UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Agronomia Eliseu Maciel
Programa de Pós-Graduação em Agronomia
Área de Concentração em Fitomelhoramento

Tese

Resistência à podridão-parda no pessegueiro

Maximiliano Dini

Pelotas, 2019

MAXIMILIANO DINI

Resistência à podridão-parda no pessegueiro

Tese apresentada ao Programa de Pós-Graduação em Agronomia, da Faculdade de Agronomia Eliseu Maciel, da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área do conhecimento: Fitomelhoramento).

Orientadora: Dra. Maria do Carmo Bassols Raseira

Co-orientador: Dr. Bernardo Ueno

Pelotas, 2019

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

D585r Dini, Maximiliano

Resistência à podridão-parda no pessegueiro / Maximiliano Dini ; Maria do Carmo Bassols Raseira, orientadora ; Bernardo Ueno, coorientador. — Pelotas, 2019.

237 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2019.

1. *Prunus persica*. 2. *Monilinia spp.*. 3. Resistência genética. 4. Herdabilidade. I. Raseira, Maria do Carmo Bassols, orient. II. Ueno, Bernardo, coorient. III. Título.

CDD : 634.25

Elaborada por Dafne Silva de Freitas CRB: 10/2175

Maximiliano Dini

Resistência à podridão-parda no pessegueiro

Requisito parcial para obtenção do grau de Doutor em Ciências, Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas.

Data da Defesa: 09/08/2019

Banca Examinadora:

Dra. Maria do Carmo Bassols Raseira (Presidente)

Pesquisadora, Melhoramento em Fruticultura, Embrapa Clima Temperado. Professora colaboradora do PPGA, área de concentração em Fitomelhoramento da Universidade Federal de Pelotas, Pelotas, RS, Brasil.

Dr. Leandro José Dallagnol

Professor, Departamento de Fitossanidade, Fitopatologia – Coordenador do Laboratório de Interação Planta-Patógeno, Universidade Federal de Pelotas, Pelotas, RS, Brasil.

Dr. Gustavo Giménez

Pesquisador, Melhoramento em Hortricultura – Diretor do Programa Nacional de Pesquisa em Produção Hortícola, INIA Las Brujas, Canelones, Uruguai.

Dr. Rodrigo Cezar Franzon

Pesquisador, Melhoramento em Fruticultura e Fruteiras nativas do Sul do Brasil, Embrapa Clima Temperado, Pelotas, RS, Brasil.

Dra. Caroline Marques Castro

Pesquisadora, Recursos genéticos, melhoramento de plantas e biologia molecular, Embrapa Clima Temperado. Professora colaboradora do PPGA, área de concentração em Fitomelhoramento da Universidade Federal de Pelotas, Pelotas, RS, Brasil.

Agradecimentos

À vida por todos os desafios e as pessoas que colocou no meu caminhar, por tudo o que ela me deu, por todas as coisas boas, objetivos e metas cumpridas.

À Universidade Federal de Pelotas pela oportunidade de realizar o doutorado no Programa de Pós-Graduação em Agronomia, Área de Fitomelhoramento.

À CAPES e ao PPGA pelo fornecimento da bolsa de doutorado.

À Embrapa Clima Temperado por possibilitar a realização deste trabalho, pela utilização da infraestrutura e apoio.

Ao INIA Las Brujas por permitir minha ausência para completar meus estudos de Pós-Graduação, especialmente aos incentivadores Jorge Soria e Roberto Zoppolo. ¡Muchas gracias!

À Dra. Maria do Carmo Bassols Raseira, minha orientadora, guia, exemplo a seguir, por me brindar todos seus ensinamentos, tanto técnicos quanto éticos, sem sombra de dúvidas, um exemplo de pessoa, capacidade, humildade e dedicação.

Ao meu co-orientador Bernardo Ueno, agradeço pela sua disponibilidade e dedicação sempre que necessitei.

À l'équipe de l'INRA d'Avignon, en particulier Bénédicte Quilot-Turion, Marie, Marie-Noëlle Corre, Véronique Signoret et Laure Heurtevin. Merci beaucoup !

À minha família, por entenderem minha ausência neste peíodo e pelo incentivo, aos meus pais Cristina Viñoly e Antonio Dini, aos meus irmãos Alexis e Vanessa, e a minha querida sobrinha Lara. ¡Muchas gracias!

A Dani, por ser parte da minha vida e do meu amadurecimento pessoal e sentimental. Obrigado por todo o amor, incentivo e apoio!!!

Aos meus colegas do Laboratório de Melhoramento de Plantas Frutíferas da Embrapa Clima Temperado, por toda a ajuda nos trabalhos e momentos vividos, especialmente a minha parceira na grande parte dos trabalhos Silvia Scariotto, e também muito especialmente a Priscila Marchi, Everton Pederzzoli, Silvia Carpenedo, Rodrigo Franzon, Gilberto Kuhn, Maicon Bönemann, Chaiane Milech, Fernanda Roch, Carolina Silveira e Robson Camargo. E do PPGA da UFPel, pelas experiências compartilhadas, discussões e pelo companheirismo em especial, aos meus irmãos Bruno Carra e Everton Sozo de Abreu. Muito obrigado a todos!!!

A vocês, toda minha gratidão, carinho, respeito e eterna amizade!

Resumo

DINI, Maximiliano. **Resistência à podridão-parda no pessegueiro.** 2019. 237f. Tese (Doutorado) – Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, RS.

A podridão-parda (*Monilinia* spp.) é a principal doença dos frutos de caroço, e incrementar a resistência genética à mesma é um dos principais objetivos dos programas de melhoramento em todo o mundo. No entanto, ainda não se dispõe de cultivares que apresentem elevado grau de resistência genética ao fungo causador da doença e o conhecimento dos mecanismos relacionados à resistência é bastante limitado. O objetivo central deste trabalho foi buscar fontes de resistência à podridão-parda, estudar a sua segregação e herança, assim como contribuir para o entendimento dos mecanismos de resistência envolvidos. Os trabalhos foram desenvolvidos no Brasil, na Embrapa Clima Temperado e na França, no INRA Avignon. No Brasil, foram estudadas 16 progênies F1 (303 *seedlings*) e seus 20 genitores, sendo 10 progênies originadas de cruzamentos recíprocos. Foram estudados os caracteres fenológicos, que mostraram alta herdabilidade, sendo a data de colheita negativamente correlacionada com a incidência da doença. As 10 populações recíprocas foram fenotipadas quanto à sua reação à *M. fructicola* nas flores, sendo a maioria dos genótipos muito suscetível. Foi testada a metodologia de fenotipagem das flores quanto à podridão, sendo recomendado utilizar flores abertas ou em estádio de balão, com uma suspensão de 20-200 conídios de *M. fructicola* por flor, realizando as avaliações 96 horas após inoculação. O conteúdo de compostos fenólicos, antocianinas e atividade antioxidante das pétalas foi correlacionado negativamente com a suscetibilidade à podridão das flores. Quanto à podridão-parda em frutos, foram inoculados com uma suspensão de *M. fructicola* ($2,5x10^4$ conídios mL^{-1}), frutos com e sem ferimentos dos *seedlings* e genitores das 16 populações. Verificou-se variabilidade quanto à suscetibilidade, com herdabilidade de média a baixa, sem evidências de efeito materno. A cv. Bolinha foi utilizada como padrão de resistência e vários dos genótipos testados foram iguais ou melhores que o padrão, sendo que diversos deles produzem frutos de melhor qualidade, demostrando o avanço genético do programa de melhoramento da Embrapa. Os experimentos conduzidos na França concentraram-se em avaliar o efeito dos ferimentos nos frutos e a sua relação com a infecção por podridão-parda (*M. laxa*). Em um primeiro trabalho, frutos feridos de cinco genótipos de nectarineiras foram inoculados com *M. laxa* em diferentes estádios de desenvolvimento, e em dois momentos em relação ao ferimento efetuado (imediatamente ou 7 horas após). No primeiro estádio, coincidente com o endurecimento do caroço, os frutos foram resistentes e foi identificada uma reação vermelha na película, associada a alguns genótipos. Essa reação foi isolada e analisada por HPLC, identificando-se o aparecimento de novos compostos nos frutos. Em outros experimentos, foi estudada a reação de frutos feridos em relação à infecção com *M. laxa*, ao conteúdo de compostos fenólicos e triterpenoides (por HPLC), e compostos voláteis (por cromatografia gasosa). No mesmo sentido, foram realizados trabalhos com *M. laxa* in vitro, e frutos feridos em condições de campo e laboratório. Os frutos feridos apresentaram modificações no padrão de seus compostos e os mesmos parecem ter influência na suscetibilidade à podridão-parda. Os resultados conduzem a recomendações aos programas de melhoramento e direcionamento de novas pesquisas.

Palavras-chave: *Prunus persica*; *Monilinia* spp.; resistência genética; herdabilidade.

Abstract

DINI, Maximiliano. **Resistance to brown rot in peach.** 2019. 237f. Thesis (PhD.) - Graduate Program in Agronomy, Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS.

