesco et al., 2017, 2018b).

In addition, *in vitro* experiments were conducted to screen the efficacy of secondary metabolites such as volatile and non-volatile compounds against *N. parvum* pathogenicity.

The activity of both strains was demonstrated through *in vitro* tests and the inhibitory capacity of both strains against the target pathogen resulted not displayed through the secretion of volatile organic compounds (VOCs), but by the production of other metabolites such as non-volatile, showing a reduction of 69.2% of the *N. parvum* colony growth. This is contrary to previously results obtained using L1 and L8 strains against other fungal pathogens (Di Francesco et al., 2015), supporting the hypothesis that these biocontrol agents act with different mechanisms on different target pathogens.

In *in vivo* experiments on wood, only L8 strain, applied 24 h before pathogen inoculation, was capable of partial disease reduction in ‘Fuji’ wood. However, ‘Gala’ cultivar, both wood and fruit, was the more susceptible to *N. parvum*. It is known that the main commercial apple cultivars have different susceptibility to pathogens, and Spotts et al. (1999) already reported that ‘Fuji’ and ‘Granny Smith’ apple fruit were the most resistant varieties to fungal pathogens with respect to ‘Gala’ and ‘Golden Delicious’.

IR spectroscopy was applied to investigate both the effects of fungal degradation on wood by examining the relative content of lignin, hemicelluloses, and cellulose (by using the bands intensity ratios

showed in Table 2) and the possible presence of non-volatile compounds secreted by *A. pullulans*, such as phenolic compounds and α and β -glucans, that could act as a chemical and physical barrier to fungal attack (Synytsya and Novak, 2014). It must be recalled that all the analyzed intensity ratios allow to estimate the relative content of the different chemical components, while the quantification of the different substances can be obtained with different analytical techniques such as chromatography (Synytsya and Novak, 2014). However, spectroscopic characterization is faster and does not require any sample treatment.

Interestingly, after 12 d from the pathogen artificial inoculation in wood, both *A. pullulans* strains displayed the capability to inhibit *N. parvum* to produce pycnidia probably due to their high influence on the degradation of the carbohydrates fraction of wood (cellulose and hemicellulose), as confirmed by the IR intensity ratios involving carbohydrates (i.e. I_{1510}/I_{1370} , I_{1510}/I_{1430} and I_{1510}/I_{1730}) that decreased in the presence of L1 and L8. The selected IR intensity ratios has been previously used in order to measure the wood decay induced by several fungal attack (Ozgenç et al., 2018; Beck et al., 2018; Lucejko et al., 2018; Pandey and Nagveni, 2007). In fact, the presence of lignin and hemicellulose can be a barrier to the hydrolytic attack by fungi to the cellulose fraction (Wang et al., 2013), therefore the calculated IR ratios can give more insight into the mechanism of wood degradation by *N. parvum*. The yeasts had a different outcome on the two apple cultivars: in ‘Fuji’ L8 strain effects were statistically different from control samples while L1 was not; in ‘Gala’ both strains proved to be effective in preserving cellulose from degradation. Tomak et al. (2013) reported an increase of the I_{1510}/I_{1370} ratio on bamboo wood exposed to brown-rot fungal attack attributed both to the degradation of the cellulosic fraction of wood and to a partial increase of lignin peaks in decayed wood due to oxidative reactions induced by reactive oxygen species. Moreover, the I_{897}/I_{1730} intensity ratio was found to generally increase in presence of both strains, even if it resulted significant only for the L1 strain on ‘Gala’ wood (Table 3), indicating an increase in cellulose content in wood in comparison to hemicellulose, which might be degraded faster than cellulose by fungal attack (Tomak et al., 2013). As a matter of fact, hemicellulose has a random and less order structure compared to cellulose, therefore it can be more easily attacked by the enzymatic hydrolysis reactions induced by fungi (Wang et al., 2013). Another factor affecting the extent of wood decay is the crystalline index of cellulose: this polymer can form both crystalline and amorphous domains that possess a different kinetic towards enzymatic hydrolysis, since amorphous domains are degraded faster compared to crystalline ones (Lucejko et al., 2018). The reported spectroscopic results cannot clearly put in correlation the antagonistic effect of both strain with the variation of cellulose crystallinity (I_{1370}/I_{2900} , Table 3), probably indicating that wood decay in presence of *N. parvum* involves both crystalline and amorphous domains of cellulose at similar rates. On the contrary, Lucejko et al. (2018) reported an increase in cellulose crystallinity following to fungal attack in consequence to a preferential metabolization of amorphous cellulose.

Together with the characterization of the chemical modifications induced on wood, IR spectroscopy allowed to study the relative content of glucans secreted by *A. pullulans*: the I_{1160}/I_{1023} , expresses the ratio between β and α glucans secreted by the yeast: L8 strain showed a significative increase of this ratio with respect to the control in ‘Fuji’ apple wood only, indicated an increased production of β -glucans, that were reported to exhibit an immune stimulatory activity (Bozoudi and Tsaltas, 2018) and that could have supported the competition of *A. pullulans* against the activity of *N. parvum*. Unfortunately, due to the nature of the matrix (i.e. wood) it was not possible to gain insight into phenolics content, identified by typical IR bands (Heneczkowski et al., 2001) that, unfortunately, overlap with stronger bands arising from the vibration of the functional groups present in wood components, in particular lignin (Heneczkowski et al., 2001). As far as the *in vivo* assay on fruits, results partially confirmed the above-mentioned observation: ‘Gala’ confirmed to be the most susceptible of tested cultivars, but

conversely both L1 and L8 strains inhibited the pathogen growth showing a high efficacy (on average 60%). It is well known that BCAs can control diseases by a combination of different mechanisms of action, and their effectiveness is always related to the pathogen-host interactions. Taken together, our data demonstrate that *A. pullulans* L1 and L8 strains exerted efficacy against *N. parvum* through different modes of action, especially through antibiosis by the production of non-volatile secondary metabolites. Moreover, both strains were able to colonize the wood surface, an essential requirement for biocontrol activity of *N. parvum* in field. More investigations are required to verify a possible L1 and L8 endophytic attitude, thus providing an alternative supplement to environmental-friendly disease management. The present study shows that application of L1 and L8 *A. pullulans* strains could play an essential role in the biological control of *N. parvum* of apple or a good conjunction system with other organic control tactics.

More investigations are necessary to understand the exact contribute of both strains in the control of the tested fungal pathogen, such as the non-volatile compounds characterization, in order to improve their efficacy especially with regard to the study of their mechanisms of action and possible application.

CRediT authorship contribution statement

C. Rusin: Writing - original draft, Data curation. **A. Di Francesco:** Conceptualization, Methodology, Writing - original draft, Data curation, Writing - review & editing. **M. Di Foggia:** Writing - original draft, Data curation, Methodology. **S. D'Aquino:** Supervision. **A. Rombolà:** Conceptualization, Supervision. **V. Tugnoli:** Data curation, Supervision. **R. Vasconcelos Botelho:** Supervision. **E. Baraldi:** Conceptualization, Writing - review & editing, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2019.04.015>.

References

- Alfonzo, A., Conigliaro, G., Torta, L., Burruano, S., Moschetti, G., 2009. Antagonism of the endophytic *Bacillus subtilis* strain AG1 to fungal pathogens that cause tracheomycotic deterioration of vine wood. *Phytopathol. Mediterr.* 48, 186–187. <https://doi.org/10.14601/Phytopathol.Mediterr-2886>.
- Beck, G., Thybringer, E.E., Thygesen, L.G., 2018. Brown-rot fungal degradation and deacetylation of acetylated wood. *Int. Biodeterior. Biodegrad.* 135, 62–70. <https://doi.org/10.1016/j.ibiod.2018.09.009>.
- Bester, W., Crous, W.P., Fourie, P.H., 2007. Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* species. *Aust. Plant Pathol.* 36, 73–77. <https://doi.org/10.1071/AP06086>.
- Bozoudi, D., Tsaltas, D., 2018. The multiple and versatile roles of *Aureobasidium pullulans* in the viti-vinicultural sector. *Fermentation* 4, 85–99. <https://doi.org/10.3390/fermentation4040085>.
- Cloete, M., Fourie, P.H., Damm, U., Crous, P.W., Mostert, L., 2011. Fungi associated with dieback symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. *Phytopathol. Mediterr.* 50, S176–S190.
- Delgado-Cerrone, L., Mondino, P., Alaniz, S., 2016. *Botryosphaeriaceae* species associated with stem canker, dieback and fruit rot on apple in Uruguay. *Eur. J. Plant Pathol.* 146, 637–655. <https://doi.org/10.1007/s10658-016-0949-z>.
- Dennis, C., Webster, J., 1971. Antagonistic properties of species-groups of *Trichoderma* – I. Production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.* 57, 25–39. [https://doi.org/10.1016/S0007-1536\(71\)80077-3](https://doi.org/10.1016/S0007-1536(71)80077-3).
- Di Francesco, A., Ugolini, L., Lazzeri, L., Mari, M., 2015. Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. *Biol. Control* 81, 8–14. <https://doi.org/10.1016/j.biocontrol.2014.10.004>.
- Di Francesco, A., Ugolini, L., D'Aquino, S., Pagnotta, E., Mari, M., 2017. Biocontrol of *Monilinia laxa* by *Aureobasidium pullulans* strains: insights on competition for nutrients and space. *Int. J. Food Microbiol.* 248, 32–38. <https://doi.org/10.1016/j.jfoodmicro.2017.02.007>.
- Di Francesco, A., Calassanzio, M., Ratti, C., Mari, M., Folchi, A., Baraldi, E., 2018a. Molecular characterization of the two postharvest biological control agents *Aureobasidium pullulans* L1 and L8. *Biol. Control* 123, 53–59. <https://doi.org/10.1016/j.biocontrol.2018.05.005>.
- Di Francesco, A., Mari, M., Ugolini, L., Baraldi, E., 2018b. Effect of *Aureobasidium pullulans* strains against *Botrytis cinerea* on kiwifruit during storage and on fruit

- nutritional composition. *Food Microbiol.* 72, 67–72. <https://doi.org/10.1016/j.fm.2017.11.010>.
- Heneczowski, M., Kopacz, M., Nowak, D., Kuzniar, A., 2001. Infrared spectrum of some flavonoids. *Acta Pol. Pharm.* 58, 415–420.
- Kotze, C., Niekerk, J., van Halleen, F., Mostert, L., Fourie, P., 2011. Evaluation of biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. *Phytopathol. Mediterr.* 50, 247–263. https://doi.org/10.14601/Phytopathol_Mediterr-8960.
- Lucejko, J.J., Mattonai, M., Zborowska, M., Tamburini, D., Cofta, G., Cantisani, E., Kudela, J., Cartwright, C., Colombini, M.P., Ribechini, E., Modugno, F., 2018. Deterioration effects on wet environments and brown rot fungus *Coniophora puteana* on pine wood in the archaeological site of Biskupin (Poland). *Microchem. J.* 138, 132–146. <https://doi.org/10.1016/j.microc.2017.12.028>.
- Lundberg, A., Unestan, T., 1980. Antagonism against *Fomesannosus*. Comparison between different test methods “in vitro” and “in vivo”. *Mycopathologia* 70, 107–115.
- Oliveira, M., Varanda, C., Félix, M.R., 2016. Induced resistance during the interaction pathogen x plant and the use of resistance inducers. *Phytochem. Lett.* 15, 152–158. <https://doi.org/10.1016/j.phytol.2015.12.011>.
- Ozgenc, O., Durmaz, S., Boyaci, I.H., Eksi-Kocak, H., 2018. ATR-FTIR spectroscopic analysis of thermally modified wood degraded by rot fungi. *Drezwno* 61 <https://doi.org/10.12841/wood.1644-3985.247.02>. 201–201.
- Pandey, K.K., Nagveni, H.C., 2007. Rapid characterization of brown and white rot degraded chir pine and rubber wood by FTIR spectroscopy. *Eur. J. Wood Wood Prod.* 65, 477–481. <https://doi.org/10.1007/s00107-007-0181-9>.
- Prodorutti, D., Cainelli, C., Gualandri, V., Profaizer, D., Dallago, A., Branz, A., Delaiti, L., Pertot, I., Angeli, G., 2012. Moria e deperimento del melo in Trentino. *Atti Giornate Fitopatologiche* 2, 619–620.
- Rampazzo, M., Di Foggia, M., 2018. The sunk-panel book-binding of a Renaissance Venetian Commissione Dogale: the scientific examination of the decoration materials. *Herit. Sci.* 6, 14–26. <https://doi.org/10.1186/s40494-018-0180-7>.
- Rooney-Latham, S., Soriano, M., 2016. First report of *Neofusicoccum nonquiescens* causing branch dieback of apple in California. *Plant Dis.* 100, 1012. <https://doi.org/10.1094/PDIS-01-12-0019-PDN>.
- Sakalidis, M.L., Slippers, B., Wingfield, B.D., St, J., Hardy, G.E., Burgess, T.I., 2013. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum*-*N. ribis* species complex. *Divers. Distrib.* 19, 873–883. <https://doi.org/10.1111/ddi.12030>.
- Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., Droby, S., 1999. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. *Postharvest Biol. Technol.* 17, 189–199. [https://doi.org/10.1016/S0925-5214\(99\)00036-8](https://doi.org/10.1016/S0925-5214(99)00036-8).
- Slippers, B., Wingfield, M.J., 2007. *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol. Rev.* 21, 90–106. <https://doi.org/10.1016/j.fbr.2007.06.002>.
- Slippers, B., Boissin, E., Phillips, A.J.L., Groenewald, J.Z., Wingfield, M.J., Postma, A., Burgess, T., Crous, P.W., 2013. Phylogenetic lineages in the botryosphaeriales: a systematic and evolutionary framework. *Stud. Mycol.* 76, 31–49. <https://doi.org/10.3114/sim0020>.
- Spagnolo, A., Larignon, P., Magnin-Robert, M., Hovasse, A., Cilindre, C., Van Dorsselaer, A., Clément, C., Schaeffer-Reiss, C., 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. *Int. J. Mol. Sci.* 15, 9644–9669. <https://doi.org/10.3390/ijms15069644>.
- Spotts, R.A., Cervantes, L.A., Mielke, E.A., 1999. Variability in postharvest decay among apple cultivars. *Plant Dis.* 83, 1051–1054. <https://doi.org/10.1094/PDIS.1999.83.11.1051>.
- Synytysya, A., Novak, M., 2014. Structural analysis of glucans. *Ann. Transl. Med.* 2, 17–30. <https://doi.org/10.3978/j.issn.2305-5839.2014.02.07>.
- Tomak, E.D., Topaloglu, E., Gumuskaya, E., Yildiz, U.C., Ay, N., 2013. An FT-IR study of the changes in chemical composition of bamboo degraded by brown-rot fungi. *Int. Biodeterior. Biodegrad.* 85, 131–138. <https://doi.org/10.1016/j.ibiod.2013.05.029>.
- Thomidis, T., Michailides, T.J., Efstathia, E., 2011. *Neofusicoccum parvum* associated with fruit rot and shoot blight of peaches in Greece. *Eur. J. Plant Pathol.* 131, 661. <https://doi.org/10.1007/s10658-011-9840-0>.
- Úrbez-Torres, J.R., 2011. The status of *Botryosphaeriaceae* species infecting grapevines. *Phytopathol. Mediterr.* 50, S5–S45. https://doi.org/10.14601/Phytopathol_Mediterr-9316.
- von Arx, J.A., 1987. *Plant-pathogenic Fungi*. J. Cramer, Berlin, Germany.
- Wang, W., Yuan, T., Cui, B., Dai, Y., 2013. Investigating lignin and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis. *Bioresour. Technol.* 134, 381–385. <https://doi.org/10.1016/j.biortech.2013.02.042>.
- Wu, S., 2017. Using *Burkholderia* sp. as a Biocontrol Agent in Kiwifruit Plants Against *Neofusicoccum parvum* (Ph.D. Thesis). Lincoln University, New Zealand.
- Zhang, D., Spadaro, D., Garibaldi, A., Gullino, M.L., 2010. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. *Biol. Control* 54, 172–180. <https://doi.org/10.1016/j.biocontrol.2010.05.003>.

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Characterization of apple cultivar susceptibility to *Neofusicoccum parvum* Brazilian strains

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Abstract Due to climate change, *Neofusicoccum parvum* is currently emerging as a new pathogen of Rosaceae plant species. This increases the need of gaining knowledge on the pathogenicity of this fungus, particularly on apple cultivars of considerable economic importance. In this study, the virulence and temperature dependence of *N. parvum* isolates was assayed on ‘Gala’ and ‘Fuji’ apple fruit and wood. At 25 °C and 30 °C, ‘Gala’ fruits showed a higher susceptibility to all fungal strains than ‘Fuji’, whereas low infection severity was shown at 15 °C

and 20 °C on both cultivars. Infrared spectroscopy revealed that ‘Fuji’ fruit tissues displayed a higher content of phenolic compounds and pectin esterification degree, as factors possibly correlated with fruit susceptibility. When strain virulence was assayed on wood, no significant difference in susceptibility was detected, as also shown by SEM analysis. The virulence of *N. parvum* was also tested by assaying the activity of lytic enzymes such as cellulase, polygalacturonase, polymethylgalacturonase, and xylanase.

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Introduction

Botryosphaeriaceae (Theiss & P. Syd.) are cosmopolitan fungal species pathogens of a broad range of both annual and perennial plant hosts (Slippers and Wingfield 2007). The taxonomy of *Botryosphaeriaceae* is complex and distinction of members based on morphological aspects is very difficult due to the high similarity among species (e.g. *Botryosphaeria* (Ces. & De Not.) and *Neofusicoccum* (Crous, Slippers and Phillips) species produce very similar conidia (Phillips et al. 2013). For this reason, molecular analyses are often required for species identification (Slippers et al. 2013). On the other hand, pathogens belonging to this family can grow fast on fruit crops, causing significant economic losses worldwide (Coakley et al. 1999). Among *Botryosphaeriaceae* species reported to be pathogenic in fruit crops, *Neofusicoccum parvum* is one of the common species, in warmer geographical areas (Úrbez-Torres 2011; Sakalidis et al. 2013). Some years ago, Sakalidis et al. (2013) confirmed the widespread diffusion of *N. parvum* showing its virulence on about 90 plant hosts worldwide. Generally, *N. parvum* is reported as the causal agent of grapevine dieback (Úrbez-Torres 2011; Taylor et al. 2005; Luque et al. 2009; Spagnolo et al. 2014) causing plant losses up to 50% (Bertsch et al. 2013). In particular, *N. parvum* symptoms are associated with orange/brown stripes located in the longitudinal direction in the outer xylem of wood plants. These symptoms are often associated with the foliar symptoms (Mugnai et al. 1999) suggesting that the pathogen is capable of producing phytotoxic metabolites which can migrate systemically into the host plant. *Botryosphaeriaceae* can cause severe symptoms also in apple fruit consisting of a soft light-colored rot called “white rot” (Khon and Hendrix 1983). In Brazil, *N. parvum* is recognized as an emerging pathogen for apple crops, raising concerns about protection strategies (Melzer and Berton 1986; Hansen et al. 2007). Nevertheless, its ecological role in plant communities is poorly studied (Slippers et al. 2007).

In order to gain knowledge on the infection mechanism of this fungus in apple tree and fruit we set out to study the virulence of five *N. parvum* Brazilian strains at

different temperatures and on different cultivars by in vitro and in vivo assays respectively. Scanning Electron Microscopy (SEM) was performed on apple twigs artificially infected with the pathogen in order to assess the infection timings. The activities of major lytic enzymes were quantified by in vitro agar plate assays and infrared spectroscopy analysis of infected apples was performed to detect metabolic profiles likely associated with cultivar susceptibility.

Materials and methods

Pathogen

After field observations, *N. parvum* strains were isolated from cankers of internal wood necrosis of ‘Gala’ apple trees, located in different experimental orchards of the State University of Mid-Western of Paraná (Brazil) (Supplemental Fig. 1). Wood necrotic parts were inoculated on Potato Dextrose Agar (PDA, 39 g L⁻¹ of distilled water) (Sigma, St. Louis, MO, USA) at 25 °C for 5 d. Then, fungi were morphologically selected, purified and grown on Malt Extract Agar plates (MEA, 50 g L⁻¹ of distilled water) (Sigma, St. Louis, MO, USA). From each experimental orchard, three pure cultures were isolated.

The strains NP1, NP2, NP3, NP4, and NP5 were selected for their high virulence on apple fruit and identified by morphological analysis and sequencing of ribosomal DNA ITS regions. PCR was performed using universal primers ITS1 (GCCGTAGGTGAACC TGCGG) and ITS4 (GCCTCCGCTT ATTGATATGC) directly from mycelia in pure culture, using the protocol described by Iotti and Zambonelli (2006). PCR reactions were conducted in 50 µL volume reactions containing 10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 150 µM of each dNTP, 300 µM of each primer, and 1.5 U of TaKaRa Taq DNA polymerase (Takara, Otsu, Japan). Twenty micrograms of Bovine Serum Albumin (BSA) (Fermentas, Vilnius, Lithuania) were added to the reaction tubes containing the mycelial fragments before adding the other PCR reagents. The amplification reactions were carried out in a T gradient thermal cycler (Biometra, Gottingen, Germany) with an initial denaturation at 95 °C for 6 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final step of 72 °C for 10 min. PCR products were run on 1% agarose gels, stained with ethidium

bromide and visualized under UV light. The amplified DNA was purified by Nucleospin Extracts II Kit (Macherey–Nagel, Germany) and finally sequenced by Eurofins Genomics (Luxemburg). The ITS sequences were compared to those of the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>) using the BLASTN search.

Culture substrates

NP1, NP2, NP3, NP4, and NP5 strains were grown on MEA, Apple Agar (AA, 15 g agar technical and 250 mL of a commercial apple juice per 1000 mL of distilled water), PDA in Petri dishes (90 mm) with/without four autoclaved woody sticks (70 mm) (Supplemental Fig. 2) and incubated at 25 °C with 12-h dark and 12-h light cycles for 8 d, to verify the most suitable condition for conidia production. For each medium, 10 plates were considered as replicates.

