Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v44i1.55850

Peach and nectarine susceptibility to brown rot and protocol optimization to evaluate *Monilinia fructicola* sporulation

Maximiliano Dini[1,2*], Maria do Carmo Bassols Raseira[3], Silvia Scariotto[4], Silvia Carpenedo[3] and Bernardo Ueno[3]

[1]Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa Postal, 354, 96010-900, Capão do Leão, Rio Grande do Sul, Brazil. [2]Instituto Nacional de Investigación Agropecuaria, Programa Nacional de Investigación en Producción Frutícola, Estación Experimental INIA Las Brujas, Rincón del Colorado, Canelones, Uruguay. [3]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. [4]Universidade Tecnológica Federal do Paraná, Campus Pato Branco, Pato Branco, Paraná, Brazil. *Author for correspondence. E-mail: mdini@inia.org.uy

ABSTRACT. The fungus *Monilinia fructicola,* which causes brown rot in fruits, is one of the main peach pathogens. The emergence of fungicide-resistant fungus isolates, as well as the attempt to reduce sprays, favors adoption of other control strategies. Among them, one of the most important is genetic resistance. This study was carried out aiming to evaluate the susceptibility of 16 peach and 4 nectarine genotypes to brown rot, as well as to evaluate how well the sporulation area and diameter correlate with number of spores in the lesions. Both wounded and non-wounded fruits were inoculated with 10 µL of *M. fructicola* suspension. Wounded fruits from all genotypes (nectarines and peaches) showed susceptibility to *M. fructicola*, from 92 to 100% of incidence. The disease incidence was between 18 and 100% when non-wounded fruits were inoculated. High variability was detected for the fungus sporulation, in both wounded and non-wounded fruits, with ranges between 16 to 96% and 0 to 94%, respectively. The fungus sporulation was variable among the genotypes (between 0.1 to 96.0 conidia per mm^2) and it is positively correlated with the diameter and area of sporulation. The genotypes Conserva 947, Conserva 1662, Conserva 672, Conserva 1600, and 'Bolinha', are the ones with less susceptible to brown rot.

Keywords: *Prunus persica*; genetic resistance; screening.

Received on September 16, 2020.
Accepted on January 20, 2021.

Introduction

The fungus *Monilinia fructicola* (Winter) Honey, the causing agent of brown rot, is one of the main pathogens on the peach culture (*Prunus persica* (L.) Batsch), in Brazil and worldwide. The damages may occur at any time of the peach cycle, starting at blooming until postharvest. The disease symptoms are blossom blight, twig cankers and mainly fruit rots. The fruits are more resistant in early stages of development, however different types of injuries favor the pathogen entrance, causing the disease. Under optimum conditions for the disease (high humidity, and mild temperatures), these symptoms may be visible within 48 hours after infection (May-de-Mio, Moreira, Monteiro, & Justiniano Júnior, 2008; May-de-Mio, Garrido, Ueno, & Fajardo, 2014).

Brown rot is mainly controlled through fungicides sprays (Thomidis, Michailides, & Exadaktylou, 2009). However, the increasing concern about the environment preservation and workers and consumers health (Baró-Montel et al., 2019; Elshafie, Mancini, Camele, De Martino, & De Feo, 2015), as well as the emergence of fungicide resistant pathogen populations (Luo et al., 2010; Hily, Singer, Villani, & Cox, 2011; Zhu, Bryson, & Schnabel, 2012; Chen, Yuan, Schnabel, & Luo, 2017; Fu, Tian, Pei, Ge, & Tian, 2017), result in the demand for others control strategies such as genetic resistance. However, the use of this strategy is limited in commercial orchards because, although there are significant differences in susceptibility among available genotypes, commercial cultivars that are resistant to brown rot are not yet available (Santos & Ueno, 2014). The Brazilian peach cultivar Bolinhapresents a lower susceptibility than other cultivars already evaluated (Feliciano, Feliciano, & Ogawa, 1987, Santos, Raseira, & Zanandrea, 2012; Fu, Burrell, Linge, Schnabel, & Gasic, 2018). However, poor fruit quality and productivity discouraged its cultivation.

The fruit resistance to brown rot is a quantitative, polygenic trait (Martinez-Garcia et al., 2013; Pacheco et al., 2014; Baró-Montel et al., 2019). This type of gene action delays the epidemic development in the

orhcard, even when the genotype is susceptible to it (Dallagnol & Araujo Filho, 2018), and can be quantified based on the monocyclic processes, such as infection efficiency, latent period, rate of lesion and sporulation expansion (Kushalappa & Gunnaiah, 2013).

Sporulation studies has great epidemiological importance in the advancement of fungal diseases (Rios & Debona, 2018) and in the case of brown rot its main incidence is as secondary inoculum (May-de-Mio et al., 2014; Oliveira Lino et al., 2016). Knowledge of sporulation capacity of *M. fructicola* in fruits of a particular genotype is necessary to be able to recommend its planting, especially under Brazilian growing conditions which are predisposing to the disease (high temperatures, occurrence of rains and humidity during the growing season) (May-de-Mio et al., 2014). Previous work carried out with a large number of peaches of Brazilian cultivars (screening) indicated that conidia counting on infected fruits was effective but was labor and time demanding (Joel Fortes, personal communication), although in some specific studies it is used to know and compare the conidia density of *Monilinia* spp. (Gell, De Cal, Torres, Usall, & Melgarejo, 2009; Sun, Gao, Liu, & Wang, 2009). The most recent literatures use other types of variables to measure this parameter, they use the area covered by sporulation, sporulation diameter, number or percentage of fruits with sporulation (Scariotto, Santos, & Raseira, 2015; Garcia-Benitez, Melgarejo, De Cal, & Fontaniella, 2016; Obi, Barriuso, Moreno, Giménez, & Gogorcena, 2017; Obi, Barriuso, Usall, & Gogorcena, 2019).

The present study was carried out aiming to evaluate the susceptibility of peach and nectarines genotypes to brown rot; as well as to check how accurate the measurement of the sporulation surface correlates with the spores counting, thus simplifying the evaluation proccess.