Brown rot (*Monilinia* spp.) is the main disease of stone fruits and increasing genetic resistance to it is one of the main objectives of breeding programs worldwide. However, peach cultivars with high degree of genetic resistance to the disease-causing fungus are not yet available, and knowledge of resistance-related mechanisms is quite limited. The main objective of this work was to search for brown rot resistance sources, to study its segregation and inheritance, as well as to contribute to the understanding of the resistance mechanisms involved. Some experiments were developed at Embrapa, in Brazil and others, at INRA, in France. In Brazil, 16 F1 progenies (303 seedlings) and their 20 parents were studied being 10 of the progenies originated from reciprocal crosses. Phenological traits were studied showing high heritability, being harvest date negatively correlated with disease incidence. Flowers of 10 reciprocal populations were phenotyped for *M. fructicola* reaction, and most genotypes were very susceptible. The flower phenotyping methodology for rot was tested and based on the results, it was recommended to use open or balloon flowers with a suspension of 20-200 conidia of *M. fructicola*, performing the evaluations 96 hours after inoculation. The content of phenolic compounds, anthocyanins and antioxidant activity of the petals was negatively correlated with the susceptibility to flowerblight. In fruits of seedlings and parents of the 16 populations, brown rot reaction was evaluated through inoculation of wounded and unwouded fruits with a *M. fructicola* suspension ($2{,}5 \times 10^4$ conídios mL^{-1}). There was variability in susceptibility, with heritability medium to low, without evidence of maternal effect.The cv. Bolinha was used as the resistance standard. Several of the tested genotypes were equal or better than the standard, being some of them, producers of better quality fruits, demonstrating the genetic progress of the Embrapa breeding program. The experiments conducted in France focused on the effect of wounds on fruits and their relationship with brown rot infection. In a first study, injured fruits from five nectarine genotypes were inoculated with *M. laxa* at different stages of development and at two moments, in relation to the wound (immediately or 7 hours after it was made). In the first stage, coincident with the pit hardening, the fruits were resistant and a red reaction, associated with some genotypes, was identified in the epidermis. This reaction was isolated and analyzed by HPLC, identifying the appearance of new compounds in the fruits. In other experiments, the reaction of injured fruits in relation to *M. laxa* infection, to the phenolic compounds and triterpenoids content (by HPLC), and to volatile compounds (by gas chromatography) was studied. On the same direction, tests were carried out with *M. laxa* in vitro and fruits injured under field and laboratory conditions. Injured fruits show changes in the pattern of their compounds and they seem to influence the susceptibility to brown rot. The results lead to recommendations for breeding programs and direct new research projects.

Key-words: *Prunus persica*; *Monilinia* spp.; genetic resistance; heritability.

Lista de Figuras

Artigo 1

Figure 1	Full bloom histograms (expressed in days from June 1st). Frequency of seedlings for all progenies and all parents used on the study (A), individual F1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Temperate Agriculture, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil…………………………………………	65
Figure 2	Harvest date histograms (expressed in days from June 1st). Frequency of seedlings for all progenies and all parents used on the study (A), individual F1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Temperate Agriculture, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil………..	66
Figure 3	Fruit development period histograms (expressed in days). Frequency of seedlings for all progenies and all parents used on the study (A), individual F1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Temperate Agriculture, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil…………………………………………	67
Figure 4	Parental and F1 progenies and F1 reciprocal, with their respective median values (parentheses) for the full bloom (A), harvest date (B) (expressed in days from June 1st) and fruit development period (C) (expressed in days). Significance was tested with the Mann-Whitney test in the contrasts between F1 vs. F1 reciprocal, female parental (Pf) vs. male parental (Pm), Pf + Pm vs. F1, Pf vs. F1, Pm vs. F1, Pf + Pm vs. F1 reciprocal, Pf vs. F1 reciprocal, and Pm vs. F1 reciprocal…………………	69

Artigo 2

Figure 1	Severity scale used to evaluate the blossom blight in peach flowers artificially inoculated by spraying……………………………………………	78
Figure 2	Average temperatures and daily rains in July and August of 2015 and 2016, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil…..	79
Figure 3	Blossom blight incidence in peach genotypes submitted or not to *M. fructicola* inoculation, evaluated after 72 and 120 hours. The columns correspond to the average of two harvest seasons (2015 and 2016) and the vertical bars in each column refer to the standard error………………..	82
Figure 4	Severity of blossom blight in F1 progenies and their parents inoculated with *M. fructicola* and evaluated 72 and 120 hours after inoculation, years 2015 and 2016. The severity scale used (0 to 4) is detailed in Table 1 and in Figure 1..…………………………………………………………....	84

Artigo 3

Figure 1	Scale used for the evaluation of infection and severity of blossom blight on artificially inoculated peach flowers with spray…………………………...	94

Figure 2 Means of total phenolic compounds (A) expressed as mg Chlorogenic acid equivalent per 100 g of tissue (mg CAE 100 g^{-1}), total anthocyanins (B) expressed as mg Cyanidin 3-glucoside equivalent per 100 g of tissue (mg C3GE 100g^{-1}), and total antioxidant activity (C) expressed as µg Trolox equivalent per g of tissue (µg TE g^{-1}) of the flower petals of four peach genotypes…….. 99

Artigo 4

Figure 1 Narrow-sense heritability (h^2) estimated by regression midparent and the mean of all the progenies, for lesion diameter (LD) and sporulation diameter (SPD). The slope coefficient is the value of the h^2 estimating. In the cycle 2016-2017... 110

Artigo 5

Figure 1 Brown rot incidence and sporulation presence 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……………………………................................... 127

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)………………….. 130

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……………………………….……………………………………. 131

Artigo 6

Figure 1 Brown rot incidence (BRI) on wounded and non-wounded fruits of parents (A) and progenies (B) average for three and two seasons (2015-16; 2016-17; 2017-18 and 2016-17; 2017-18, respectively). 'Bolinha' was included as control... 139

Figure 2 Relative frequency histograms of 16 progenies classified by lesion area (LA) expressed as percentage of the fruit that was affected by the brown rot lesion, on wounded (W) and non-wounded (NW) fruits. The mean values of female (P_f) and male (P_m) parents for each population are indicated. Embrapa Peach Breeding Program, 2015-2016, 2016-2017 and 2017-2018 growing seasons...…………………………………………… 140

Figure 3 Sporulation presence (SPP) on wounded and non-wounded fruits of parents (A) and progenies (B) average for three and two seasons (2015-16; 2016-17; 2017-18 and 2016-17; 2017-18, respectively). 'Bolinha' was included as control... 142

Figure 4 Relative frequency histograms of 16 progenies classified by sporulation area (SPA) expressed as a percentage of the fruit that was affected by the brown rot sporulation, on wounded (W) and non-wounded (NW) fruits. The mean values of female (Pf) and male (Pm) parents for each population are indicated. Embrapa Peach Breeding Program, 2015-2016, 2016-2017 and 2017-2018 growing seasons……………………………………. 143

Artigo 7

Figure 1 Lesion (A) and sporulation (B) diameter in five nectarine genotypes inoculated with *M. laxa* immediately after wounded (gray line) and inoculated 7 hours after wounded (black line), in three stages of fruit development. date1 = May 28th, 11 to 15 weeks after full bloom (WAFB); date2 = June 20th, 15 to 19 WAFB; date3 = corresponds to the ripening date of each genotype (20 to 26 WAFB)……………………………………….. 163
Figure 2 *Monilinia laxa* infection probability (A), infection delay (B) and progression rate (C) in five nectarine genotypes inoculated with *M. laxa* immediately after wounded (plain lines) or inoculated seven hours after wounded (dashed lines), in three stages of fruit development. First date (05/28), 11 to 15 weeks after full bloom (WAFB); second date (06/20), 15 to 19 WAFB; third its ripening date of each genotype (20 to 26 WAFB)…. 164
Figure 3 Brown rot in 'Zephyr' fruit at three development stages, inoculated with *Monilinia laxa* immediately after wounding and 7 hours after wounding, at three fruit development stages: date1 = May 28th 15 weeks after full bloom (WAFB); date2 = June 20th (19 WAFB); date3 = July 23th, ripening stage (21 WAFB). Photos were taken 240 hours after inoculation……….. 166
Figure 4 HPLC-identified compounds in immature fruit (11 to 15 weeks after full bloom) skin samples from five nectarine genotypes (C207, C116, F115, H165 and 'Zephyr'), with reactionless and red reaction, after wounding and inoculation with *Monilinia laxa*, either immediately and seven hours after…………………………………………………………………………… 169

Figure 5 HPLC spectrum of the two main compounds, Eriodictyol-7-glucoside (A) and Naringenin-7-glucoside syn.: Prunin (B), present in the red reaction area, detected in wounded immature fruits (11 to 15 weeks after full bloom) infected immediately after wounding…………………………………….. 170

Artigo 8

Figure 1 Lesion and sporulation diameter in 'Zephyr' and C216 nectarine, inoculated with *M. laxa* 130 days after full bloom, immediately and seven hours after wounded………………………………………………………………. 188
Figure 2 Boxplot of the HPLC-DAD peaks area of compounds identified in fruits of 'Zephyr' (June 19, 2018) and C216 nectarine (July 03, 2018), subjected to wounded (W) or non-wounded (NW). Abbreviations of compounds in Table 1…………………………………………………………………………… 189
Figure 3 Results from PCA analysis, projection of volatile compounds detected by GC-MS in fruits of 'Zephyr' (June 18, 2018) and C216 nectarine (July 02, 2018), subjected to wounded or non-wounded…………………………… 196

