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Phenolic compounds identified in apricot branch tissues and their role in the control of *Monilinia laxa* growth

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ABSTRACT

Secondary metabolites, such as phenolics, are plant defence substances. In the present study, the impact of *Monilinia laxa* inoculation under controlled conditions on phenolic content of apricot branches was investigated. A bi-parental hybrid population issued from Bergeron and Bakour cultivars (BerBa) and consisting of 192 hybrids was studied. The susceptibility of the BerBa population to *M. laxa* was evaluated by measuring the length of the necrosed tissues and the concentration of fungal DNA by qPCR in the branches 8 days post-inoculation. The results exhibit significant differences between the two parental cultivars in terms of necrosis length, which confirm their different susceptibility to the pathogen. A considerably high content of 2′,6′-dihydroxy-4′–methoxyacetophenone hexoside was found in branch tissues of Bakour (tolerant parental cultivar), amounting to 1.72 mg/g and 1.41 mg/g of fresh weight in non-inoculated and inoculated samples, respectively. The content of this compound in Bergeron branch tissues was several times lower, amounting to 0.24 mg/g and 0.14 mg/g of fresh weight in non-inoculated and inoculated samples, respectively. In the inoculated branches, scopolin was almost twice as abundant in the tolerant parental cultivar branch tissues as in the susceptible ones. In general, after inoculation a lower content of phenolic compounds was observed for hybrids with longer necrosed tissue. A Principal Component Analysis showed that at 8 days post-inoculation *M. laxa* concentration in the branches and the content of phenolic compounds, such as scopolin and chlorogenic acids, were negatively correlated. Additionally, the antifungal activity of pure phenolic compounds against *M. laxa* mycelial growth and spore germination was investigated. At a concentration of 500 mg/L, up to 40 % inhibition of *M. laxa* mycelial growth by scopoletin, up to 60 % inhibition by 2′,4′,6′-trihydroxyacetophenone and total inhibition by 2′-hydroxy-4′-methoxyacetophenone was observed. Scopoletin and 2′,4′,6′-trihydroxyacetophenone also inhibited spore germination by about 50 %. These results can serve to improve breeding programs aiming to develop apricot cultivars resistant to brown rot blossom blight.

1. Introduction

Plants have developed manifold strategies for defence against pathogens. A mechanical barrier composed of bark or waxy cuticle serves as the first line of defence (Aoun, 2017). Additionally, plants produce a vast array of secondary metabolites protecting them from pathogens, including fungi. These metabolites are present in different organs and tend to accumulate in the external tissues on the plant surface (Harborne, 2009). Plants' secondary metabolites can be divided into different groups – based on their chemical structure – such as terpenes, phenolics, and nitrogen- or sulphur-containing compounds. They can exist as constitutive compounds – defence chemicals present in the plant before contact with a pathogen – or as phytoalexins – antifungal chemicals newly produced after infection (Grayer and Kokubun., 2001). As a reaction to fungal infection, plants can also release compounds which are normally stored as glycosides. Due to the cell disruption

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caused by the fungi, the stored compounds come into contact with hydrolysing enzymes present in other compartments and liberate aglycones with antifungal activity (Lattanzio et al., 2006).

Brown rot caused by the ascomycete fungi *Monilinia laxa* (Aderhold and Ruhland), *Monilinia fructicola* (Winter) or *Monilinia fructigena* (Aderhold and Ruhland) is one of the most important diseases for stone fruits worldwide (Holb, 2008; Obi et al., 2018a). It affects peaches, cherries, apricots, plums and other fruit trees, such as apples and pears (Hrustić et al., 2012). *M. laxa* and *M. fructigena* had a negative economic impact and caused heavy losses in Central Europe (Oliveira Lino et al., 2016), while *M. fructicola* was more present in Asia and North and South America (Hrustić et al., 2012). Recent review article stated that the ubiquitous pattern of spread of different *Monilinia* species does not allow anymore to affirm their distribution in specific regions (Obi et al., 2018a). *M. laxa* incidence constitutes one of the most impactful problems for organic apricot production in Switzerland (Christen et al., 2012). *M. laxa* mainly affects branches and flowers (Byrde and Willetts, 1977; Holb, 2006), whereas *M. fructigena* and *M. fructicola* mainly affect fruit of apricot trees (Hrustić et al., 2012).

M. laxa spores overwinter in fruit mummies and cankers on a tree and form a source of infection in the following year (Oliveira Lino et al., 2016). The spores are dispersed by wind and rain, enter the flower, move down the branch in the sap and infect other aerial parts of the plant. Necrosis and cankers develop on the attacked branches, impeding the circulation of the sap and desiccating branches (Holb, 2008). On apricot trees, gum exudates are very often observed close to these cankers (Hrustić et al., 2012). *M. laxa* needs high air humidity and low temperature (ideally 10–12 °C) for sporulation and infection of branches and flowers (Tamm and Fluckiger, 1993). There are two main attacks by the fungus during the year. One is at bloom time, when the opened flowers are very susceptible to infection, especially if favourable weather conditions are present (Holb, 2008; Tresson et al., 2020). In the case of unfavourable weather conditions, the infection may remain latent and visual decay symptoms develop after harvesting (Obi et al., 2018a). The second attack takes place on fruit during the harvest period through wounds in the fruit or by contact with already infected fruit. These two infection periods are independent of each other and controlled by different mechanisms (Wagner et al., 2005).

To prevent *Monilinia* infection, apricot orchards are treated with chemical fungicides during bloom and harvest time to avoid the spread of the fungus into flowers and fruit. Notwithstanding their efficacy, there is an urgent need in Europe to reduce the use of chemical pesticides (Frische et al., 2018). Efforts are made to satisfy consumers' demand for residue-free fruit by using environmentally friendly solutions. The development of brown rot resistant cultivars could be an ideal strategy for brown rot disease control (Obi et al., 2018a). No source of total resistance to brown rot blossom blight is currently known; nevertheless, some cultivars may have promising tolerance levels (Bassi and Audergon, 2006; Nicotra et al., 2006; Trandafirescu and Teodorescu., 2006). In order to acquire new apricot cultivars resistant to *Monilinia* infection, knowledge on the mechanism and source of tolerance is essential.

Hitherto, tolerance to *Monilinia* has mainly been studied in peach fruit. Fruit cuticle constitutes the first mechanical and chemical barrier against the entrance of the pathogen (Oliveira Lino et al., 2016). Another source of tolerance in fruit is the thickness of the epidermis (Hrustić et al., 2012); the thicker the epidermis, the more tolerant the cultivar, as was clearly demonstrated in Bolinha, a peach variety (Feliciano et al., 1987). Moreover, it has been observed that peach fruit susceptibility to *Monilinia* spp. increases during ripening, with immature fruit being the most tolerant to brown rot (Mari et al., 2003). This might be linked to immature fruit hardness or its pH, which hinders fungus penetration (Obi et al., 2018b). Additionally, it has been shown that the content of phenolic compounds in peach fruit diminishes with ripening (Lee and Bostock., 2007; Villarino et al., 2011). Taking into account the fact that the content of chlorogenic acid and neochlorogenic acid has been negatively correlated with the incidence of brown rot, its lower concentration at later fruit development stages might explain higher susceptibility of ripe peaches (Villarino et al., 2011). Lee and Bostock (2007) proposed that phenolic compounds and other antioxidants might contribute to brown rot resistance by inhibition of fungal cutinase synthesis, necessary to penetrate the intact surface of fruit. Negative correlations have also been found between the content of other phenolics in peach (such as anthocyanins and total phenolics in peel tissue), and brown rot disease incidence and severity (Obi et al., 2019). More recently, ascorbate content has been proposed to contribute to tolerance to brown rot in peach (Obi et al., 2020).