Material and methods

The study was performed at Embrapa Clima Temperado, in the laboratories of fruit breeding and phytopathology, in Pelotas, Rio Grande do Sul State, Brazil (31º40' S and 52º26' W; 57 m altitude). Fruit susceptibility to brown rot was tested for 20 different genotypes (among cultivars and selections), four nectarines: 'Sunmist', Necta 506, Necta 532, and Necta 540 and 16 peaches: 'Chimarrita', Cascata 1359, Cascata 1577, 'BRS Rubimel', TX2D163, 'Maciel', 'Atenas', 'Bolinha', 'Cerrito', Conserva 655, Conserva 572, Conserva 947, Conserva 1526, Conserva 1600, and Conserva 1662 and a whipping type selection (Chorão). Except for 'Sunmist' (University of Florida) and TX2D163 (Texas A&M University), all genotypes are from the Embrapa Peach Breeding Program. All genotypes are cultivated in the same area (Embrapa work collection), under the same cultural management. Trees were spaced 2 m between trees and 5 m between rows, conducted in an open vase and with three trees (between 10 and 14-year-old) per genotype obtained by budding (clones).

The experimental design was completely randomized, and each genotype was considered as one treatment, with four replications of five fruits. The inoculation was made on wounded fruits in 2015-2016, 2016-2017, and 2017-2018 harvest seasons, and non-wounded fruits in the 2016-2017 and 2017-2018 harvest seasons.

The fungus isolate was obtained from mummified fruits infected by *M. fructicola,* collected at four different peach orchards of Embrapa Clima Temperado. From these, fragments of approximately 5 mm were transferred to Petri dishes containing Potato Dextrose Agar (PDA) culture media and incubated for seven to ten days, in a growth chamber at 25 ± 2°C and 12 hours of light. Contamination with other fungi or bacteria was eliminated by successive cultures until the pure culture was obtained. The four fungal isolate was stored in test tubes with PDA culture medium in a cold chamber (4 ± 1°C). Whenever necessary, the fungus was cultured on ripe peach fruits, purified again and transferred to Petri dishes with PDA. The conidia were removed from the cultures of *M. fructicola* with seven to ten days of incubation, with a brush and 10 mL of distilled water. The suspension was then filtered, and the concentration of conidia was determined using an optical microscope and hemocytometer. Finally, the inoculum used was prepared under aseptic conditions on the day of inoculation, using the same proportion of the four strains of *M. fructicola*.

Fruits at commercial ripening stage were harvested from the four quadrants of the plant. They were selected for absence of apparent damages by insects and/or infection. Subsequently, they were submitted to disinfestation by immersion in 70% alcohol for one minute, followed by three minutes in a 0.5% sodium hypochlorite solution. After this period, they were washed tree times in distilled water. Fruits were arranged into transparent plastic boxes (24 × 23 × 10 cm), being five per box, placed over plastic rings. The boxes were previously disinfested with 70% alcohol and the bottom of them was lined with filter paper moistened with sterilized distilled water.

The inoculation was made by pippetting the inoculum suspension on fruits without wounding in a previously marked location in the equatorial region. For the inoculation on wounded fruits (wound

penetration of 1 mm into the fruits with the syringe tip), it was used an inoculation syringe of 100 µL coupled to a repeating dispenser 50x (Hamilton®). In both, wounded and non-wounded fruits, 10 µL suspention of *M. fructicola* (2.5×10^4 conidia mL^{-1}) with Tween-80® (0.1 g L^{-1}) was inoculated in the equatorial region of each fruit (Crisosto, Gradziel, Ogundiwin, Bostock, & Michailides, 2009; Martínez-García et al., 2013; Scariotto et al., 2015; Fu et al., 2018; Obi et al., 2019).

After inoculation, the fruits were stored in the boxes and incubated in a growth chamber, with 23 ± 1°C temperature, 75% relative humidity and 12 hours of light. The evaluations were performed 72 hours after inoculation (hAI), considering as infected the fruits that presented characteristic disease lesions. The severity was evaluated by measuring the lesion diameter (LD), with a digital caliper, and using the average of two perpendicular measurements. Presence of sporulation (SPP) was also evaluated (% of fruits with sporulation), and if so, sporulation diameter (SPD) was measured, in the same way as the LD. The LD and SPD in wounded fruits data were subjected to Pearson correlation.

Lesion (LA) and sporulation (SPA) areas were calculated, considering as circular shape, by the formula: $A = (\pi \times D1 \times D2) / 4$, being D1 and D2, the diameters of each perpendicular measurements (Pazolini, Santos, Citadin, Storck, & Flores, 2016). Likewise, the area of one side of the fruit was calculated using the measures of fruit diameter and height. From these results, the percentage of fruit affected by the lesion and sporulation was calculated.

In 2017-2018, three skin samples of 5 mm diameter were collected from five inoculated wounded fruits, on the sporulation zone of each of them, using a cork borer. If sporulation was not visible, the samples were taken from the area with brown rot lesion. The samples were maintained in vials with 1 mL of lactic acid until the evaluation. Conidia counting was determined, in duplicate, using an optical microscope and the hemocytometer with vials previously shaken for 30 seconds. Sporulation mm^{-2} (SP mm^{-2}) was calculated by multiplying the average concentration of conidia (conidia mL^{-1}) by the volume of storage media and dividing it by the sample area (mm^2), expressed in conidia per mm^2 (Kadish, Grinberger, & Cohen, 1990). The SP mm^{-2} was submitted to Sperman correlation with the other sporulation variables measured on the same harvest season (SPP, SPD, and SPA).

The data were submitted to analysis of variance (ANOVA) and the means of the genotypes were grouped by the Scott-Knott test ($p \leqslant 0.05$). For the statistical analysis, the data expressed in percentage were transformed into $\arcsin \sqrt{x / 100}$, to meet the assumptions of homogeneity of the variances and normality of the residues.

The divergence among genotypes was evaluated by the UPGMA hierarchical grouping method (Unweighted Pair-Group Method using Arithmetical Averages) applied to genotype averages for all analyzed variables regarding lesion and sporulation, grouping the genotypes for to brown rot resistance in the fruit. The average Euclidean distance was used as a dissimilarity measure, and the fitness between the matrices and clustering was estimated by the cophenetic correlation coefficient (CCC). The cut-off point was defined as half of the average Euclidean distance.