Artigo 9

Figure 1	Brown rot lesion growth in 'Zephyr' immature fruits wounded and immediately inoculated with *Monilinia laxa*, which were submitted to three previous conditions: unwounded; wounded on one side in the laboratory; wounded on one side when still on the tree………………………..………..	217
Figure 2	*Monilinia laxa* sporulation growth in 'Zephyr' immature fruits wounded and immediately inoculated, which were submitted to three previous conditions: unwounded; wounded on one side in the laboratory; wounded on one side when still on the tree…………….………………………………	218
Figure 3	Boxplots of the delay, progression rate and diameter at 240 hours after inoculation (hai) of *Monilinia laxa* lesion and sporulation, inoculated immediately after wounded in 'Zephyr' immature fruits, in three conditions: unwounded previously (red); previously wounded in the laboratory on one side of the fruit (green); previously wounded in the tree on one side of the fruit (blue). ^{ns} not significantly significantly by F-test at $p \leq 0.01$………………………………………………………………..	219
Figure 4	Reaction red detected in immature fruits wounded and immediately inoculated with *Monilinia laxa*. A: Red reaction is highlighted in the inoculated wound on the left side of the fruit, on the right side of the fruit multiple wounds without inoculation reactionless. B: Infection above red reaction in the inoculated wound on the left side of the fruit, on the right side of the fruit multiple wounds without inoculation reactionless. C: Red reaction in the inoculated wound on the left side of the fruit, on the right side of the fruit unwounded and reactionless………………………………..	220
Figure 5	*Monilinia laxa* inoculated in open Petri dishes with PDA, placed inside a sealed plastic box in three conditions: without fruits, with unwounded fruits and with wounded fruits. Means of the diameter of the colony of *M. laxa* 168 hours after inoculation followed by different letters are significantly different by the Tukey's test at $p \leq 0.01$………………………..	221
Figure 6	*Monilinia laxa* lesion and sporulation growth in 'Zephyr' immature fruits wounded and immediately inoculated with *M. laxa*, in two conditions: previously with wounded fruits in a closed box for 7 hours, and without the presence of wounded fruits ………………………………………………	222
Figure 7	Boxplots of the delay, rate and diameter at 240 hours after inoculation (hai) of *Monilinia laxa* lesion and sporulation, inoculated immediately after wounded in 'Zephyr' immature fruits, in three conditions: with wounded fruits in a closed box for 7 hours (red), and without the presence of wounded fruits ns, ** and ***, not significantly ($p > 0.01$), significantly at $p \leq 0.01$ and $p \leq 0.001$ by F-test. ………………………..….	223

Lista de Tabelas

Artigo 1

Table 1 Parents of F1 progenies and number of seedlings of each progeny, in the Peach Breeding Program at Embrapa Temperate Agriculture, Pelotas, Rio Grande do Sul, Brazil.. 64

Table 2 Broad-sense (H^2) and narrow-sense (h^2) estimated heritability for full bloom (FB), harvest date (HD) and fruit development period (FDP) in peach progenies of the Peach Breeding Program at Embrapa Temperate Agriculture, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.. 68

Table 3 Spearman´s correlation between phenological characters (full bloom, harvest date and fruit development period) and percentage of brown rot incidence in peach progenies orchard of the Peach Breeding Program at Embrapa Temperate Agriculture, in 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.................... 70

Artigo 2

Table 1 Grading scale for assessing the severity of the blossom blight in peach.. 77

Table 2 Descriptive statistics of the variables of incidence and severity of blossom blight in 10 reciprocal peach progenies and their parents, evaluated after 72 and 120 hours, with and without artificial inoculation, in the years 2015 and 2016, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.. 81

Artigo 3

Table 1 ANOVA summary: Effects of conidial concentration of *Monilinia fructicola* (plot), phenological flower stage (subplot), genotype (sub-subplot), doubles interactions and triple interaction for incidence and severity of blossom blight in peach, evaluated at 72, 96 and 120 hours after inoculation (hai).. 95

Table 2 Incidence and severity according to conidial concentration of *Monilinia fructicola* (CC) and genotype, evaluated at 72, 96 and 120 hours after inoculation (hai).. 96

Table 3 Incidence and severity according to conidial concentration of *Monilinia fructicola* (CC) and phenological flower stage (PFS), evaluated at 72, 96 and 120 hours after inoculation (hai)... 97

Table 4 Incidence and severity according to suspension genotype and phenological flower stage (PFS), evaluated at 72, 96 and 120 hours after inoculation (hai).. 98

Table 5 Spearman´s correlation between phenolic compounds (PC), anthocyanins (Ant), antioxidant activity (AA), incidence (Inc) and severity (Sev) of blossom blight.. 100

Artigo 4

Table 1	Progeny F1, parents and number of seedlings of the Embrapa peach breeding program, Pelotas, Rio Grande do Sul, Brazil……………………..	106
Table 2	Descriptive statistics of incidence and severity of brown rot in 16 peach progenies and 20 parental genotypes evaluated in the 2015-2016 and 2016-2017 cycles, in Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil…………………………………………………………………….	108
Table 3	Averages of incidence and severity of lesion and sporulation of brown rot in peach, evaluated for 16 progenies and 20 parents, for two years at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil………….	109

Artigo 5

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, Brazil……………………………………………………………………………...	128
Table 3	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, Brazil……………………………………………………	129
Table 3	Spearman's correlation between sporulation variables in 2017-2018 season. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil..	130

Artigo 6

Table 1	Progeny identification, parents and number of seedlings of each progeny, in the Peach Breeding Program at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil……………………………………………………	136
Table 2	Broad-sense (H^2) and narrow-sense (h^2) estimated heritability for incidence and severity of *M. fructicola* on wounded and non-wounded peach fruits, Embrapa Peach Breeding Program, Pelotas, Rio Grande do Sul, Brazil……………………………………………………………………...	144
Table 3	Genetic advancement by selection of genotypes better than or equal to 'Bolinha'………………………………………………………………………..	145

Artigo 7

Table 1	ANOVA summary: Effects of genotype (Gen), inoculation time (InocTime), development stage (DevStage) and interactions for *Monilinia laxa* lesion and sporulation INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France…………………………………….	165
Table 2	Compounds identified in HPLC, wavelength (λ), retention time (RT), lambda maximum (λ max), abbreviation used and analysis of variance using the peaks area and contrast between red reaction and reactionless, in immature wounded fruits (11 to 15 weeks after full bloom). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France…………………………………………………………...…………..	168

Artigo 8

Table 1	Compounds identified by HPLC analysis, compounds family, wavelength (λ), lambda maximum (λ max), retention time (RT) and abbreviation used, 2018 season. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France…………………………………………………	190
Table 2	ANOVA summary of the compounds identified by HPLC-DAD, using the peaks area in C216 and 'Zephyr' (Factor: Genotype) in immature fruits subjected to wounded or non-wounded (Factor: Treatment) and samples collected at 1 and 7 hours (Factor: Hour), 2018 season. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon. France…….	191
Table 3	Volatile compounds, abbreviation and retention time, identified by GC-MS in immature fruits of 'Zephyr' (June 18, 2018) and C216 nectarine (July 02, 2018), subjected to wounded or non-wounded and samples collected at 7 hours. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France…………………………………………………	192
Table 4	ANOVA summary of the volatile compounds identified by GC-MS in immature fruits of C216 and 'Zephyr' (Factor: Genotype), subjected to wounded or non-wounded (Factor: Treatment), year 2018. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France…..	194
Table 5	Eigenvectors and correlations of the principal components (PC) with the original variables (volatile compounds detected by GC-MS analysis). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France……………………………………………………………...	195

Sumário

1 Introdução geral 15

2 Projeto de Pesquisa 19

2.1 Título 19

2.2 Introdução 19

2.3 Problema 21

2.4 Hipóteses 22

2.5 Objetivos 23

2.5.1 Objetivo Geral 23

2.4.2 Objetivos Específicos 23

2.5 Material e métodos 23

2.5.1 Material 23

2.5.2 Metodologia 26

2.5.3 Resultados esperados 37

2.6 Cronograma 39

2.7 Recursos necessários 41

2.7.1 Material de consumo 41

2.7.2 Outras despesas 41

2.7.3 Orçamento total 41

2.8 Referências bibliográficas 42

3 Relatório do trabalho de campo 46

4 Artigos desenvolvidos 50

4.1 Artigo 1. Peach phenological characters: heritability, maternal effect and correlation with brown rot 51

4.2 Artigo 2. Blossom blight resistance in peach: heritability and progeny segregation 71

4.3 Artigo 3. Blossom blight resistance in peach: phenotyping and antioxidants content in petals 91

4.4 Artigo 4. Heritability and segregation of resistance to brown rot in peach fruits 104

4.6 Artigo 5. *Monilinia fructicola* lesion and sporulation in peach and nectarine fruits 113

4.5 Artigo 6. Breeding peaches for brown rot resistance in Embrapa 132

4.7 Artigo 7. Fruit reaction to brown rot at different development stages and inoculation time after wounded 155

4.8 Artigo 8. Reaction of wounded nectarine fruits: brown rot infection, phenolic and volatile compounds 181

4.9 Artigo 9. Influence of wounded nectarine fruit on *Monilinia laxa* infection....213

5 Considerações finais 226

6 Referências (Introdução geral)....230

Apêndices....233

1 Introdução geral

A fruticultura de clima temperado apresenta grande importância no contexto mundial de frutas. No Brasil, ela vem se expandindo tanto em área cultivada quanto em produtividade, tendo em vista o grande potencial de mercado. Como atividade econômica, envolve mais de cinco milhões de pessoas que trabalham de forma direta ou indireta no setor (FACHINELLO et al., 2011).

O pessegueiro [*Prunus persica* (L.) Batsch] é a terceira mais importante espécie de clima temperado, depois da macieira e pereira. A sua produção mundial atingiu mais de 24,7 milhões de toneladas em 2017, sendo a China o maior produtor com 58% da produção total. Nesse mesmo ano, o Brasil figurou no 12° lugar, com uma produção de 248.583 toneladas e uma área cultivada de 17.118 ha (FAO, 2019). O valor da produção de pêssegos no Brasil atingiu 414,5 milhões de reais, no ano 2017 (IBGE, 2019).

No Brasil, o pêssego e a nectarina são produzidos principalmente nos estados do Sul e em parte dos estados do Sudeste, onde as condições edafoclimáticas favorecem a exploração comercial. Foi no Rio Grande do Sul que, em razão das condições climáticas, da proximidade às indústrias de conserva, e do programa de melhoramento genético para cultivares adaptadas, o cultivo do pessegueiro mais cresceu (FRANZON; RASEIRA, 2014). Assim, o Rio Grande do Sul é o principal produtor, com cerca de 69% da produção nacional, ocupando mais de 12,5 mil hectares, e atingindo um valor da produção de 212,8 milhões de reais para o ano 2017. O município de Pelotas (RS) destaca-se como maior produtor nacional de pêssegos, sendo responsável por mais de 19% da produção total do país, em 2017 (IBGE, 2019).