As described above, *M. laxa* enters into the flower and can then move down the branch. Christen et al. (2012) observed that the progression of the fungus within the branches and the severity of symptoms, i.e. blighted shoots and twigs, vary significantly between cultivars. In other pathosystems showing damage to the woody parts a possible role of phenolics in defence mechanism was investigated. Lambert et al. (2012) investigated phenolic compounds in woody parts of foliar cuttings in grapevine defence against trunk disease agents. The content of stilbene oligomers was significantly higher in plants infected with *Neofusicoccum parvum* than in control plants. Moreover, Conrad et al. (2017) found four phloem flavonoids to be biomarkers of coast live oak's resistance to *Phytophthora ramorum,* an invasive pathogen causing sudden oak death.

However, the mechanism of tolerance to *Monilinia* attack in branches has not been highlighted. Specifically, to our knowledge, there is no literature data on the effect of phenolic compounds' content in apricot branches on an *M. laxa* infection. Therefore, the objectives of this study are (1) to identify and quantify the phenolic compounds in branch tissues of parental cultivars and a bi-parental population in both uninfected (control) and infected branches and (2) to investigate a possible association between these compounds and the level of resistance to brown rot blossom blight.

2. Materials and methods

2.1. Plant material

An F1 bi-parental apricot population of 192 descendants (named BerBa) from a cross between the parental genotypes Bergeron and Bakour was developed in 2004 by the Gotheron Experimental Unit for Integrated Research (UERI) of the Institut National de Recherche Agronomique (INRA, Saint-Marcel-lès-Valence, France) and the trees were planted in the experimental field in the same year. Bakour is a Tunisian cultivar tolerant to *Monilinia,* and Bergeron is a French cultivar susceptible to *Monilinia* (Parveaud et al., 2011).

2.2. Reagents

All solvents used were of analytical grade or higher. $CaCO_3$, the yeast extract Bacto (concentrate of the water-soluble portion of autolysed *Saccharomyces cerevisiae* cells), potato dextrose agar (PDA) and agar were obtained from Fisher Scientific (Hampton, USA), whereas lactic acid was obtained from AppliChem (Darmstadt, Germany). Formic acid, 2-propanol, methanol, acetic acid, 5-*O*-caff ;eoylquinic acid (5-CQA), (+/-)-catechin, scopoletin, 2′-hydroxy-4′methoxyacetophenone and 2′,4′,6′-trihydroxyacetophenone were purchased from Sigma-Aldrich (Saint Louis, USA). HPLC-grade methanol (Macron Fine Chemicals) was acquired from Avantor (Radnor, USA). Roti-C/I for extraction of nucleic acids (chloroform/isoamyl alcohol at a ratio of 24:1), ethanol, *p*-coumaric acid, Na_2EDTA, Na_2SO_3, Tris, NaCl, polyvinylpyrrolidone K25 (PVP K25), cetrimonium bromide (CTAB) and Tris/EDTA solution were obtained from Carl Roth (Karlsruhe, Germany).

Deionised water used for the mobile phase and in each aqueous solution in HPLC analyses was obtained using a Millipore Milli-Q-50 18 mΩ system (Darmstadt, Germany); the other solutions were prepared with water purified using an Ultra Clear RO EDI reverse osmosis device

(Evoqua Water Technologies, Pittsburgh, USA).

2.3. Inoculation with Monilinia laxa under controlled conditions

2.3.1. Monilinia laxa strain

A wild type of *M. laxa,* isolated from apricot flowers in the field of the UERI Gotheron and validated in a laboratory, was used for the inoculation. A plug of fungus mycelium 5 mm in diameter was grown in darkness for 7 days at 23 °C on Petri dishes in 25 mL of PDA medium per dish (Tamm and Fluckiger, 1993). The PDA medium was prepared by mixing 19.5 g of PDA in 500 mL of water, sterilised at 121 °C for 15 min in an autoclave (Systec VX-95, Linden, Germany).

2.3.2. Inoculation of detached branches

When flower buds were in the C–D phenological stage – the phase between bud break and the opening of sepals (Viti et al., 2013) – one-year branches from each genotype of the BerBa population trees were cut and transported from the fields of UERI Gotheron to a laboratory at the Agroscope Research Centre in Conthey, Switzerland. Branches were 25–30 cm long with the basal diameter between 0.5 and 1.5 cm. All the flowers were removed from the branches, a 2 cm-long strip of the bark was cut from each branch with a scalpel and disinfected with ethanol. The wound was inoculated with a 4 mm diameter plug of mycelium (without spores) from 7-day old colonies of *M. laxa*, and the branch was covered with blotting paper and Parafilm. Three inoculated and three non-inoculated (control) branches per genotype were kept under controlled conditions – 20 °C, 80 % relative humidity, in darkness – in a Phytotron chamber (Clitec, Küssnacht, Switzerland). The extent of infection was evaluated 8 days post-inoculation (8 dpi) by measuring the length of necrosed tissues in each branch after removing the bark on both sides of the wound with a potato peeler. The test was conducted in 2019 and was preceded by preliminary tests in 2017 and 2018, carried out to define the optimal sampling time post-inoculation and sampling distance from the inoculation point.

2.4. Simultaneous extraction of DNA and phenolics from apricot branch tissues

At 8 dpi, branch tissue samples were collected from each inoculated and non-inoculated branch. Each sample, consisting of two 3 cm pieces (around 100 mg), cut with a potato peeler above and below the inoculation plug, was placed in one compartment of a U-form extraction bag (Bioreba, Reinach, Switzerland) and stored at -80 °C. In total, three inoculated samples and three negative controls per hybrid were collected.

On the day of analysis, each frozen sample was homogenised with a 2.5 mL EB1 solution composed of 25 mM Na_2EDTA and 10 mM Na_2SO_3 and ground quickly in a Homex 6 grinder (Bioreba). This homogenate was split for extraction of DNA and soluble phenolics.

2.4.1. Extraction of DNA and quantification of Monilinia laxa DNA

900 µL of the EB1 extract was withdrawn from the homogenate and 450 µL of it was transferred to a 2 mL screw cap tube and mixed with 690 µL of 1.6x CTAB buffer (composed of 200 mM Tris, 2.8 M NaCl, 50 mM Na_2EDTA, 4 % CTAB and 2 % PVP K25, adjusted to pH 8.0 with HCl). The extract was then incubated at 70 °C for 20 min. After cooling to room temperature, 600 µL of chloroform/isoamylalcohol was added to the tube, shaken vigorously for 2 s three times and centrifuged at 20 000 *g* for 2 min (Eppendorf Centrifuge 5425, Hamburg, Germany). An 800-µL portion of the clear supernatant was mixed with 600 µL of 2-propanol and incubated at room temperature for 60 min. Then, DNA was precipitated at 20 000 *g* for 2 min, and the pellet was washed once with 700 µL of 70 % ethanol. After evaporation of the remaining ethanol at 70 °C, the DNA pellet was finally dissolved in 100 µL of 0.1x Tris/EDTA buffer solution (1 mM Tris and 0.1 mM Na_2EDTA, at a pH of 8.0).