Results and discussion

When the fruits were inoculated after being wounded, all genotypes were susceptible to brown rot, with incidence range (BRI) from 92 to 100% (Figure 1). When fruits were not wounded at the inoculation, there was high variability, with some genotypes presenting less than 30% BRI (Conserva 947, Conserva 672, and 'Bolinha') and others more than 90% (Necta 540, Chorão, Cascata 1359, and Cascata 1577). Baró-Montel et al. (2019) in a study with *Monilinia fructicola* inoculation, observed a slight difference on brown rot incidence in non-wounded fruits. Other studies with inoculated non-wounded fruits had averages between 60 and 100% (Obi et al., 2017) and between 50 and 100% (Obi et al., 2019).

Regarding to SPP, high variability was detected for both inoculation methods: inoculation in wounded and non-wounded fruit it ranged from 16 and 96% and 0 and 94%, respectively (Figure 1). Selections Conserva 947 (16%), Conserva 1662 (25%), Conserva 672 (28%), and 'Bolinha' (38%) stand out as the genotypes with the least fruit SPP when inoculated after being wounded. On non-wounded fruits, two more genotypes showed less than 3% of fruits with SPP (Conserva 947, Conserva 1662, 'Bolinha', Conserva 672, Conserva 1600, and 'Maciel').

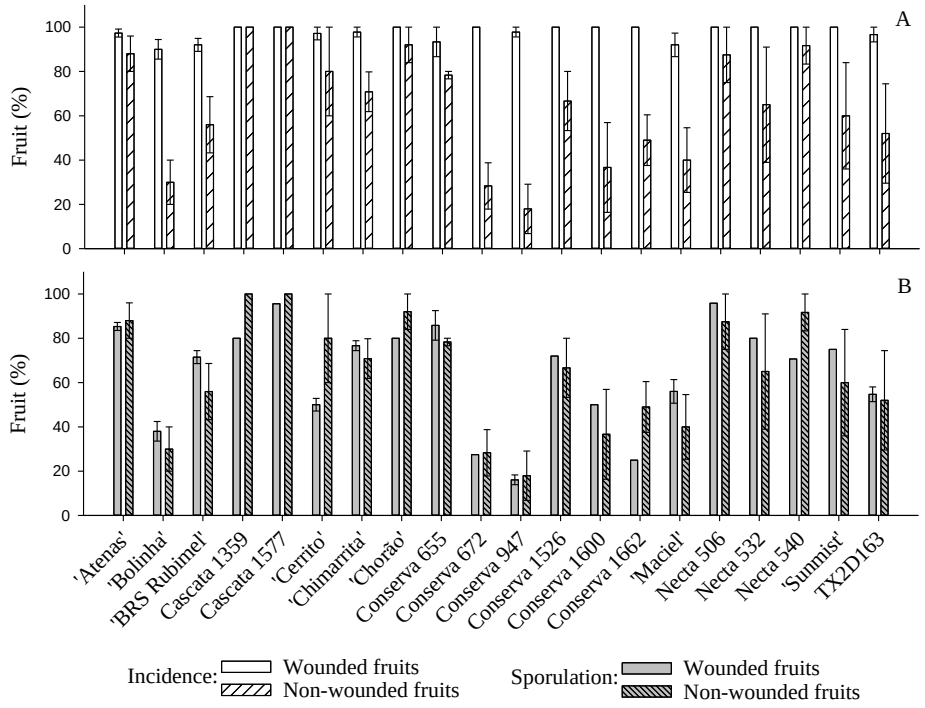

Figure 1. Brown rot incidence (A) and sporulation (B) in 20 peach genotypes inoculated with *Monilinia fructicola* in wounded and non-wounded fruits, evaluated at 72 hours after inoculation. The columns correspond to the average values of three harvest seasons (2015-2016, 2016-2017 and 2017-2018) for wounded fruits and two harvest seasons (2016-2017 and 2017-2018) for non-wounded fruits. The vertical bars in each column refer to standard error. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil.

ANOVAs performed between genotypes for LD and LA were highly significant ($p < 0.0001$) for both wounded and non-wounded fruits, and for all harvest seasons. Wounded inoculated fruits of the evaluated genotypes showed averages LD between 12.7 and 44.0 mm and LA between 10.2 and 81.8% (Table 1). Applying Scott-Knott grouping test for LD, four groups were identified on the first harvest season and three in the second and third harvest seasons. The genotype which presented the lowest averages for LD in the three harvests seasons was Conserva 947. For LA, five groups were obtained in the first harvest season and four in the second and third. The genotypes identified in the lowest LA group, during the three harvest seasons were Conserva 947, 'Bolinha', Conserva 1600, Conserva 1662, and Conserva 672, with intervals between 10.2 and 25.0% (Table 1). Fu et al. (2017) reported averages between 12 and 34 (wounded fruits) and between 0 and 26 (non-wounded fruits) of disease severity index, being this index the product of the average of LD and the disease incidence.

It is important to note that the variable LA was more efficient than LD to differentiate the levels of susceptibility, since it allows to correct the lesion area according to fruit size. Some genotypes, as 'Sunmist', Chorão and Necta 532, which produce small fruits, presented higher values of LA, even if LD is not in the highest group. On the other hand, large fruits such as Conserva 672, 'Cerrito', Conserva 655, 'BRS Rubimel', and 'Maciel', presented in general, lower LA than LD values. This allowed to allocate these genotypes in groups of less susceptibility in comparison to when LD was used as a variable of severity. Similar results were obserbed by Walter et al. (2004), who performed a screening for brown rot resistance in apricot fruits (*Prunus armeniaca*), observed that the LA, measure after 72h AI, was the variable that best discriminated the genotypes.

The variability was even higher among genotypes in the case of non-wounded fruits, with values of LD and LA ranging from 0.0 to 41.6 mm and from 0.0 to 79.5%, respectively. But, the experiment accuracy was lower, with coefficients of variation between 62.1 and 84.1%. The genotypes located in the lower susceptibility group with lower values for both LD and LA were Conserva 947, 'Bolinha', Conserva 1600, Conserva 672, and 'Maciel' (Table 1). The cultivar Bolinha is known for having the lowest LD in several other studies performed in non-wounded fruits (Feliciano et al., 1987; Santos et al., 2012; Scariotto et al., 2015; Fu et al., 2018).