Wood and fruit

Hardwood twigs of one year old (50 cm) were collected from ‘Fuji’ and ‘Gala’ plants of Bologna University experimental fields located in Cadriano (Bologna, Italy) in February 2018, stored at 5 °C and used for experiments within 5 d from collection. ‘Fuji’ and ‘Gala’ apples (*Malus domestica* L. Borkh) were obtained from an orchard located in Sala Bolognese (Bologna, Italy). Harvested fruits were stored at 0 °C and used for experiments within 5 d after harvest. Fruits were wounded by a sterile nail (3 × 3 × 3 mm) on the equator (one wound per fruit).

In vivo experiment: virulence in wood

One hundred and fifty twigs of ‘Gala’ and ‘Fuji’ apples were cut into 30 cm long segments, rinsed with tap water, surface disinfected with 70% alcohol and air dried. Three wounds were made between buds position on each twig using a sterile blade and a mycelium agar plug (4 mm in diameter) from a 8 d-old colony of each strain was placed in the wound and wrapped with sterile cotton wetted by 5 mL of sterile water and covered with Parafilm® (Pechiney Plastic Packaging, USA) (Fig. 1 a). For each apple cultivar, 15 twigs were inoculated with the above mentioned five fungal strains. Negative control was constituted by no inoculated twigs. Twigs were soaked in 200 mL of sterile water in upright position and incubated at room temperature (20 °C),

with 12-h dark and 12-h light at 60% of relative humidity (RH). The lesions were evaluated 12 d after the inoculation and measured by the software ImageJ 1.x (Wisconsin, USA) (Schneider et al. 2012). Conidia from inoculated twigs of both apple cultivars were collected with 2 mL of distilled water and counted through a microscope (Nikon Eclipse TE2000-E). The experiment was repeated twice.

Scanning Electron Microscope observation in wood

The virulence of *N. parvum* strain NP1 on wood was investigated with SEM observations of inoculated ‘Gala’ and ‘Fuji’ twigs as previously described. The control twigs were not inoculated. The SEM observations were carried out 3, 6, and 12 d after the twig inoculations. A little portion of tissue (3 × 3 × 3 mm), corresponding to the inoculated wound, was excised from treated twigs and observed under SEM (Zeiss DSM 962 microscope at 30 kV). Three samples from each twig were fixed in a phosphate buffer (pH 7.4) containing glutaraldehyde (3%). Before observation, the fixed tissues were rinsed three times with phosphate buffer (pH 7.4), dried by washing with increasing concentrations of ethanol, stuck on aluminium stubs, and coated with gold palladium (Di Francesco et al. 2017). Nine samples derived from three twigs of the same cv were examined for each sampling time (for a total of 54 samples).

In vivo experiment: virulence in apple fruit

Fungal virulence displayed by the isolate NP1 was also tested on apple fruit and the influence of different temperatures was assayed to evaluate the effect on the strain virulence on apples measured as lesion diameter.

‘Gala’ and ‘Fuji’ fruits were inoculated with *N. parvum* NP1 conidia suspensions (1×10^4 conidia mL⁻¹). Conidial suspensions were prepared by washing *N. parvum* pycnidia, artificially inoculated on ‘Gala’ and ‘Fuji’ apple twigs, with sterile distilled water containing 0.05% (v/v) Tween 80, quantified with a hemocytometer and diluted to the required concentration.

Each fruit was wounded with a sterile needle and inoculated with 20 µL of a pathogen suspension. The control consisted of inoculated fruit with sterile water. Apples were kept at 15 °C, 20 °C, 25 °C and 30 °C at 90–95% RH. The lesion diameters were measured after 7 d from the inoculation. The experiment was conducted

Fig. 1 ‘Gala’ apples twigs were wounded and inoculated with *Neofusicoccum parvum* (NP1 strain) mycelium agar plug, wrapped with sterile cotton wetted by 5 mL of sterile water and covered with Parafilm. After 12 d from the artificial inoculation are noticeable: pycnidia (a), conidia (14 μm length on average) (b) and symptoms (c)



with 15 fruits per temperature and cultivar and was repeated twice (for a total of 480 fruits).

Neofusicoccum parvum pathogenesis enzyme activity

Cellulase (endo-1,4- β -glucanase), pectic enzyme (polygalacturonase and polymethylgalacturonase) and xylanase enzyme activities of *N. parvum* were evaluated 3, 9, 24, and 48 h after inoculation.

Assays were performed in Petri dishes containing modified agar medium (Di Francesco et al. 2018). Three mycelium plugs (4 mm) from 8 d-old fungal colony were placed equidistant in the medium. Control plates for each enzyme-pathogen combination were inoculated with MEA plugs without mycelium. After incubation at 25 °C, enzymes activities were determined with specific colorimetric methods visualizing a clear zone of substrate degradation around the mycelium plugs. The diameter of degradation halos (mm) was measured with a ruler. For each incubation time for pathogen and control, four plates (replicates) were considered.

For cellulase activity, the agar medium consisted in PYE (peptone: 0.5 g; yeast extract: 0.1 g; agar: 16 g L⁻¹) supplemented with 0.5% Na-carboxymethylcellulose (Sigma-Aldrich, St. Louis, MO, USA) (Mugnai et al. 1997). The halo produced by endo-1,4- β -glucanase activity of each fungus was visualized by staining plates with a 0.2% Congo red water solution for 15 min, then discolored with NaCl 1 M. Polygalacturonase activity

was determined through the Eriksson and Pettersson culture medium (Eriksson and Petterson 1975) enriched with the sodium salt of polygalacturonic acid (Sigma-Aldrich) from citrus fruit (0.5%) and agarized with low calcium Oxoid No. 1 agar (2%, Oxoid, Basingstoke, UK). The medium was sterilized following the procedure of Ayers et al. (1966) and then the pH was adjusted to 5 and 8 respectively by adding NaOH 1 M. Polymethylgalacturonase activity was determined through the above cited culture medium enriched with Sigma citrus pectin (0.5%) and agarized with calcium-rich Oxoid No. 3 agar (2%). The growth media was sterilized following the procedure of Durrands and Cooper (1988) and the pH was adjusted to 8 by adding HCl 1 M. The halo produced by pectic enzyme activities was visualized by 1% cetylmethyl ammonium bromide (CTAB) dissolved in distilled water, heated to 30 °C. For the xylanase assay, the medium contained 0.5% beech wood xylan (Sigma-Aldrich) in a minimal medium consisting of NaNO₃ 0.3%; KH₂PO₄ 0.1%; MgSO₄ 0.05%; yeast extract 0.1%; agar 1.2% (St Leger et al. 1997). The clearing halo was visualized by staining with Congo red, then de-stained with NaCl and measured as previously described. Each assay was repeated twice.

ATR spectroscopy

Three sterile flasks containing 50 mL of Malt Extract Broth (MEB, 50 g L⁻¹ of distilled water) were

inoculated each with 15 plugs of *N. parvum* NP1 mycelium 8 d-old, and incubated at 25 °C in a rotary shaker (250 rpm) for 8 d. The culture was centrifuged at 5000×g for 20 min at 4 °C and the mycelium was washed with distilled water and filtered through two layers of miracloth. Then, ‘Gala’ and ‘Fuji’ fruit slices (70 mm thickness) were inoculated with 1 g of *N. parvum* mycelium.

After 3, 9, 24, and 48 h, *N. parvum* mycelium was removed and apple slices were stored at –80 °C and suddenly lyophilized (FD-10 Freezing Dryer, Lab kits, H.K.) for 10 d to avoid water spectroscopic interferences. The control consisted in not inoculated apple slices.

The IR/ATR spectra of fruits at different times of both cultivars were compared in order to ascertain how *N. parvum* infection affected the chemical composition of fruits. Moreover, the IR/ATR spectra of the pulp of apple fruits displayed increasing differences compared to their control references with the inoculation time, therefore, only the longest inoculation time spectra are discussed.

IR spectra were recorded with a Bruker ALPHA series FT-IR spectrophotometer (Bruker, Ettlingen, Germany) equipped with a crystal diamond cell for attenuated total reflectance (ATR). The spectra were collected from 4000 to 400 cm^{–1} and averaged over 100 scans (resolution = 4 cm^{–1}) on three different samples for each inoculation time.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA). Separation of means was performed using the least significant difference (LSD) test, at $P < 0.05$. Data were reported as mean values ± standard error (SE) of two independent experiments. All analyses were performed with the software Statgraphic Plus Version 2.1 (USA).

Results

Pathogen

The [sequence analysis](#) of the ITS rDNA regions identified the five pathogen strains used in this study as *N. parvum* with a [sequence homology](#) percentage of 99% with the relative accession number KY053054.

The results of molecular characterization were compared with *N. parvum* morphological identification keys, showing a significant match.

Conidia production

Neofusicoccum parvum strains used in our tests, showed the ability to grow on different culture substrates, such as MEA, PDA, and Apple Agar. On these substrates, fungi grew very fast reaching complete colonization of the plate (90 mm) in only 5 d, however they did not produce pycnidia, in any of the different substrate (Table 1), even after 15 d (data not shown). On the other hand, when grown for 12 d on ‘Gala’ and ‘Fuji’ apple twigs (Fig. 1 a), all the strains produced pycnidia, but no statistical difference was detected among their concentrations, except for NP1 strain grown on ‘Gala’ apple twigs (Fig. 1 b and c and Table 1). At this incubation time (12 d), apple twigs showed great lesions only in correspondence of the fungus inoculation point and a large amount of black pycnidia appeared under the Parafilm layer (Fig. 1 a, b). Pycnidia derived from wood lesions contained conidia of an average length of 14 µm, without showing statistically significant differences between the two apple cultivars (data not shown). On the contrary, statistically significant difference was shown between conidia concentration detected on ‘Gala’ and ‘Fuji’ twigs (Table 1), with the first cultivar (‘Gala’) resulting the most suitable substrate for the pathogen strains (3.07×10^4 conidia mL^{–1} on average). The obtained conidia were inoculated on MEA plate and the mycelium growth and morphology were very similar to those of the original *N. parvum* isolates inoculated on twigs. After 8 d of incubation in MEA plates, mycelium plugs were placed on artificial wounds of new apple twigs treated as above and similar wood lesions after 12 d were detected containing pycnidia, fully satisfying Koch’s postulates, and supporting protocol reproducibility. The *N. parvum* isolates similarly colonized woody tissues of the two tested apple cultivars. After 12 d, blackish-brown discolorations were observed on woody tissues of both apple cultivars twigs (Fig. 1 c). Lesions were equally extended (about 20 mm²) in both cultivars. No clear and significant pathogenicity/virulence difference in the inoculated twigs was detected among the tested strains, and for this reason, one strain (NP1) for subsequent analyses was chosen.

Table 1 Culture substrates used to induce *Neofusicoccum parvum* strains to produce conidia (MEA: Malt Extract Agar, PDA: Potato dextrose agar, AA: Apple Agar, SS: Sterile Sticks, ‘Gala’ Twigs, ‘Fuji’ Twigs)

Strain	Culture substrate						Gala twigs	Fuji twigs
	MEA	PDA	AA	MEA + S.S.	PDA + S.S.	AA+S.S.		
NP1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.10×10^4 cell mL ⁻¹ ± 6.2bA	1.53×10^4 cell mL ⁻¹ ± 3.3aB
NP2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.07×10^4 cell mL ⁻¹ ± 0.6aA	1.52×10^4 cell mL ⁻¹ ± 5.2aB
NP3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.07×10^4 cell mL ⁻¹ ± 8.9aA	1.47×10^4 cell mL ⁻¹ ± 8.8aB
NP4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.06×10^4 cell mL ⁻¹ ± 9.9aA	1.44×10^4 cell mL ⁻¹ ± 9.0aB
NP5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.07×10^4 cell mL ⁻¹ ± 5.2aA	1.47×10^4 cell mL ⁻¹ ± 8.3aB

Conidia concentration values are the means of 15 repetitions (3 wounds) each per strain. Values within the same column followed by different lowercase letters are significantly different ($P < 0.05$) according to the LSD test. Values within the same row followed by different uppercase letters are significantly different ($P < 0.05$) according to the LSD test

n.d., not detected

SEM analysis

Scanning Electron Microscopy (SEM) observations of ‘Gala’ and ‘Fuji’ apple twigs inoculated with *N. parvum* NP1, revealed that after 3 d of incubation at 25 °C the pathogen started to germinate and colonize the tissue surface (Fig. 2 Aa). At 6 d (Fig. 2 Ab) after inoculation the SEM observations displayed a great increase of the mycelium with respect to the 3th day. In addition, the scanning observation at higher magnification (500 ×) emphasized the difference between the healthy and the tissue colonized by the fungus (Fig. 4 **panel).

Six days later (Fig. 2 Ac), the pathogen hyphae already severely invaded the twigs tissues (cross section). No differences were observed between ‘Gala’ and ‘Fuji’ twigs about the *N. parvum* virulence (Fig. 2B (a, b, c)).

Virulence on apple fruit

After 7 d, the strain NP1 did not show any disease symptoms at 15 °C on both apple cultivars, whereas at 30 °C the fungal pathogen displayed the highest severity (60 mm and 71 mm for ‘Fuji’ and ‘Gala’ respectively) (Fig. 3). Moreover, NP1 displayed a higher virulence in ‘Gala’ apples with respect to ‘Fuji’ both at 25 °C and 30 °C. The fungus pathogenicity was limited at 20 °C.

Enzymes activity

The pectinase, polygalacturonase, xylanase, and cellulase pathogenesis enzymes activity of *N. parvum* strain NP1 was evaluated after 3, 9, 24 and 48 h of fungal growth. All the examined fungal enzyme activities started to be detectable at 3 h from the inoculation on the substrate, gradually increasing in time. At 24 and 48 h from the inoculation, the fungal strain showed the highest enzymes activity (Fig. 4). Xylanase resulted the highest enzymatic activity with respect to the other, displaying a very intense and large halo areas, already from 3 h of inoculation (200 mm²). Only cellulase activity appeared to decrease after 48 h from the inoculation. On the other hand, pectinase resulted in the tested fungal time of growth the lowest enzymatic activity with respect to the others CWDEs.

ATR spectroscopy

IR/ATR spectra gave useful information to gain insight the chemical variation of affected fruits. In particular, the ratio between the peaks areas centered at 1730 and 1610 cm⁻¹ was used to monitor the esterification degree of pectins (Supplemental Fig. 3). Figure 5 shows the spectrum of ‘Gala’ slices after 48 h from inoculation (in black) and the spectrum difference between this and the control at the same time (in blue). Positive peaks in the difference spectrum can be correlated to an increased

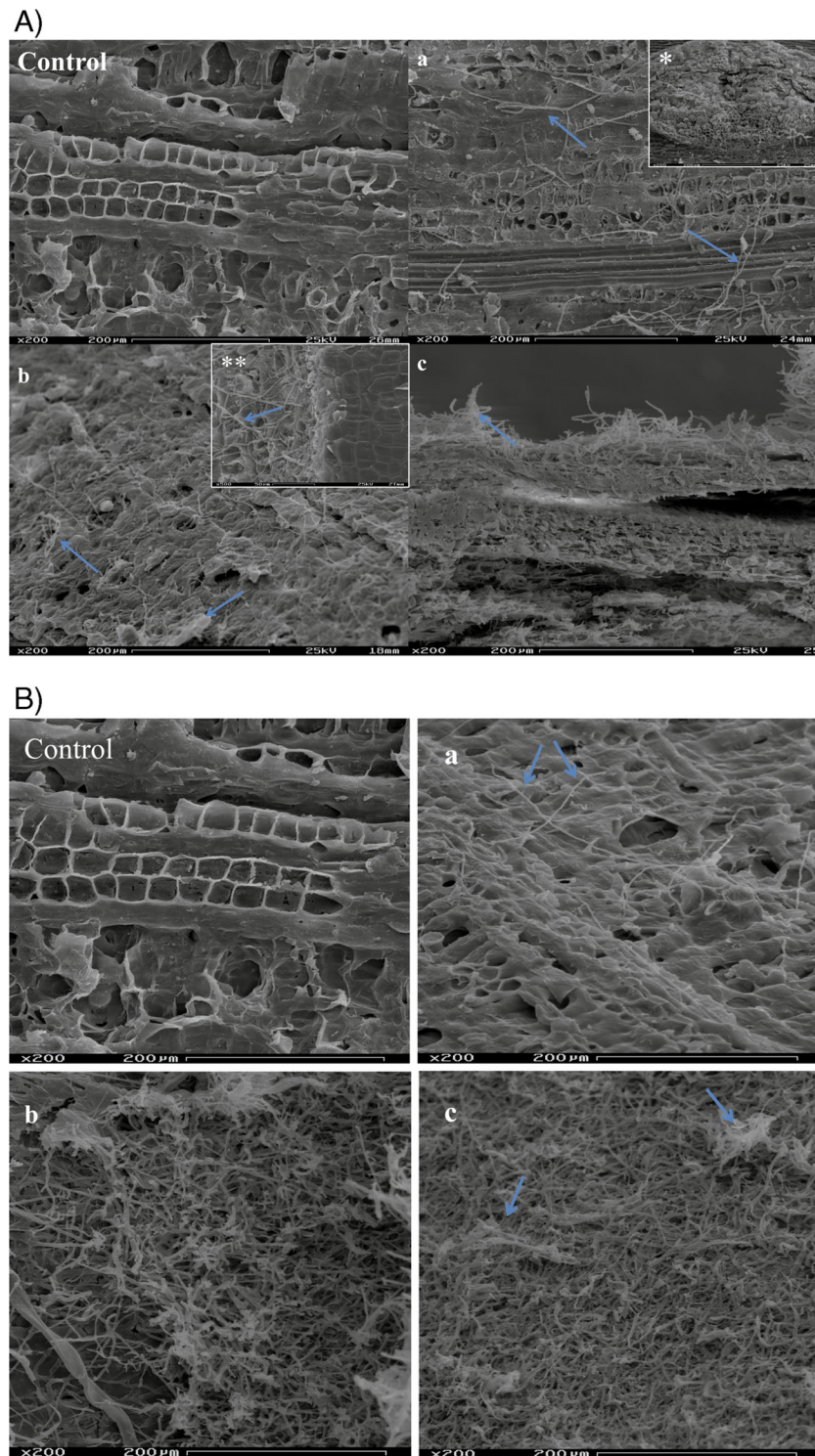
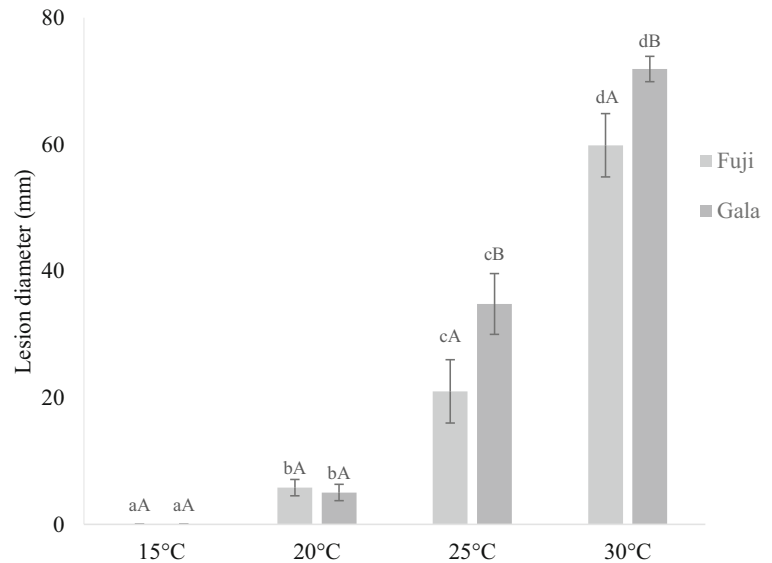


Fig. 2 Scanning electron micrographs (SEM) of ‘Gala’ (A) and ‘Fuji’ (B) twigs (surface and cross section) inoculated and not (Control) with *Neofusicoccum parvum* NP1 strain at different times from the inoculation (3d (a), 6d (b), 12d (c)). SEM shown, 200 x magnification in all images. *Specifics of the hyphae fungus

penetration in twig tissue at 3 d from the artificial inoculation. **Specifics (500 ×) of the hyphae fungus colonization in twig tissue at 6 d from the artificial inoculation. Light blue arrows pointed NP1 mycelium

Fig. 3 Lesion diameter (mm) of *Neofusicoccum parvum* NPI strain on apple fruit artificially inoculated after one week. Fruit wounds were inoculated with 20 μ L of *Neofusicoccum parvum* conidia suspension (10^4 conidia mL^{-1}). Fruits were kept at 15°C, 20°C, 25°C and 30°C for 7 d. Data are the means of fruits for each incubation temperature. Lower case: analysis per cultivar - Upper case: analysis between cultivar; different letters indicate significant differences according to LSD test, $P < 0.05$



concentration of phytochemical compounds, while a negative peak is related to a decreased concentration. Actually, several peaks related to the esterification

degree of pectins turned out to be positive in the difference spectrum: 1737, 1370, 1280, 1231, 1119, 991 and 799 cm^{-1} . More in detail, the peaks at 1737 and