DNA was analysed by duplex qPCR for both plant 18S-RNA-DNA (Oberhänsli et al., 2014) and *M. laxa* ITS-5.6S RNA-DNA (Van Brouwershaven et al., 2010). Real-time primers and their corresponding fluorogenic hydrolysis probes, specific for *M. laxa* and the host, were synthesised by Microsynth AG (Balgach, Switzerland) and combined in a duplex assay. The probe Mlx_ITS_P (Table 1) was laid at the same position of the ITS1 regions as the original probe, P2_fgn/lx/ps (Van Brouwershaven et al., 2010), but increased in length by 7 bases to meet the requirements for using a standard blackhole quencher (BHQ1) without needing to use the proprietary MGB-modified quencher (Applied Biosystems, Waltham, USA). The duplex TaqMan qPCR assay was run in a volume of 13 µL using 1 µL of DNA, 6.5 µL of Kapa Probe Fast Universal Mix (Merck, Darmstadt, Germany) and 5.5 µL of water containing the primers and probes at the concentrations indicated in Table 1. The DNA concentration of the plant tissue extract was in the range of 50−100 ng/µL. Samples were run in a 72-well rotor (Rotorgene Q, Qiagen, Hilden, Germany) during 45 cycles in a two-step cycling protocol of denaturation for 5 s at 95 °C and annealing/elongation for 20 s at 60 °C after an initial activation of the hot start DNA polymerase for 3 min at 95 °C. FAM and ROX signals were recorded after the completion of each annealing/elongation step. The fungal DNA was quantified with a standard curve obtained with three dilutions of 1, 10, and 100 pg of pure *M. laxa*-DNA per reaction, which was calculated for each run of the qPCR. The fungal DNA concentration was then normalized with a quantification curve of a dilution series of plant extracts. The results were expressed as a weight ratio of fungal DNA to plant DNA (pg/ng).

2.4.2. Extraction, identification and quantification of soluble phenolics

900 µL of 4 % acetic acid in methanol (v/v) was added to the remaining EB1 extract in the U-form extraction bags and mixed by grinding again in the Homex grinder for 10 s. 1 mL of extract was transferred into a 1.5 mL Eppendorf tube and centrifuged at 20 000 *g* for 2 min. The supernatant was stored at -20 °C until subjected to HPLC analysis. To correct for possible fluctuations in the analysed volume, an internal standard (*p*-coumaric acid) was added to the extraction solvent.

A chromatographic system (Waters, Milford, USA) was composed of a 2707 autoinjector, 1525 binary pump and 2998 photodiode array detector and operated by Empower 3 software. 10 µL of the branch tissue extract was injected onto a Kinetex C18 column (5 µm, 100 Å, 150 × 4.6 mm; Phenomenex, Torrance, USA) and eluted with a gradient of 0.1 % (v/v) formic acid in water (A) and methanol containing 0.1 % (v/v) formic acid (B). The elution started with 80 % of A for 5 min,

Table 1
Primers (forward/reverse [F/R]) and probes (P) for duplex TaqMan qPCR assay for detection of the ITS1-5.8S rRNA gene of *Monilinia laxa* and the 18S rRNA gene of apricot tissue. The probes were labelled at their 5′ ends with fluorophores FAM and ROX and at their 3’ ends with their corresponding blackhole quenchers BHQ1 and BHQ2.

Primer/ probe	Sequence 5′–3’	Final concentration (nM)
Mon139_F	CACCCTTGTGTATTATTACTTTGTTGCTT	300
Mon139_R	CAAGAGATCCGTTGTTGAAAGTTTTAA	300
Mlx_ITS_P	FAM-CCTTGTATGCTCGCCAGAGAATAATCAA-BHQ1	100
Md_F	AGAGGGAGCCTGAGAAACGG	50
Md_R	CAGACTCATAGAGCCCGGTATTG	50
Md_P	ROX-CCACATCCAAGGAAGGCAGCAGGCG-BHQ2	50

followed by a linear gradient leading to 70 % of A at 10 min, kept isocratic for 10 min and increased to 90 % B within the next 2 min. The elution at 90 % of B continued until 27 min and at 28 min switched back to the initial solvent. The flow rate was 0.8 mL/min. The column was re-equilibrated between sample injections with A for 7 min. A diode array detection was performed by scanning over a wavelength range from 210 to 600 nm. The chromatograms were recorded at 280, 320 and 340 nm. The identification of phenolic compounds was carried out by comparison of the retention times and UV spectra of the samples with those obtained for the standards (Kosińska et al., 2013). Compounds, for which no standards were available, were identified based on their UV and MS spectra. MS spectra were obtained using a Q Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, USA) in both positive and negative ESI modes.

The quantification was performed using calibration with external standards and normalisation with an internal standard (*p*-coumaric acid). The calibration curves were obtained by injection of the external standard solutions at varying concentrations, ranging from 5 to 450 µg/mL, and containing a constant amount of internal standard, under the same conditions as for the analysed samples. 5-CQA, (+/-)-catechin and scopoletin contents were calculated in reference to their standards. Isomers of caff ;eoylquinic acids were quantified based on the standard curve of 5-CQA, scopolin was quantified based on scopoletin, and epicatechin and procyanidin dimers were quantified based on the standard curve of (+/-)-catechin. The acetophenone derivatives were quantified with the calibration curve of 2′,4′,6′-trihydroxyacetophenone.

2.5. In vitro antifungal activity against Monilinia laxa

The effect of five pure phenolic compounds on mycelial growth and spore germination was evaluated according to procedure of Celar and Kos (2016). The compounds tested or their derivatives were found in the branch tissues of apricots.

2.5.1. Mycelial growth test

Pure phenolic compounds were dissolved individually in deionised water at different concentrations and added at a ratio of 1:9 to agar cooled to 50 °C. The range of final concentration in the medium was 12.5–500 mg/L for 2′,4,’6′-trihydroxyacetophenone, 2′-hydroxy-4′-methoxyacetophenone and scopoletin and 12.5–250 mg/L for 5-CQA and catechin. Each concentration was tested in triplicate. For the control plates, sterilised water was added to the medium at a ratio of 1:9. The agar plates were equidistantly inoculated with three inverted mycelial plugs of 5 mm diameter, which were cut from the edge of actively growing 7-day-old colonies. Plates were incubated for 72 h at 23 °C in darkness. After this period, the diameters of the mycelia were measured.

2.5.2. Spore germination test

To obtain spores for the test, *M. laxa* was grown in a V8 medium specific for *Monilinia* sporulation (Hu et al., 2011). Briefly, 100 mL of vegetable juice V8 (Campbell’s), 0.5 g of $CaCO_3$,1 g of glucose, 1 g of Bacto yeast extract and 10 g of agar were mixed, then water was added to equal 500 mL. The pH of the medium was adjusted to 5.2 with lactic acid, and the medium was autoclaved at 121 °C for 15 min. The fungus was grown at 12 °C with a photoperiod of 12 h/day and 10 h/night for 15 days (Tamm and Fluckiger, 1993). After evaluating the vegetative growth, 5 mL of *M. laxa* spores (concentration of 10^5 spores/mL) were added onto agar plates containing one of the five phenolic compounds in a range of concentration of 25–400 mg/L and incubated in darkness for 24 h at 23 °C (for the control, agar without phenolic compounds was used). Subsequently, spores were suspended in water and a portion of 50 µL was transferred to a Thoma Neubauer haemocytometer (Assistent, Sondheim, Germany), and 100 spores were counted under a microscope with an NIS Elements BR 2.30 image analyser (Wild Heerbrugg, Switzerland). The number of germinated spores with 100 was recorded. The test was carried out in triplicate per concentration and compound.

2.5.3. Percentage of inhibition

The percentage of mycelial growth and spore germination inhibition by phenolic compounds was calculated according to the equation

$$I\ (\%) = [(C - P) / C] \times 100,$$

where *I,* is the percentage of mycelial growth/germination inhibition, *C,* is the diameter of the mycelium/number of spores germinated without phenolic compounds (control) and *P,* is the diameter of the mycelium/number of spores germinated with phenolic compounds.