When the inoculation was made without wounding, the SPD and SPA averages were between 0.0 to 36.8 mm and 0.0 to 69.5%, respectively. Testing several peach genotypes to *M. laxa*, Obi et al. (2017) obtained LD averages between 38.0 to 62.5 mm in one experiment, and 48.9 to 56.5 mm in another, in two years of evaluation (Obi et al., 2019). The high values of LD reported in the mentioned studies may be due to the longer incubation time (120h AI) and/or the high susceptibility of the genotypes when compared to the Embrapa`s genotypes.

Table 1. Means of lesion diameter (LD) and lesion area (LA) evaluated 72 hours after inoculation in wounded (2015-2016, 2016-2017 and 2017-2018 seasons) and non-wounded (2016-2017 and 2017-2018 seasons) fruits, in Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil[(1)].

Genotype	2015-2016		2016-2017				2017-2018			
	Wounded		Non-wounded		Wounded		Non-wounded		Wounded	
	LD[(2)] (mm)	LA[(3)] (%)	LD (mm)	LA (%)	LD (mm)	LA (%)	LD (mm)	LA (%)	LD (mm)	LA (%)
'Atenas'	36.7 c	47.4 c	17.3 b	15.7 b	38.9 c	46.5 c	32.5 b	34.0 b	31.7 c	32.6 b
'Bolinha'	20.1 a	12.5 a	8.1 a	5.8 a	20.9 a	19.7 a	6.5 a	4.6 a	20.4 b	14.4 a
'BRS Rubimel'	35.0 c	31.5 b	6.2 a	3.7 a	30.1 b	37.5 b	34.9 b	45.2 b	28.8 c	31.6 b
Cascata 1359	30.0 b	36.6 c	25.3 c	31.0 b	28.6 b	36.3 b	24.0 b	31.2 b	31.5 c	36.9 c
Cascata 1577	25.8 b	29.6 b	41.3 c	57.0 c	38.8 c	60.1 d	41.6 b	58.5 b	33.6 c	43.0 c
'Cerrito'	25.7 b	23.5 a	16.1 b	15.1 b	27.9 b	25.0 a	14.6 a	11.8 a	32.7 c	31.1 b
'Chimarrita'	37.1 c	42.4 c	14.2 b	9.5 b	40.0 c	46.5 c	32.0 b	41.1 b	35.6 c	44.0 c
'Chorão'	33.4 c	73.2 e	41.3 c	69.5 d	35.6 c	69.7 d	23.9 b	42.5 b	33.1 c	79.5 d
Conserva 655	33.3 c	27.4 b	14.0 b	9.1 b	42.8 c	44.8 c	15.1 a	8.8 a	22.2 b	16.3 a
Conserva 672	27.1 b	22.9 a	6.9 a	5.8 a	25.1 b	19.1 a	8.5 a	9.1 a	22.4 b	15.3 a
Conserva 947	17.3 a	10.2 a	0.0 a	0.0 a	19.5 a	13.3 a	10.5 a	7.9 a	12.7 a	12.7 a
Conserva 1526	28.0 b	21.5 a	15.7 b	12.5 b	44.0 c	56.0 c	12.4 a	8.8 a	38.2 c	44.0 c
Conserva 1600	23.5 a	14.0 a	0.0 a	0.0 a	24.8 b	24.9 a	7.6 a	5.8 a	24.4 b	19.3 a
Conserva 1662	20.8 a	13.0 a	12.2 b	12.0 b	25.9 b	19.4 a	18.2 a	12.6 a	17.4 a	7.85 a
Conserva 655	33.3 c	27.4 b	14.0 b	9.1 b	42.8 c	44.8 c	15.1 a	8.8 a	22.2 b	16.3 a
Conserva 672	27.1 b	22.9 a	6.9 a	5.8 a	25.1 b	19.1 a	8.5 a	9.1 a	22.4 b	15.3 a
Conserva 947	17.3 a	10.2 a	0.0 a	0.0 a	19.5 a	13.3 a	10.5 a	7.9 a	12.7 a	12.7 a
'Maciel'	41.2 d	38.8 c	1.26 a	0.4 a	24.5 b	18.6 a	17.1 a	14.1 a	14.6 a	9.35 a
Necta 506	35.6 c	60.4 d	10.1 b	8.9 b	40.2 c	64.6 d	16.0 a	16.8 a	36.0 c	55.3 c
Necta 532	42.2 d	81.8 e	30.6 c	46.4 c	38.3 c	63.6 d	33.0 b	52.3 b	35.0 c	79.5 d
Necta 540	33.9 c	50.5 d	27.7 c	40.3 c	34.3 c	52.1 c	31.7 b	48.1 b	25.5 b	25.5 b
'Sunmist'	31.3 c	59.9 d	31.5 c	50.9 c	31.1 b	62.2 d	32.2 b	55.0 b	33.4 c	57.7 c
TX2D163	29.0 b	27.5 b	17.5 b	13.6 b	27.0 b	22.2 a	0.0 a	0.0 a	29.8 c	32.4 b
CV (%)	23.2	38.9	78.5	84.1	25.3	43.4	62.1	78.9	35.9	48.1

[(1)]Means followed by the same letter in the column belong to the same group by the Scott-Knott's clustering test at $p \leqslant 0.05$. [(2)]LD = evaluated in two perpendicular measures by fruits. [(3)]LA = percentage of the one face of the fruit lesioned by *Monilinia fructicola*.

All ANOVAs performed for SPD, SPA, and SP mm^{-2}, were highly significant ($p < 0.0001$) for both wounded and non-wounded fruits and for all evaluated seasons (Table 2).

Considering SPD, five groups were formed by Scott-Knott teste, in the first harvest, four in the second and three in the third. The genotypes that remained in the group with lower SPD and with the lowest averages during the three harvest seasons were Conserva 947, 'Bolinha', Conserva 1600, and Conserva 1662. For SPA, four groups were stated in the three harvests and the least susceptible were the same ones with the addition of selection Conserva 672, all with less than 7.3% (Table 2).

On non-wounded fruits, the variability among genotypes was high, with values ranging from 0.02 to 29.8 mm and from 0 to 37.8%, for SPD and SPA, respectively. However, the coefficients of variation were extremely high, between 125.8 and 143.9%, which can be explained by the large number of fruits without fungus sporulation. The genotypes in the lowest susceptibility group, with the lowest values for both SPD and SPA in the two evaluated seasons, were Conserva 947, 'Bolinha', Conserva 1600, Conserva 1662, Conserva 672, and 'Cerrito' (Table 2).