O crescimento da cultura do pessegueiro, deve-se, em parte, ao melhoramento genético. No Brasil, os programas de melhoramento começaram no início da década de 1950, no Instituto Agronômico de Campinas, em São Paulo. Alguns anos depois, um programa similar começou no estado do Rio Grande do Sul. Este último foi coordenado inicialmente pela Secretaria de Agricultura do Rio Grande do Sul. No entanto, no final da década de 50´, o programa foi transferido para Pelotas (RS) e coordenado por uma instituição federal. Com o advento da Embrapa em 1973, o programa não só foi preservado, mas incrementado (RASEIRA et al., 2008). O grande número de cultivares lançados por este e outros institutos de pesquisa, principalmente públicos, garante uma produção qualitativa, além de oferecer opções para o cultivo em regiões de clima temperado e subtropicais (RASEIRA; CENTELLAS-QUEZADA, 2003; CITADIN et al., 2014).

A podridão-parda é a doença economicamente mais importante desta cultura. Pode ser causada por três espécies do gênero *Monilinia*: *M. laxa* (Aderh. & Ruhl.) Honey, é predominante na Europa, China, África do Sul, Chile, América do Norte e Oriente Médio; *M. fructicola* (Wint.) Honey, é importante na América do Sul e América do Norte, e mais recentemente presente na Europa e na Ásia; enquanto *M. fructigena* (Aderh. & Ruhl.) Honey, presente na Europa e na China, é a de menor distribuição e importância (OGAWA et al., 1995; ADASKAVEG et al., 2008; HU et al., 2011). O período da incidência da doença se estende desde a floração até a pós-colheita, e os principais sintomas são a podridão das flores, cancro dos ramos e podridões nos frutos imaturos e maduros, sendo esta última a fase fenológica mais suscetível (OGAWA et al., 1995; ADASKAVEG et al., 2008; MAY-DE-MIO et al., 2008, 2014).

Sob condições climáticas amenas, úmidas e chuvosas, esta doença pode causar perda total da cultura e, na tentativa de reduzir essas perdas, os produtores necessitam aplicar fungicidas até mesmo semanalmente, e intensificar as aplicações na floração e na pré-colheita (MONDINO et al., 2010; OGAWA et al, 1995). Atualmente, a crescente preocupação com o meio ambiente e a saúde de produtores e consumidores (BARÓ-MONTEL et al., 2019; ELSHAFIE et al., 2015), assim como a ocorrência de isolados de fungos resistentes às principais moléculas fungicidas utilizadas (LUO et al., 2010; HILY et al., 2011; ZHU et al., 2012; CHEN et al., 2017) enfatizaram a importância de outras estratégias de controle, como a resistência genética, para reduzir as aplicações de agroquímicos. Esta é a maneira mais eficiente de controlar a doença, reduzindo os custos de produção e o impacto ambiental.

A seleção de genótipos resistentes ainda é muito limitada devido ao desconhecimento de boas fontes de resistência ou imunidade (RASEIRA; FRANZON, 2014). A resistência do pessegueiro à *Monilinia* é uma característica quantitativa (poligênica), considerada como um caráter de difícil transmissão dos genitores para as progênies e altamente influenciada pelo ambiente (WAGNER JÚNIOR et al. 2003, 2005; RASEIRA; FRANZON, 2014). No entanto, existem diferenças significativas na suscetibilidade entre os genótipos disponíveis (ADASKAVEG et al. 2008; SANTOS; UENO, 2014).

Há evidências de que não há correlação entre a resistência na flor e nos frutos (FABIANE, 2011; SANTOS et al., 2012; WAGNER JUNIOR et al., 2003, 2005), portanto, a seleção de genótipos resistentes deve ser feita de forma independente (RASEIRA; FRANZON, 2014; WAGNER JUNIOR et al., 2003, 2005).

As plantas reagem ao estresse ativando vários mecanismos diferentes, dependendo do estádio de desenvolvimento, intensidade e duração do estresse e do tipo de órgão/tecido (TOSETTI et al., 2013). Dentre os estresses abióticos, encontram-se os danos mecânicos, como por exemplo, os ferimentos físicos. Estes podem ocorrer no campo e/ou após a colheita, por multiplos fatores. Respostas fisiológicas, bioquímicas e moleculares ao ferimento em plantas resultam em alterações metabólicas que visam diferentes finalidades, entre elas podem ser citadas: cicatrizar e recuperar o tecido ferido, colocar barreiras mecânicas a organismos invasores patógenos ou oportunistas e, ativar mecanismos de defesa contra organismos invasores (ZHOU; THORNBURG, 1999; BRUXELLES; ROBERTS, 2001; CHEONG et al., 2002; SHANKER; VENKATESWARLU, 2011). Essas mudanças envolvem a modulação seletiva da expressão gênica, sendo que vários genes relacionados à ferida já foram identificados e sua expressão estudada (MITSUDA et al., 2007; KOO; HOWEA, 2009; TRINIDADE et al., 2011; TSABALLA et al., 2015), também em relação a hormônios como etileno, ácido abscísico e ácido jasmônico (BIRKENMEIER; RYAN, 1998; LEÓN et al., 2001; BROEKAERT et al. 2006; KOO; HOWEA, 2009). Vários dos genes identificados codificam moléculas de sinalização, proteínas regulatórias (fatores de transcrição), modulando a expressão de outros genes de resposta. Os genes de resposta, modulados pelos fatores de transcrição, codificam principalmente para proteínas efetoras, incluindo aquelas que melhoram a resistência ou a recuperação de células de estresse ou dano, como proteínas de choque térmico, enzimas modificadoras da parede celular, metabólitos secundários e proteínas relacionadas à patogênese (CHEONG et al., 2002).

Em comparação com outros órgãos/tecidos da planta, pouca informação está disponível sobre os mecanismos moleculares de frutos e como eles reagem aos ferimentos. No amadurecimento da fruta, as feridas causam um aumento na taxa de respiração e na produção de etileno, e geralmente levam ao amolecimento da polpa, ruptura da membrana, escurecimento, senescência acelerada, perda de peso e água e desenvolvimento de doenças microbianas (TOIVONEN; BRUMMELL, 2008). No caso de frutos de pessegueiro, já foram estudadas as respostas moleculares e bioquímicas ao ferimento no mesocarpo de frutos de pessegueiro, em produtos minimamente processados (TOSETTI et al., 2013), porém nunca foram relacionadas com a resistência à infecção com *Monilinia*.

Os mecanismos envolvidos na resistência, assim como o conhecimento dos parâmetros genéticos, fenotípicos e ambientais que influenciam direta ou indiretamente os mesmos, são de fundamental importância para o delineamento dos programas de melhoramento do pessegueiro e frutas de caroço, em geral, visando a obtenção de cultivares geneticamente superiores quanto à resistência à podridão-parda.

Diante do exposto, este trabalho apresentou como objetivo principal: buscar fontes de resistência à podridão-parda e estudar a sua segregação e herança, assim como contribuir para o entendimento dos mecanismos de resistência envolvidos.

2 Projeto de Pesquisa

2.1 Título

Resistência da flor e do fruto à podridão-parda em genótipos de *Prunus persica.*

2.2 Introdução

A fruticultura é uma atividade econômica com amplo desenvolvimento e em constante crescimento no mundo. No ano de 2013, foram produzidas mais de 37 milhões de toneladas de frutas, sendo o Brasil o terceiro maior produtor mundial, atrás apenas da China e da Índia (FAOSTAT, 2016). O Brasil apresenta regiões com diferentes condições edafoclimáticas, o que permite o cultivo de diversas espécies frutíferas, as quais representam uma atividade geradora de empregos e renda (PICOLOTTO, 2009).

A fruticultura de clima temperado apresenta grande importância no contexto mundial de frutas. No Brasil, ela vem se expandindo tanto em área cultivada quanto em produtividade, tendo em vista o grande potencial de mercado (RASEIRA et al., 2014). Como atividade econômica envolve mais de cinco milhões de pessoas que trabalham de forma direta ou indireta no setor (FACHINELLO et al., 2011).

A cultura do pessegueiro (*Prunus persica* (L.) Batsch) vem crescendo em todo o mundo, pelo aumento no consumo de frutos in natura e produtos industrializados (SANTOS et al., 2012). O grande número de cultivares garante uma produção qualitativa, além de adaptar-se para o cultivo em regiões de clima temperado e subtropicais (RASEIRA e CENTELLAS-QUEZADA, 2003).

A produção mundial de pêssegos atingiu mais de 21 milhões de toneladas em 2013, sendo a China o maior produtor com mais do 55% da produção total. O Brasil figurava em 13° lugar com uma produção de 217.706 toneladas e uma área cultivada de 18.091 ha. No Mercosul, destacam-se Chile e Argentina como os maiores produtores seguidos pelo Brasil (FAOSTAT, 2016). O valor da produção de pêssegos no Brasil atingiu 312.059 mil reais para o ano 2013 (IBGE, 2015).

Nesse país, o pêssego e a nectarina são produzidos principalmente nos estados do Sul e em parte dos estados do Sudeste, onde as condições edafoclimáticas favorecem a exploração comercial. Foi no Rio Grande do Sul que, em razão das condições climáticas, proximidade às indústrias de conserva, e ao melhoramento genético de cultivares adaptados, o cultivo do pessegueiro mais cresceu (FRANZON e RASEIRA, 2014). Assim, Rio Grande do Sul é o principal produtor, com cerca de 63% da produção nacional, ocupando mais de 13 mil hectares, e atingindo um valor da produção de 141.144 mil reais para o ano 2013. Na região Sul, o município de Pelotas/RS, destaca-se como maior produtor nacional de pêssegos, sendo responsável por mais de 13% da produção total do país (IBGE, 2015).