2.6. Statistical analysis

Data were presented as means ± standard deviations. A statistical analysis was performed using XLSTAT 2019.3.2 software (Addinsoft, New York, USA). The difference in content of phenolic compounds and necrosis length between parental cultivars was analysed with two-sample *t*-test. For the results of the whole F1 population, the assumption of the residual’s normality was tested with the Shapiro-Wilk test and the assumption of homoscedasticity with the Bartlett’s test. Since the assumption of normal distribution and homoscedasticity were violated, non-parametric alternative to ANOVA - Kruskal-Wallis test followed by Dunn test was applied to analyse the results. Statistical significance was set at the $p < 0.05$. To identify patterns in data Principal Component Analysis (PCA) was carried out.

3. Results

3.1. Necrosis length as a measure of susceptibility to Monilinia laxa

The evaluation of *M. laxa* infection under controlled conditions was performed by the measurement of necrosis length on the inoculated branches at 8 dpi. Fig. 1 presents the results of necrosis measurements for two parental cultivars of the BerBa population. An average necrosis of 2 mm and 63 mm was observed for Bakour and Bergeron cultivars, respectively. The results show a statistically significant difference in necrosis length between the two parental cultivars ($p = 0.013$) and are consistent with the expected high susceptibility of the Bergeron cultivar and high tolerance of the Bakour cultivar to *M. laxa*.

Fig. 2 illustrates the results of the necrosis length in the branches of the whole BerBa population at 8 dpi. Large differences between hybrids were observed, with the results ranging from 0 to 69 mm. Bakour and

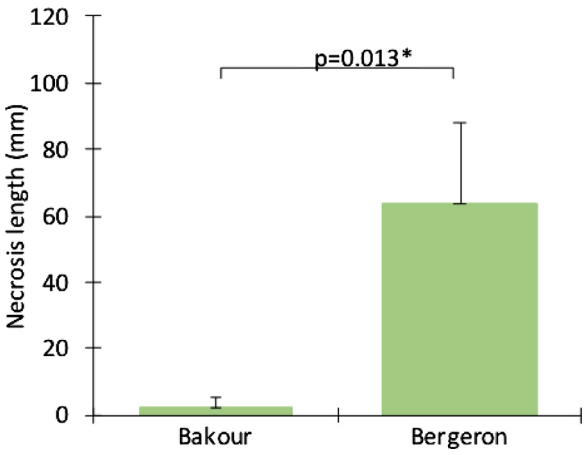

Fig. 1. Necrosis length in the branches of the parental cultivars inoculated with *Monilinia laxa* at 8 days post-inoculation. Results are expressed as an average of $n = 3$ replicates; error bars represent standard deviation. Significance of difference was evaluated by two-sample *t*-test.

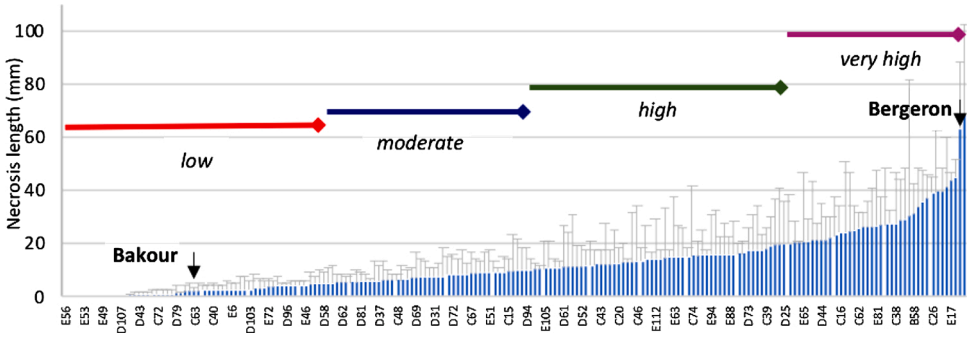

Fig. 2. Necrosis length in the branches of the individual hybrids of the BerBa population inoculated with *Monilinia laxa* at 8 days post-inoculation. Results are expressed as an average of $n = 3$ replicates; error bars represent standard deviation. The arrows indicate the parental cultivars. The hybrids were sorted into one of four categories, based on the necrosis length. The range of each category was indicated by the horizontal bars.

Bergeron cultivars are localised at two extremes of the whole population. For further analysis, the hybrids of the BerBa population were grouped into four categories (as illustrated in Fig. 2) according to their susceptibility to *M. laxa*, measured by necrosis length: *low* for necrosis up to 5 mm, *moderate* for necrosis ranging from 5 to 10 mm, *high* for 10–20 mm and *very high* for necrosis longer than 20 mm. No necrosis was observed on branches in the controls (without inoculation) in the whole BerBa population.

3.2. *Monilinia laxa* concentration in branch tissues

In order to confirm the presence of *M. laxa* in the inoculated branches, the DNA of branch samples was extracted and quantified by qPCR. The fungus concentration was expressed as the amount of *M. laxa* DNA in relation to the amount of DNA of apricot tissue. The average fungus concentration of the hybrids in each category differed significantly (Fig. 3). The hybrids in the *low* category (necrosis up to 5 mm) showed the lowest concentration of *M. laxa* DNA amounting to around 22 pg/ng. The concentration of fungus DNA was significantly higher in the *moderate* and *high* categories, 52 and 75 pg/ng, respectively; and even higher for the *very high* category, for which it reached 133 pg/ng of apricot DNA.

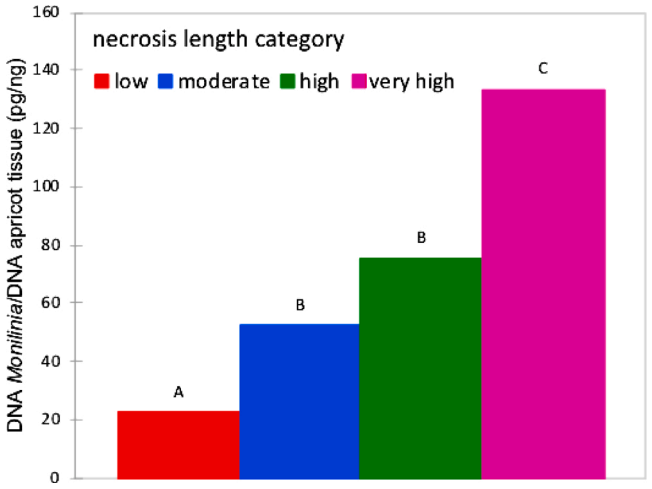

Fig. 3. Average concentration of *Monilinia laxa* DNA for individual necrosis length categories. The DNA was quantified in the branch tissues at 8 days post-inoculation. The assignment of hybrids into categories was indicated in Fig. 2. Each category consists of between 37 and 58 hybrids. Bars with different letters correspond to means significantly different at $p < 0.05$ (Kruskal- Wallis followed by Dunn test).

3.3. Content of phenolics in the branches

Phenolic compounds from the class of hydroxycinnamic acids (chlorogenic acid (5-CQA) and its isomers - neochlorogenic and cryptochlorogenic acids, commonly named as chlorogenic acids), flavanols (catechins and procyanidin dimers), coumarins (scopoletin and its glucoside scopolin) and acetophenones were identified in the branch tissues of the BerBa population, both in the non-inoculated and inoculated branches (Table 2). Altogether, 11 compounds were quantified. Concerning the Bakour and Bergeron cultivars, the flavanols and chlorogenic acids were the predominant compounds in the branch tissues. There were no significant differences in the content of phenolics in the branch tissues of parental cultivars before and after inoculation ($p \geq 0.05$). In the control samples (not inoculated with *M. laxa*), the content of only 3 compounds – procyanidin dimer (2), 3-*O*-caff ;eoylquinic acid (3-CQA) and 2′,6′-dihydroxy-4′–methoxyacetophenone hexoside (hereafter referred to as acetophenone hexoside) – were significantly different between branch tissues of Bakour and Bergeron cultivars ($p < 0.05$). The content of the same three compounds, as well as scopolin, differed in branch tissues of inoculated branches of two parental cultivars. A higher content of procyanidin dimer (2) and 3-CQA was observed in Bergeron cultivar branch tissues compared to Bakour. A considerably high content of acetophenone hexoside was found in Bakour cultivar branch tissues, amounting to 1.72 mg/g of fresh weight (FW) and 1.41 mg/g FW in non-inoculated and inoculated samples, respectively. In contrast, the content of this compound in Bergeron branch tissues was several times lower, amounting to 0.24 mg/g and 0.14 mg/g FW in non-inoculated and inoculated samples, respectively. Similarly, in the samples of infected branches, the content of scopolin was significantly higher ($p < 0.05$) in tolerant parental cultivar branch tissues than in susceptible one. An aglycone of scopolin – scopoletin – was not detected in Bakour nor in Bergeron cultivar branch tissues; nonetheless, it was present in a wide range of concentrations in BerBa hybrids, and, in general, its content was higher in the branch tissues after inoculation with *M. laxa*.