In the inoculation without wound, the lower development of the disease may be due to the delay in infection and/or in the lower probability of conidia to succeed in fruit penetration. As a consequence, the LA is smaller, so SPA will also be smaller. It should be pointed out that in the case of non-wounded fruits inoculation, although the fruits are visually intact, there may have microcracks from where the fungus can infect. In the case of *M. fructicola* seems that the wound is necessary for the infection to exist (Lee & Bostock, 2006; Oliveira et al., 2016). Thus, the number of conidia that would have success in infecting, in case of wound inoculation would be much higher in relation to inoculation without wound, since the deposition of the conidia would have to be coincident with the place where the micro-wound is located (Michailides & Morgan, 1997).

There was a high correlation (0.84) between LD and SPD of fruits inoculated with wound. An even higher correlation (0.959) was obtained by Obi et al. (2019). However, in the present study, the correlation was not higher due to the great presence of fruits with lesions, but without fungus sporulation (concentration of points on the Y axis) (Figure 2). The main genotypes that had a high percentage of fruits with lesions but without sporulation were Conserva 947, Conserva 1662, Conserva 672, 'Bolinha', 'Cerrito', and Conserva 1600 (Figure 1 and Tables 1 and 2).

Table 2. Means of sporulation diameter (SPD) and sporulation area (SPA) evaluated 72 hours after inoculation in wounded fruits for three seasons (2015-2016, 2016-2017 and 2017-2018), and conidia number evaluated in 2017-2018 harvest season, in Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil[(1)].

Genotype	2015-2016		2016-2017				2017-2018				
	Wounded		Non-wounded		Wounded		Non-wounded		Wounded		
	SPD[(2)] (mm)	SPA[(3)] (%)	SPD (mm)	SPA (%)	SPD (mm)	SPA (%)	SPD (mm)	SPA (%)	SP mm^{-2}[(4)] (conidia mm^{-2})	SPD (mm)	SPA (%)
'Atenas'	22.3 c	21.3 b	6.4 b	5.0 a	23.8 c	21.0 b	15.5 b	10.6 b	12.4 a	18.7 b	13.0 b
'Bolinha'	5.4 a	1.9 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.2 a	5.7 a	2.3 a
'BRS Rubimel'	19.9 c	11.5 a	0.0 a	0.0 a	11.8 b	10.1 a	20.0 b	18.1 c	45.1 b	16.0 b	12.9 b
Cascata 1359	16.4 b	12.0 a	13.9 c	11.5 b	17.0 c	12.7 a	12.4 b	11.3 b	3.2 a	15.8 b	11.4 b
Cascata 1577	16.7 b	12.1 a	29.8 d	28.5 c	28.2 d	32.1 b	29.1 b	27.6 c	47.5 b	19.4 b	18.3 b
'Cerrito'	8.7 a	5.1 a	0.0 a	0.0 a	13.9 b	9.4 a	0.0 a	0.0 a	57.5 b	16.0 b	10.4 b
'Chimarrita'	23.3 c	18.1 b	0.0 a	0.0 a	23.9 c	20.1 b	20.4 b	22.2 c	37.2 b	25.1 c	24.2 c
'Chorão'	23.9 c	42.9 c	26.9 d	37.8 d	24.7 c	39.0 c	4.3 a	6.4 a	96.0 c	26.1 c	50.3 d
Conserva 655	19.5 c	10.1 a	5.6 b	2.9 a	23.8 c	15.5 a	5.0 a	2.1 a	27.1 a	13.9 b	6.4 a
Conserva 672	13.9 b	7.3 a	0.0 a	0.0 a	4.0 a	2.3 a	0.0 a	0.0 a	12.4 a	2.8 a	1.1 a
Conserva 947	1.3 a	0.7 a	0.0 a	0.0 a	0.0 a	0.0 a	2.3 a	1.9 a	0.1 a	0.0 a	0.0 a
Conserva 1526	14.1 b	7.4 a	3.0 b	1.1 a	29.7 d	26.8 b	0.0 a	0.0 a	78.9 c	22.8 c	17.7 b
Conserva 1600	5.6 a	2.0 a	0.0 a	0.0 a	4.7 a	3.2 a	2.9 a	2.1 a	2.4 a	5.0 a	2.0 a
Conserva 1662	5.8 a	2.1 a	0.0 a	0.0 a	1.5 a	1.1 a	0.0 a	0.0 a	0.6 a	0.0 a	0.0 a
'Maciel'	28.1 d	18.5 b	0.0 a	0.0 a	4.1 a	2.0 a	1.3 b	0.68 a	19.3 a	1.0 a	0.5 a
Necta 506	27.9 d	37.2 c	2.9 b	2.8 a	29.2 d	38.0 c	5.8 a	5.6 a	59.8 c	25.6 c	28.2 c
Necta 532	36.8 e	69.5 d	17.4 c	15.6 b	33.0 d	52.4 d	16.6 b	16.8 d	20.6 a	21.1 c	38.2 d
Necta 540	22.8 c	24.4 b	14.2 c	15.0 b	18.6 c	20.4 b	15.5 b	16.5 c	5.2 a	14.6 b	11.4 b
'Sunmist'	23.3 c	39.8 c	12.3 c	17.4 b	22.7 c	41.7 c	14.7 b	20.8 c	46.9 b	26.6 c	39.3 d
TX2D163	11.5 b	15.1 b	3.3 b	1.8 a	8.8 b	5.2 a	0.0 a	0.0 a	13.6 a	13.0 b	9.5 b
CV (%)	49.9	74.9	125.8	143.9	65.6	89.0	127.7	143.3	77.6	73.0	87.3

[(1)]Means followed by the same letter in the column belong to the same group by the Scott-Knott's clustering test at $p \leq 0.05$. [(2)]SPD = evaluated in two perpendicular measures by fruits; [(3)]SPA = percentage of the one face of the fruit affected by *Monilinia fructicola* sporulation; [(4)]SP mm^{-2} = Sporulation mm^{-2}, evaluated in five fruits per genotype in three samples of 5 mm of diameter per fruit, expressed in conidia per mm^2.