O crescimento da cultura do pessegueiro no Brasil, em parte, deve-se ao melhoramento genético. Os programas de melhoramento genético do pessegueiro no Brasil datam de pouco antes de 1950 (RASEIRA e FRANZON, 2014), com muitas cultivares próprias, liberadas (mais de 100) e algumas introduzidas de outros países e avaliadas pelos institutos de pesquisa do país. No Rio Grande do Sul, em 1953 foi iniciado o programa de melhoramento genético nesta cultura, atualmente centrado na atual Embrapa Clima Temperado (RASEIRA et al., 2014).

Entre as principais doenças da cultura, está a podridão-parda causadora de danos durante todo o ciclo. Esta doença pode ser causada por três espécies do gênero *Monilinia*: *M. laxa* (Aderh. & Ruhl.) Honey de importância na Europa, África do Sul, Chile e Iraque; *M. fructigena* (Aderh. & Ruhl.) Honey presente na Europa, mas de menor distribuição e importância; *M. fructicola* (Wint.) Honey considerada a espécie causadora de podridão-parda mais importante na cultura do pessegueiro no Brasil e em grande parte do mundo (OGAWA et al., 1995; AGRIOS, 1998; ADASKAVEG et al., 2008).

As perdas econômicas causadas por esta doença incluem as perdas pela podridão da fruta, além da redução do rendimento pelo ataque às flores e ramos da brotação à colheita, assim como pelo aumento dos custos de seu controle (OGAWA et al, 1995; MONDINO et al., 2010). Neste contexto é crescente a busca pela utilização de resistência genética, como método de controle da doença, reduzindo a dependência do uso de fungicidas no seu manejo. Esta é um dos objetivos prioritários dos programas de melhoramento do pessegueiro, no mundo.

Neste sentido, o melhoramento genético visando a obtenção de novas cultivares que atendam a qualidade dos frutos, produção, adaptação climática, e apresentem bom comportamento produtivo frente às principais doenças é de muita importância para a expansão da cultura.

2.3 Problema

O conhecimento dos parâmetros genéticos, fenotípicos e ambientais que influenciam direta ou indiretamente nos caracteres de importância econômica em pessegueiro, são de fundamental importância para o delineamento dos programas de melhoramento desta frutífera, pois permitem antever a possibilidade de sucesso com a seleção de diferentes genótipos em diferentes ambientes.

Não se encontra referência à existência de efeitos maternos em caracteres de importância econômica no pessegueiro, e também não estão totalmente esclarecidos os valores da herdabilidade de vários destes caracteres nas populações que se trabalha no Brasil.

Dentre os caracteres agronômicos do pessegueiro, de importância econômica está o ciclo, período de tempo entre a plena floração e o início da maturação. A importância do ciclo está na possibilidade de serem desenvolvidas cultivares de ciclo curto com florações tardias, escapando às geadas e, ao mesmo tempo com maturação precoce, o que traz benefícios para o produtor como a comercialização antecipada com maior valor comercial e custos de produção mais baixos pelo curto período de tempo que a fruta está na árvore, além de menores riscos.

Outros caracteres de muita importância no pessegueiro são os referentes a resistência a doenças. A podridão-parda é a principal doença do pessegueiro no Brasil e no mundo, causando severas perdas aos fruticultores. Os principais sintomas dessa doença são a podridão das flores, cancros nos ramos e podridões nos frutos, estendendo os prejuízos econômicos desde a floração até pós-colheita dos frutos. Normalmente o controle dessa doença depende de várias aplicações de fungicidas intensificadas na floração e na pré-colheita. Nestas últimas décadas, o incremento na preocupação com a saúde dos consumidores, devido a possíveis resíduos de agrotóxicos nos frutos e o aumento na severidade da legislação fitossanitária enfatizam outras estratégias de controle, como a resistência genética. Esta é a forma mais eficiente e a melhor alternativa de controle da doença, além de reduzir o custo de produção e o impacto ambiental, quer seja pela redução no número de aplicação de fungicidas ou pela eliminação de seu uso. A seleção de genótipos resistentes é ainda limitada, apesar de estar em muitos programas de melhoramento genético do pessegueiro no mundo, pela escassez de boas fontes de resistência.

O desenvolvimento de novas cultivares de pêssego é um processo que envolve a geração de grandes populações de indivíduos, a partir das quais os melhores genótipos são selecionados, sendo um processo de elevado custo financeiro e de tempo. A grande maioria das características de interesse são relacionadas ao fruto, portanto, é necessário cultivar todos os indivíduos originários dos cruzamentos até que produzam pelo menos duas ou três safras, para sua correta avaliação e seleção. Se estiveram disponíveis marcadores moleculares, indicadores da presença da característica de interesse, eles podem ser utilizados para a seleção antecipada de genótipos, acelerando e melhorando a eficiência do processo de melhoramento genético. Porém, a utilização de seleção assistida por marcadores moleculares nos programas de melhoramento de pessegueiro, ainda é incipiente, e não existem marcadores moleculares validados para a característica de resistência do fruto à principal doença do pêssego, a podridão-parda.

Diante do exposto, este trabalho visa: estimar a herdabilidade de diferentes caracteres que se busca incorporar nas novas progênies, dando uma ideia do progresso que se poderá esperar, e da quantidade de gerações necessárias para atingir determinado objetivo; verificar se existe ou não efeitos maternos para estes caracteres; buscar novas fontes de resistência para podridão-parda em flores e/ou frutos; e por último, tentar validar potencias marcadores moleculares relacionados à resistência à podridão-parda nos frutos.

2.4 Hipóteses

O caráter ciclo no pessegueiro tem alta herdabilidade, e selecionando-se os genitores pelo fenótipo possibilita rápido ganho genético para este caráter.

Existem diferenças entre genótipos de pessegueiro quanto à resistência à podridão-parda na flor e no fruto, e está característica tem média herdabilidade permitindo bom progresso através da seleção dos genitores pelo fenótipo.

É possível que haja efeito materno no pessegueiro, para características como ciclo e resistência à podridão-parda em flores e/ou frutos. Desta maneira a distribuição dos indivíduos de populações derivadas de um cruzamento serão mais semelhantes ao fenótipo do genitor feminino e o ganho genético para esse caráter é maior quanto mais próximo seja o genitor feminino ao objetivo que se deseja atingir.

Existem marcadores moleculares ligados à resistência à podridão-parda nos frutos, podendo-se utilizar os mesmos em processo de seleção assistida por marcadores, acelerando e aumentando a eficiência de um programa de melhoramento genético de pessegueiro que tenha esta característica como alvo.

2.5 Objetivos

2.5.1 Objetivo Geral

O presente trabalho tem como objetivo central buscar fontes de resistência a podridão-parda, e estimar a herdabilidade e verificar possível existência de efeito materno em alguns caracteres de importância econômica, em pessegueiro (*Prunus persica* (L.) Batsch).

2.4.2 Objetivos Específicos

a) Estimar a herdabilidade do caráter ciclo no pessegueiro, no sentido amplo e restrito;
b) Testar a reação de diversos genótipos de pessegueiro à podridão-parda em flores e frutos, buscando encontrar novas fontes de resistência à doença;
c) Verificar a possível existência de efeito materno no caráter ciclo e resistência à podridão-parda em flores e/ou frutos;
d) Realizar a fenotipagem quanto a incidência e severidade de ataque da podridão-parda em frutos nas populações segregantes e nos respectivos genitores;
e) Validar ou rejeitar potenciais marcadores moleculares SNPs ligados à resistência à podridão-parda em frutos.

2.5 Material e métodos

2.5.1 Material

O experimento será conduzido nas safras 2016-2017 e 2017-2018, aproveitando também os dados obtidos da safra 2015-2016, na área experimental da Embrapa Clima Temperado, Pelotas/RS, localizada na zona Sul do Rio Grande do Sul, sob latitude de 31° 46’ S, longitude 52° 20’ W e altitude de 57 metros. O clima de Pelotas é classificado como subtropical úmido, sofrendo influência marítima pela proximidade do oceano Atlântico, a qual se manifesta na elevada umidade atmosférica com uma umidade relativa média anual de 80,7% e verões e invernos com temperaturas amenas, onde a temperatura média anual é de 17,8°C. A precipitação média anual é de 1367 mm, com chuvas regularmente distribuídas durante todo o ano (UFPEL, 2015). Na sede da Embrapa Clima Temperado (Monte Bonito) a média histórica desde o ano 1984-2010 do acumulado de Horas de Frio (≤7,2°C) é de 342 HF (EMBRAPA, 2015).

Serão avaliadas progênies originárias de primeira geração (F_1) de hibridações dirigidas, realizadas nos anos 2008, 2009 e 2012, provenientes de cruzamentos recíprocos entre os genitores. Serão avaliados também os genitores dessas progênies, dos quais se têm disponíveis três plantas por cultivar ou seleção. Essas plantas foram obtidas por enxertia (clones). Os genitores utilizados serão as cultivares ´Chimarrita`, ´Maciel` e ´Cerrito`, e as seleções Cascata 1055, Conserva 672, Conserva 947, Conserva 1526, Conserva 1600 e Conserva 1662. As dez progênies F_1 e seus genitores, bem como a quantidade de indivíduos disponíveis por progênie, encontram-se na Tabela 1.

Tabela 1 – Progênies F_1, genitores e número de plantas disponíveis dos cruzamentos recíprocos.