The content of phenolic compounds in the BerBa population differed greatly, especially for 5-CQA, catechin and procyanidin dimer (2). The content of the predominant compound in the population – 5-CQA – ranged from 1.87 to 12.1 mg/g FW and from 1.40 to 18.3 mg/g FW for non-inoculated and inoculated branches, respectively. The mean content of the sum of flavanols amounted to around 10 mg/g FW for both the control samples and inoculated branches. The content of acetophenone hexoside oscillated between 0.04 and 4.46 mg/g FW and between 0.10 and 5.55 mg/g FW for non-inoculated and inoculated branches, respectively. The inoculation caused an increase in the mean scopoletin content of the BerBa population from 0.03 to 0.30 mg/g and in the maximum scopoletin content of the BerBa population from 0.22 to 1.42 mg/g FW of branch tissue. An accumulation of scopoletin in the branches as a result of *M. laxa* infection can be presumed based on these results.

Table 2
Phenolic compound content in the tissues of non-inoculated branches and Monilinia laxa–inoculated branches.

Phenolic compound	Non-inoculated branch tissues (mg/g FW)					Inoculated branch tissues (mg/g FW)				
	Bakour (T)*	Bergeron (S)*	Population#			Bakour (T)*	Bergeron (S)*	Population#		
			min	max	mean§			min	max	mean§
catechin	2.07 ± 0.33	2.85 ± 1.39	0.56	8.26	3.21[b]	2.05 ± 0.41	3.99 ± 1.78	0	10.1	4.00[a]
epicatechin	0.75 ± 0.23	0.74 ± 0.66	0	1.73	0.56[a]	0.61 ± 0.06	1.17 ± 0.10	0	1.83	0.44[b]
procyanidin dimer (1)	1.93 ± 0.06	1.38 ± 0.47	0.28	4.14	1.89[a]	2.13 ± 0.54	1.38 ± 0.21	0	5.10	1.90[a]
procyanidin dimer (2)	**2.15 ± 0.32**	**4.29 ± 0.71**	0.20	9.58	3.85[a]	**2.37 ± 0.24**	**5.08 ± 0.70**	0	9.48	3.78[a]
neochlorogenic acid (3-CQA)	**0.10 ± 0.03**	**0.39 ± 0.30**	0	1.16	0.24[b]	**0.14 ± 0.17**	**0.99 ± 0.81**	0	1.48	0.33[a]
cryptochlorogenic acid (4-CQA)	0.12 ± 0.03	0.66 ± 0.33	0.09	1.68	0.54[a]	0.13 ± 0.05	0.86 ± 0.34	0.07	1.60	0.55[a]
chlorogenic acid (5-CQA)	2.35 ± 0.25	5.55 ± 2.45	1.87	12.1	5.45[a]	3.63 ± 2.52	15.7 ± 7.51	1.40	18.3	6.10[a]
scopolin	0.70 ± 0.04	0.53 ± 0.12	0.39	1.76	0.92[a]	**0.63 ± 0.06**	**0.33 ± 0.08**	0.05	1.76	0.74[b]
scopoletin	nd	nd	0	0.22	0.03[b]	nd	nd	0	1.42	0.30[a]
2′,6′-dihydroxy-4′–methoxy acetophenone hexoside	**1.72 ± 0.16**	**0.24 ± 0.06**	0.04	4.46	2.13[a]	**1.41 ± 0.54**	**0.14 ± 0.01**	0.10	5.55	1.89[b]
hydroxyacetophenone derivative	0.19 ± 0.01	nd	0	0.55	0.22[a]	0.08 ± 0.09	0.02 ± 0.04	0	0.76	0.19[b]

T - tolerant to M. laxa.
S - susceptible to M. laxa.
*average ± standard deviation, n = 3, values in bold are significantly different at $p < 0.05$ between two parental cultivars within the same treatment.
#results for 192 hybrids.
§population mean with different letters are significantly different at $p < 0.05$.
CQA - caff ;eoylquinic acid.

Table 3
Phenolic compound content in the tissues of branches of BerBa population for which significant differences between samples before and after inoculation with *Monilinia laxa* were noted according to Kruskal-Wallis analysis followed by Dunn test.

Phenolic compound	Category*	Mean content of phenolic compound		Effect of inoculation
		Non-inoculated branches	Inoculated branches	
scopolin	high	0.929	0.665	↓
	very high	0.956	0.420	↓
scopoletin	low	0.034	0.214	↑
	moderate	0.019	0.299	↑
	high	0.035	0.353	↑
	very high	0.033	0.352	↑
2,6-dihydroxy-4–methoxy acetophenone hexoside	very high	2.286	1.326	↓
hydroxyacetophenone derivative	very high	0.223	0.119	↓

*The assignment of hybrids into categories was indicated in Fig. 2.
↑increase.
↓decrease.

In order to take into account the changes in the phenolic compound content evoked by the *M. laxa* inoculation, the results obtained for 4 different categories before and after inoculation were compared. The categories and phenolic compounds, for which significant differences between non-inoculated and inoculated samples were observed, were compiled in the Table 3. For the hybrids in the *very high* category, significant decrease in the content of three phenolic compounds i.e. scopolin, acetophenone hexoside and acetophenone derivative was noted after inoculation. The content of scopolin diminished after inoculation also for the *high* category. Interestingly, the content of scopoletin was significantly higher in the branches after inoculation for all four categories of hybrids, which may suggest its release from scopolin as a result of inoculation. When the correlation between the ratio of the content of each individual phenolic compound in inoculated versus non-inoculated samples and the necrosis length was analysed, negative correlations were obtained for scopolin and acetophenone hexoside, with correlation coefficients amounting to -0.648 and -0.438, respectively ($p < 0.0001$). Specifically, the higher the increase in scopolin content in the branch tissues, due to inoculation with *M. laxa*, the lower the necrosis length observed. Additionally, a ratio of scopolin content in inoculated versus non-inoculated samples was highly positively correlated ($r = 0.828$; $p < 0.0001$), with a ratio of acetophenone hexoside content.