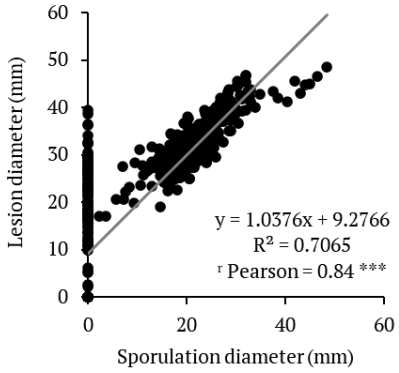

Figure 2. Correlation between sporulation and lesion diameter in wounded fruits of 20 peaches genotypes inoculated with *Monilinia fructicola* evaluated over three seasons (2015-2016, 2016-2017, and 2017-2018), Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil. *** = significant at $p \leq 0.001$.

The variability for SP mm^{-2} was also very large among genotypes, with intervals between 0.1 and 96.0 conidia per mm^2 of fruit surface. The Scott-Knott grouping test separated the genotypes into three groups, being the lowest SP mm^{-2} group composed by genotypes with less than 27.1 conidia per mm^2 whereas the highest SP mm^{-2} group was composed by Necta 506, Conserva 1526, and Chorão, with 59.8, 78.9, and 96.0 conidia per mm^2, respectively (Table 2). Quantifying the number of conidia in fruits of different apricot genotypes, Walter et al. (2004) found values from 10 to 140 and from 20 to 270 conidia per mm^2, when inoculated with *M. laxa* and *M. fructicola*, respectively.

The genotypes Cascata 1359, Necta 540, and Necta 532 (3.2, 5.2, and 20.6 conidia per mm^2, respectively), presented high values for LD, SPD, LA and SPA, indicating that, although the susceptibility to brown rot in these genotypes was high, the SP mm^{-2} was low. Genotypes that had brown rot lesion without fungus sporulation have great importance in epidemiological terms for the disease, reducing the secondary inoculum available in the orchard (May-de-Mio et al., 2014; Rios & Debona, 2018). On the other hand, in fruits of 'Cerrito', whose values for LD, SPD, LA, and SPA were generally low, but their SP mm^{-2} was high (57.5 conidia per mm^2) (Table 2).

The four variables studied in the 2017-2018 season related to sporulation were positively correlated among them ($p < 0.001$) (Table 3). The fact that these variables are significantly and positively correlated represents a great advantage when evaluating a large number of fruits of different genotypes (screening), since spore counting under a microscope is a time-consuming and expensive task, and often difficult to do.

Table 3. Spearman's correlation between sporulation variables in 2017-2018 season. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil[(1)].

Sporulation variables	Sporulation mm^{-2} (conidia per mm^2)	% of fruit with sporulation	Sporulation diameter (mm)	Area covered by sporulation (%)
Sporulation mm^{-2}	-	2.00^{-04}	1.20^{-03}	1.60^{-03}
% of fruit with sporulation	0.74	-	4.30^{-06}	2.10^{-06}
Sporulation diameter	0.67	0.84	-	1.30^{-11}
Area covered by sporulation	0.66	0.85	0.96	-

[(1)]Values above and below the diagonal give the *p*-value and Spearman's correlation coefficients, respectively.

Using a UPGMA analysis with all the studied variables, it was found nine groups (cut-off point = 0.82), with a CCC of 0.78 (Figure 3).

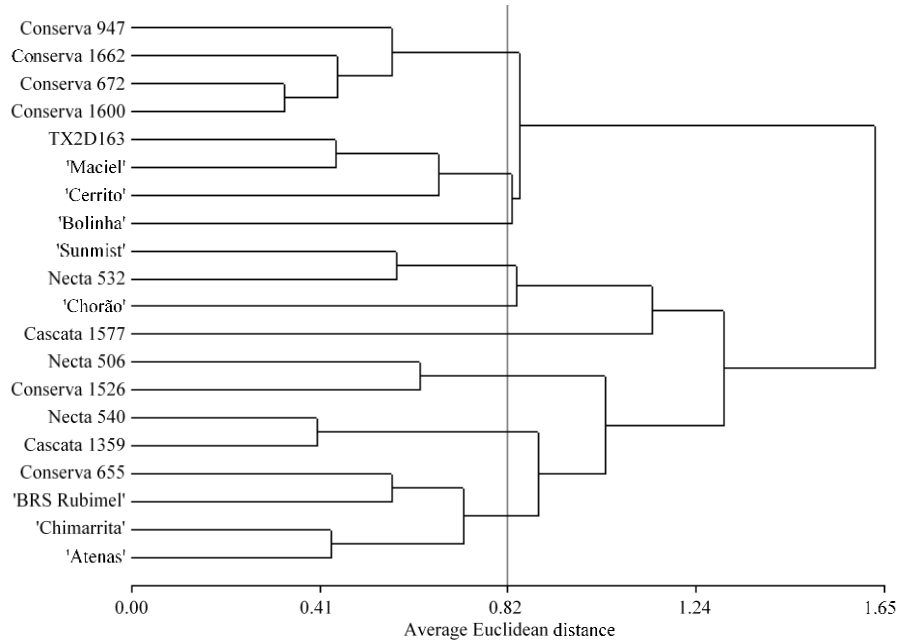

Figure 3. Dendrogram representing analysis of conglomerates among 20 evaluated genotypes, obtained by Unweighted Pair-Group Method using Arithmetic averages (UPMGA). Average Euclidean distance based on the means of incidence and severity of the lesion and sporulation of brown rot in fruits. Cut-off point was 0.82, corresponding to half the average Euclidean distance. Cophenetic correlation coefficient = 0.78. Analyzed data of the 2015-2016, 2016-2017, and 2017-2018 harvest seasons. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul State, Brazil.

The genotypes Conserva 947, Conserva 1662, Conserva 672, and Conserva 1600 are in the group with lowest averages in most of the variables related to the lesion and sporulation of *M. fructicola*. 'Bolinha' was the only genotype in the second group that also presented low values in these variables, but it was allocated on a separate group, because its lowest average BRI in wounded fruits. The selections Conserva 947 and Conserva 1662 have cv. Bolinha and a Bolinha seedling respectively as one of their ancestors whereas the selections Conserva 672 and Conserva 1600 have cv. Aldrighi in their genealogy. Furthermore, the cv. Bolinha is believed to come from the cv. Aldrighi, suggesting that the common ancestor of all these genotypes goes back to the latter cultivar which could be transmitting the low susceptibility to brown rot.