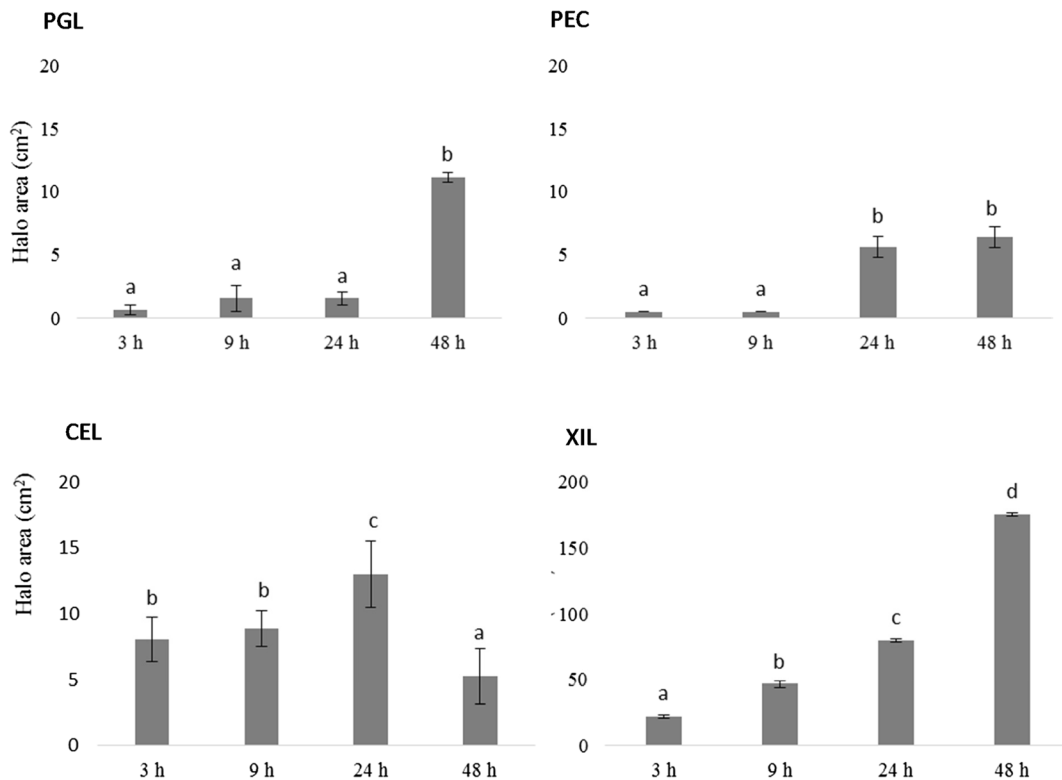


Fig. 4 Polygalacturonase (PGL), Pectinase (PEC), Cellulase (CEL) and Xylanase (X 1 IL) activity (halo- cm^2) of *Neofusicoccum parvum* NP1, after 3h, 9 h, 24 h and 48 h of incubation at 25°C. Each value is the mean of three replicates $\pm$

standard errors. Data were analyzed for significant differences ($P < 0.05$) by analysis of variance (ANOVA) followed by LSD test. Within the same enzyme the same letters represent no significant differences according to LSD test ($P < 0.05$)

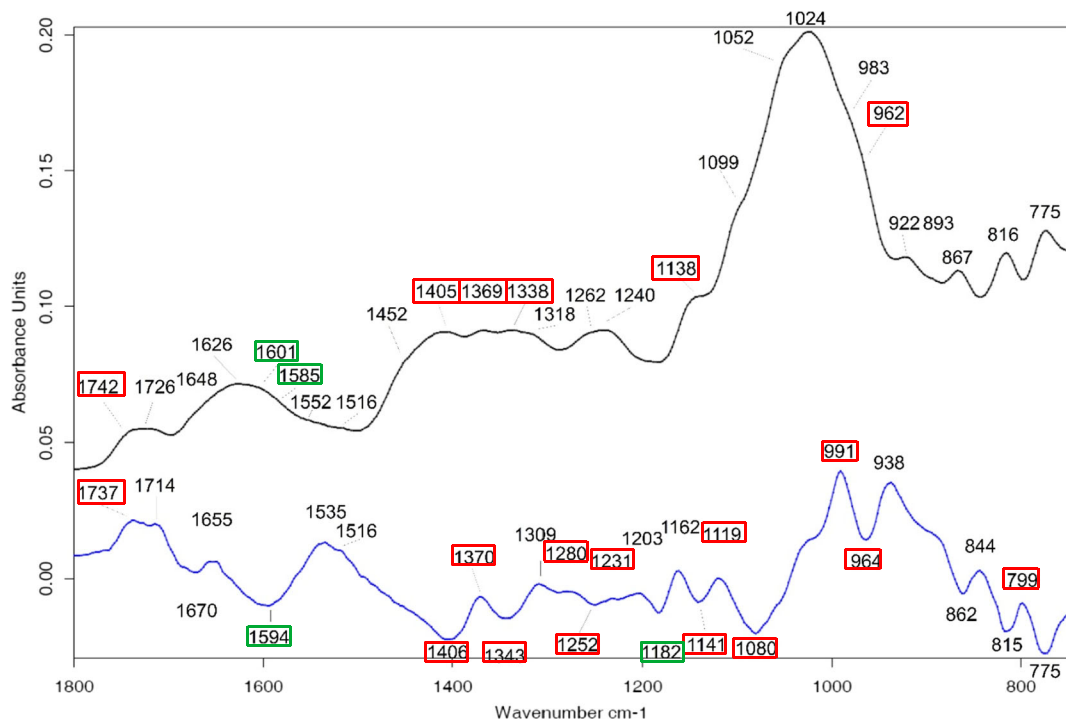


Fig. 5 Infrared spectrum of ‘Gala’ apple fruit at 48 h after inoculation (black 1 spectrum). The blue spectrum is the difference between the inoculated sample at 48 h and its control. Red boxes: peaks attributed to pectin; green boxes: peaks attributed to phenolics

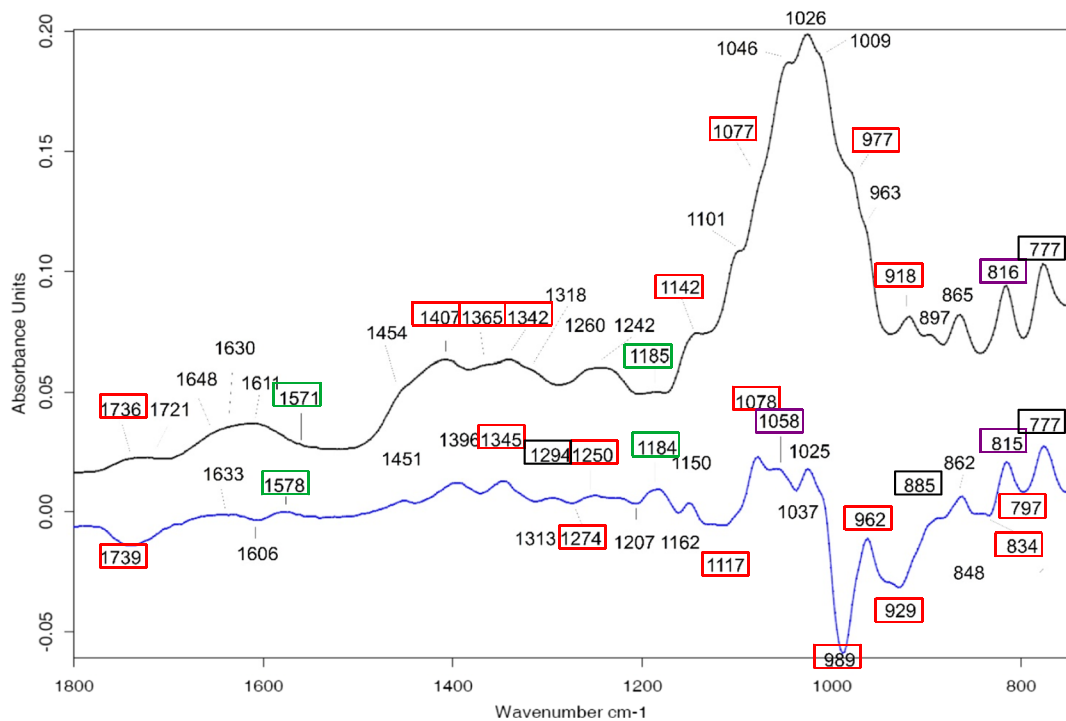


Fig. 6 Infrared spectrum of ‘Fuji’ apple fruit at 48 h after inoculation (black 1 spectrum). The blue spectrum is the difference between the inoculated sample at 48 h and its control. Red boxes:

peaks attributed to pectin; green boxes: peaks attributed to phenolics; purple boxes: peaks attributed to hemicellulose; black boxes: peaks attributed to glucose

1119 cm^{-1} are typical of acetylated pectins, whose concentration increases in infected ‘Gala’ apples compared to control. Moreover, other bands attributable to pectin vibrations changed their relative intensities or their wavenumber (Synytsya et al. 2003): 1406, 1343, 1252, 1141, 1080 and 964 cm^{-1} , indicating chemical changes into pectin structure induced by the fungal presence. On the other hand, peaks related to phenolic compounds (i.e. flavonoids, lignins and ferulate esters) showed a marked decrease with respect to the control: in particular the bands at 1594 and 1182 cm^{-1} (Heneczowski et al. 2001).

‘Fuji’ apples’ spectrum after 48 h from inoculation, showed other variations in the difference spectrum (blue spectrum, Fig. 6), indicating different chemical variations induced by fungal attack. For example, the esterification degree of pectins decreased (Synytsya et al. 2003): 1739, 1274, 1117, 989, 929 and 834 cm^{-1} bands showed a negative peak. Some of these peaks indicate that pectin in this cv is mainly methylated: i.e. the 1739 and the 989 cm^{-1} bands are indicative of methyl esterification (Barros et al. 2002). As in ‘Gala’, other pectin-related bands are affected by inoculation: 1365, 977, 918 and 834 cm^{-1} . Conversely, typical peaks related to phenolic compounds increased after fungal inoculation (Bonora et al. 2009): 1578 and 1184 cm^{-1} . Other features typical of the IR/ATR spectra of inoculated ‘Fuji’ slices are the increase in intensity of bands attributable to hemi-cellulose (1058 and 815 cm^{-1}) and those related to free monosaccharides, in particular to glucose moieties (i.e. 1294, 885 and 777 cm^{-1}) (Schulz and Baranska 2007).

Discussion

The increase of temperatures and the wide variety of commercialized apple cultivars (Di Francesco et al. 2019) are contributing factors in the emergence of different fungal pathogens such as *Botryosphaeraceae* spp. in Europe (Carlucci et al. 2009; Spagnolo et al. 2011). Between *Botryosphaeraceae* species, *N. parvum* and *Neofusicoccum luteum* resulted the most aggressive (Pérez et al. 2010; Adesemoye et al. 2014; Chen et al. 2014; Delgado-Cerrone et al. 2016).

Our study aimed to characterize the infection mechanism of *N. parvum* as causal agent of fruit and wood diseases of apple. This fungus is representing a real concern in South America and

Europe particularly in Trentino Alto Adige region (Italy), causing severe losses for apple production (Prodorutti et al. 2012; Javier-Alva et al. 2009; Molina-Gayosso et al. 2012; Marques et al. 2013). The present study showed that the pathogenicity of the strain NP1 in apple fruit and wood was highly influenced by the temperature (25 °C/30 °C) and the host chemical composition, especially considering the fruit. In effect, NP1 strain did not show differences of virulence between cultivars in the apple wood colonization. These results can be partially confirmed by Yang et al. (2017), who demonstrated that shoots inoculated with conidia derived from an in vitro production method had larger lesions than those inoculated with conidia from *in planta* production. In fact, as Amponsah et al. (2008), we could not produce spores by in vitro assays from our *N. parvum* isolates but only through the twig inoculation.

Conversely between apple fruit cultivars, where ‘Gala’ apples showed a higher susceptibility to *N. parvum* with respect to ‘Fuji’, displaying lesion diameters 1.5-fold larger. This finding can be related to a different biochemical response to fungal attack as revealed by ATR/IR spectroscopy. In fact, ‘Fuji’ apples showed a higher content of phenolic compounds compared to the control fruits, while ‘Gala’ showed a decrease of the corresponding IR bands. Boyer and Liu (2004) reported a higher total phenolic and flavonoid content in ‘Fuji’ compared to ‘Gala’ apples. Several reports highlighted the role of phenolic compounds such as ferulic acid and its derivatives in fruit defense response against fungi attack in different crops (Bonora et al. 2009; Lambert et al. 2012; Srivastava et al. 2013). Moreover, the two apple cultivars showed an opposite trend of the pectins esterification degree that is reported to be affected by the fungal attack (Bonora et al. 2009): usually the esterification degree decreases in response to fungal infection by the action of pectin methyl esterases secreted by fungi (Kikot et al. 2009; Vallette-Collet et al. 2003); in fact de-esterification increases the susceptibility of the cell wall structure by favoring the spreading of the attack (Lionetti et al. 2012). Our results showed that ‘Gala’ preferentially displayed the typical peaks of acetylated pectins that increase with time after inoculation, therefore a less effective response to fungi, while ‘Fuji’ showed the typical peaks of methylated pectins. In fact, it is known that the main commercial apple cultivars displayed different susceptibility to pathogens supporting previous data reported by Spotts et al. (1999), where ‘Fuji’ and ‘Granny Smith’ apple were the most resistant varieties to apple fungal pathogens with respect to ‘Gala’ and ‘Golden’ cvs. Overall, significant differences

on susceptibility to *Botryosphaeriaceae* fungi were detected among stone fruit cultivars by Beckman and Reilly (2005) with the cv ‘Summergold’ as the most susceptible and the ‘Redskin’ the least, but no significant differences in virulence between fungal strains were observed in accordance with our results.

Doubtless, *N. parvum* NP1 strain phytopathogenic behaviour is related to the high production of degradative and oxidative enzymes, often simultaneously secreted (Esteves et al. 2014) such as cellulases and xylanases. Fungi associated with wood diseases may be related to different fungal activities such as the production of phytotoxic compounds (Stempien 2017), and enzymes. As the latter, the enzymatic activities could be associated with the fungal behaviour: low extracellular cellulolytic activities are indicative of a symbiotic lifestyle and low polysaccharide degrading activity accompanied by high proteolytic and lipolytic activities may reflect an adaptation to a non-pathogenic lifestyle (Esteves et al. 2014). In our case, *N. parvum* was able to degrade the carbohydrate components of cell walls and exhibit mainly cellulase and xylanase activities starting from 3 h after the inoculation, explaining its high and rapid wood decay capability. Furthermore, results showed that our strain particularly exerted xylanase enzyme activity, known to be responsible for the degradation of polysaccharides by *Phaeoacremonium minimum* (esca disease) (Stempien 2017). These results are confirmed by the increase of IR bands related to free monosaccharides (in particular glucose) that were found in both apple cultivars. Moreover, the increase of hemicellulose bands in ‘Fuji’ spectra could be related to an increased resistance of this cultivar to fungal xylanase activity. On the contrary, pectic and polygalacturonic enzymatic activities, principally involved in the degradation of pectic polymers of fruits and consequence of soft-rot symptoms, were less effective than the above-mentioned cellulase and xylanase enzymes. This fact is probably due to *N. parvum* ability to shift between endophytic and pathogenic phases (Amponsah et al. 2011; Sakalidis et al. 2011; Baskarathevan et al. 2012; Ramírez-Suero et al. 2012; Fernandes et al. 2014; Abou-Mansour et al. 2015). Mugnai et al. (1999) also described how some fungal secreted compounds in the wood could be translocated to the leaves and fruits, inducing the typical white rot symptoms. Therefore, more investigations on different apple fruit and wood cultivars are necessary also to better clarify the relationship between the

increase of fungal virulence and the climate change to prevent the real risk of new epidemics in several geographical regions.

Compliance with ethical standards

Conflict of interest This work was not financed by grants. The authors declare no conflict of interest. This article does not contain any work conducted on animal or human participants.

References

- Abou-Mansour, E., Débieux, J. L., Ramírez-Suero, M., Bénard-Gellon, M., Magnin-Robert, M., Spagnolo, A., et al. (2015). Phytotoxic metabolites from *Neofusicoccum parvum*, a pathogen of *Botryosphaeria* dieback of grapevine. *Phytochemistry*. <https://doi.org/10.1016/j.phytochem.2015.01.012>.
- Adesemoye, A. O., Mayorquin, J. S., Wang, D. H., Twizeyimana, M., Lynch, S. C., & Eskalen, A. (2014). Identification of species of *Botryosphaeriaceae* causing bot gummosis in citrus in California. *Plant Disease*. <https://doi.org/10.1094/PDIS-05-13-0492-RE>.
- Amponsah, N. T., Jones, E. E., Ridgway, H. J., & Jaspers, M. V. (2008). Production of *Botryosphaeria* species conidia using grapevine green shoots. *New Zealand Plant Protection*, 61, 310–305.
- Amponsah, N. T., Jones, E. E., Ridgway, H. J., & Jaspers, M. V. (2011). Identification, potential inoculum sources and pathogenicity of species of the *Botryosphaeriaceae* associated with grapevine dieback disease in New Zealand. *European Journal of Plant Pathology*. <https://doi.org/10.1071/AP05018>.
- Ayers, W. A., Papavizas, G. C., Diem, A. F., Durrands, P. K., & Cooper, R. M. (1966). Polygalacturonate transeliminase and polygalacturonic acid role of pectinases in vascular wilt disease as deterlacturonase production by *Rhizoctonia solani*. *Phytopathology*, 56, 1006–1011.
- Barros, A. S., Mafra, I., Ferreira, D., Cardoso, S., Reis, A., Lopes de Silva, J. A., et al. (2002). Determination of the degree of methyl esterification of pectic polysaccharides by FT-IR using an outer product PLS1 regression. *Carbohydrate Polymers*. [https://doi.org/10.1016/S0144-8617\(02\)00017-6](https://doi.org/10.1016/S0144-8617(02)00017-6).
- Baskarathevan, J., Jaspers, M. V., Jones, E. E., & Ridgway, H. J. (2012). Development of isolate-specific markers for *Neofusicoccum parvum* and *N. luteum* and their use to study rainwater splash dispersal in the vineyard. *Plant Pathology*. <https://doi.org/10.1111/j.1365-3059.2012.02675.x>.
- Beckman, T. G., & Reilly, C. C. (2005). Relative susceptibility of peach cultivars to fungal gummosis (*Botryosphaeria dothidea*). *Journal of the American Pomological Society*, 59, 111–116.
- Bertsch, C., Ramirez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., et al. (2013). Grapevine trunk diseases: Complex and still poorly understood. *Plant Pathology*. <https://doi.org/10.1111/j.1365-3059.2012.02674.x>.

- Bonora, S., Francioso, O., Tugnoli, V., Prodi, A., Di Foggia, M., Righi, V., et al. (2009). Structural characteristics of ‘Hayward’ kiwifruit from elephantiasis-affected plants studied by DRIFT, FT-Raman, NMR, and SEM techniques. *Journal of Agricultural and Food Chemistry*. <https://doi.org/10.1021/jf9002957>.
- Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal*. <https://doi.org/10.1186/1475-2891-3-5>.
- Carlucci, A., Lops, F., Raimondo, M. L., Gentile, V., Mucci, M., & Frisullo, S. (2009). The *Botryosphaeria* species from vineyards of Apulia. *Phytopathologia Mediterranea*, 48, 180–180.
- Chen, S. F., Morgan, D. P., Hasey, J. K., Anderson, K., & Michailides, T. J. (2014). Phylogeny, morphology, distribution, and pathogenicity of *Botryosphaeriaceae* and *Diaporthaceae* from English walnut in California. *Plant Disease*. <https://doi.org/10.1094/PDIS-07-13-0706-RE>.
- Coakley, S. M., Scherm, H., & Chakraborty, S. (1999). Climate change and plant disease management. *Annual Review of Phytopathology*. <https://doi.org/10.1146/annurev.phyto.37.1.399>.
- Delgado-Cerrone, L., Mondino, P., & Alaniz, S. (2016). *Botryosphaeriaceae* species associated with stem canker, die-back and fruit rot on apple in Uruguay. *European Journal of Plant Pathology*, 146, 637–655. <https://doi.org/10.1007/s10658-016-0949-z>.
- Di Francesco, A., Ugolini, L., D’Aquino, S., Pagnotta, E., & Mari, M. (2017). Biocontrol of *Monilinia laxa* by *Aureobasidium pullulans* strains: Insights on competition for nutrients and space. *International Journal of Food Microbiology*. <https://doi.org/10.1016/j.ijfoodmicro.2017.02.007>.
- Di Francesco, A., Mari, M., & Roberti, R. (2018). Defense response against postharvest pathogens in hot water treated apples. *Scientia Horticulturae*. <https://doi.org/10.1016/j.scientia.2017.09.039>.
- Di Francesco, A., Cameldi, I., Neri, F., Barbanti, L., Folchi, A., Spadoni, A., & Baraldi, E. (2019). Effect of apple cultivars and storage periods on the virulence of *Neofabraea* spp. *Plant Pathology*. <https://doi.org/10.1111/ppa.13074>.
- Durrands, P. K., & Cooper, R. M. (1988). Selection and characterization of pectinase-deficient mutants of the vascular wilt pathogen *Verticillium dahliae*. *Physiological and Molecular Plant Pathology*. [https://doi.org/10.1016/S0885-5765\(88\)80029-8](https://doi.org/10.1016/S0885-5765(88)80029-8).
- Eriksson, K., & Petterson, B. (1975). Extracellular enzyme system utilized by the fungus *Sporotrichum pulverulentum* (*Chrysosporium lignorum*) for the breakdown of cellulose. *European Journal of Biochemistry*, 51, 193–206.
- Esteves, A. C., Saraiva, M., Correia, A., & Alves, A. (2014). Botryosphaeriales fungi produce extracellular enzymes with 2 biotechnological potential. *Canadian Journal of Microbiology*. <https://doi.org/10.1139/cjm-2014-0134>.
- Fernandes, I., Alves, A., Correia, A., Devreese, B., & Esteves, A. C. (2014). Secretome analysis identifies potential virulence factors of *Diplodia corticola*, a fungal pathogen involved in cork oak (*Quercus suber*) decline. *Fungal Biology*. <https://doi.org/10.1016/j.funbio.2014.04.006>.
- Hansen, J., Sato, M., Kharecha, P., Russell, G., Lea, D. W., Siddall, M. (2007). Climate change and trace gases. Philosophical transactions. *Series A, mathematical, physical, and engineering sciences*. <https://doi.org/10.1098/rsta.2007.2052>.
- Heneczowski, M., Kopacz, M., Nowak, D., & Kuzniar, A. (2001). Infrared spectrum analysis of some flavonoids. *Acta Poloniae Pharmaceutica*, 58, 415–420.
- Iotti, M., & Zambonelli, A. (2006). A quick and precise technique for identifying ectomycorrhizas by PCR. *Mycological Research*, 110, 60–65.
- Javier-Alva, J., Gramaje, D., Alvarez, L. A., & Armengol, J. (2009). First report of *Neofusicoccum parvum* associated with dieback of mango trees in Peru. *Plant Disease*. <https://doi.org/10.1094/PDIS-93-4-0426B>.
- Khon, F. C., & Hendrix, F. F. (1983). Influence of sugar content and pH on development of white rot on apples. *Plant Disease*, 67, 410–412.
- Kikot, G. E., Hours, R. A., & Alconada, T. M. (2009). Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: A review. *Journal of Basic Microbiology*. <https://doi.org/10.1002/jobm.200800231>.
- Lambert, C., Bisson, J., Waffo-Teguo, P., Papastamoulis, Y., Richard, T., Corio-Costet, M. F., et al. (2012). Phenolics and their antifungal role in grapevine wood decay: Focus on the *Botryosphaeriaceae* family. *Journal of Agricultural and Food Chemistry*. <https://doi.org/10.1021/jf303290g>.
- Lionetti, V., Cervone, F., & Bellincampi, D. (2012). Methyl esterification of pectin plays a role during plant-pathogen interactions and affects plant resistance to diseases. *Journal of Plant Physiology*. <https://doi.org/10.1016/j.jplph.2012.05.006>.
- Luque, J., Martos, S., Aroca, A., Raposo, R., & Garcia-Figueres, F. (2009). Symptoms and fungi associated with declining mature grapevine plants in Northeast Spain. *Journal of Plant Pathology*. <https://doi.org/10.4454/jpp.v91i2.968>.
- Marques, M. W., Lima, N. B., Morais Jr., M. A., Barbosa, M. A. G., Souza, B. O., Michereff, S. J., et al. (2013). Species of *Lasiodiplodia* associated with mango in Brazil. *Fungal Diversity*. <https://doi.org/10.1007/s13225-013-0231-z>.
- Melzer, R. R., & Berton, O. (1986). Incidência de *Botryosphaeria berengeriana* cultura da macieira (*Malus domestica*) no estado de Santa Catarina, Brasil. *Fitopatologia Brasileira*, 11, 891–898.
- Molina-Gayosso, E., Silva-Rojas, H. V., García-Morales, S., & Avila-Quezada, G. (2012). First report of black spots on avocado fruit caused by *Neofusicoccum parvum* in Mexico. *Plant Disease*. <https://doi.org/10.1094/PDIS-08-11-0699>.
- Mugnai, L., Surico, G., & Sfalanga, A. (1997). Produzione di enzimi esocellulari da parte di funghi del legno di viti colpite da “mal dell’esca”. *Micologia italiana*, 1, 11–22.
- Mugnai, L., Graniti, A., & Surico, G. (1999). Esca (black measles) and brown wood streaking two old and elusive diseases of grapevines. *Plant Disease*. <https://doi.org/10.1094/PDIS.1999.83.5.404>.
- Pérez, C. A., Wingfield, M. J., Slippers, B., Altier, N. A., & Blanchette, R. A. (2010). Endophytic and canker-associated *Botryosphaeriaceae* occurring on non-native Eucalyptus and native *Myrtaceae* trees in Uruguay. *Fungal Diversity*. <https://doi.org/10.1007/s13225-009-0014-8>.
- Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M. J., Groenewald, J. Z., et al. (2013). The *Botryosphaeriaceae*: Genera and species known from culture. *Studies in Microbiology*. <https://doi.org/10.3114/sim0021>.