The content of 11 phenolic compounds within the whole BerBa population after inoculation is presented in relation to the necrosis length categories (Fig. 4). For all the compounds, apart from scopoletin, a lower content in the branches was observed for hybrids with longer necrosis. In general, the average content of all ten compounds was significantly higher in the *low* category than in the *very high* category. Significant differences in scopolin content were noted between branches with the extent of necrosis shorter than 10 mm, those in the *high*, and *very high* categories. The average content of scopolin amounted to 0.94 and 0.83 mg/g FW for the *low* and *moderate* categories, respectively, whereas the average content for the *high* category was 0.67 mg/g FW. A lower average content of scopolin, i.e. 0.42 mg/g FW, was noted in hybrids in the *very high* category, the most susceptible to *M. laxa*. Similar tendencies were noted for the content of acetophenone hexoside, 4-CQA, procyanidin dimer (2), and hydroxacetophenone deriv. The branch tissues of hybrids in the *very high* category contained on average around 1.33 mg/g FW of acetophenone hexoside and procyanidin dimer (2), whereas those in the *low* and *medium* category contained more than 2 mg/g FW

3.4. Multivariate statistical analysis

The principal component analysis aims to represent the variation present in the dataset using a small number of components. The first two components represent 54.2 % of variation in the dataset, comprising necrosis length, the content of 11 phenolic compounds and fungus concentration at 8 dpi. The first principal component explains about 42.45 % of the total variation, and the second principal component an additional 11.75 %. Fig. 5 shows the relationships between the original variables and the first two components, whereby each variable is represented by a vector. The direction and length of the vectors determine to what extent particular variables affect the main components. The first component correlates strongly with scopolin, procyanidin (2) and acetophenones content, whereas the second component correlates with scopoletin content. The fact that two variables are placed next to each other indicates a strong positive correlation, as can be seen for the content of two acetophenones or chlorogenic acids and scopolin. In contrast, variables located on opposite sides (i.e. *M. laxa* concentration, necrosis length and the content of phenolic compounds, such as scopolin

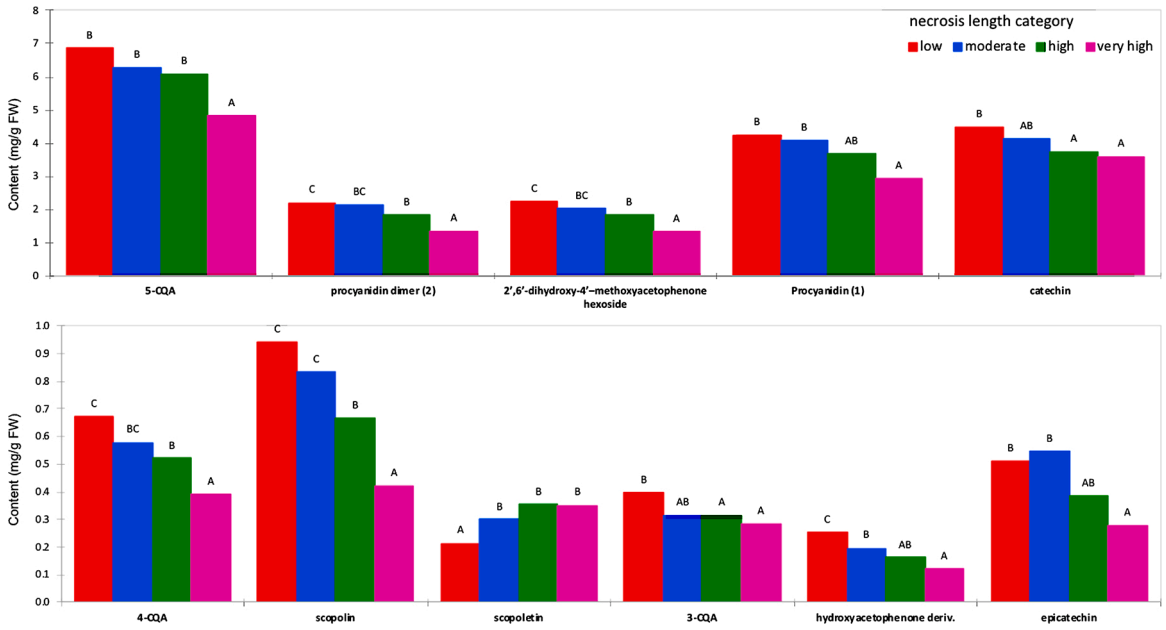

Fig. 4. The average content of phenolic compounds in the branch tissues at 8 days post-inoculation with *Monilinia laxa* for individual necrosis length categories. The categories were defined in Fig. 2. Each category consists of between 37 and 58 hybrids. Bars with different letters correspond to significantly different means: $p < 0.05$ (Kruskal-Wallis analysis followed by Dunn test).

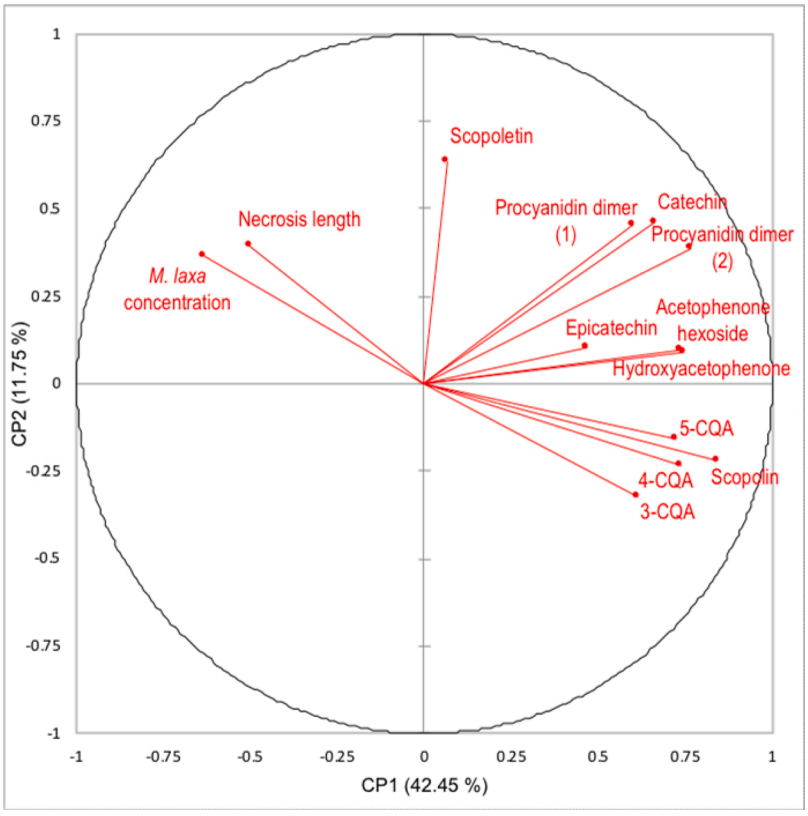

Fig. 5. Variable factor map of the principal component analysis (for the first two principal components), based on the content of 11 phenolic compounds, necrosis length and *Monilinia laxa* concentration in the branch tissues of 192 hybrids of BerBa population analysed at 8 days post-inoculation. Each variable is represented by a vector.

and chlorogenic acids) are negatively correlated. Vectors perpendicular to each other indicate a lack of correlation; therefore, necrosis length shows no correlation with flavanol content.

3.5. *In vitro antifungal activity of phenolics*

The phenolic compounds identified in the branches of apricot branches were only partially available as pure standards; therefore, the

analysis of the direct antifungal activity *in vitro* was not feasible for all of them. The effect of pure phenolic compounds on *M. laxa* mycelial growth was evaluated for 5-CQA, catechin and scopoletin (present as aglycone and glycoside in the branch tissues of the BerBa population) and two hydroxyacetophenones available as pure standards. No inhibition of mycelial growth was noted for 5-CQA and catechin up to a concentration of 250 mg/L in the agar medium. In contrast, three compounds exerted mycelial growth inhibition against *M. laxa*: at the maximal concentration of 500 mg/L up to 40 % inhibition by scopoletin, up to 60 % inhibition by 2′,4′,6′-trihydroxyacetophenone and total inhibition by 2′-hydroxy-4′-methoxyacetophenone was observed (Fig. 6).

The same five pure phenolic compounds were also evaluated for their effect on *M. laxa* spore germination. Up to 50 % inhibition of spore germination was observed for scopoletin at a concentration of 400 mg/L and up to 40 % inhibition for 2′,4′,6′-trihydroxyacetophenone at a concentration of 200 mg/L (Fig. 7). No inhibition of spore germination was noted for 5-CQA, catechin or 2′-hydroxy-4′-methoxyacetophenone up to a concentration of 400 mg/L in the medium.