The genotypes TX2D163, 'Maciel', and 'Cerrito' established a third group with similar values to the genotypes of the two previous groups with non-wounded fruits. However, when the fruits were inoculated after wounding they presented higher values for LD, SPD, LA, and SPA, as well as SP capacity was higher than the genotypes that presented the best results.

The genotypes Conserva 655, 'BRS Rubimel', 'Chimarrita', and 'Atenas' formed a fourth and intermediary group, for all evaluated conditions. Another intermediary group allocated the genotypes Necta 506 and

Conserva 1526, which presented similar values to the previous ones in wounded fruits, but in non-wounded fruits presented lower values mainly for SPP, LD, and SPD.

It is interesting to mention that Necta 540 and Cascata 1359 genotypes presented high susceptibility to brown rot but were allocated in an independent group due to the low values of SP mm^{-2} (3.2 and 5.2 conidia per mm^2, respectively). It suggests that even though these genotypes are very susceptible to brown rot their SP mm^{-2} to propagate the disease is low. The most susceptible genotypes, considering all the evaluated parameters were 'Sunmist', Necta 532, Cascata 1577, and Chorão.

The use of more than one parameter and different inoculation techniques (with and without wound), may be important for the evaluation of brown rot resistance in peach. The best results were obtained with the genotypes Conserva 947, Conserva 1662, Conserva 672, Conserva 1600, and 'Bolinha'. The four selections have fruit quality far superior to 'Bolinha' (fruit size, total soluble solids, industrial potential) and the fruits do not fall before ripening as occurs with 'Bolinha', what shows the progress regarding brown rot resistance by the Embrapa Peach Breeding Program. Conserva 947 was not released just because it is a late-type cultivar and growers prefer the early ones. However, when the interest for late maturing cultivars for processing arises it would be released. Conserva 672 does not have high productivity but it is extensively used as a parent for its phytosanitary aspects and the Conserva 1600 and Conserva 1662 are still under study.

Conclusion

Lesion and sporulation area distinguish genotypes better in terms of *M. fructicola* reaction than lesion and sporulation diameter. The lesion diameter is positively correlated to the sporulation. The sporulation mm^{-2} is positively correlated to the sporulation variables (diameter, area and % of fruit with sporulation). Genotypes such as Conserva 947, Conserva 1662, Conserva 672, 'Bolinha', 'Cerrito', and Conserva 1600 have a high percentage of fruits with lesions but without *M. fructicola* sporulation. Conserva 947, Conserva 1662, Conserva 672, and Conserva 1600 genotypes presented better results regarding brown rot resistance, being similar to 'Bolinha'.

Acknowledgements

To the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) for providing scholarship funds to the first author and, Embrapa staff, especially Everton Pederzolli, Maicon Bönemann and Gilberto Kuhn for their assistance in the experiments.

References

Baró-Montel, N., Eduardo, I., Usall, J., Casals, C., Arús, P., Teixidó, N., & Torres, R. (2019). Exploring sources of resistance to brown rot in an interspecific almond × peach population. *Journal of the Science of Food and Agriculture*, *99*(8), 4105-4113. DOI: https://doi.org/10.1002/jsfa.9640

Chen, S., Yuan, N., Schnabel, G., & Luo, C. (2017). Function of the genetic element ‘Mona’ associated with fungicide resistance in *Monilinia fructicola*. *Molecular Plant Pathology*, *18*(1), 90-97. DOI: https://doi.org/10.1111/mpp.12387

Crisosto, C., Gradziel, T., Ogundiwin, E., Bostock, R., & Michailides, T. J. (2009). *Development of predictive tools for brown and sour rot resistance in peach and nectarines* (p. 87-93). California Tree Fruit Agreement 2009 Annual Research Report. Retrieved on Aug. 18, 2020 from https://ucanr.edu/repository/fileaccess.cfm?article=92551&p=NSGVHQ

Dallagnol, L. J., & Araujo Filho, J. V. (2018). Uma visão geral da resistência genética da planta a microrganismos. In L. J. Dallagnol (Ed.), *Resistência genética de plantas a patógenos* (p. 13-64). Pelotas, RS: UFPel.

Elshafie, H. S., Mancini, E., Camele, I., De Martino, L., & De Feo, V. (2015). *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. *Industrial Crops and Products*, *66*, 11-15. DOI: https://doi.org/10.1016/j.indcrop.2014.12.031

Feliciano, A., Feliciano, A. J., & Ogawa, J. M. (1987). *Monilinia fructicola* resistance in peach cultivar Bolinha. *Phytopathology*, *77*(6), 776-780, 1987. DOI: https://doi.org/10.1094/Phyto-77-776

Fu, W., Burrell, R., Linge, C. S., Schnabel, G., & Gasic, K. (2018). Breeding for brown rot (*Monilinia* spp.) tolerance in Clemson University Peach Breeding Program. *Journal of the American Pomological Society*, *72*(2), 94-100. DOI: https://doi.org/10.17660/ActaHortic.2021.1304.10

Fu, W., Tian, G., Pei, Q., Ge, X., & Tian, P. (2017). Evaluation of berberine as a natural compound to inhibit peach brown rot pathogen *Monilinia fructicola*. *Crop Protection*, *91*, 20-26. DOI: https://doi.org/10.1016/j.cropro.2016.09.008

Garcia-Benitez, C., Melgarejo, P., De Cal, A., & Fontaniella, B. (2016). Microscopic analyses of latent and visible *Monilinia fructicola* infections in nectarines. *PLoS ONE*, *11*(8), 1-16. DOI: https://doi.org/10.1371/journal.pone.0160675

Gell, I., De Cal, A., Torres, R., Usall, J., & Melgarejo, P. (2009). Conidial density of *Monilinia* spp. on peach fruit surfaces in relation to the incidences of latent infections and brown rot. *European Journal of Plant Pathology*, *123*(4), 415-424. DOI: https://doi.org/10.1007/s10658-008-9378-y

Kadish, D., Grinberger, M., & Cohen, Y. (1990). Fitness of metalaxyl-sensitive and metalaxyl resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. *Phytopathology*, *80*(2), 200-205. DOI: https://doi.org/10.1094/Phyto-80-200