Cruzamento recíproco	**Progênie F_1**	**Genitores** ♀ ♂	**N° plantas**
1	2008.159*	Conserva 1526 × Cerrito	7
	2009.38	Cerrito × Conserva 1526	23
2	2012.26	Cascata 1055 × Chimarrita	18
	2012.43	Chimarrita × Cascata 1055	25
3	2012.49	Conserva 672 × Conserva 1526	18
	2012.61	Conserva 1526 × Conserva 672	7
4	2012.52	Conserva 947 × Conserva 1600	17
	2012.66	Conserva 1600 × Conserva 947	12
5	2012.68	Conserva 1662 × Maciel	24
	2012.88	Maciel × Conserva1662	17

(*) Identificação interna da Embrapa.

As cultivares e as seleções estão plantadas no campo experimental da Embrapa Clima Temperado (Sede), em espaçamento de 2 a 3 m entre plantas e de 5 a 6 m entre linhas, dependendo do pomar. Os *seedlings* estão plantados a campo, em espaçamento de 0,50 m entre plantas e 5 m entre linhas.

Com a finalidade de estimar a herdabilidade no sentido restrito (h^2), se incluirão mais progênies F_1 e seus genitores, para aumentar a precisão da estimativa, os mesmos descrevem-se na Tabela 2.

Tabela 2 – Progênie F_1, genitores e número de plantas dos cruzamentos adicionais.

Progênie F_1	**Genitores** ♀ ♂	**N° plantas**
2012.31*	Cascata 1359 × Cascata 1577	19
2012.46	Chorão × Maciel	25
2012.99	Necta 506 x Sunmist	20
2012.107	Necta 532 × Necta 480	25
2012.111	Necta 540 × Morena	25
2012.114	BRS Rubimel × TX2D163	21

(*) Identificação interna da Embrapa.

Na Tabela 3, são apresentadas as principais características das cultivares ou seleções utilizadas como genitores nos cruzamentos que serão utilizados neste estudo, assim como os principais objetivos da hibridação.

Tabela 3 – Principais características dos genitores das progênies F_1, e objetivos da hibridação.

Progênie F_1	Genitores e suas principais características		Principais objetivos da hibridação
	♀	♂	
2008.159	**Conserva 1526** (Pêssego conserva polpa amarela, resistência (a campo) à podridão-parda dos frutos)	**'Cerrito'** (Pêssego conserva polpa amarela, adaptação geral e baixa necessidade em frio)	Resistência à podridão-parda dos frutos e baixa necessidade em frio
2009.38	**'Cerrito'**	**Conserva 1526**	
2012.26	**Cascata 1055** (Pêssego de mesa polpa branca, boa firmeza, resistência à podridão-parda das flores)	**'Chimarrita'** (Pêssego de mesa polpa branca, bom tamanho, forma e baixa acidez)	Produtividade, resistência a podridão-parda das flores, firmeza da polpa e baixa acidez
2012.43	**'Chimarrita'**	**Cascata 1055**	
2012.49	**Conserva 672** (Pêssego conserva polpa amarela, bom tamanho, sabor e cor da polpa)	**Conserva 1526** (Pêssego conserva polpa amarela, resistência (a campo) à podridão-parda dos frutos)	Resistência à podridão-parda dos frutos e maturação de média estação a tardia
2012.61	**Conserva 1526**	**Conserva 672**	
2012.52	**Conserva 947** (Pêssego conserva polpa amarela, resistência à podridão-parda dos frutos; maturação tardia)	**Conserva 1600** (Pêssego conserva polpa amarela, resistência à podridão-parda dos frutos)	Resistência à podridão-parda dos frutos e época de maturação tardia.
2012.66	**Conserva 1600**	**Conserva 947**	
2012.68	**Conserva 1662** (Pêssego conserva polpa amarela, boa cor de polpa)	**'Maciel'** (Pêssego dupla finalidade polpa amarela, boa qualidade de frutos)	Precocidade ou média estação e boa qualidade de frutos
2012.88	**'Maciel'**	**Conserva1662**	
2012.31	**Cascata 1359** (Pêssego de mesa polpa amarela, baixa acidez, floração tardia, altos sólidos solúveis e boa firmeza)	**Cascata 1577** (Pêssego de mesa polpa branca, cor da película, baixa acidez e floração tardia)	Floração tardia, firmeza dos frutos e baixa ou média acidez
2012.46	**Chorão** (Pêssego de mesa polpa amarela, habito crescimento tipo "chorão")	**'Maciel'** (Pêssego dupla finalidade polpa amarela, boa qualidade de frutos)	Hábito de crescimento e melhor qualidade de frutas
2012.99	**Necta 506** (Nectarina polpa amarela, boa aparência externa)	**'Sunmist'** (Nectarina polpa branca, boa porcentagem de cor vermelho na película, tamanho de frutas)	Tamanho de frutas e cor da película e polpa branca
2012.107	**Necta 532** (Nectarina polpa amarela, baixa acidez, doce e caroço solto)	**Necta 480** (Nectarina polpa amarela, não fundente, coloração de fundo da película alaranjada)	Firmeza, sabor e cor da pelicula
2012.111	**Necta 540** (Nectarina polpa amarela, baixa acidez e caraço solto)	**'Morena'** (Nectarina polpa amarela, forma dos frutos e boa porcentagem de cor vermelho na película)	Forma, cor da película e adaptação
2012.114	**'BRS Rubimel'** (Pêssego polpa amarela, qualidade de frutos, baixa acidez e firmeza da polpa)	**TX2D163** (Pêssego de mesa polpa amarela, boa porcentagem de cor vermelho na película)	Firmeza da polpa, baixa acidez e cor da película

As práticas culturais (poda, raleio, adubações, aplicações de agrotóxicos, entre outras) nos pomares em que estão as árvores a serem utilizadas no trabalho, serão feitas na forma usual recomendada pela Embrapa Clima Temperado, e efetuadas por seus funcionários. Faremos o acompanhamento das mesmas, especialmente as referidas ao controle de doenças e insetos, assim como as aplicações de agrotóxicos.

2.5.2 Metodologia

A metodologia será desenvolvida para cada um dos experimentos por separado.

2.5.2.1 Experimento 1: Estimativa da herdabilidade do caráter ciclo do pessegueiro e observação de possível efeito materno neste caráter.

O trabalho será desenvolvido nas instalações e Laboratório de Melhoramento de Plantas Frutíferas da Embrapa Clima Temperado, em Pelotas/RS, nas safras 2016-2017 e 2017-2018, e aproveitando os dados da safra 2015-2016.

Serão observadas populações F_1 oriundas de hibridações, assim como seus genitores (Tabela 1 e 2), descritos no ponto 2.5.1.

Para a obtenção do ciclo do pessegueiro, serão feitas observações em cada planta individualmente, acompanhando sua fenologia e determinando as datas de floração e início de maturação. Será registrado o início de floração (10% flores abertas), plena floração (50% flores abertas) (CENTELLAS QUEZADA, 2000) e as datas de início de maturação (quando será realizada a primeira colheita). O principal fator para determinar a colheita será a cor de fundo dos frutos e a mesma será feita com os frutos em estádio de firme maturação (maturação comercial) (FERREIRA, 1976). O ciclo será calculado através do intervalo em dias entre plena floração e a data da primeira colheita (CÔRREA, 2007).

Será estimada a herdabilidade no sentido amplo e no sentido restrito. A variância observada quanto ao caráter ciclo no pessegueiro entre os três clones de um mesmo genitor deve-se ao efeito ambiental, e a média das variâncias dos genitores será utilizada como a variância ambiental média (σ_e^2). A variância observada entre plantas pertencentes a uma mesma população, quanto ao comprimento do ciclo será a variância fenotípica total (σ_p^2), ou seja, o efeito genético mais ambiental. A variância genética (σ_g^2) será calculada subtraindo a variância ambiental da variância total de cada população (CENTELLAS QUEZADA, 2000;

WAGNER JÚNIOR, 2003; CÔRREA, 2007). O cálculo da herdabilidade no sentido amplo (H^2), será estimado dividindo-se a variância genética de cada população pela variância total, como se indica na seguinte fórmula (ALLARD, 1960; GRIFFITHS et al., 2002):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

Onde:

H^2; herdabilidade no sentido amplo

σ_g^2; variância genética

σ_g^2; variância ambiental

σ_g^2 + σ_g^2 = σ_p^2; variância fenotípica total

Serão construídos distribuições de frequência e os respectivos histogramas dos dados de ciclo, medidos em dias entre plena flor e início da maturação, considerando ciclo curto até 100 dias, médio de 101 a 140 dias e longo mais de 140 dias (CÔRREA, 2007). O efeito materno será avaliado comparando a população de um dos cruzamentos com seu cruzamento recíproco, através do teste do qui-quadrado (ELER, 2014), utilizando a porcentagem do total de indivíduos de cada progênie por classe, já que o número dos mesmos não é igual. Também para testar a hipótese de efeito materno, efetuara-se a comparação entre as médias de comprimento do ciclo pelo teste t a 5% de significância para duas amostras independentes. Testando os seguintes contrastes: P_1 versus P_2, P_1 vs. F_1, P_1 vs. F_1 recíproco, P_2 vs. F_1, P_2 vs. F_1 recíproco, F_1 vs. F_1 recíproco (LONDERO et al., 2009).

A estimativa da herdabilidade no sentido restrito (h^2), será obtido pela regressão linear entre os valores médios do ciclo (em dias) dos genitores e os valores médios do ciclo (em dias) das progênies (CENTELLAS QUEZADA, 2000; FALCONER e MACKAY, 2001; GRIFFITHS et al., 2002; CÔRREA, 2007; ELER, 2014).

Também será calculada a correlação entre os dados de ciclo observados nas três safras de avaliações, para ter uma ideia da repetibilidade do caráter (CRUZ e REGAZZI, 1997; CÔRREA, 2007).