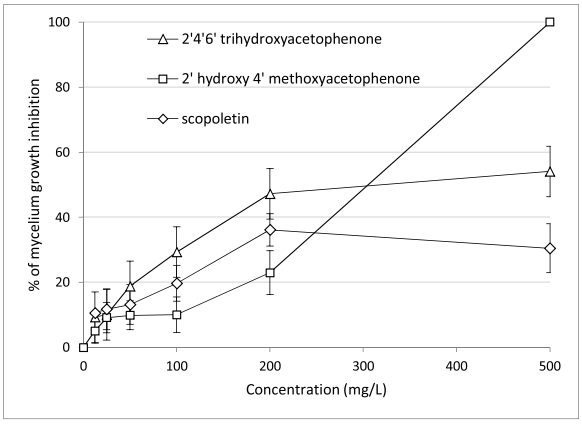

Fig. 6. Inhibition of *Monilinia laxa* mycelial growth by pure phenolic compounds.

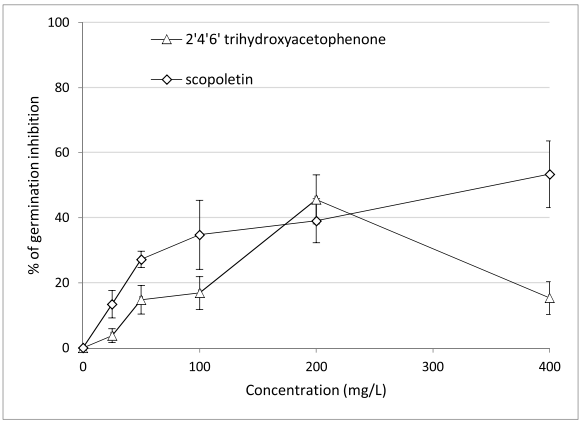

Fig. 7. Inhibition of *Monilinia laxa* spore germination by pure phenolic compounds.

4. Discussion

An adequate assessment of the symptoms caused by a fungal pathogen is crucial for a precise diagnosis of an infection and for a differentiation between genotypes that are tolerant or susceptible to brown rot blossom blight (Christen et al., 2012). Traditional assessment methods of *Monilinia* infection were based on visual inspections of tree branches in the field (Jonáš et al., 2017). However, the incidence of the fungus infection is very dependent on climatic conditions, such as precipitation, relative humidity or temperature, especially in the period of full blossom (Mari et al., 2003; Holb, 2008; Sződi et al., 2008). In the present study, detached branches of the BerBa population were artificially inoculated under controlled conditions and the necrosis length was measured at 8 dpi. Artificial inoculations with *M. laxa* had previously been conducted on blossoms and twigs of apricots (Corazza, 1983; Nicotra et al., 2006; Christen et al., 2012) and sweet cherries (Tamm et al., 1995), as well as on apricot fruit (Pascal et al., 1994; Vávra et al., 2017), plum fruit (Pascal et al., 1994) and peach fruit (Pascal et al., 1994; Villarino et al., 2011; Villalobos et al., 2016; Obi et al., 2017, 2020). In the present study, the length of necrosis in inoculated branches at 8 dpi was used as a measure of apricot susceptibility to *M. laxa*. As previously reported, there is a clear link between the length of necrosis or canker in artificially inoculated branches and the susceptibility of apricot cultivars to *Monilinia* (Crossa-Raynaud, 1969; Trandafirescu and Teodorescu., 2006). A similar method was also successfully applied to measure susceptibility of grapevine to trunk diseases (Lambert et al., 2012; Travadon et al., 2013). The inoculation under controlled conditions and necrosis length measurement carried out in the present study established differences within the BerBa population and placed the two parental cultivars, Bergeron and Bakour, at the two extremes of the range of results obtained. This distribution of this trait would be useful for developing QTLs (quantitative trait loci) to find the genetic regions responsible for resistance to *M. laxa*. This type of segregation suggests that resistance to brown rot blossom blight is a polygenic genetic trait, dominated by several genes (Crossa-Raynaud, 1969; Nicotra et al., 2006; Martinez-Garcia et al., 2013).

The quantitative PCR result confirmed the presence and proliferation of the pathogen in the necrotic tissue. The average fungal concentration differed significantly between progenies in four necrosis length categories. However, compared to the very quick method of measuring necrosis length, the much more laborious quantitative PCR was less reliable to depict the grade of susceptibility of individual host cultivars due to a higher variability (results not shown).

Phenolic compounds act as defence components (Harborne, 2009; Delvas et al., 2011; Ribera and Zuñiga., 2012; War et al., 2012; Sun et al., 2014; Martinez et al., 2017; Ullah et al., 2019). Most of the literature data on apricots' phenolic compounds concern their content in fruit. Research on the content of phenolic compounds in apricot branch tissues is rather scarce so far. However, it is known that branch phenolics act as a chemical barrier in grapevine defence against wood decay fungi (Lambert et al., 2012). Chlorogenic acids, scopoletin and scopolin, hydroxyacetophenones and catechin, epicatechin and their dimers were identified in the branch tissues of the BerBa population. Similar components were reported for branch tissues of various *Prunus* species (Feucht and Schmid., 1979; Santamour and Riedel., 1994; Prasad, 1999; Usenik et al., 2006; Raturi et al., 2011). More precisely, the flavanols were the predominant compounds, constituting about 5–12 % of the branch tissue dry weight (Feucht and Schmid., 1979), whereas chlorogenic acids were less concentrated and varied between 1.2 and 0.2 %. Scopolin, and sometimes scopoletin, occurred in the bark of numerous *Prunus* species (Santamour and Riedel., 1994). Prasad (1999) reported the presence of 4-*O*-glycosyloxy-2-hydroxy-6-methoxyacetophenone in the roots of *P. armeniaca*. Similar component, 2-*O*-β-D-glucopyranoside-6-hydroxy-4-methoxyacetophenone, was identified in *P. persica* stem bark (Raturi et al., 2011). Usenik et al. (2006) evaluated the content of phenolic compounds in the branch cambium of apricot cultivars after grafting. The content of catechin reported, ranging from 1.80 to 10.2 mg/g FW, was comparable to that observed in the present study.

The link between phenolic compound's content and susceptibility to brown rot caused by *M. laxa* was previously studied, but only in fruit. Chlorogenic acid and neochlorogenic acid content in peach fruit was negatively correlated with the incidence of brown rot for each cultivar studied (Villarino et al., 2011). However, the effect of phenolic content in apricot branches on the susceptibility to *M. laxa* has not yet been

reported in the literature. An increase in the scopoletin content in branches after inoculation with *M. laxa* was observed in the present study. The contents of procyanidin dimer (2) and 3-CQA were higher in the branches of the *M. laxa* susceptible cultivar Bergeron than in the *M. laxa* tolerant cultivar Bakour. Generally, tolerance is related to high content of phenolics, nevertheless there is no agreement yet on the defensive role of specific compounds (Harborne, 2009). However, analysis of the whole population shows that higher content of 10 phenolic compounds was linked to lower susceptibility to *M. laxa*. This suggests that the phenolics play an important role in the defence against brown rot blossom blight in apricots.