Kushalappa, A. C., & Gunnaiah, R. (2013). Metabolo-proteomics to discover plant biotic stress resistance genes. *Trends in Plant Science*, *18*(9), 522-531. DOI: https://doi.org/10.1016/j.tplants.2013.05.002

Hily, J. M., Singer, S. D., Villani, S. M., & Cox, K. D. (2011). Characterization of the cytochrome b (*cyt b*) gene from *Monilinia* species causing brown rot of stone and pome fruit and its significance in the development of QoI resistance. *Pest Management Science*, *67*(4), 385-396. DOI: https://doi.org/10.1002/ps.2074

Lee, M., & Bostock, R. M. (2006). Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. *Phytopathology*, *96*(10), 1072-1080. DOI: https://doi.org/10.1094/phyto-96-1072

Luo, C. X., Hu, M. J., Jin, X., Yin, L. F., Bryson, P. K., & Schnabel, G. (2010). An intron in the cytochrome b gene of *Monilinia fructicola* mitigates the risk of resistance development to QoI fungicides. *Pest Management Science*, *66*(12), 1308-1315. DOI: https://doi.org/10.1002/ps.2016

Martínez-García, P. J., Parfitt, D. E., Bostock, R. M., Fresnedo-Ramírez, J., Vazquez-Lobo, A., Ebenezer, A., ... Crisosto, C. H. (2013). Application of Genomic and Quantitative Genetic Tools to Identify Candidate Resistance Genes for Brown Rot Resistance in Peach. *PLoS One*, *8*(11), 1-12. DOI: https://doi.org/10.1371/journal.pone.0078634

May-de-Mio, L. L., Moreira, L. M., Monteiro, L. B., & Justiniano Júnior, P. R. (2008). Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. *Tropical Plant Pathology*, *33*(3), 227-234. DOI: https://doi.org/10.1590/S1982-56762008000300008

May-de-Mio, L. L., Garrido, L. R., Ueno, B., & Fajardo, T. V. M. (2014). Doenças da cultura do pessegueiro e métodos de controle. In M. C. B. Raseira, J. F. M. Pereira, & F. L. C. Carvalho (Eds.), *Pessegueiro* (p. 355-432). Brasília, DF: Embrapa.

Michailides, T. J., & Morgan, D. P. (1997). Influence of fruit-to-fruit contact on the susceptibility of French prune to infection by *Monilinia fructicola*. *Plant Disease*, *81*(12), 1416-1424. DOI: https://doi.org/10.1094/PDIS.1997.81.12.1416

Obi, V. I., Barriuso, J. J., Moreno, M. A., Giménez, R., & Gogorcena, Y. (2017). Optimizing protocols to evaluate brown rot (*Monilinia laxa*) susceptibility in peach and nectarine fruits. *Australasian Plant Pathology*, *46*(2), 183-189. DOI: https://doi.org/10.1007/s13313-017-0475-2

Obi, V. I., Barriuso, J. J., Usall, J., & Gogorcena, Y. (2019). Breeding strategies for identifying superior peach genotypes resistant to brown rot. *Scientia Horticulturae*, *246*, 1028-1036. DOI: https://doi.org/10.1016/j.scienta.2018.10.027

Oliveira Lino, L., Pacheco, I., Mercier, V., Faoro, F., Bassi, D., Bornard, I., & Quilot-Turion, B. (2016). Brown rot strikes *Prunus* fruit: an ancient fight almost always lost. *Journal of Agricultural and Food Chemistry*, *64*(20), 4029-4047. DOI: https://doi.org/10.1021/acs.jafc.6b00104.

Pacheco, I., Bassi, D., Eduardo, I., Ciacciulli, A., Pirona, R., Rossini, L., & Vecchietti, A. (2014). QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F1 progeny. *Tree Genetics & Genomes*, *10*(5), 1223-1242. DOI: https://doi.org/10.1007/s11295-014-0756-7

Pazolini, K., Santos, I., Citadin, I., Storck, L., & Flores, M. F. (2016). Sampling plan for assessing brown rot severity in peaches subjected to different plant extracts. *Revista Caatinga*, *29*(3), 519-527. DOI: https://doi.org/10.1590/1983-21252016v29n301rc

Rios, J. A., & Debona, D. (2018). Efeito epidemiológico da resistência de hospedeiro. In L. J. Dallagnol (Ed.), *Resistência genética de plantas a patógenos* (p. 126-149). Pelotas, RS: UFPel.

Santos, J., Raseira, M. C. B., & Zanandrea, I. (2012). Resistência à podridão-parda em pessegueiro. *Bragantia*, *71*(2), 219-225. DOI: https://doi.org/10.1590/S0006-87052012005000022

Santos, J., & Ueno, B. (2014). Controle da podridão-parda no Brasil. In M. S. Mitidieri, & J. A. Castillo (Eds.), *Manejo de la podredumbre morena (Monilinia fructicola y M. laxa) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina* (p. 73-77, Repositorio Institucional Biblioteca Digital). Santiago de Chile, CH: INTA DIGITAL..

Scariotto, S., Santos, J., & Raseira, M. C. B. (2015). Search for resistance sources to brown rot in Brazilian peach genotypes. *Acta Horticulturae*, *1084*, 211-216. DOI: https://doi.org/10.17660/ActaHortic.2015.1084.29

Sun, M. H., Gao, L, Liu, X. Z., & Wan, J. L. (2009). Fungal sporulation in two-stage cultivation. *Mycosystema*, *28*(1), 64-72.

Thomidis, T., Michailides, T., & Exadaktylou, E. (2009). Contribution of pathogens to peach fruit rot in northern Greece and their sensitivity to iprodione, carbendazim, thiophanate-methyl and tebuconazole fungicides. *Journal of Phytopathology*, *157*(3), 194-200. DOI: https://doi.org/10.1111/j.1439-0434.2008.01469.x

Walter, M., Mclaren, G. F., Fraser, J. A., Frampton, C. M., Boyd-Wilson, K. S. H., & Perry, J. H. (2004). Methods of screening apricot fruit for resistance to brown rot caused by *Monilinia* spp. *Australasian Plant Pathology*, *33*, 541-547. DOI: https://doi.org/10.1071/AP04062

Zhu, F., Bryson, P. K., & Schnabel, G. (2012). Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. *Pest Management Science*, *68*(7), 1003-1009. DOI: https://doi.org/10.1002/ps.3255