- Prodorutti, D., Cainelli, C., Gualandri, V., Profaizer, D., Dallago, A., Branz, A., et al. (2012). Moria e deperimento del melo in Trentino. *Atti Giornate Fitopatologiche*, 2, 619–620.
- Ramírez-Suero, M., Chong, J., Farine, S., Kiefe-Mazet, F., Pensec, F., Gacougnole, I., et al. (2012). Effect of *Neofusicoccum parvum* and *Diplodia seriata* extra-cellular compounds on defence gene expression in *Vitis vinifera* cv. Chardonnay and cv. Gewurztraminer. *Phytopathologia Mediterranea*, 51, 438–438.
- Sakalidis, M. L., Hardy, G. E. S. J., & Burgess, T. I. (2011). Class III endophytes, clandestine movement amongst hosts and habitats and their potential for disease; a focus on *Neofusicoccum australe*. *Australasian Plant Pathology*, 40, 510–521. <https://doi.org/10.1007/s13313-011-0077-3>.
- Sakalidis, M. L., Slippers, B., Wingfield, B. D., G. E. St. J. Hardy, Burgess, T. I. (2013). The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum*–*N. ribis* species complex. Diversity and Distributions, <https://doi.org/10.1111/ddi.12030>.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675 PMID: 22930834.
- Schulz, H., & Baranska, M. (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. *Vibrational Spectroscopy*. <https://doi.org/10.1016/j.vibspec.2006.06.001>.
- Slippers, B., & Wingfield, M. J. (2007). *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. *Fungal Biology Reviews*. <https://doi.org/10.1016/j.fbr.2007.06.002>.
- Slippers, B., Smit, A., Crous, P. W., Countinho, T. A., Wingfield, B. D., & Wingfield, M. J. (2007). Taxonomy, phylogeny and identification of *Botryosphaeriaceae* associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology*. <https://doi.org/10.1111/j.1365-3059.2006.01486.x>.
- Slippers, B., Boissin, E., Phillips, A. J. L., Groenewald, J. Z., Wingfield, M. J., Postma, A., et al. (2013). Phylogenetic lineages in the Botryosphaeriales: A systematic and evolutionary framework. *Studies in Mycology*, <https://doi.org/10.3114/sim0020>.
- Spagnolo, A., Marchi, G., Peduto, F., Phillips, A. J. L., & Surico, G. (2011). Detection of *Botryosphaeriaceae* species within grapevine woody tissues by nested PCR, with particular emphasis on the *Neofusicoccum parvum*/*N. ribis* complex. *European Journal of Plant Pathology*. <https://doi.org/10.1007/s10658-010-9715-9>.
- Spagnolo, A., Larignon, P., Magnin-Robert, M., Hovasse, A., Cilindre, C., Van Dorsselaer, A., et al. (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*. <https://doi.org/10.3390/ijms15069644>.
- Spotts, R. A., Cervantes, L. A., & Mielke, E. A. (1999). Variability in postharvest decay among apple cultivars. *Plant Disease*. <https://doi.org/10.1094/PDIS.1999.83.11.1051>.
- Srivastava, P., Andersen, P. C., Marois, J. J., Wright, D. L., Srivastava, M., & Harmon, P. F. (2013). Effect of phenolic compounds on growth and ligninolytic enzyme production in *Botryosphaeria* isolates. *Crop Protection*. <https://doi.org/10.1016/j.cropro.2012.09.015>.
- St Leger, R. J., Joshi, L., & Roberts, D. W. (1997). Adaptation of proteases and carbohydrases of saprophytic: Phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. *Microbiology*, 143, 1983–1992.
- Stempien, E. (2017). Grapevine *Botryosphaeria* dieback fungi have specific aggressiveness factor repertory involved in wood decay and stilbene metabolism. *PLoS One*. <https://doi.org/10.1371/journal.pone.0188766>.
- Synitsya, A., Copikova, J., Matejka, P., & Machovic, V. (2003). Fourier transform Raman and infrared spectroscopy of pectins. *Carbohydrate Polymers*. [https://doi.org/10.1016/S0144-8617\(03\)00158-9](https://doi.org/10.1016/S0144-8617(03)00158-9).
- Taylor, A., Hardy, G. E. St. J., Wood, P., & Burgess, T. (2005). Identification and pathogenicity of *Botryosphaeria* species associated with grapevine decline in Western Australia. *Australasian Plant Pathology*. <https://doi.org/10.1071/AP05018>.
- Úrbez-Torres, J. R. (2011). The status of *Botryosphaeriaceae* species infecting grapevines. *Phytopathologia Mediterranea*. https://doi.org/10.14601/Phytopathol_Mediterr-9316.
- Vallette-Collet, O., Cimerman, A., Reignault, P., Levis, C., & Boccara, M. (2003). Disruption of *Botrytis cinerea* pectin methyl esterase gene Bcpme I reduces virulence on several host plants. *Molecular Plant-Microbe Interactions*. <https://doi.org/10.1094/MPMI.2003.16.4.360>.
- Yang, Y. L., Turner, J., Stephens, J., Campbell, R. E., & Walter, M. (2017). Comparison of *in vitro* and *in planta* sporogenesis in *Neofusicoccum* species from blueberry. *New Zealand Plant Protection*, 70, 203–208.

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Control of the fungi *Lasiodiplodia theobromae*, the causal agent of dieback, in cv. syrah grapevines

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ABSTRACT. Grapevine trunk diseases are among the most important limiting factors of worldwide viticulture. In this context, we aimed to verify the effect of chemical fungicides, biological agents and plant extracts on the control of *Lasiodiplodia theobromae* in pruning wounds and their physiological effects in cv. Syrah grapevines. Plant extracts (clove, cinnamon, garlic, rosemary and lemongrass), commercial fungicides (difeconazole, tebuconazole, mancozeb, sulfur, pyraclostrobin, fosetyl-Al, and azoxystrobin), chitosan, *Trichoderma harzianum* and *Bacillus subtilis* were used for the *in vitro* trials. The protection of pruning wounds in the potted vines was studied using fosetyl-Al, tebuconazole, *Trichoderma harzianum*, *Bacillus subtilis*, garlic extract and clove extract. The experiments were carried out through two vegetative cycles: 2015/2016 and 2016/2017. The length of wood discoloration, pathogen re-isolation percentage, fresh mass of the pruning material, peroxidase activity, fluorescence, chlorophyll index, phenological stages, shoot length and leaf area were evaluated. The treatments with clove, garlic extract, tebuconazole, pyraclostrobin, mancozeb, fosetyl-Al and *B. subtilis* reduced mycelial growth by more than 90%. In the grapevines, the use of *T. harzianum* decreased the re-isolation of *L. theobromae*, but no differences were verified for the other plant evaluations. We concluded that the use of *T. harzianum* would be a potential option for wound protection without altering the physiological aspects of cv. Syrah grapevines.

Keywords: *Trichoderma harzianum*; pruning; protection; biological control.

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Introduction

Grapevine trunk diseases (GTDs) are among the most important limiting factors of viticulture in the world. Several trunk fungi may be responsible for these diseases, affecting both *Vitis vinifera* L. and *Vitis labrusca* cultivars. Among these fungi, the genus *Lasiodiplodia* is the causal agent of grapevine dieback, which is also referred to as black dead arm or Botryosphaeria canker. In symptomatic plants, *Lasiodiplodia theobromae* was reported to be the most prevalent (Úrbez-Torres, 2011). In Brazil, *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl (Correia et al., 2013) and *Lasiodiplodia brasiliense* (Correia et al., 2015) were isolated from grapevine decline symptomatic plants.

These pathogens usually infect plants via pruning wounds and grafting points (Úrbez-Torres & Gubler, 2009); furthermore, they can be passed from the grapevine mother plants to the young through grafting or other processes of propagation at nurseries (Gramaje & Armengol, 2011). The infection is also favoured by conditions that reduce plant vigour, such as frost, high temperatures in the summer months, poor nutrition and poorly conducted pruning, and is associated with several disease symptoms, including foliar chlorosis, stunted growth, the dieback of shoots, spurs and side-branches, canker of the trunk or side-branches, wedge-shape cankers in the vascular tissue and mortality (Larignon & Dubos, 2001).

Strategies to reduce the incidence of vine trunk pathogens are limited to the management of the crop, using healthy plants, disposing of contaminated parts, pre-pruning or double pruning (Weber, Trouillas, & Gubler, 2007). Several works have been carried out *in vitro* and *in vivo* to obtain products that promote the control of these pathogens, such as chemical or biological products. However, the efficacy ranges broadly

according to the species studied (Rolshausen et al., 2010; Amponsah, Jones, Ridgway, & Jaspers, 2012; Díaz & Latorre, 2013).

The use of some biological control agents for the control of GTD fungi has also been tested, demonstrating the *in vitro* and *in vivo* efficacy of these agents. Alfonzo, Conigliaro, Torta, Burruano, and Moschetti (2009) evaluated *in vitro* *Bacillus subtilis*, getting control of *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, *Verticillium dahliae* and *Botryosphaeria rhodina*. Using biological control agents triggers the colonization of the plants' wounds, preventing the entry of trunk pathogens. Thus, these agents become a plant-protection option over time (Mutawila, Fourie, Halleen, & Mostert, 2011). Mutawila, Halleen, and Mostert (2016) reported that the application of *Trichoderma* species on grapevine pruning wound surfaces reduces wound infection by trunk pathogens. The protection of wounds with chemical or biological products can also reduce the presence of disease in the area (Halleen, Fourie, & Lombard, 2010; Díaz & Latorre, 2013; Sosnowski, Loschiavo, Wicks, & Scott, 2013).

The application of plant extracts can be an alternative method for disease control with lower environmental impacts. In vines, the use of garlic extract had an effect on the *in vitro* control of *Botryosphaeria dothidea*, *Diplodia seriata*, *Eutypa lata*, *Ilyonectria macrodidyma*, and *Phaeoacremonium aleophilum* and inhibited the development of *D. seriata* and *P. chlamydospora* in grapevine branches (Cobos et al., 2015).

As described, various management methods, including chemicals, biological control, natural molecules and material sanitation, have been tested against pathogens causing diseases of the stems of vines. Despite the reported experiments and the wide range of products registered for grapevines in Brazil, until now, there are no registered products for the control of *L. theobromae*.

In this context, this work aimed to verify the effect of chemical fungicides, biological agents and plant extracts on the control of *Lasiodiplodia theobromae* in pruning wounds and its physiological effects on grapevine plants cv. Syrah.

Material and methods

In vitro trials

Trials were carried out at the Phytopathology Laboratory of UNICENTRO (Guarapuava, Paraná State, Brazil) using an isolate of *Lasiodiplodia theobromae* (CMM 307) from the fungal collection of the Federal University of Pernambuco obtained by the isolation of symptomatic vines (*Vitis vinifera*) in Petrolina, Pernambuco State, Brazil.

For the tests with plant extracts, flower buds of clove (*Caryophyllus aromaticus* L.), bark cinnamon (*Cinnamomum zeylanicum*), fresh bulbs of garlic (*Allium sativum* L.), dried leaves of rosemary (*Rosmarinus officinalis*), and lemongrass (*Cymbopogon citratus*) were applied. The plant extracts were prepared using 30 g of plant material in 100 mL of distilled water, shredding the mixture in a blender, and subsequently filtering the mixture through a cheesecloth. A commercial garlic extract was also used (Natualho®, ai: 70%). The plant extracts were added to a PDA culture medium (potato dextrose agar, K25-610102, KASVI, Lot: 100713203) to obtain final concentrations of 0, 5, 10, 15, and 20% (v/v).

To test the volatile substances, the same plant extracts were used at a concentration of 20% of each extract, adapted from the methodology of Celoto, Papa, Sacramento, and Celoto (2008), using bipartite Petri dishes.

For the experiment with commercial products, the following treatments were added to the PDA culture medium: difeconazole (Score®, 1 mL L⁻¹, ai: 250 g L⁻¹), tebuconazole (Folicur® 200 EC, 8.75 mL L⁻¹, ai: 200 g L⁻¹), mancozeb (Dithane®, 3 g L⁻¹, ai: 800 g kg⁻¹), sulfur (Kumulus®, 3 g L⁻¹, ai: 800 g kg⁻¹), pyraclostrobin (Comet®, 5.33 mL L⁻¹, ai: 250 g L⁻¹), fosetyl-Al (Aliette®, 2.5 g L⁻¹, ai: 450 g L⁻¹), azoxystrobin (Amistar®, 0.24 g L⁻¹, ai: 500 g L⁻¹), chitosan (Fish Fétil Quitosana®, 16 mL L⁻¹, ai: 2%), *Bacillus subtilis* (Serenade®, 80 mL L⁻¹, ai: 13.68 g L⁻¹), *Trichoderma harzianum* (Ecotrich®, 4 g L⁻¹, ai: 300 g kg⁻¹) and *Trichoderma harzianum* (Predatox®, 4 mL L⁻¹, ai: 20 g L⁻¹).

The biological control agents *Bacillus subtilis* and *Trichoderma harzianum* were submitted to direct pairing tests with the pathogen and further evaluation of the volatile metabolites; this was performed using bipartite Petri dishes filled with PDA medium in the two compartments. First, the biological control agents were inserted in a partition of the plate. After 48h of recording, the mycelial discs of the phytopathogen were inserted into the other partition of the dish.

In all the trials, mycelial discs of 5 mm diameter of an isolated *Lasiodiplodia* were transferred to the centre of the Petri dishes containing culture medium with the treatments and incubated in a culture chamber at 25°C under 12h day/night photoperiods. The experimental design was completely randomized with five replications.

The evaluation of mycelial growth was performed daily by diameter measurements (cm) of the colony with a digital pachymeter 8" (ZAAS Precision, São Paulo, Brazil). From the evaluations of mycelial growth, the area under the mycelial growth curve (AUCMG) was estimated, adapted from Campbell and Madden (1990), and for the antagonism test, the index of antagonism following the methodology of Campanile, Ruscelli, and Luisi (2007) was used. The data were analysed using analysis of variance (ANOVA), a mean comparison by using a Scott-Knott test at 5% and a regression analysis for the doses. The percentage inhibition of each treatment was calculated in comparison with the control, and the means comparison was performed by a t-test at 5%. The statistical analyses were performed using the statistical software ASSISTAT Version 7.7 (Silva & Azevedo, 2016).

In vivo trials

The *in vivo* trials were conducted in a greenhouse in Guarapuava, Paraná State, Brazil (25°33' S and 51°29' W, altitude of 1,095 m). Nursery grapevines of cv. Syrah clone 174 (*Vitis vinifera*) grafted on rootstock 'Paulsen 1103' were transplanted into 3 L pots containing 2 L of soil, 0.9 L of organic compost and 0.1 L of dolomitic limestone.

The experiments were performed in two seasons: summer pruning and winter pruning. The grapevines were pruned at the height of the seventh bud in the vegetative period of the plant during the second week of December (summer 2015) and at the third bud spurs during the first week of August (winter 2016). The pruning scissors were disinfected with 70% (v/v) alcohol. Then, at the pruning wound sites, the treatments were applied with a micropipette, applying 2 mL of solution per wound (Díaz & Latorre, 2013).

The treatments used were an uninoculated control (water), inoculated control (water), fosetyl-Al (Aliette®, 2.5 g L⁻¹, ai: 450 g L⁻¹), tebuconazole (Folicur® 200 EC, 8.75 mL L⁻¹, ai: 200 g L⁻¹), *Bacillus subtilis* (Serenade®, 80 mL L⁻¹, ai: 13.68 g L⁻¹), *Trichoderma harzianum* (Ecotrich®, 4 g L⁻¹, ai: 300 g kg⁻¹), plant extracts of clove (*Caryophyllus aromaticus* L., dose 20%) and plant extracts of garlic (*Allium sativum* L., dose 20%).

For each pruning season, 24h after the application of the treatments, the fresh pruning wounds were inoculated with a mycelial disc (5 mm in diameter), taken from the PDA cultures of *L. theobromae*, which was placed upside down over the fresh pruning cut and wrapped with Parafilm M (Pechiney Plastic Packaging) to avoid rapid dehydration. For the control, without inoculation, the PDA discs without fungi colonization were used.

The experimental design was in randomized blocks with eight treatments, six replicates and an experimental plot consisting of one pot.

In August (winter 2016), 230 days after the first season treatments, and in January (summer 2017), 156 days after the second season treatment, spurs were removed from the vines and returned to laboratory for assessment. Then, the branches were cut longitudinally, and the lesions, i.e., the length of wood discoloration (LWD) extending from the point of inoculation, were measured with a digital pachymeter 8" (ZAAS Precision, São Paulo, Brazil). To confirm that the lesions were caused by trunk pathogens, 12 small pieces of discoloured wood from the margin of each lesion were surface-sterilized following the methodology of Almança et al. (2013), in which the pruned branches were disinfected with 70% alcohol for 30 seconds, with 3.5% sodium hypochlorite for 2 minutes and again immersed in 70% alcohol for 30 seconds and placed in PDA. The percent re-isolation was calculated.

The plants inoculated with *L. theobromae* were also examined for the presence of physiological changes. In both trial periods, the fresh mass (g) of the pruning material (precision balance model M2202, BEL Equipamentos Analíticos) and the chlorophyll index, with results expressed in ICF values (Falker chlorophyll index) according to Falker, (2008) (chlorophyll meter ClorofiLOG® model CFL 1030, Falker Automação Agrícola, Porto Alegre, Brazil) and chlorophyll *a* fluorescence (Fv/Fm - maximum PSII quantum yield - measured with a modulated fluorometer (PAM-2500 model, Walz, Effeltrich, Germany)) were evaluated.

After summer pruning, after 24h of inoculation, the activity of the guaiacol peroxidase enzyme was analysed in a UV-visible spectrophotometer (UV-1800 model, Shimadzu, Kioto, Japan) at 480 nm (Urbanek,

Kuzniak-Gebarowska, & Herka, 1991). A unit of GPX activity was defined as the change of 1.0 unit of absorbance (480 nm) per milligram of soluble protein per minute (UA mg P⁻¹ min.⁻¹).

After winter pruning, were also evaluated, the length of the branches (measured monthly with a tape measure), the phenological stages according to Eichhorn and Lorenz (1984) and the leaf area, determined with the model leaf area LI 3100C (Licor, Nebraska, United States of America) installed on bench, and the results were expressed in cm².