In previous studies, both scopoletin, scopolin and numerous hydroxyacetophenone derivatives were shown to play a significant role in various pathosystems. For example, an accumulation of both scopoletin and scopolin was found in aerial parts of morning glories after interaction with *Fusarium oxysporum* (Shimizu et al., 2005). Higher levels of scopoletin accumulated in young leaves of wild tobacco plants accounted for their strong resistance against *Alternaria alternata* (Sun et al., 2014; Li and Wu, 2016). *Botrytis cinerea* induced the accumulation of scopoletin and pathogenesis-related proteins in the resistant cultivar of *Nicotiana tabacum* cv. Petit Havana (El Oirdi et al., 2010). The fungitoxic effect of scopolin, related coumarins and hydroxyacetophenone on *Sclerotinia sclerotiorum* was recognised as a way to overcome sunflower head rot (Prats et al., 2006, 2007). Acetophenone glycoside was previously identified as an antifungal agent and isolated from the methanol extract of the roots of red currants (Chevalley et al., 2001). 2′, 6′-dihydroxy-4′-methoxyacetophenone was identified as phytoalexin in the roots of burnet (*Sanguisorba minor*) after fungal inoculation with *B. cinerea* (Kokubun et al., 1994). Flavan-3-ol content increased in black poplar stems infected with *Plectosphaerella populi* over the course of infection compared to non-infected controls (Ullah et al., 2019). Several reports support the contribution of catechin to plant resistance against *Fusarium graminearum*. Catechin concentration increased in some resistant naked barley seeds following *Fusarium* inoculation. Higher amounts of catechin were recorded in *Fusarium* head blight–resistant barley genotypes compared to susceptible ones (Gauthier et al., 2015). Eight individual flavonols, three flavanols and two chlorogenic acids (neochlorogenic acid and chlorogenic acid) in the leaves of black currants played a significant role in the resistance to foliar diseases (Vagiri et al., 2017).

To evaluate possible antifungal activity of phenolic compounds in apricot branch tissues, an *in vitro* assay for measurement of mycelial growth and spore germination inhibition was conducted. Villalobos et al. (2016) report that *M. laxa* mycelial growth was inhibited by more than 65 % by aqueous ethanol extract of defatted soy flour, which contained isoflavones and phenolic acids, such as syringic acid, coumaric acid and ferulic acid. The same study tested treatment of peach fruit with an extract of soy flour before inoculation with *M. laxa* and observed a clear reduction of the growth of *M. laxa* compared to untreated fruit. In the present study, chlorogenic acid at a concentration up to 250 mg/L showed no inhibition of *M. laxa* mycelial growth. Spore germination of *M. laxa* was not affected by chlorogenic acid at a concentration up to 400 mg/L. This is in line with data showing that the germination of *M. laxa* spores was not affected by chlorogenic acid at concentrations of up to 500 ppm (Villarino et al., 2011). According to these authors, despite the lack of direct antifungal effect, a higher concentration of chlorogenic acid in the fruit contributed to a higher resistance of peach cultivars to brown rot infection by interfering with the production of fungal melanin. Lee and Bostock (2007) studied the resistance of peach fruit to *M. fruticola and* proposed that the high concentration of chlorogenic acid in fruit from genotypes with high levels of disease resistance might contribute to brown rot resistance by inhibiting production of fungal cutinases, necessary for the fungus to penetrate the intact surface of fruit. Pure chlorogenic or caffeic acid at a concentration of 5 mM did not inhibit spore germination or mycelial growth of cultured *M. fructicola* (Bostock et al., 1999). However, chlorogenic acted as a biofungicide against phytopathogenic fungi *Fusarium solani eumartii, S. sclerotiorum, B. cinerea, Verticillium dahliae* and *Cercospora sojina* (Martinez et al., 2017). At a rather high concentration of more than 1 g/L, it inhibited spore germination and reduced mycelial growth, probably by altering the permeability of the cell membrane, which causes cell lysis and loss of cell content. Catechin showed no inhibition of mycelial growth in the present study. The addition of catechin and proanthocyanidin to culture media at a high concentration of 2 g/L reduced the mycelial growth of *P. populi* (Ullah et al., 2019). In addition, *Fusarium culmorum* growth was inhibited by catechin, epicatechin and, to a lesser extent, by their dimers (Skadhauge et al., 1997).

As mentioned above, the role of hydroxyacetophenones and coumarins in different pathosystems has been revealed. Their antifungal activities *in vitro* were also studied. 2′,6′-dihydroxy-4′-methoxyacetophenone inhibited spore germination of *B. cinerea* and *Phomopsis perniciosa* with an EC_{50} of 45 and 410 μM, respectively (Kokubun et al., 1994). In the present study, 2′,4′,6′-trihydroxyacetophenone inhibited both spore germination and mycelial growth of *M. laxa*, whereas 2′-hydroxy-4′-methoxyacetophenone only inhibited mycelial growth. Even though the acetophenones tested do not have exactly the same structure as those identified in apricot branches and their inhibiting activity might differ, it gives an important insight into possible role of this group of compounds in the control of *M. laxa* growth. Scopoletin inhibited spore germination and mycelial growth of *M. laxa* up to 53 % (at 400 mg/L) and up to 36 % (at 200 mg/L), respectively. Scopoletin affected *B. cinerea* in a dose-dependent manner with 90 % spore germination inhibition and 25 % mycelial growth inhibition, at a concentration of 500 mg/L (El Oirdi et al., 2010). At similar concentrations (480−530 mg/L), both scopolin and scopoletin inhibited mycelial growth of *A. alternata* by 90 % (Sun et al., 2014) and *Sclerotinia* germ tube growth by up to 60 % (Prats et al., 2006). Glycosylation is a common modification of plant secondary metabolites and has been proposed to be involved in the detoxification and storage of secondary metabolites. Glycosides are hydrolysed by β-glucosidase, which is induced in both fungus and host during the infection process and becomes more toxic to the fungal pathogen. Therefore, scopolin was initially considered as a storage form of scopoletin; nonetheless, its antifungal activity was revealed in the aforementioned studies. Therefore, scopolin is probably both a defence compound and a reservoir from which plants can produce other antifungal substances. The lower capacity to accumulate scopolin might differentiate susceptible from resistant cultivars. It was shown not only that susceptible cultivars accumulate less scopolin but also that its release is delayed compared to resistant cultivars (Aoun, 2017). In the presented study, the content of scopolin in the progenies of *high* and *very high* categories decreased after inoculation of branches with *M. laxa,* whereas the content of scopoletin increased for all categories. Therefore, it might be speculated that aglycone of scopolin – scopoletin was released as defence mechanism.

5. Conclusions

The development of resistant/tolerant apricot cultivars is an important strategy for fighting against pathogens and enabling production of high-quality fruit with respect to environmental issues. The present study established a link between a high content of some phenolics in branches and lower susceptibility to *M. laxa*, expressed as shoot necrosis length. Apart from having direct antifungal activity, phenolics are able to inhibit pathogens' production of enzymes and synthesis of melanin and therefore impede fungal infection. The direct antifungal activity of those compounds on *M. laxa* mycelial growth and spore germination has been revealed in the present study. A synergistic antifungal effect of some components cannot be ruled out, especially in view of simultaneous accumulation of scopolin and acetophenone hexoside observed in response to *M. laxa* infection. This might suggest that synthesis of those substances is controlled by the same factor. Based on the

variability observed in the necrosis length and the content of the individual phenolic compounds in the bi-parental population, a QTL analysis will be applied for assessing the genetic components involved in *M. laxa* resistance.

CRediT authorship contribution statement

Jorge Del Cueto: Methodology, Investigation, Writing - original draft. **Agnieszka Kosinska-Cagnazzo:** Validation, Formal analysis, Writing - original draft, Visualization. **Patrick Stefani:** Methodology, Investigation, Writing - review & editing. **Julien Héritier:** Methodology, Investigation. **Guillaume Roch:** Resources. **Thomas Oberhänsli:** Methodology, Writing - review & editing. **Jean-Marc Audergon:** Conceptualization, Methodology, Resources. **Danilo Christen:** Funding acquisition, Conceptualization, Methodology, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

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