Os dados obtidos neste experimento 1 serão utilizados para testar possíveis correlações com os dados de incidência e severidade à podridão-parda dos experimentos 2 e 3, da seguinte maneira: data de floração com incidência e intensidade da podridão-parda em flores, data de colheita com incidência e intensidade da podridão-parda em frutos, comprimento do ciclo com incidência e intensidade da podridão-parda em frutos.

2.5.2.2 Experimento 2: Reação de flores de diferentes genótipos de pessegueiro à podridão-parda (*Monilinia fructicola*).

O trabalho será desenvolvido nas instalações da Embrapa Clima Temperado, Laboratório de Melhoramento de Plantas Frutíferas e Laboratório de Fitopatologia, em Pelotas/RS, nas safras 2016-2017 e 2017-2018, e serão aproveitados os dados da safra 2015-2016, já coletados.

Para testar a reação de flores de diferentes genótipos de pessegueiro à podridão-parda, será utilizada a técnica de flores destacadas, citada por Fabiane (2011) como a técnica mais fácil e prática para ser adotada em um estudo de resistência à podridão-parda em flores. Será avaliada a incidência e a severidade da *Monilinia fructicola*. As flores serão das mesmas progênies F_1 e seus genitores referidas no item 2.5.1 (Tabela 1 e 2).

Para os indivíduos das progênies F_1 serão inoculadas 12 flores, divididas em três repetições, com parcelas de quatro flores de cada genótipo. Para cada genótipo serão observadas mais quatro flores, sem inoculação, correspondendo ao controle, para ter uma noção da proporção do inóculo latente, que vem do campo. Também serão avaliados os genitores de cada uma dessas progênies F_1, dos quais se dispõe de três clones de cada um. Para esses genitores serão avaliadas 16 flores por clone, distribuídas da mesma forma que os indivíduos *seedlings*.

O número de plantas a ser utilizadas por cruzamento poderá ser inferior ao número de indivíduos disponíveis por progênie, já que são plantas de três anos em sua maioria, e nem todas terão número suficiente de ramos para avaliar flores e, posteriormente, frutos.

O delineamento experimental será inteiramente casualizado, considerando-se cada genótipo diferente como um tratamento, ou seja, cada indivíduo dos *seedlings* é considerado como um tratamento, bem como seus genitores. As flores serão colocadas em caixas plásticas (50cm x 35cm x 10cm) previamente lavadas com água e hipoclorito. Nas caixas serão colocadas placas de espuma fenólica (Green-up) lavadas com água corrente por 30 minutos, onde em cada célula (2,5cm x 2,5cm x 3,8cm) da espuma se colocara uma flor com uma pequena porção de ramo,

O isolado do fungo será obtido dos pomares de pessegueiros da Embrapa Clima Temperado (Pelotas/RS). Serão colhidas de quatro locais diferentes, múmias produzidas pelo fungo *Monilinia fructicola*. Essas frutas mumificadas contém as estruturas de sobrevivência do fungo, da safra anterior. Das múmias serão retirados pequenos fragmentos de aproximadamente 5 mm de tamanho e transferidos para placas de Petri contendo meio de cultura BDA (Batata Dextrose Ágar) e incubados em sala de crescimento a 25±2°C, por sete a dez dias, a 12 horas de luz. A contaminação com outros fungos ou bactérias será eliminada através de sucessivas repicagens até a obtenção de cultura pura. A cultura pura do fungo será conservada em tubos de ensaio, com meio de cultura BDA, em câmara fria a 4±1°C. Sempre que for necessário, repicar-se-á o mesmo sobre frutos maduros de pessegueiro para voltar a isolar o fungo em placas de Petri com meio de cultura BDA. Todas as manipulações do fungo serão feitas na câmara de fluxo laminar do Laboratório de Fitopatologia da Embrapa Clima Temperado e sempre serão mantidas as identidades das quatro estirpes, dependendo do local de coleta.

Para a preparação do inóculo, serão retirados os conídios das placas de Petri com o crescimento da *M. fructicola,* de sete a dez dias, dos quatro diferentes locais. Será adicionada água destilada e com ajuda de um pincel serão retirados os conídios. Depois a suspensão é filtrada com papel de filtro e se determina a concentração de conídios, contados em microscópio óptico, com auxílio de câmara de Neubauer. Será ajustada a suspensão de *M. fructicola* à concentração de 1,0 x 10^5 conídios mL^{-1} (FELICIANO et al. 1987; FABIANE, 2011; WAGNER JÚNIOR et al. 2011; SANTOS et al., 2012). Por último se tomam partes iguais das diluições dos quatro isolados e se misturam, tendo assim um inóculo composto. Ao final se faz a certificação de que a mistura esteja na concentração desejada, com o microscópio óptico e a câmara de Neubauer.

Para a inoculação, primeiro serão coletados ramos produtivos dos indivíduos a testar, com gemas no estado de botão rosado. Os ramos serão preparados eliminando flores velhas ou danificadas e mantidos em baldes com água, em câmara fria por 48 horas, com o objetivo de uniformizar a floração (SANTOS et al., 2012), e evitar parte da contaminação com o patógeno (LUO et al., 2001; MAY-DE MIO et al., 2008). Após 48 horas na câmara, os ramos são deixados por mais 24 horas à temperatura ambiente, para que as flores abram (antese).

Depois dessas 72 horas, serão escolhidas 16 flores no estado de flor aberta e colocadas na espuma fenólica previamente lavada e hidratada. A inoculação será com borrifador, com 0,8mL aproximadamente de suspensão conidial (1,0 x 10^5 conídios mL^{-1}) de *M. fructicola,* por caixa de espuma fenólica (FABIANE, 2011; SANTOS et al., 2012), contendo 100 flores aproximadamente.

Depois da inoculação, as flores serão conservadas em espuma fenólica dentro de bandejas com água, cobertas por saco plástico e colocadas em câmara de crescimento (Fitotron), com temperatura controlada 23±1°C (SANTIAGO, 2013), e fotoperiodo de 12 horas de luz. Após 72 e 120 horas, será avaliada visualmente a incidência e a severidade da podridão-parda nas flores, considerando-se como infectadas aquelas flores que apresentaram pétalas com mancha necrótica (FABIANE, 2011; WAGNER JÚNIOR 2003; SANTOS et al., 2012), e a severidade será avaliada conforme a seguinte escala (Tabela 4 e Figura 1).

Tabela 4 – Escala de notas que será utilizada para a avaliação da severidade da podridão-parda nas flores de pessegueiro, utilizando como modelo os desenhos da Figura 3.

Nota	Critério
0	Não apresenta infecção
1	Manchas necróticas nas pétalas cobrindo >1% ≤20%
2	Manchas necróticas nas pétalas >21% ≤40%
3	Manchas necróticas nas pétalas >41% ≤60%
4	Manchas necróticas nas pétalas >61%

Não serão avaliadas infecções nas anteras e ou pistilo, por serem os órgãos mais sensíveis a fungos (OGAWA et al. 1995, MAY-DE MIO et al., 2014) e apresentarem crescimento de outros gêneros de fungos tais como *Cladosporium*, *Penicillium*, *Alternaria* e *Botrytis* (OGAWA et al., 1995; MAY-DE MIO et al., 2008), já que o trabalho será desenvolvido com material sem uma desinfecção previa e existe uma influência do inóculo natural do campo.

A escala a ser utilizada (Tabela 3) foi feita visando sua praticidade na hora da avaliação. Os limites da escala que determinam as notas correspondem à porcentagem da área de uma flor com presença de manchas necróticas. Esses limites da escala, foram transformados em uma escala de figuras (Figura 3), com a ajuda do programa ImageJ (IMAGEJ, 2015). Esta escala foi feita com base em diferentes fotografias de flores avaliadas às 72 e 120 horas de inoculadas com *M. fructicola*, nas mesmas condições que o experimento. Portanto, o padrão desta escala é para flores inoculadas artificialmente com borrifador de gota fina, 1,0 x 10^5 conídios mL^{-1} e aproximadamente 0,8 mL por caixa (50cm x 35cm x 10cm) com espuma fenólica, contendo 100 flores aproximadamente.

Para os cálculos estatísticos e a estimativa da herdabilidade será utilizada a porcentagem de incidência e a severidade da podridão-parda nas flores, está última variável previamente transformada para que cumpra com as pressuposições de normalidade.

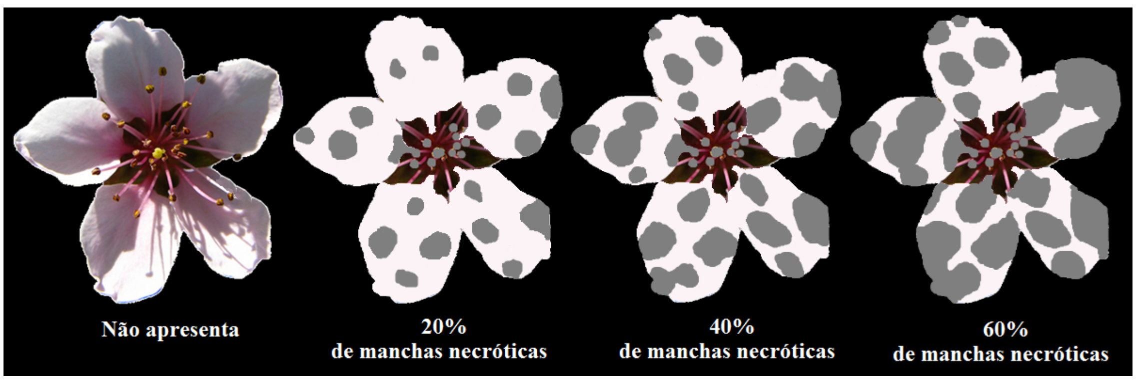


Figura 1 – Escala utilizada para a avaliação da severidade da podridão-parda nas flores de pessegueiro inoculadas artificialmente com borrifador.