The data were analysed with an analysis of variance and a mean comparison by a Scott-Knott test at 5% significance using the statistical programme ASSISTAT Version 7.7 (Silva & Azevedo, 2016).

Results and discussion

In vitro trials

Antifungal activity is considered to be high when aqueous extracts provide inhibition equal to or greater than 50% (Venturoso et al., 2011). Thus, the results obtained in the experiment demonstrated the antifungal efficiency of cinnamon, clove and garlic. These treatments had a quadratic effect according to dose, with inhibitions between 90 and 100% of the mycelial growth of the pathogens at all the doses used (Figure 1a). The antifungal activities of clove and garlic extracts was proven, demonstrating the potential of these two extracts for the *in vitro* control of *L. theobromae*. The effect of garlic extract on the *in vitro* control of disease-causing fungi such as *D. seriata* and *P. chlamydospora* in grapevines was also reported by Cobos et al. (2015).

The cinnamon and rosemary extracts had quadratic effects according to dose. The highest inhibition using a 20% dose of cinnamon was verified with a reduction of 98.5%, and for the rosemary extract, the maximum inhibition was verified at a 10% dose (-24%). The lemongrass extract had a linear effect according to dose and a maximum inhibition at 20% (-31%) (Figure 1b).

The action of the extracts could be directly related to its composition. In addition to the efficiency of the extracts when added to the culture medium, the diffusion of traces of volatile compounds may induce or inhibit germination or growth or trigger changes in the development of plants and fungi (French, 1992). Volatile oils, essential oils, and ethereal oils or essences are complex mixtures of volatile substances, lipophilic, usually odoriferous and liquid, and soluble in apolar organic solvents such as ether but have limited solubility in water (Simões & Spitzer, 2001), indicating that the extraction of the aqueous extract carries a lesser amount of active principles. The lower drag of the active principles may explain the low efficiency of the volatile compounds in this experiment.

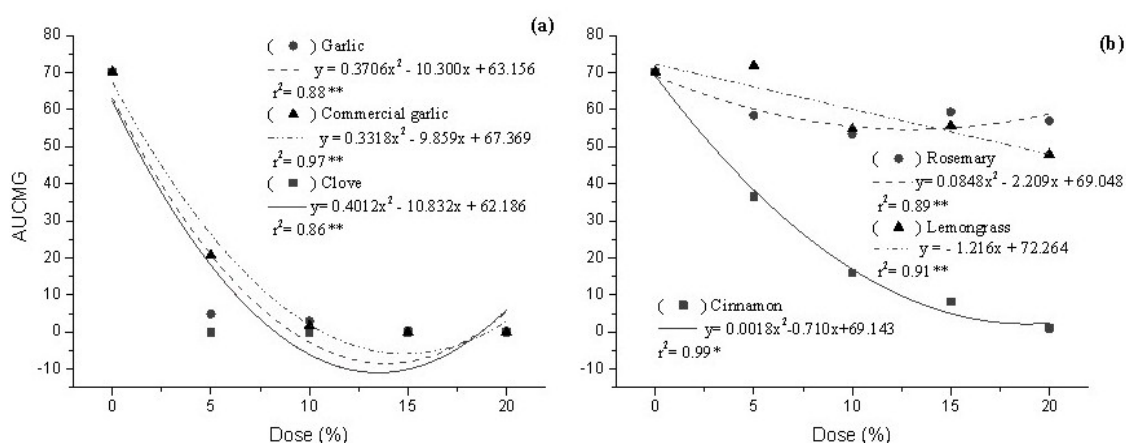


Figure 1. Area under the curve of the mycelial growth (AUCMG) of *Lasiodiplodia theobromae* subjected to different plant extracts. ** significant ($p < 0.01$). Guarapuava, Paraná State, Brazil, 2016.

The majority of the chemical treatments and biological control agents decreased the mycelial growth of *L. theobromae* (Figure 2a). The treatments tebuconazole, mancozeb, pyraclostrobin, fosetyl-Al, *B. Subtilis*, and *T. harzianum* (ai: 300 g kg⁻¹) were the most effective, followed by difeconazole and *T. harzianum* (ai: 20 g L⁻¹). The treatments with sulfur, azoxystrobin, and chitosan did not differ from the control, which showed the highest mycelial growth.

The volatiles of the plant extracts from cinnamon and garlic reduced the AUCMG, and the greatest reduction was obtained from the extracts of cinnamon (-35%) (Figure 2b). Although the effectiveness of control by the clove volatiles was not verified, they were efficient when diluted in culture medium, indicating that direct contact with the pathogen is necessary.

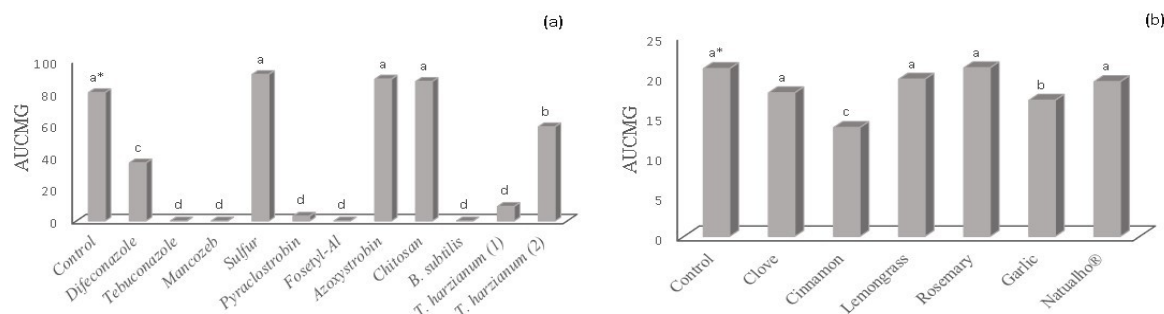


Figure 2. Area under the curve of the mycelial growth (AUCMG) of *Lasiodiplodia theobromae* submitted to treatments with chemical products and biological control agents (a) and to different volatile plant extracts (b). *Means followed by the same letter do not differ significantly by the Scott-Knott test ($p \leq 0.05$). (1) Commercial product with 300 g kg⁻¹ of active ingredient, (2) Commercial product with 20 g L⁻¹ of active ingredient. Guarapuava, Paraná State, Brazil, 2016.

These results corroborate those obtained by Amponsah et al. (2012), who reported the efficacy of carbendazim, flusilazole, methyl thiophanate, tebuconazole, iprodione, fenarimol, procymidone, mancozeb, and chlorothalonil in inhibiting mycelial growth of *Neofusicoccum australe*, *N. Luteum*, and *Diplodia mutila*, the causal agents of GTD. The efficacy of tebuconazole on the *in vitro* control of pathogens has also been reported for the control of Botryosphaeriaceae by Sosnowski et al. (2013).

Another chemical active ingredient with potential control is fosetyl-Al. In addition to the efficiency demonstrated *in vitro*, fosetyl-Al has the capacity to translocate into the plant via both the xylem and phloem (Kimati, 2011), which allows the use of this product in the protection of wounds. The *in vitro* efficiency of fosetyl-Al was verified in the control of *Phomopsis* spp. by Mostert and Crous (2000).

No significant differences were verified in the reduction of area under the curve of mycelial growth (AUCMG) of *L. theobromae* subjected to treatments with volatile metabolites of biological control agents (data not shown). Regarding the antagonist test, the biological control agents inhibited the growth of *L. theobromae*, obtaining an index of antagonism of 66.4 and 62.2% for *B. subtilis* and *T. harzianum*, respectively, with no significant difference between them.

The use of *B. subtilis* has also been reported to be effective in controlling *L. theobromae* on rubber trees (Sajitha, Florence, & Dev, 2014). Santos et al. (2016) observed that the antagonistic indices of *B. subtilis* were higher than those of *T. harzianum* in the control of *Dactylonectria macrodidyma*, a causal agent of black foot disease. The same authors reported the efficiency of the volatiles of biological antagonists for the tested pathogen. In this experiment, the volatiles of the biological control agents were not efficient in controlling *L. theobromae*, confirming that the effectiveness of the treatments can vary among fungal species.

Considering the results of the tests *in vitro*, the raw aqueous extracts of garlic and clove, the chemicals tebuconazole, mancozeb, fosetyl-Al, pyraclostrobin, and difeconazole, and the biological control agents *T. harzianum* and *B. subtilis* have potential for use in the control of *Lasiodiplodia theobromae* in grapevines.

In vivo trials

In the present experiment, the means of re-isolation of the inoculated plants without wound protection treatments were similar in both seasons, indicating that the cultivar is susceptible to the entry of pathogens in pruning wounds at different periods of the year, making it necessary to protect these wounds in any pruning condition.

Nevertheless, in general, infection with fungi that cause trunk diseases is favoured by conditions that reduce plant vigour, such as frost, high temperatures in the summer months, malnutrition and poorly conducted pruning (Larignon & Dubos, 2001). Another important factor is the spread and increase of infection rates when pruning is performed. Úrbez-Torres and Gubler (2011) observed that

the susceptibility in California vineyards of cvs. Chardonnay and Cabernet Sauvignon to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum* was significantly higher when inoculation was performed in early winter rather than at the end of winter.

According to our findings, the plants treated with garlic extract after summer pruning differed from all the treatments, showing an increase in the area of internal vascular discoloration (Figure 3a). Nevertheless, for vines inoculated in winter pruning, the inoculated control and the treatments with tebuconazole and *T. harzianum* differed from the other treatments, obtaining the largest length of wood discoloration (Figure 3b).

For the inoculation performed in the summer pruning, there was a reduction in the percent re-isolation for the treatment with *T. harzianum* (Figure 3c). Similarly, in the winter pruning, the use of *T. harzianum* also decreased the percent of re-isolation of the pathogen, not differing from the control without inoculation and the treatment with the clove extract (Figure 3d).

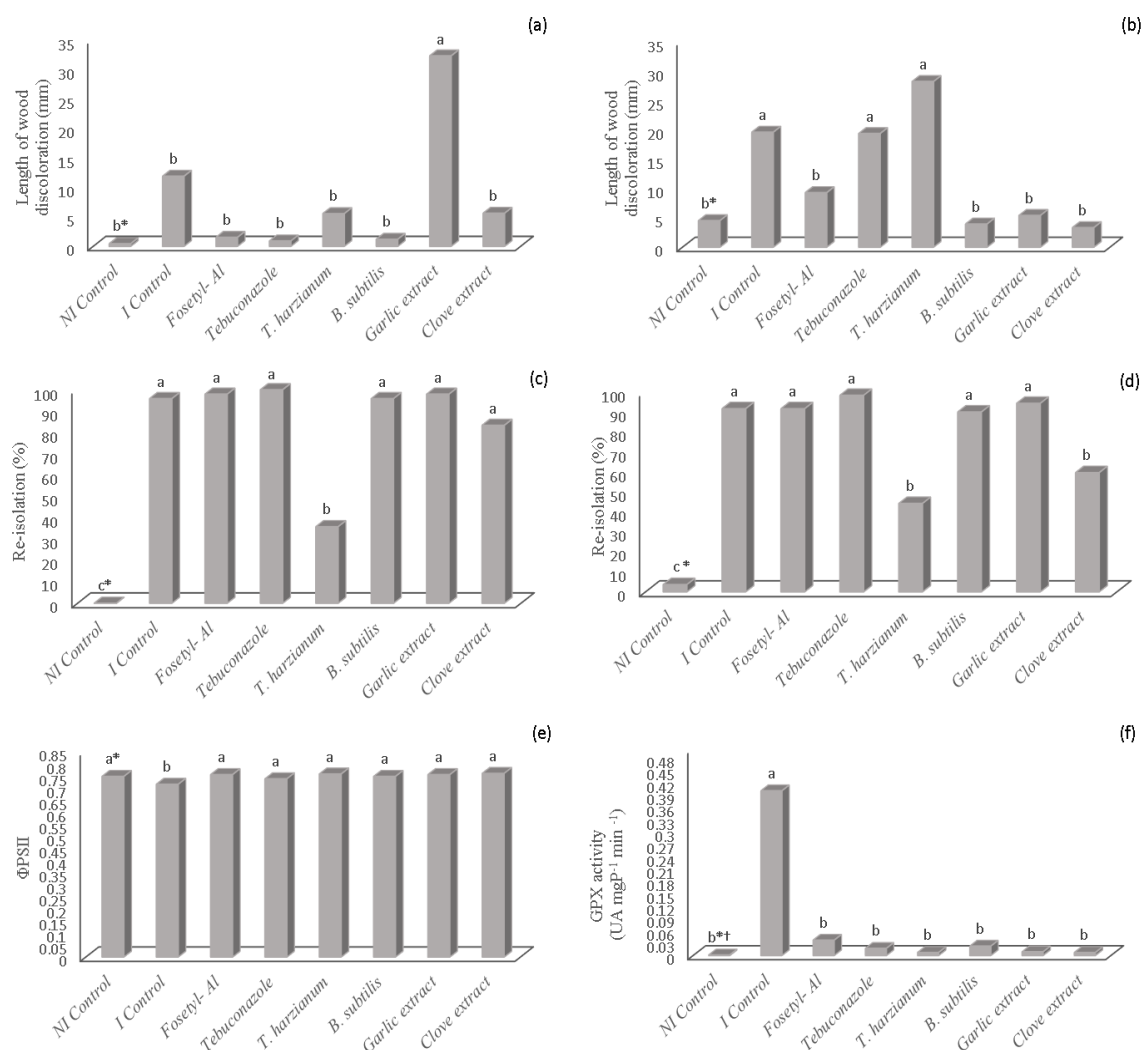


Figure 3. Length of wood discoloration (mm) 230 days after summer pruning inoculation (a), 156 days after winter pruning inoculation (b), percent re-isolation after summer pruning (c), percent re-isolation after winter pruning (d), PSII quantum yield (Fv/Fm) after summer pruning inoculation (e), and peroxidase activity (UA mg P⁻¹ min⁻¹) 24 h after summer pruning inoculation (f) of vines inoculated with *Lasiodiplodia theobromae* in cv. Syrah. †Data transformed by $\sqrt{x}$. *Means followed by the same letter do not differ significantly by the Scott-Knott test ($p \leq 0.05$). ns: not significant. NI: not inoculated. I: inoculated. Guarapuava, Paraná State, Brazil, 2017.

Some researchers have reported the effectiveness of chemical products in pruning wound protection to pathogenic fungi of the vine trunk and decreasing vascular symptoms (Díaz & Latorre, 2013; Sosnowski et al., 2013). Wound protection can also be performed with plant extracts and biological control agents. The use of wound protection decreases the severity of the disease. The use of fosetyl-Al, *B. subtilis*, garlic extracts and clove extracts reduced the severity of the pathogen after winter pruning,

but the garlic extract did not have the same effect in the different periods of inoculation, different from the reports of Cobos et al. (2015) that verified positive effects of wound treatment with garlic extract.

According to Mutawila et al. (2011), the effectiveness of protection based on *Trichoderma* spp. depends on the ability of vine pruning wound colonization by these fungi, and it depends on the interaction with the vine and is more or less effective according to cultivar. After the infection by pathogens in the wounds, the expression of external symptoms can occur; however, the appearance of symptoms is not often observed in the first year of infection, or the symptoms can be observed in one year and are more tenuous or even nonexistent in the following year (Mundy & Manning, 2011). Generally, grapevine decline symptoms are expressed in adult vineyards that are 7 years or older (Larignon & Dubos, 2001). In addition to the difficulty of symptom appearance in the early years, the external symptoms of the presence of the pathogen *Lasiodiplodia* tend to be easily confused with the presence of other pathogens or may result from causes that are not yet known (Úrbez-Torres, 2011).

There was no significant difference in the variables fresh mass of the pruning material, phenological stages and leaf area (data not shown). The presence of *L. theobromae* led to a significant reduction in chlorophyll *a* fluorescence in plants of the inoculated control relative to the other treatments (Figure 3e) and showed higher guaiacol peroxidase activity than all the other treatments (Figure 3f).

The inoculated plants, those without the use of a wound protection treatment, had shorter canes at the evaluations at 77, 119 and 156 days after winter pruning (DAWP) relative to the other treatments (Figure 4).

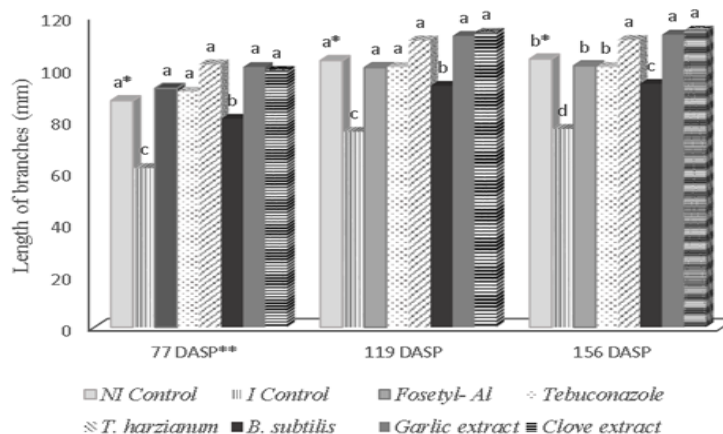


Figure 4. Length of the canes (cm) after inoculation in winter pruning of vines inoculated with *Lasiodiplodia theobromae* in cv. Syrah.

*Means followed by the same letter do not differ significantly by the Scott-Knott test ($p \leq 0.05$). ns: not significant. **DAWP: days after the winter pruning. NI: not inoculated. I: inoculated. Guarapuava, Paraná State, Brazil, 2017.

Physiological stress in association with GTD fungi may contribute to the expression of symptoms in vines (Mundy & Manning, 2011). Among these parameters, peroxidase activity is indicative of intercellular stress due to the activation of the plant's antioxidant system to relieve oxidative stress. The increase in guaiacol peroxidase (GPX) activity in the inoculated control indicates that the entry of this fungus into the plants promotes stress. Another parameter, which confirms the stress caused by this pathogen in vines, was the lower chlorophyll *a* fluorescence, which was represented by the Fv/Fm ratio. It is also possible that the peroxidase and fluorescence evaluations of photosynthesis indicated a level of stress in the plant before they resulted in external symptoms. Letousey et al. (2010) observed that Chardonnay grapevine plants decreased their photosynthesis levels one week before the onset of apoplexy symptoms caused by Esca's disease, suggesting that the vine perceives some signs of the presence of the pathogen (probably toxins) and reacts by decreasing photosynthesis and triggering defence mechanisms. This level of stress caused a decrease in vine growth, reducing the cane length in some evaluations.

Conclusion

Plants infected with fungi that cause diseases of the vine wood alter their metabolism, regardless of the presence of symptoms, such as changes in chlorophyll fluorescence indices, peroxidase activity, and cane growth.

Regarding the management of *L. theobromae* in the grapevine cv. Syrah, the use of *T. harzianum* proved to be an effective option in the protection of wounds, reducing the mycelium growth *in vitro*, re-isolation of inoculated vines not altering the physiological conditions of the plant.

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References

- Almança, M. A. K., Abreu, C., Scopel, F., Benedetti, M., Halleen, F., & Cavalcanti, F. R. (2013). *Evidências morfológicas da ocorrência de Phaeomoniella chlamydospora em videiras no Estado do Rio Grande do Sul* (Comunicado Técnico, 134). Bento Gonçalves, RS: Embrapa Uva e Vinho.
- Alfonzo, A., Conigliaro, G., Torta, L., Burruano, S., & Moschetti, G. (2009). Antagonism of the endophytic *Bacillus subtilis* strain AG1 to fungal pathogens that cause tracheomycotic deterioration of vine wood. *Phytopathologia Mediterranea*, 48(1) 186-187. DOI: 10.14601/Phytopathol_Mediterr-2886
- Amponsah, N. T., Jones, E., Ridgway, H. J., & Jaspers, M. V. (2012). Evaluation of fungicides for the management of Botryosphaeria dieback diseases of grapevines. *Pest Management Science*, 68(5), 676-683. DOI: 10.1002/ps.2309.
- Campanile, G., Ruscelli, A., & Luisi, N. (2007). Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by *in vitro* and *in planta* tests. *European Journal of Plant Pathology*, 117(3), 237-246. DOI: 10.1007/s10658-006-9089-1
- Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. New York, US: John Wiley & Sons.
- Celoto, M. I. B., Papa, M. D. F. S., Sacramento, L. V. S. D., & Celoto, F. J. (2008). Atividade antifúngica de extratos de plantas a *Colletotrichum gloeosporioides*. *Acta Scientiarum. Agronomy*, 30(1), 1-5. DOI: 10.4025/actasciagron.v30i1.1104
- Cobos, R., Mateos, R. M., Álvarez-Pérez, J. M., Olego, M. A., Sevillano, S., González-García, S., ... Coque, J. J. (2015). Effectiveness of natural antifungal compounds to control infection through pruning wounds by grapevine trunk disease pathogens. *Applied and Environmental Microbiology*, 81(18), 6474-6483. DOI: 10.1128/AEM.01818-15
- Correia, K. C., Câmara, M. P. S., Barbosa, M. A. G., Sales Jr., R., Agustí-Brisach, C., Gramaje, D., ... Michereff, S. J. (2013). Fungal trunk pathogens associated with table grape decline in Northeastern Brazil. *Phytopathologia Mediterranea*, 52(2), 380-387. DOI: 10.14601/Phytopathol_Mediterr-11377
- Correia, K. C., Silva, M. A., Morais Jr., M. A., Armengol, J., Phillips, A. J. L., Câmara, M. P. S., & Michereff, S. J. (2015). Phylogeny, distribution and pathogenicity of *Lasiodiplodia* species associated with dieback of table grape in the main Brazilian exporting region. *Plant Pathology*, 65(1), 92-103. DOI: 10.1111/ppa.12388
- Díaz, G. A., & Latorre, B. A. (2013). Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. *Crop Protection*, 46, 106-112. DOI: 10.1016/j.cropro.2013.01.001
- Eichhorn, K. W., & Lorenz, D. H. (1984). Phaenologische entwicklungsstadien der rebe. *European and Mediterranean Plant Protection Organization*, 14(2), 295-298.
- Falker. (2008). Manual do medidor eletrônico de teor clorofila (ClorofiLOG/CFL 1030). Retrieved on Nov. 4, 2017 from <http://www.falker.com.br/download.php>
- French, R. C. (1992). Volatile chemical germination stimulators of rust and other fungal spores. *Mycologia*, 84(3), 277-288. DOI: 10.2307/3760180
- Gramaje, D., & Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Disease*, 95(9), 1040-1055. DOI: 10.1094/PDIS-01-11-0025
- Halleen, F., Fourie, P., & Lombard, P. (2010). Protection of grapevine pruning wounds against *Eutypa lata* by biological and chemical methods. *South African Journal for Enology & Viticulture*, 31(2), 125-132. DOI: 10.21548/31-2-1409

- Kimati, H. (2011). Controle químico. (4a ed.). In L. Amorim, J. A. M. Rezende, & A. Bergamin Filho (Eds.), *Manual de fitopatologia: Princípios e conceitos* (p. 343-365). São Paulo, SP: Agronomica Ceres.
- Larignon, P., & Dubos, B. (2001). The villainy of black dead arm. *Wines Vines*, 82(1), 86-89.
- Letousey, P., Baillieul, F., Perrot, G., Rabenoelina, F., Boulay, M., Vaillant-Gaveau, N., ... Fontaine, F. (2010). Early events prior to visual symptoms in the apoplectic form of grapevine esca disease. *Phytopathology*, 100(5), 424-431. DOI: 10.1094/PHYTO-100-5-0424
- Mostert, L., & Crous, P. (2000). Phomopsis (dead arm): new facets of this well-known vine disease. *Wynboer Oktober*, 135(1), 15-17.
- Mundy, D., & Manning, M. (2011). Physiological response of grapevines to vascular pathogens: a review. *New Zealand Plant Protection*, 64, 7-16. DOI: 10.30843/nzpp.2011.64.5974
- Mutawila, C., Fourie, P., Halleen, F., & Mostert, L. (2011). Grapevine cultivar variation to pruning wound protection by *Trichoderma* species against trunk pathogens. *Phytopathologia Mediterranea*, 50(4), 264-276. DOI: 10.14601/Phytopathol_Mediterr-8981
- Mutawila, C., Halleen, F., & Mostert, L. (2016). Optimisation of time of application of *Trichoderma* biocontrol agents for protection of grapevine pruning wounds. *Australian Journal of Grape and Wine Research*, 22(2), 279-87. DOI: 10.1111/ajgw.12218
- Rolshausen, P. E., Úrbez-Torres, J. R., Rooney-Latham, S., Eskalen, A., Smith, R. J., & Gubler, W. D. (2010). Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. *American Journal of Enology and Viticulture*, 61(1), 113-119.
- Sajitha, K., Florence, E. M., & Dev, S. A. (2014). Screening of bacterial biocontrols against sapstain fungus (*Lasiodiplodia theobromae* Pat.) of rubberwood (*Hevea brasiliensis* Muell. Arg.). *Research in Microbiology*, 165(7), 541-548. DOI: 10.1016/j.resmic.2014.07.002
- Santos, R. F. D., Heckler, L. I., Lazarotto, M., Garrido, L. R., Cecilia, R., & Blume, E. (2016). *Trichoderma* spp. and *Bacillus subtilis* for control of *Dactylonectria macrodidyma* in grapevine. *Phytopathologia Mediterranea*, 55(2), 293-300. DOI: 10.14601/Phytopathol_Mediterr-18048
- Silva, F. D. A. S., & Azevedo, C. A. V. D. (2016). The Assistat Software Version 7.7 and its use in the analysis of experimental data. *African Journal of Agricultural Research*, 11(39), 3733-3740. DOI: 10.5897/AJAR2016.11522
- Simões, C. M. O., & Spitzer, V. (2001). Óleos voláteis. (3a. ed.). In C. M. O. Simões, E. P. Schenkel, G. Gosmann, J. C. P. Mello, L. A. Mentz, & P. R. Petrovick (Eds.), *Farmacognosia: da planta ao medicamento* (p. 387-416) Porto Alegre, RS; Florianópolis, SC: UFRGS/EDUFSC.
- Sosnowski, M. R., Loschiavo, A. P., Wicks, T. J., & Scott, E. S. (2013). Evaluating treatments and spray application for the protection of grapevine pruning wounds from infection by *Eutypa lata*. *Plant Disease*, 97(12), 1599-1604. DOI: 10.1094/PDIS-02-13-0201-RE
- Urbanek, H., Kuzniak-Gebarska, E., & Herka, K. (1991). Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiologiae Plantarum*, 7(13), 43-50.
- Úrbez-Torres, J. R. (2011). The status of Botryosphaeriaceae species infecting grapevines. *Phytopathologia Mediterranea*, 50(4), 5-45. DOI: 10.14601/Phytopathol_Mediterr-9316
- Úrbez-Torres, J. R., & Gubler, W. (2009). Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease*, 93(6), 584-592. DOI: 10.1094/PDIS-93-6-0584
- Úrbez-Torres, J. R., & Gubler, W. (2011). Susceptibility of grapevine pruning wounds to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum*. *Plant Pathology*, 60(2), 261-270. DOI: 10.1111/j.1365-3059.2010.02381.x
- Venturoso, L., Bacchi, L. M. A., Gavassoni, W. L., Conus, L. A., Pontim, B. C. A., & Bergamin, A. C. (2011). Antifungal activity of plant extracts on the development of plant pathogens. *Summa Phytopathologica*, 37(1), 18-23. DOI: 10.1590/S0100-54052011000100003
- Weber, E. A., Trouillas, F. P., & Gubler, W. D. (2007). Double pruning of grapevines: a cultural practice to reduce infections by *Eutypa lata*. *American Journal of Enology and Viticulture*, 58(1), 61-66.

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Agronomic, Physiological, and Post-Harvest Aspects of Different Blueberry Cultivars Treated with Silicon

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Abstract

The objective of this study was to evaluate the effect of foliar application of silicon in different blueberry (*Vaccinium myrtillus*) cultivars. The treatments were installed in a factorial design with six replications, and consisted of three different cultivars (Brightwell, Beckblue and Climax) with or without regular application of silicon via foliar, using silicon oxide at 1.5 g L⁻¹. It was evaluated the following plant attributes: leaf area, chlorophyll fluorescence, and phenylalanine ammonia lyase activity, meanwhile the following postharvest attributes were evaluated: fruit number, diameter and weight, yield, pH, content of soluble solids, titratable acidity, contents of phenolic compounds and anthocyanins, and activity of peroxidase. Vegetative development of blueberry plants in response to Si presented higher yield, leaf area, phenylalanine ammonia lyase activity, and higher chlorophyll fluorescence relationships such as maximum photochemical efficiency and PSII potential activity, as well as less stressed plants, especially in cultivars Climax and Brightwell. For post-harvest physicochemical characteristics, silicon foliar application improved fruit weight and diameter for cvs. Beckblue and Climax, and fruit number for cv. Brightwell. All cultivars, in response to silicon application increased the titratable acidity, the levels of phenolic compounds, the level of anthocyanins, whereas peroxidase activity decreased. The performance of the evaluated blueberry cultivars was better through silicon application, with greater performance of vegetative growth, fruit yield, and fruit quality, especially in cultivar Climax, which was the most suitable in terms of fruit yield in this study.

Keywords: anthocyanins; phenolic compounds, plant nutrition, productivity, *Vaccinium myrtillus*

Introduction

The blueberry (*Vaccinium myrtillus*) fruits are important due its organoleptic characteristics and the benefits to human health, related to the prevention of diseases, such as cardiovascular (Eladwy et al., 2018), neurodegenerative (Debom et al., 2016), cancer (Davidson et al., 2018; Lin et al., 2019) and inflammation (Li et al., 2016; Kalt et al., 2020). Many of the benefits that blueberries can provide were assigned to a large amount of anthocyanin, which is one of the species with the highest content among the fruits and vegetables (Kalt et al., 2001; Gündüz et al., 2015; Kalt et al., 2020). Besides the fresh consumption, these fruits can be destined for the preparation of teas, pies, cakes, puddings, cookies, ice cream, jellies, jams, as well as processed foods (Pritts et al., 1992).

In 2020, world production was 851 thousand tons of blueberries (Faostat, 2022). The three largest producing

countries in the 2020 season were the United States, Peru and Canada, which accounted for 35%, 21% and 17% of world production respectively (Faostat, 2022). In Brazil, there are still no official data on the cultivation of blueberry, however, it is esteemed that there are about 400 ha planted, divided between the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, and Minas Gerais (Cantuarías-Avilés et al., 2014). The main limiting factor for the expansion of the blueberry crop in Brazil is the cold requirement to overcome dormancy, which varies from 300 to 1100 hours per year (with temperatures ≤ 7.2 °C), depending upon the cultivar (Cantuarías-Avilés et al., 2014). In southern Brazil, some regions could meet these conditions, such as the region of Guarapuava, which on average, can reach about 300 hours of cold in winter season (Botelho et al., 2006), which is suitable for some blueberry cultivars being these cultivars Aliceblue, Beckblue, Bluegem, Brightwell, Climax, and Powderblue

(Rabteye) (Cantuarias-Avilés et al., 2014).

Regarding the better performance of plants, minerals can be used to influence the levels of organic compounds through their action in biochemical or physiological processes (Ferreira et al., 2006). Silicon (Si) in plants is a stress-protective mineral nutrient (Moradtalab et al., 2018), as it is a beneficial nutrient in plant physiology and development (Menegale et al., 2015). In plants, Si absorption was in the form of monosilicic acid (H_4SiO_4), mainly (Menegale et al., 2015). Si benefits metabolic functions in stressed plants, through the formation of an outer protective layer composed of silica, and the reactivity of the absorbed silicon with heavy metal ions and other plant compounds (Rodrigues & Datnoff, 2015).

The application of Si has beneficial effects on many fruit crops, but there is still little information about the effect of Si on blueberry. The application of Si in passion fruit (*Passiflora edulis*) increased the photosynthetic rate of the plant (Costa et al., 2018), and in strawberry (*Fragaria × ananassa*) its improved pulp firmness and increased the level of anthocyanins in fruits (Munaretto et al., 2018). In tomato (*Solanum lycopersicum*) a similar result was observed, where the application of Si increased pulp firmness, fruit yield, soluble solids, vitamin C, and lycopene contents (Marodin et al., 2014; Marodin et al., 2016).

Based on the above information, we hypothesize that Si application can potentially improve blueberry production and quality. The objective of this study was to evaluate the effect of Si on the physiological, agronomic, and on postharvest attributes of different blueberry cultivars.

Material And Methods

Site description

The experiment was carried out in Guarapuava, Paraná, Brazil (25°23'S, 51°27'W, and altitude of 1,120 m) under humid subtropical mesothermic climate (Cfb-Köppen) (Alvares et al., 2013), and the average chilling accumulation in the region of Guarapuava is about 308.6 hours (Botelho et al., 2006). The soil of the experimental area is a very clayey Typic Hapludox (USDA Soil Survey), corresponding to Latossolo Bruno according to the Brazilian soil classification system (Embrapa, 2013).

Blueberry plants with 3 years old in a 3 × 1 m spacing were drip irrigated. The weeds were removed and controlled periodically. Phytosanitary control was conducted using Bordeaux mixture, calcium polysulfide (lime sulfur), and Neem oil, whenever necessary.

Experimental design and evaluations

The experimental design consisted of blocks in a randomized factorial 3 × 2 (cultivars × Si treatment), which consisted of three cultivars (Beckblue, Climax e Brightwell) and two Si treatments (with and without Si application), with six replications and one plant per experimental unit.

Silicon (Si) applications were conducted weekly with an electric backpack sprayer, between November 2018 and March 2019, totaling 21 applications. Si source was silicon oxide (SiO_2) (98% of SiO_2), applied at a concentration of 1.5 g L⁻¹ in aqueous solution. Fruit harvesting began on Dec. 18, 2018 (after 7 Si applications), being conducted every two days.

Soon after harvest, fruit diameter, average fruit mass and productivity per plant were determined. Then, they were stored at -18 °C for further physicochemical analyses. To evaluate the single leaf area, ten leaves of each plant were taken randomly and measured with leaf area meter (LI-3100; Li-COR Biosciences Inc.). The chlorophyll fluorescence was measured on fully expanded leaves three weeks after the final Si application in the morning of sunny days (between 08 to 11h a.m.). A Portable Chlorophyll Fluorometer (PAM-2500; Walz) were used. Prior to this measurement, plants were dark adapted for 20 minutes and measurements were performed. From the fluorescence in response to the first light pulses, the following parameters were obtained: F_o (initial fluorescence), F_m (maximum fluorescence), F_v (variable fluorescence), and the following parameters were calculated: F_v/F_o (PSII potential activity) and F_v/F_m (maximum photochemical efficiency).

Protein concentration was determined in leaves and fruits by the method of Bradford, 1976). Phenylalanine ammonia lyase (PAL) activity was evaluated based on the difference in absorbance resulting from the conversion of phenylalanine to trans-cinnamic acid based on the method of (Umesha, 2006). For the evaluation of Protein and PAL, expanded leaves of plants from each experimental plot were collected in the morning, wrapped in aluminum foil, cooled in liquid nitrogen, placed in a Styrofoam box with ice for transport to the laboratory, and subsequently stored in a freezer at -80°C, samples were collected after the last application of silicon. Pulp was macerated and was analyzed for soluble solids content (SS) with digital refractometer model Pal-1 (Atago). The titratable acidity (TA) was determined as follow: 10 g of the blueberry pulp were diluted in 50 ml of distilled water, and this solution was titrated with a standard solution of 0.1 mol L⁻¹ NaOH until it reached pH 8.2. Polyphenol levels of fruit peels was analyzed according to method of (Singleton et al., 1999) using Folin Ciocalteu reagent and calibration curve with

gallic acid. Absorbance readings was obtained at 740 nm (UV 1800, Shimadzu).

The evaluations of anthocyanin contents were conducted in the fruit peel using the differential pH method (Giusti & Wrolstad, 2001; 2003). Delphinidin-3-O-glucoside was the major anthocyanin in blueberry. Peroxidase enzyme (POD) activity on fruit pulp was determined according to (Kar and Mishra, 1976), with determination of the enzymatic activity constituted the conversion of guaiacol into tetraguaiacol.

In **Figure 1** is presented the data of rainfall and maximum and minimum temperatures during the experiment. Meteorological data were obtained from a meteorological station (SIMEPAR/Brazil) located around 100 m far from the experiment.

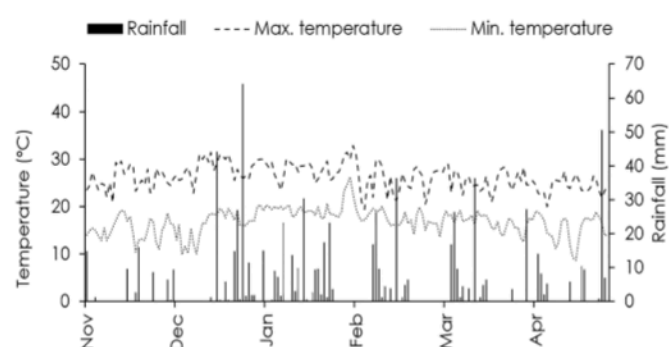


Figure 1. Precipitation (mm), maximum, and minimum temperature (°C) recorded on 2018/2019 growing season in Guarapuava, Paraná, Brazil.

Statistical analysis

The data were submitted to the Shapiro-Wilk test ($p \leq 0.05$) to verify the normality and homoscedasticity of the variance, and when they did not meet this condition, they were transformed according to Box-Cox test and then subjected to analysis of variance (ANOVA). Data was submitted to analysis of variance ($\alpha = 0.05$) in a factorial scheme (3×2). When significant, treatments means were classified by the Tukey test ($\alpha = 0.05$).

Results and Discussion

Leaf applications of Si increased leaf area in all cultivars evaluated (**Figure 2A**). The leaf area of cv. Climax was higher compared to other cultivars for both treatments (Figure 2A). The initial fluorescence (F_o) presented an interaction between the cultivars and the application of Si, where within cv. Blueberry the treatment with Si reduced the F_o , while there was no difference in the other cultivars (Figure 2B). The cultivar Beckblue presented the highest initial fluorescence emission (F_o) (Figure 2B).

Variable fluorescence (F_v) presented interaction between cultivars and Si application (Figure 2C). The Beckblue cultivar, compared to the other cultivars,

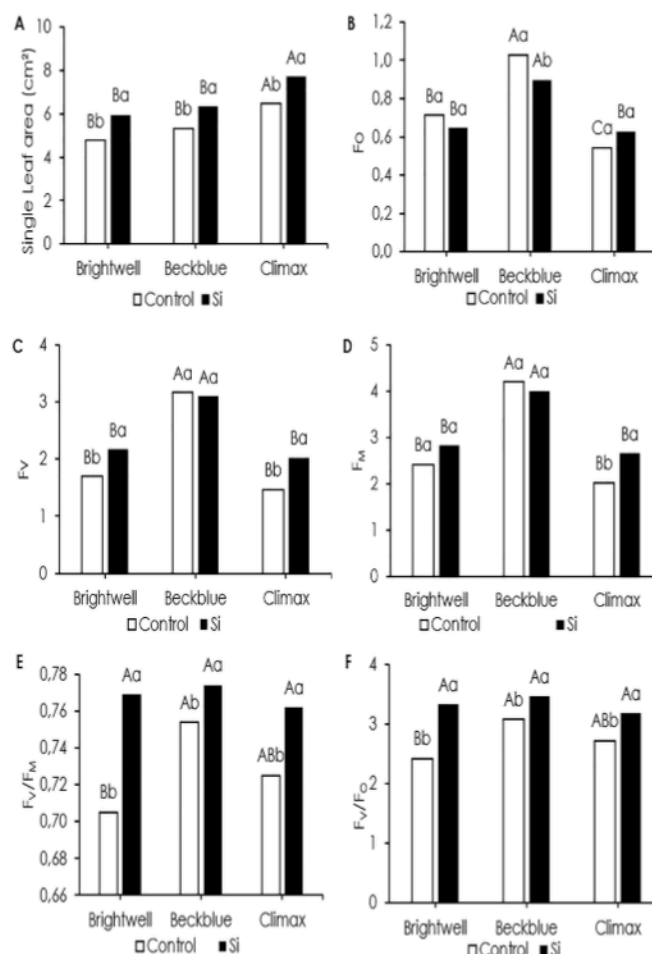


Figure 2. Leaf area (A), initial fluorescence - F_o (B), variable fluorescence - F_v (C), maximum fluorescence - F_m (D), maximum primary photochemical efficiency - F_v/F_m (E), PSII potential activity - F_v/F_o (F) of different blueberry cultivars treated or not with silicon in Guarapuava, Paraná, Brazil. *Same lowercase letters mean no differences between silicon treatments; and same uppercase letters means no differences between blueberry cultivars.

presented the highest F_v , but this attribute did not differ in response to Si, whereas other cultivars presented an increase in F_v when treated with Si (Figure 2C).

Maximum fluorescence (F_m) presented interaction between cultivars and Si application (Figure 2D). The cultivar Beckblue presented the highest F_m , but no difference in Si treatment, meanwhile the cultivars Climax and Brightwell presented higher F_m with the application of Si.

The F_v/F_m ratio presented interaction between cultivars and Si application (Figure 2E). The F_v/F_m ratio increased in the Si application for all cultivars. Within control treatment, F_v/F_m ratio presented the following order between cultivars: Beckblue $\geq$ Climax $\geq$ Brightwell, meanwhile within Si treatment there was no significant difference between the cultivars (Figure 2E).

The F_v/F_o ratio presented interaction between cultivars and Si application (Figure 2F). The F_v/F_o ratio increased in the Si application for all cultivars. Within

control treatment, F_v/F_o ratio presented the following order between cultivars: Beckblue $\geq$ Climax $\geq$ Brightwell, meanwhile within Si treatment there was no significant difference between the cultivars (Figure 2F).

The PAL enzyme activity presented interaction between Si application and cultivars (Figure 3A). The Si application improved PAL activity in cvs. Beckblue and Climax. Within control treatment PAL enzyme activity presented the following order between cultivars: Brightwell = Climax > Beckblue; meanwhile within Si treatment presented the following order between cultivars: Climax > Beckblue > Brightwell (Figure 3A).

The number of fruits presented interaction between Si application and cultivars (Figure 3B). The Si application improved fruit number only for cv. Brightwell (+25%). Within control treatment, the fruit number presented the following order between cultivars: Climax > Beckblue = Brightwell; meanwhile within Si treatment the decreased sequence was Climax $\geq$ Brightwell $\geq$ Beckblue (Figure 3B).

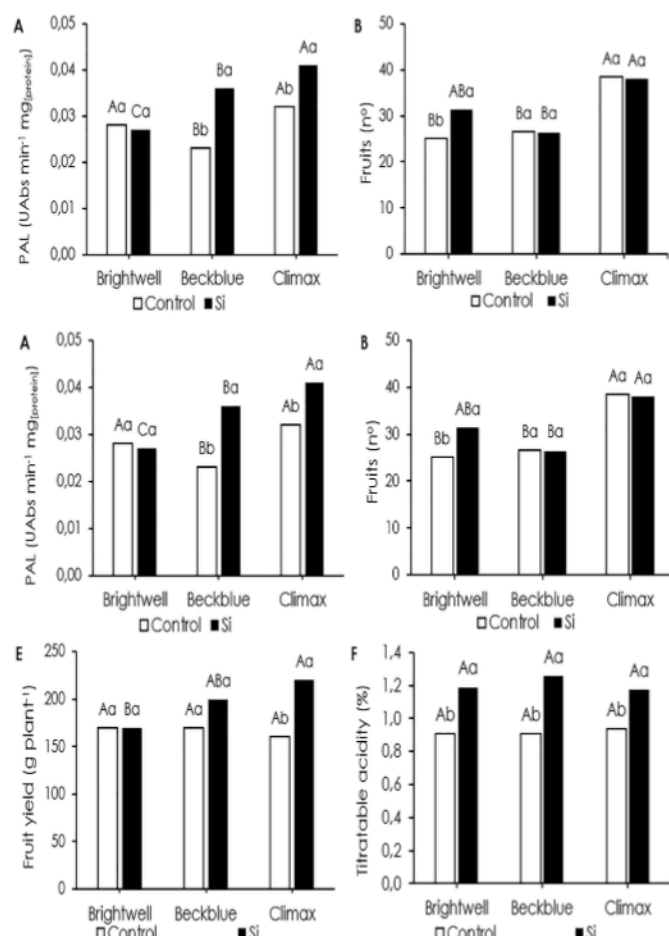


Figure 3. PAL (A), number of fruits (B), fruit diameter (C), fruit weight (D), yield (E), titratable acidity (F) of different blueberry cultivars treated with silicon in Guarapuava, Paraná, Brazil. *Same lowercase letters mean no differences between silicon treatment; and same uppercase letters means no differences between blueberry cultivars.

The fruit diameter presented interaction between Si application and cultivars (Figure 3C). The application of Si increased the fruit diameter of cvs. Beckblue and Climax, at 10% and 14.5%, respectively, while in cv. Brightwell there was no difference (Figure 3C). Within control treatment the diameter presented no differences between cultivars, meanwhile within Si treatment the diameter presented the following order between cultivars: Beckblue $\geq$ Climax $\geq$ Brightwell (Figure 3C).

The average fruit weight presented interaction between Si application and cultivars (Figure 3D). Foliar application of Si increased the average fruit weight for cultivars Beckblue and Climax, by 22% and 34%, respectively, while in Brightwell there was no difference (Figure 3D). Within the control treatment, the average fruit weight had no difference among cultivars, meanwhile with Si application, the average fruit weight presented the following order: Climax $\geq$ Beckblue $\geq$ Brightwell (Figure 3D).

The fruit yield presented interaction between Si application and cultivars (Figure 3E). The application of Si improved fruit yield Climax and Beckblue cultivars (Figure 3E). Within control treatment, fruit yield presented no difference among cultivars, meanwhile within Si treatment was as follows: Climax $\geq$ Beckblue $\geq$ Brightwell (Figure 3E). The titratable acidity did not differ between cultivars both with and without Si application, however, the application of Si increased acidity for all cultivars (Figure 3F).

The content of phenolic compounds differed between cultivars and with the application of Si (Figure 4A). Within the cultivars, the content of phenolic compounds was as follows: Brightwell = Climax > Beckblue (Figure 4A). The application of Si increased the content of phenolic compounds by 20%, 21% and 24% for the cultivars Brightwell, Beckblue and Climax, respectively (Figure 4A). There were no significant differences between cultivars for anthocyanin content in the control treatment (Figure 4B). The application of Si increased the concentration of anthocyanins in the fruit epidermis for all cultivars. Among the cultivars, the anthocyanin content presented the following order: Climax = Brightwell > Beckblue (Figure 4B).

POD activity presented interaction between Si application and cultivars (Figure 4C). Within cultivars, Si application reduced POD activity only for the cvs. Climax and Beckblue in 54% and 60%, respectively (Figure 4C). Without Si application, cultivars presented the following order: Climax > Beckblue > Brightwell; meanwhile with Si application was as follows: Climax > Beckblue = Brightwell (Figure 4C).

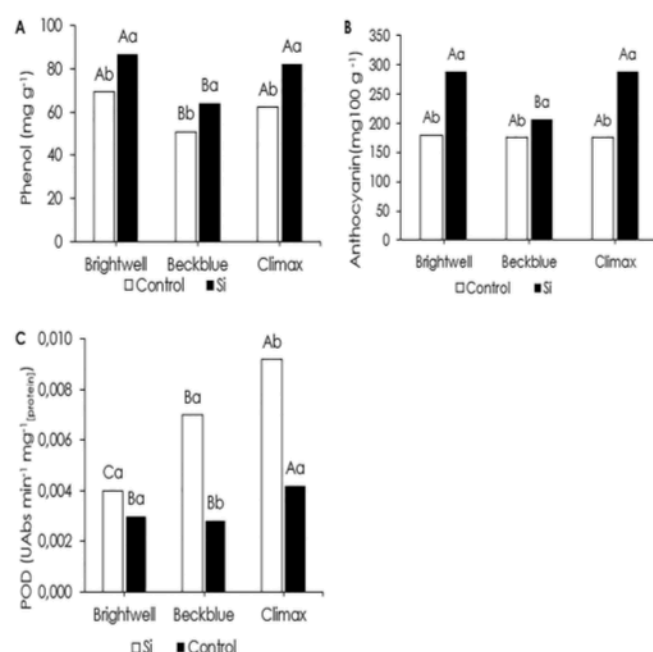


Figure 4. Total phenols (A), anthocyanins (B) and POD activity (C) of different blueberry cultivars treated with silicon. *Same lowercase letters mean no differences between silicon treatment; and same uppercase letters means no differences between blueberry cultivars.

The greater leaf area in response to Si may be related to the deposit of this element below the cuticle, which can provide a more erect plant architecture, thus improve light interception and, therefore, with greater potential for photosynthesis (Silva et al., 2013). Si accumulation below the cuticle can also reduce water loss through transpiration, which benefits net photosynthesis (Silva et al., 2013). The application of Si through various sources was also reported to increase the leaf area of sugarcane (*Saccharum officinarum*) (Sobral et al., 2011), physalis (*Physalis angulata*) (Assis et al., 2013) and passion fruit (*Passiflora edulis*) (Costa et al., 2018).

The greater leaf area in response to Si in all of the cultivars can be related to the deposit of this element below the cuticle, which can provide a more erect plant architecture, thus improving light interception and, therefore, more potential for photosynthesis (Silva et al., 2013). Si accumulation below the cuticle can also reduce water loss through transpiration, which benefits photosynthesis (Silva et al., 2013). The increase of leaf area after the application of Si through various sources was also observed in sugarcane (*Saccharum officinarum*) (Sobral et al., 2011), physalis (*Physalis angulata*) (Assis et al., 2013) and passion fruit (*Passiflora edulis*) (Costa et al., 2018).

Higher values of F_0 indicate low efficiency in the transfer of excitation energy between pigment molecules of photosystem II light collectors (PSII) (Goltsev et al., 2016).

They can also indicate lower energy transfer efficiency to the PSII reaction center after the dissociation of the antenna complex from PSII and damage to the thylakoids (Goltsev et al., 2016). Si treatment reduced F_0 significantly for one of the cultivars in this study (Beckblue), meaning that the mineral improved energy use compared to the control. In rice (*Oryza sativa*) plants, it was also reported that the application of Si reduced fluorescence emission (F_0) (Tatagiba et al., 2016).

The FV parameter is related to the maximum quantum yield of the PSII. When FV is low, it indicates the lower activity of the PSII and the dissipation of the excitation energy as heat (Goltsev et al., 2016). Thus, it improved PSII performance and lowered the dissipation of energy. Another study showed that the application of Si increased F_v in cocoa (*Theobroma cacao*) (Souza et al., 2012).

When F_M values are low, it indicates that the PSII electron acceptors cannot be fully reduced (Goltsev et al., 2016). Therefore, the lowest level of F_M indicates that the photosynthetic apparatus of plants without Si application was under more significant stress than those with Si application. Another study reported that in cocoa, higher maximum fluorescence (F_M) values were found with the application of 7.15 mL L⁻¹ of Si (Souza et al., 2012).

The F_v/F_M ratio represents the maximum primary photochemical efficiency of PSII, and for most developed plants under stress-free conditions, the maximum value is 0.83 (Pokorska & Romanowska, 2007; Goltsev et al., 2016). In our study, the mean for cultivars with Si application was 0.76, while for plants without Si application, it was 0.74 (Figure 2E). The lower F_v/F_M evidence that the plant may be under stress, which impairs PSII functions and decreases electron transfer efficiency (Goltsev et al., 2016). Through stress reduction, Si may have allowed greater photosynthetic efficiency and electron transfer in the evaluated cultivars. Other studies also reported that the application of Si increased the F_v/F_M ratio (Tatagiba et al., 2016; Souza et al., 2012), especially at the dose of 6.5 mL L⁻¹ of potassium silicate (Souza et al., 2012).

In blueberry plants submitted to foliar application of Si, the F_v/F_M ratio was slightly higher (Figure 2E), the range considered ideal for healthy plants, which is around 0.86 (Goltsev et al., 2016). Plants without Si showed an even lower F_v/F_M ratio, which shows that Si allowed a greater efficiency of the photosynthetic apparatus and in the transfer of electrons to the Climax and Brightwell cultivars through the reduction of stress.

The F_v/F_0 ratio indicates the maximum efficiency of the photochemical process in PSII and/or the potential

photosynthetic activity, and its decrease indicates a decline in the photochemical rate (Goltsev et al., 2016). Therefore, Si promoted an increase in the photochemical rate for all of the cultivars in our study. Another study also reported that the application of salicylic acid to tomato plants increased the F_v/F_o ratio (Favaro et al., 2019).

The results of better photosynthesis parameters for plants with Si application can be related to the more significant leaf area for all cultivars caused by the Si deposition below the cuticle. This deposition can change the architecture of the plant, making them more upright, capturing more sunlight, and therefore increasing the photosynthetic rate (Silva et al., 2013). Also, it is speculated that plants treated with Si have a greater capacity to transfer the excitation of energy from the pigments to the formation of reducing power (NADPH and ATP), which increases the capacity of assimilation of CO_2 in the biochemical phase of photosynthesis (Baker, 2008; Favaro et al., 2019).

The increase in PAL enzyme activity in response to Si application in blueberry is promising. PAL is involved in some biosynthetic routes of secondary metabolism, such as the synthesis of phenolic compounds as phenylpropanoids, coumarins, flavonoids, condensed tannins and lignin (Taiz et al., 2017). Another study reported that the application of Si in rice also increased the activity of the PAL enzyme, as well as peroxidase and polyphenol oxidase (Cai et al., 2008). The increase in PAL activity may be associated with the ability of Si to promote greater expression of genes encoding this enzyme (Rodrigues & Datnoff, 2015).

The higher production of lignin gives more mechanical strength to the plant, especially in vascular tissues, and favors the conduction of water and minerals through the xylem (Taiz et al., 2017). Thus, the higher fruit weight, yield and leaf area may have been indirectly benefited as a result of the higher PAL activity. Another study reported that fertilization with Si, applied both via soil and foliar improved the number of strawberry fruits (Silva et al., 2013). The application of Si was also reported to increase the diameter of fruits in sapodilla trees (*Manilkara achras*) (Lalithya et al., 2014).

Studies on the effect of foliar application of Si on strawberries have divergent results. One study reported an increase in strawberry mass (Silva et al., 2013). Meanwhile, another study reported no difference (Munaretto et al., 2018). Other studies also reported that the application of Si increased fruit yield in strawberry (Silva et al., 2013) and tomato (Marodin et al., 2014). In sapodilla plants, the application of Si modified leaf angle, resulting in a greater

number, diameter, and mass of fruits (Lalithya et al., 2014). The change in leaf angle improves light interception and, thus, more significant photosynthetic activity, with a consequent increase in metabolite translocation and plant performance (Das et al., 2019). Si increases carbon fluxes and enzyme activity in the Krebs cycle, which also leads to greater photosynthetic efficiency (Das et al., 2019).

The content of organic acids in tissue plants can increase by Si application, which influences the titratable acidity of the fruits (Silva et al., 2013). It was reported in rice that foliar application of Si could increase the content of organic acids such as citric, succinic, and malic due to increased carbon fluxes and enzyme activity in the Krebs cycle and, consequently, higher production of their metabolite intermediates (Das et al., 2019). Another study also reported that soil and foliar application of Si increased the titratable acidity in strawberries (Silva et al., 2013). In contrast, another study reported no effect of the foliar application of Si on the titratable acidity in strawberries (Munaretto et al., 2018).

Si can increase the activity of enzymes such as PAL in wheat (*Triticum aestivum*), which is related to the synthesis of phenolic compounds (Gomes et al., 2005). Si catalyzes the deamination of phenylalanine to form phenylpropanoids, which are precursors of phenolic compounds (Gunes et al., 2007; Gomes et al., 2008). In addition, Si is related to the formation of bioactive compounds in plants and the improvement of the photosynthetic process and activates secondary metabolic pathways (Gunes et al., 2007; Munaretto et al., 2018). Studies reported that Si application did not affect the levels of phenolic compounds in strawberry (Munaretto et al., 2018) and soybean (Ferreira et al., 2006) plants.

Anthocyanins are flavonoids from the compound phenolics group responsible for the blue, violet, and shades of red (Singleton, 1987). Therefore, the increase of anthocyanins is related to the increase of Phenolic compounds. Other studies also reported that foliar application of Si increased anthocyanin content in strawberry fruits (Munaretto et al., 2018; Silva et al., 2013).

The POD enzyme is related to cell protection and disease resistance through the oxidation of phenolic substances to quinones in the tissue aging process (Mohammadi & Kazemi, 2002). POD is an enzyme with an essential role in enhancing antioxidant capacity, promoting the oxidation of phenolics, forming ethylene, and improving respiratory strength (Chittoor et al., 1999). POD levels increase with fruit ripening as a defensive

response to neutralize free radicals (Chittoor et al., 1999). The decrease in POD activity in blueberries found in this study could be related to the lower stress conditions in fruits treated with Si. Si application was also reported to improve POD activity in wheat (Gomes et al., 2005) and potato (Gomes et al., 2008).

Conclusions

The performance of the evaluated blueberry cultivars was better through Silicon application, with greater performance of vegetative growth, fruit yield, and fruit quality, especially in cultivar Climax, which was the most suitable in terms of fruit yield in this study. Photosynthetic attributes, fruit yield, fluorescence attributes, and phenylalanine activity were benefited with the application of Si.

In the post-harvest aspects, foliar application of Si improved the quality of blueberry fruits, with greater mass and diameter in cultivars Beckblue and Climax, and greater number of fruits in cultivar Brightwell. In the three cultivars evaluated, the application of Si increased the titratable acidity content, the content of phenolic compounds and anthocyanins. The application of Si reduced peroxidase activity for all cultivars.

References

- Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711–728.
- Assis, F.A., Moraes, J.C., Avad, A.M., Coelho, M. 2013. The effects of foliar spray application of silicon on plant damage levels and components of larval biology of the pest butterfly *Chlosyne lacinia saundersii* (Nymphalidae). *International Journal of Pest Management* 59: 128–134.
- Baker, B. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology* 59: 89–113.
- Botelho, R.V., Ayub, R.A., Müller, M.M.L. 2006. Sommatory of chilling hours and chilling units at different regions of Paraná state. *Scientia Agraria* 7: 89–96.
- Bradford, M.M.A. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.
- Cantuaras-Avilés, T., Silva, S.R.D., Medina, R.B., Moraes, A.F.G., Alberti, M.F. 2014. Cultivo do mirtilo: atualizações e desempenho inicial de variedades de baixa exigência em frio no Estado de São Paulo. *Revista Brasileira de Fruticultura* 36: 139–147.
- Cai, K., Gao, D., Luo, S., Zeng, R., Yang, J., Zhu, X. 2008. Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. *Physiologia Plantarum* 134: 324–333.
- Chittoor, J.M., Leach, J.E., White F.F. 1999. Induction of peroxidase during defense against pathogens. In: Datta, S.K. Muthukrishnan, S. *Pathogenesis-Related Proteins in Plants*. CRC Press, Boca Raton, USA. 171–193 P.
- Costa, B.N.S., Costa, I.D.J.S., Dias, G.D.M.G., Assis, F.A.D., Pio, L.A.S., Soares, J.D.R., Pasqual, M. 2018. Morpho-anatomical and physiological alterations of passion fruit fertilized with silicone. *Pesquisa Agropecuária Brasileira* 53: 163–171.
- Das, P., Manna, I., Sil, P., Bandyopadhyay, M., Biswas, A.K. 2019. Exogenous silicon alters organic acid production and enzymatic activity of TCA cycle in two NaCl stressed indica rice cultivars. *Plant Physiology and Biochemistry* 136: 76–91.
- Davidson, K.T., Zhu, Z., Balabanov, D., Zhao, L., Wakefield, M.R., Bai, Q., Fang, Y. 2018. Beyond conventional medicine – a look at blueberry, a cancer-fighting superfruit. *Pathology and Oncology Research* 24: 733–738.
- Debom, G., Gazal, M., Soares, M.S.P., Couto, C.A.T., Mattos, B., Lencina, C., Kaster, M.P., Guisleni, G.C., Tavares, R., Braganhol, E., Chaves, V.C., Reginatto, F.H., Stefanello, F., Spanevello, R.M. 2016. Preventive effects of blueberry extract on behavioral and biochemical dysfunctions in rats submitted to a model of manic behavior induced by ketamine. *Brain Research Bulletin* 127: 260–269.
- Eladwy, R.A., Mantawy, E.M., El-Bakly, W.M., Fares, M., Ramadan, L.A., Azab, S.S. 2018. Mechanistic insights to the cardioprotective effect of blueberry nutraceutical extract in isoprenaline-induced cardiac hypertrophy. *Phytomedicine* 51: 84–93.
- Embrapa. 2013. *Sistema brasileiro de classificação de solos*. Embrapa, Rio de Janeiro, Brasil. 353 p.
- Faostat. Crops and livestock products. 2022. <http://www.fao.org/faostat/en/#data/TP> <Access em 2 Fev. 2022>
- Favaro, R., Resende, J.T.V., Gabriel, A., Zeist, A.R., Cordeiro, E.C.N., Favaro, J.L. 2019. Salicylic acid: resistance inducer to two-spotted spider mite in strawberry crop. *Horticultura Brasileira* 37: 60–64.
- Ferreira, M.M.M., Ferreira, G.B., Fontes, P.C.R., Dantas, J.P. 2006. Tomato quality as a function of nitrogen doses and organic fertilization in two seasons. *Horticultura Brasileira* 24: 141–145.
- Giusti, M.M., Wrolstad, R.E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal* 14: 227–225.
- Giusti, M., Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad, R.E. *Current Protocols in Food Analytical Chemistry*. John Wiley & Sons, Oregon, EUA. 1000 p.
- Goltsev, V., Kalaji, H.M., Paunov, M., Bqba, W., Horaczek, T., Mojski, J., Kociel, H., Allakhverdiev, S.I. 2016. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russian Journal of Plant Physiology* 63: 869–

893.

Gomes, F.B., Moraes, J.C.D., Santos, C.D.D., Goussain, M.M. 2005. Resistance induction in wheat plants by silicon and aphids. *Scientia Agricola* 62: 547-551.

Gomes, F.B., Moraes, J.C., Santos, C.D.D., Antunes, C.S. 2008. Use of silicon as inductor of the resistance in potato to *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Neotropical Entomology* 37: 185-190.

Gunes, A., Inal, A., Bagci, E.G., Coban, S., Pilbeam, D.J. 2007. Silicon mediates changes to some physiological and enzymatic parameters symptomatic for oxidative stress in spinach (*Spinacia oleracea* L) grown under B toxicity. *Scientia Horticulturae* 113: 113-119.

Gündüz, K., Serçe, S., Hancock, J.F. 2015. Variation among Highbush and Rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics. *Journal of Food Composition and Analysis* 38: 69-79.

Kalt, W., Cassidy, A., Howard, L.R., Krikorian, R., Stull, A.J., Tremblay, F., Zamora-Ros, R. 2020. Recent research on the health benefits of blueberries and their anthocyanins. *Advances in Nutrition* 11: 224-236.

Kalt, W., Ryan, D.A., Duy, J.C., Prior, R.L., Ehlenfeldt, M.K., Vander Kloet, S.P. 2001. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). *Journal of Agricultural and Food Chemistry* 49: 4761-4767.

Kar, M., Mishra, D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during Rice leaf senescence. *Physiologia Plantarum* 57: 315-319.

Lalithya, K.A., Bhagya, H.P., Choudhary, R. 2014. Response of silicon and micronutrients on fruit character and nutrient content in leaf of Sapota. *Biolife* 2: 594-598.

Li, J., Deng, R., Hua, X., Zhang, L., Lu, F., Coursey, T.G., Pflugfelder, S.C., Li, D.Q. 2016. Blueberry component pterostilbene protects corneal epithelial cells from inflammation via anti-oxidative pathway. *Scientific Reports* 6: 19408.

Lin, Y., Li, B., Zhao, J., Wei, L., Wang, Y., Wang, M., Dia, V.P., Meng, X. 2019. Combinatorial effect of blueberry extracts and oxaliplatin in human colon cancer cells. *Journal of Cellular Physiology* 234: 17242-17253.

Marodin, J.C., Resende, J.T., Morales, R.G., Faria, M.V., Trevisan, A.R., Figueiredo, A.S., Dias, D.M. 2016. Tomato post-harvest durability and physicochemical quality depending on silicon sources and doses. *Horticultura Brasileira* 34: 361-366.

Marodin, J.C., Resende, J.T., Morales, R.G., Silva, M.L., Galvão, A.G., Zanin, D.S. 2014. Yield of tomato fruits in relation to silicon sources and rates. *Horticultura Brasileira* 32: 220-224.

Menegale, M., Castro, G., Mancuso, M. 2015. Silicon: interaction with soil-plant system. *Journal of Agronomic Sciences* 4: 435-454.

Mohammadi, M., Kazemi, H. 2002. Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Science* 162: 491-498.

Moradtalab, N., Weinmann, M., Walker, F., Höglinger, B., Ludewig, U., Neumann, G. 2018. Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. *Frontiers in Plant Science* 9: 420.

Munaretto, L.M., Botelho, R.V., Resende, J.T.V., Schwarz, K., Sato, A.J. 2018. Productivity and quality of organic strawberries pre-harvest treated with silicon. *Horticultura Brasileira* 36: 40-46.

Pokorska, B., Romanowska, E. 2007. Photoinhibition and D1 protein degradation in mesophyll and agranal bundle sheath thylakoids of maize. *Functional Plant Biology* 34: 844-852.

Pritts, M.P., Hancock, J.F., Strik, B., Eames-Sheavly, M., Celentano, D. 1992. Highbush blueberry production Guide. *Northeast Regional Agricultural Engineering Service* 607: 255-7654.

Rodrigues, F.A., Datnoff, L.E. 2015. *Silicon and plant diseases*. Springer, Viçosa, Brasil. 148 p.

Silva, M.L.S., Resende, J.T.V., Trevizam, A.R., Figueiredo, A.S.T., Schwarz, K. 2013. Influence of silicon on production and fruit quality of strawberry. *Semina: Ciencias Agrarias* 34: 3411-3424.

Singleton, V.L. 1987. Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *American Journal of Enology and Viticulture* 38: 69-77.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* 299: 152-178.

Sobral, M.F., Nascimento, C.W., Cunha, K.P., Ferreira, H.A., Silva, A.J., Silva, F.B. 2011. Basic slag and its effects on the concentration of nutrients and heavy metals in sugarcane. *Revista Brasileira de Engenharia Agrícola e Ambiental* 15: 867-872.

Souza, C.A.S., Pinto, D.G., Aguilar, M.A.G., Coelho, R.L., Júnior, A.J.G., Cao, J.R., Braga, T.Z., Siqueira, P.R. 2012. Influence of silicon on the growth, the fluorescence of the chlorophyll A and in the incidence of insect pests in cacao genotypes. *Agrotrópica* 24: 31-40.

Taiz, L., Zeiger, E., Møller, I.M., Murphy, A. 2017. *Fisiologia e desenvolvimento vegetal*. Artmed, Porto Alegre, Brasil. 858 p.

Tatagiba, S.D., Damatta, F.M., Rodrigues, F.A. 2016. Silicon partially preserves the photosynthetic performance of rice plants infected by *Monographella albescens*. *Annals of Applied Biology* 168: 111-121.

Umesha, S. 2006. Phenylalanine ammonia-lyase activity in

tomato seedlings and its relationship to bacterial canker disease resistance. *Phytoparasitica* 34: 68–71.

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Overcoming dormancy in 'Laetitia' plum trees with the use of mineral oil, vegetable oil and copper sulphate

Authors: C. Rusin, K.S. Sapelli, R. Schoroeder, J.G.S. Cabral, R.V. Botelho

Keywords: catalase, chilling hours, postharvest, *Prunus salicina* L., sprouting

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Abstract:

Some regions do not provide adequate chilling for growing plum trees, requiring the use of sprouting inducers, which mostly can be highly toxic to the environment. The objective of this work was to test the efficiency of copper sulphate and vegetable and mineral oils in breaking dormancy of plum trees for use in an organic production system. In the first year (2018/2019), the greatest hastening of sprouting was observed for treatments using mineral oil 2% + vegetable oil 2% (one or two applications). Moreover, all treatments using vegetable oil and mineral oil showed greater final sprouting, differing significantly from the other treatments. In the second year (2019/2020), treatments with one application of 2% copper sulfate or 1% mineral oil + 1% vegetable oil hastened sprouting, although for final sprouting at 20 days after treatments, there were no significant differences. In both years, there was reduction in catalase activity in plant buds for most treatments with mineral and vegetable oils, except for mineral oil 2% + 2% vegetable oil (1 application) in the first year. This effect was also observed for treatments with one application of 1 or 2% copper sulphate, in both crops. In both cycles, there were frequent periods of rain during flowering, which impaired fruit set, leading to inconsistent results in relation to production aspects. The highest fruit mass was verified for the treatment with an application of 2% mineral oil + 2% vegetable oil in the first year. The treatment with an application of 2% copper sulphate showed a higher number of fruits in both years. Considering the results, the effects of two applications of 2% mineral oil + 2% vegetable oil were promising and could be a viable alternative for breaking the dormancy of 'Laetitia' plum trees in sustainable systems of fruit production.

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Almança et al. Controle de doenças e pragas da videira em vinhedos conduzidos em regime de dupla poda. INFORME AGROPECUÁRIO (BELO HORIZONTE). v.41, p.52 - 62, 2020.

Almança et al. Uso do Trichoderma na cultura da uva In: Trichoderma USO NA AGRICULTURA, ed.1. Brasília, DF: Embrapa, 2019, p. 507 – 520.

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Biology



and Species



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The vine is one of the oldest plants on the planet, whose remotes fossils date back more than 120 million years ago. It has been part of human civilization since ancient times, with many indications of its use for consumption and cultivation in various archaeological and historical sites. Until today, the vine is very present in modern society, with great socio-economic and cultural importance in many countries. The taxonomy of the genus *Vitis* has been the subject of a lot of discussion, however it is generally considered to comprise two distinct sections: *Muscadinia* and *Euvinis*. It is clear that genus *Vitis* shows an impressive genetic variability for many agronomic characteristics being strongly conditioned by the climatic and soil conditions where the plants are implanted.

Thus, written by a group of international viticulture researchers, “*Vitis: Biology and Species*” is a book that provides up-to-date reviews, overviews and summaries of current research on the most recent developments in *Vitis* species characterization, biology and composition.

This book is composed by thirteen chapters that provide current research on different topics of recent knowledge about native grape varieties from different origins, the impact of different climatic and soil conditions on vine managements, the description of the main grapevines disease and their control, grape varieties composition and the use of modern digital technologies on viticulture. By reading this book, readers can find an excellent description of the state-of-the art information and perspectives regarding to the most recent studies on the different dimensions of *Vitis* plants production. This new book is an important publication, which will be of great use to winegrowers, vine and wine scientists, students and other professionals that might be interested in reading and learning about some fascinating areas of vine research and discovery the most recent tendencies of vine biology and species.

Special Feature

The editors created a special video to provide additional information about the book. To watch the video, [click here](#).

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Reviews

“This book is a fascinating journey into the viticultural world and biodiversity, jealously guarded by man starting from the Mediterranean, in the oceans, islands and continents. The authors narrate the symbiosis of man and *Vitis*, the most generous genus, in which rural and scientific knowledge are admirably reflected and fruitful technological innovations ripen. The reading invites, with a look at the past, to appreciate the inestimable values preserved in the wine-growing landscapes and, at the same time, look with confidence to the future, inspired by the search for harmony between man and nature.” – **Adamo Domenico**



in different producing regions of the world, including different species and new technologies applied to characterize them. Addressing scientific questions for experts and professionals, but in a clear and accessible way, this book will also help technicians, producers and the general public to find answers to several aspects involving the modern cultivation of vines.” – **Professor Dr. Regina Vanderlinde, Institute of Biotechnology – University of Caxias do Sul, RS, Brazil, and President of O.I.V. – International Organisation of Vine and Wine**

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Apresentação

A mudança do período de colheita do verão chuvoso para o inverno seco, por meio do manejo da dupla poda, técnica validada pela EPAMIG, possibilitou a obtenção de uvas com melhores índices de maturação e sanidade, incrementando a qualidade dos vinhos finos de colheita de inverno (ou simplesmente denominados vinhos de inverno). Esta inovação foi concretizada por meio da parceria público-privada que deu início às pesquisas conduzidas com o objetivo de transferir o ciclo de produção da videira da primavera/verão para a estação seca de outono/inverno do Sudeste brasileiro, com elevada amplitude térmica.

Impulsionados pela notoriedade desses vinhos, novos empreendimentos vitícolas foram implantados, inclusive na região central do Brasil, ainda não explorada pela vitivinicultura. A colheita de inverno tem contribuído para mudar o cenário nacional de produção de vinhos finos, inserindo as regiões Sudeste e Centro-Oeste em uma nova fronteira vitícola.

Esta nova edição do Informe Agropecuário, Vinhos de colheita de inverno, reúne informações técnicas relevantes obtidas durante 20 anos de pesquisa com o manejo da dupla poda, com destaques para: panorama atual dos vinhedos; implantação e condução dos parreirais; resultados recentes de pesquisas sobre porta-enxertos e cultivares; controle de maturação e vinificação; viticultura de precisão; informações sobre terroir e orientações para obtenção das Indicações Geográficas e a criação da marca coletiva “Vinhos de Inverno”. Espera-se que as informações contidas nos respectivos artigos possam atender à alta demanda que existe hoje sobre todo o processo produtivo das uvas destinadas à elaboração dos “Vinhos de Inverno”.

Renata Vieira da Mota
Claudia Rita de Souza

Informe Agropecuário

Uma publicação da EPAMIG

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Pesquisa abre novos horizontes para a produção de vinhos finos

Até o início do século 21, a produção de vinho fino no Sudeste brasileiro era inexpressiva, sendo o estado do Rio Grande do Sul detentor da quase totalidade dessa produção. Condições climáticas adversas à boa maturação e à sanidade das uvas resultavam em baixa produção, pois no ciclo produtivo da videira a maturação e a colheita coincidiam com o verão chuvoso da região. Entretanto, em algumas localidades do Sudeste, o outono/inverno apresentava as condições ideais para a maturação da uva para vinificação, tais como escassez de chuvas, dias ensolarados e noites frias.

Ao avaliar este potencial, a EPAMIG deu início, a partir de 2001, a uma linha de pesquisa com a finalidade de alterar o ciclo de produção da videira, de tal forma que o período de maturação e de colheita da uva coincidissem com os meses de outono/inverno. Parcerias com empresas privadas proporcionaram avaliar a possibilidade de gerar um segundo ciclo da videira mediante duas podas anuais. O deslocamento da colheita para o período de inverno seco da Região Sudeste permitiu a evolução da maturação das uvas no campo.

Assim, surgiram os vinhos de colheita de inverno, elaborados com uvas de variedades da espécie *Vitis vinifera*, como exigido pela legislação brasileira, para produção de vinhos finos. Dentre as variedades inicialmente testadas, as que se adaptaram melhor ao manejo da dupla poda foram as uvas 'Syrah' (tinta) e 'Sauvignon Blanc' (branca), com boa expressão vegetativa e excelente potencial de produção e maturação.

Os vinhos elaborados a partir das uvas colhidas no inverno têm sua qualidade comprovada pela conquista de prêmios nacionais e internacionais, destacando-se o mais recente: Medalha de Ouro no concurso Brazil Wine Challenge 2020, concedida ao Vinho 'Sirah' EPAMIG, safra 2018, cujas uvas foram produzidas no Campo Experimental de São Sebastião do Paraíso (CESP) da EPAMIG Sul, sob o manejo da dupla poda.

Esta edição do Informe Agropecuário apresenta importantes resultados para a produção de vinhos finos no Sudeste brasileiro, demonstrando que a pesquisa é fator preponderante para o desenvolvimento agropecuário nacional.

Nilda de Fátima Ferreira Soares
Presidência da EPAMIG

Tecnologia da dupla poda impulsiona produção de vinhos finos no Sudeste brasileiro



O engenheiro-agrônomo, José Procópio Stella, é formado pela Universidade Federal de Viçosa (UFV) e atuou na Associação de Crédito e Assistência Rural de Mato Grosso (Acamart) e na Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais (Emater-MG), em Pouso Alegre. Foi superintendente operacional da Plantar, em Curvelo, MG, e gerente operacional na Sementes Viçosa, em Andradas, MG. Seguindo a vocação familiar para a vitivinicultura, atualmente é diretor-técnico da Vinícola Stella Valentino Ltda., localizada em Andradas. A Vinícola produz vinhos finos, mantendo a herança de cinco gerações da família Stella, desde a Itália até o Brasil.

IA - *A família Stella tem tradição centenária no cultivo de uvas americanas e produção de vinho. Como foi a passagem para a produção de uvas viníferas e elaboração de vinhos finos?*

José Procópio Stella - A família Stella, desde 1910, cultiva uvas americanas e híbridas e elabora vinhos comuns para o consumo próprio. Em 2002 foi feito um projeto para a produção de vinhos finos, com isso foram introduzidas 36 variedades de uvas viníferas. Depois de 18 anos de pesquisa, em parceria com a EPAMIG Sul, em Caldas, consegui-

mos produzir alguns vinhos finos, tais como: Syrah, Tempranillo e Sauvignon Blanc.

IA - *Quais foram as principais dificuldades para iniciar a produção de vinhos finos?*

José Procópio Stella - No Sudeste brasileiro, a colheita das uvas é realizada nos meses de janeiro e fevereiro. Porém, nesta época têm-se altos índices pluviométricos, o que dificulta a maturação das uvas viníferas. Então, numa parceria com a EPAMIG, foi desenvolvida a técnica da dupla poda, ou seja, transferimos a colheita para

os meses de inverno. Com isso, colhemos as uvas numa época seca, com excelente luminosidade, com amplitude térmica perfeita (temperaturas noturnas em torno de 8 °C e temperaturas diurnas em torno de 25 °C) e solo seco. O conjunto desses fatores é tudo o que precisamos para obter uma maturação plena das uvas. Assim, a grande dificuldade que era colher uva de excelente qualidade no verão, conseguimos por meio da transferência da colheita para o inverno.

IA - *E quais foram as principais vantagens nesta mudança, tanto na*

produção de uvas viníferas, quanto na elaboração de vinhos finos?

José Procópio Stella - A grande vantagem da colheita no inverno é obter uma maturação perfeita das uvas. Com isso, levamos para a vinificação uvas de excelente qualidade, o que nos coloca no mesmo patamar das grandes regiões vitivinícolas do mundo que produzem vinhos finos. Trata-se de uma grande conquista e a possibilidade de ampliação de mercados, tanto interno quanto externo.

IA - *Qual a importância da pesquisa para o setor vitivinícola do Sudeste e qual(is) seria(m) o(s) principal(ais) tema(s) que precisaria(m) ser estudado(s)?*

José Procópio Stella - A pesquisa realizada pela EPAMIG é de suma importância para o setor vitivinícola. Muito já se fez, porém precisamos da pesquisa de maneira contínua, pois sempre novos desafios surgem e daí a necessidade de investigações permanentes. Dentre os principais temas, citamos a adaptação de novas cultivares de uvas e porta-enxertos e também métodos de controle de doenças. Estes dois temas são de extrema importância para obter uma produção de qualidade.

IA - *Publicações técnico-científicas como o Informe Agropecuário, que completou 45 anos, têm contribuído para o desenvolvimento agropecuário, em especial vitivinícola?*

José Procópio Stella - A tecnologia agrícola desenvolve-se de maneira muito rápida. Visto isso, todas as publicações técnico-científicas são extremamente bem-vindas e o Informe Agropecuário sempre mostrou-se de alto nível, vindo a contribuir de maneira ímpar no setor vitivinícola.

IA - *Como é a interação entre os produtores na cadeia vitivinícola?*

José Procópio Stella - Os produtores de vinhos de inverno criaram uma associação para representá-los. Trata-se da Associação Nacional dos Produtores de Vinhos de Inverno (Anprovin). Esta Associação tem o objetivo de unir os produtores tanto no setor vitivinícola quanto no setor mercadológico. Estão sendo criados padrões de qualidade e, no futuro, a criação de um selo atestando a veracidade dos vinhos de inverno.

“

A grande vantagem da colheita no inverno é obter uma maturação perfeita das uvas. Com isso, levamos para a vinificação uvas de excelente qualidade, o que nos coloca no mesmo patamar das grandes regiões vitivinícolas do mundo que produzem vinhos finos.

”

IA - *Qual a sua opinião sobre a criação do selo “Vinhos de Inverno”?*

José Procópio Stella - O selo “Vinhos de Inverno” é uma iniciativa de extrema importância para nós produtores, confirmando a grande qualidade dos nossos vinhos. Tem também a

finalidade de disciplinar e padronizar os nossos vinhos para que mantenham alto nível de qualidade. Este selo vem para valorizar nossa produção, ampliar a divulgação desses produtos e criar uma identidade para estes vinhos.

IA - *Quais as principais dificuldades enfrentadas pelo setor vitivinícola?*

José Procópio Stella - A dificuldade do setor vitivinícola é levar até o consumidor de vinho fino a informação da qualidade desses vinhos, visto o grande trabalho de marketing dos vinhos importados. Precisamos levar ao consumidor a informação de que hoje em dia temos vinhos com a mesma qualidade dos importados. Os vinhos finos da Stella Valentino são produzidos com uvas colhidas no inverno, 100% locais e traduzem em seu paladar o que há de mais complexo e surpreendente.

IA - *O senhor já atua com enoturismo. Como é esta experiência e quais as demandas para incrementar esta atividade na região?*

José Procópio Stella - A Vinícola Stella Valentino foi criada com o objetivo principal de trabalhar com o enoturismo. A nossa produção é pequena, porém os vinhos são de altíssima qualidade e a intenção é comercializá-los dentro da própria Vinícola. Já estamos recebendo turistas desde fevereiro de 2019, os quais têm interesse em conhecer todo o processo produtivo e a história da Vinícola.



Trichoderma

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Uso do *Trichoderma* na cultura da uva

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Introdução

No Brasil, a produção de uvas está concentrada nas regiões Sul, Sudeste, Centro Oeste e Nordeste, sendo que o estado do Rio Grande do Sul representa 64,85% da área cultivada do Brasil, com 47.383 ha plantados. No estado do Rio Grande do Sul, a viticultura é realizada em diferentes regiões tais como, Serra gaúcha, Campos de cima da Serra, Campanha gaúcha e Serra do sudeste. Na Serra gaúcha atualmente são plantados aproximadamente 37.900 ha, entre uvas viníferas, americanas e híbridas, o que corresponde a 51,0% da área plantada no Brasil (Rosa, 2016; IBGE, 2018; Mello et al., 2017).

Na viticultura brasileira, um dos principais entraves é a ocorrência de doenças, acarretando perdas qualitativas e quantitativas na produção de uvas, vinhos, sucos e derivados. Dentre essas doenças, as fúngicas representam a sua maioria e ocorrem em todas as regiões produtoras, mesmo que estas estejam situadas em diferentes climas e condições ambientais. De maneira geral as doenças fúngicas mais importantes tendem a ser as mesmas nestas regiões, sendo as principais o míldio (*Plasmopara viticola*), o oídio (*Erysiphe necator*), a antracnose (*Elsinoe ampelina*), a escoriose (*Phomopsis viticola*), a podridão-cinzenta (*Botryotinia fuckeliana*), a podridão da uva madura (*Glomerella cingulata*) e as doenças de tronco, tais como: fusariose (*Fusarium oxysporum* f.sp. *herbemontis*), esca (*Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Fomitiporia* spp.) Petri (*Phaeomoniella chlamydospora*, *Phaeoacremonium* spp.), pé-preto (*Cylindrocarpon* spp., *Ilyonectria* spp., *Campylocarpon* spp., *Dactylonectria* spp., entre outras) e podridão-descendente (*Botryosphaeria* spp., *Neofusicoccum* spp., *Lasiodiplodia* spp.) (Sonego et al., 2005; Almança et al., 2015; Almança et al., 2013a; Cavalcanti et al. 2013).

As doenças de tronco da videira em especial, tem causado prejuízos na viticultura em diversos países desde a produção de mudas até estabelecimento dos vinhedos, causando declínio e morte das plantas (Fontaine et al., 2016; Almança et al., 2013a; Cavalcanti et al., 2013). No Brasil, em levantamento realizado por Rusin et al (2015) em vinhedos da Serra gaúcha, observou-se que em média, 90% das plantas com sintomas externos de declínio e morte apresentavam a presença de pelo menos uma espécie de fungo causador de doença de tronco. A ocorrência destas doenças traz a necessidade de reposição de plantas ou renovação completa do vinhedo, dependendo do percentual de plantas afetadas e idade do vinhedo, o que acarreta no aumento do custo de produção e diminuição da renda do produtor. Fontaine et al. (2016) demonstraram que com o avanço da idade dos vinhedos a ocorrência destas doenças aumenta e a produção de uva diminui, sendo que a partir dos 15 anos as perdas podem ultrapassar 50% da produção.

Outro grupo de doenças importante, é o das podridões de cacho. *Botryotinia fuckeliana* (forma sexuada *Botrytis cinerea*) tem ocorrência em todas as regiões produtoras de videira e tem sido considerado um dos principais agentes causadores dessas podridões, causando redução de produção e qualidade das uvas. Este impacto ocasiona uma má qualidade dos vinhos produzidos alterando a composição, aumentando a presença de compostos tóxicos que afetam as leveduras, inibindo o processo de fermentação e diminuindo a intensidade de cor (Fedrizzi et al., 2011; Agudelo-Romero et al., 2015). Assim como nas demais doenças fúngicas, o uso de fungicidas químicos é a principal estratégia no controle de controle da podridão-cinzenta (Shtienberg, 2007; Molitor et al., 2012), entretanto, nas podridões de cacho, além disso, recomenda-se utilizar medidas de manejo como desfolha, poda verde e adubação nitrogenada adequada (Wilcox, 2014). Contudo, o desenvolvimento de produtos de controle biológico tem possibilitado novas possibilidades de manejo destas doenças com menor impacto ambiental.

Várias espécies do fungo *Trichoderma* têm elevada capacidade de controle de fungos habitantes de solo e, em algumas situações, também destaca-se no controle de fungos causadores de doenças da parte aérea. Neste sentido, a utilização deste agente de biocontrole poderia ser realizada em viveiros no processo de produção de mudas e também nos vinhedos.

Com relação ao controle de fungos de solo, uma possibilidade para o manejo do pé-preto é a utilização de *Trichoderma* spp. em viveiros e no momento do plantio. Na África do Sul, Halleen et al. (2001) observaram que porta-enxertos inoculados com *Trichoderma harzianum* apresentaram uma menor incidência de *Cylindrocarpus* spp. com resultados semelhantes aos fungicidas utilizados. Além disto, observou um aumento na massa total de raízes nos porta-enxertos com presença de *T. harzianum*.

Adicionalmente, alguns trabalhos evidenciaram a possibilidade de controle de fungos causadores de doenças de tronco na parte aérea das plantas de videira. Kexiang et al. (2001), relataram que isolados de *Trichoderma* spp. foram eficientes sobre espécies de