O cálculo da H^2 para o caráter resistência à podridão-parda em flores será feito da mesma forma que foi descrito no experimento 1 para o caráter ciclo (item 2.5.2.1). O cálculo da estimativa da h^2, será obtido pela regressão linear entre os valores médios dos genitores e os valores médios dos indivíduos das progênies (GRIFFITHS et al., 2002).

Na avaliação se incluirá a seleção Conserva 655 por ser mencionada por Fabiane (2011) como um genótipo suscetível à podridão-parda na flor. Assim, a mesma será utilizada como uma testemunha nas avaliações estatísticas. A seleção Cascata 1055, além de ser genitor de um dos cruzamentos recíprocos da avaliação, será utilizada como uma testemunha com menor suscetibilidade à podridão-parda (FABIANE, 2011).

Os dados serão submetidos à análise da variância, e no caso de serem significativamente diferentes, serão utilizados testes para comparar as médias e uma possível análise de agrupamentos dos genótipos para a característica resistência à podridão-parda na flor. A infecção natural será estimada pela avaliação das flores sem inoculação artificial.

A existência ou não de efeito materno será avaliado comparando a população de um dos cruzamentos com seu cruzamento recíproco. Efetuara-se a comparação entre as médias pelo teste t a 5% de significância para duas amostras independentes. Testando os seguintes contrastes: P_1 versus P_2, P_1 vs. F_1, P_1 vs. F_1 recíproco, P_2 vs. $F_{1,}$ P_2 vs. F_1 recíproco, F_1 vs. F_1 recíproco (LONDERO et al., 2009).

Na safra 2016-2017, serão coletadas flores de alguns genótipos, que mostraram diferenças maiores na safra anterior, para testes com diferentes concentrações de inóculo, procurando determinar uma concentração ideal para melhor diferenciação entre genótipos.

2.5.2.3 Experimento 3: Reação de frutos de diferentes genótipos de pessegueiro à podridão-parda (*Monilinia fructicola*).

O trabalho será desenvolvido nas instalações da Embrapa Clima Temperado, Laboratório de Melhoramento de Plantas Frutíferas e Laboratório de Fitopatologia, em Pelotas/RS, nas safras 2016-2017 e 2017-2018, além de aproveitar os dados da safra 2015-2016, já coletados.

Para testar a reação de frutos de diferentes genótipos de pessegueiro à podridão-parda, será utilizada a técnica de inoculação de frutos com deposição de gota com ferimento (CRISOSTO et al. 2007; PASCAL et al., 1994) e sem ferimento da fruta (PASCAL et al., 1994; SANTOS et al., 2012), avaliando a incidência e a severidade da *M. fructicola*. Serão avaliados frutos de populações F_1 oriundas de hibridações recíprocas entre elas, e seus genitores descritos no item 2.5.1 (Tabela 1), além das populações adicionais (Tabela 2).

Serão avaliadas amostras de 5 frutos por *seedling*, por tratar-se de plantas de três anos em sua maioria. Para os genitores de cada progênie, dos quais se dispõe de três indivíduos geneticamente iguais, serão avaliados cinco frutos por clone, totalizando 15 frutos por genitor.

O delineamento experimental será inteiramente casualizado, considerando-se cada genótipo diferente como um tratamento, ou seja, cada *seedling* e cada um dos genitores é considerado como um tratamento diferente. Cada fruto será considerado como uma repetição, totalizando 5 repetições por tratamento.

O isolado do fungo será obtido da mesma forma descrita no item anterior (podridão-parda em flores).

Para a preparação do inóculo serão utilizados frutos maduros previamente desinfestados, se repicará o fungo das placas de Petri com o crescimento da *M. fructicola*, das quatro diferentes origens, onde foram colhidas frutas mumificadas. Após 72 a 120 horas de crescimento do fungo, com água destilada e ajuda de um pincel se tiram os conídios, que depois serão filtrados separadamente, por origem, com papel de filtro e se determina a concentração de conídios, contados em microscópio óptico, com auxílio de câmara de Neubauer. Por separado, será ajustada a suspensão de *M. fructicola* à concentração de 2,5 x 10^4 conídios mL^{-1} (CRISOSTO et al. 2007; 2009; SANTIAGO, 2013), por último se tomam partes iguales das diluições e se misturam, tendo assim um inóculo composto.

Os frutos em estádio de firme maturação (maturação comercial) serão colhidos dos quatro quadrantes da planta, da parte interna e externa da copa. O principal fator para determinar a colheita será a cor de fundo dos frutos. Depois de colhidos será realizada uma seleção dos mesmos, no laboratório de Melhoramento Genético da Embrapa Clima Temperado, quanto à ausência de danos mecânicos, de insetos e/ou infecção aparente. Posteriormente, serão submetidos a uma desinfestação, primeiramente em imersão em álcool ao 70% por um minuto, seguido por imersão em hipoclorito (cloro ativo 0,2%) por três minutos, seguindo-se um descanso de 10 minutos, para logo serem lavados três vezes, com água destilada e esterilizada. Os frutos serão dispostos em caixas plásticas transparentes de 24cm x 23cm x 10cm, em número de cinco por caixa, sobre anéis de plástico. As caixas serão previamente desinfetadas com álcool ao 70% e a base delas será forrada com papel filtro umedecido com água destilada (WAGNER JÚNIOR et al, 2011; SCARIOTTO, 2016).

A inoculação será feita através da deposição de gota nos frutos, tanto com ferimento quanto sem ferimento. Para ambos será utilizada uma microseringa de 100µL acoplada em um dispensador de repetição 50x (Hamilton®) (SANTIAGO, 2013; SCARIOTTO, 2016). O volume utilizado será de 10µL de suspensão de conídios na concentração de 2,5 x 10^5 conídios mL^{-1} (CRISOSTO et al., 2007; 2009;

MARTÍNEZ-GARCÍA et al., 2013; SCARIOTTO, 2016). Para a inoculação com ferimento a penetração será de 1mm, injetando-se a mesma quantidade que para a inoculação sem ferimento.

Depois da inoculação, os frutos serão conservados nas caixas e as mesmas serão colocadas em câmara de crescimento (Fitotron), com temperatura controlada 23±1°C, e fotoperiodo de 12 horas de luz. Após 72 e 120 horas, será avaliada visualmente, a incidência e a severidade da podridão-parda nos frutos, considerando-se como infectados o número de frutos que apresentem a doença, e a severidade será avaliada medindo o diâmetro da lesão, utilizando-se para isso um paquímetro digital e utilizando a média de duas medições perpendiculares (SANTOS et al., 2012; MARTÍNEZ-GARCÍA et al., 2013). Também será quantificado o índice de esporulação, para o que se observará a presença ou não de esporulação, e no caso de apresentar será medida a área que abarca a esporulação da mesma forma que para a medição da lesão (SCARIOTTO, 2016).

Também será calculada a área (cm^2) da lesão e da esporulação pela formula S= (π x C x L) / 4, sendo S a área da lesão ou esporulação, C o comprimento e L a largura (MAFFIA et al., 2007). A partir dessas mensurações calcular-se-á a porcentagem do total de uma face do fruto (metade do fruto) que foi afetado pela lesão e pela esporulação. Estas últimas variáveis, expressas em porcentagem, são independentes do tamanho do fruto, o que é particularmente importante neste caso que se estão avaliando genitores de pessegueiros que são cultivares e seleções muitas vezes de bom tamanho de frutos e também se estão avaliando suas progênies, onde muitos destes indivíduos podem produzir frutos de tamanho consideravelmente menores.

Para os cálculos estatísticos e a estimativa da herdabilidade será utilizada a porcentagem de incidência, a porcentagem de frutos com esporulação, a média do tamanho e da área da lesão, a média do tamanho e da área com esporulação do fungo nos frutos, assim como a porcentagem média da área do fruto que foi afetado pela lesão e a esporulação.

A herdabilidade no sentido amplo será estimada da mesma forma que nos casos anteriores, detalhado no item 2.5.2.1, e o cálculo da estimativa da herdabilidade no sentido restrito, será obtido pela regressão linear entre os valores médios dos genitores e os valores médios dos indivíduos das progênies (GRIFFITHS et al., 2002).

Serão incluídas na avaliação, as cultivares ´Bolinha` e ´Atenas`, o primeiro por ser um genótipo que apresenta resistência horizontal à podridão-parda no fruto (FELICIANO et al., 1987; WAGNER JUNIOR, 2003; SANTOS et al. 2012), e a segunda por ser mencionado por Fabiane (2011) e Wagner Júnior et al. (2011) como um dos genótipos mais suscetíveis, em um trabalho com 30 genótipos testados. Estas duas cultivares serão consideradas como testemunhas, nas avaliações estatísticas.

Os dados serão submetidos à análise da variância, e no caso de dar significativa, será feita a comparação de médias pelo teste de Dunnett e um possível análise de agrupamentos dos genótipos para a característica resistência à podridão-parda no fruto. A existência ou não de efeito materno será avaliado comparando a população de um cruzamento com seu cruzamento recíproco. Efetuara-se a comparação entre as médias pelo teste t a 5% de significância para duas amostras independentes. Testando os seguintes contrastes: P_1 versus P_2, P_1 vs. F_1, P_1 vs. F_1 recíproco, P_2 vs. F_1, P_2 vs. F_1 recíproco, F_1 vs. F_1 recíproco (LONDERO et al., 2009).

Na safra 2016-2017, serão coletadas amostras de frutas inoculadas de alguns genótipos que apresentaram diferenças significativas, na safra anterior, quanto a diâmetro da esporulação, classificando-os por época de colheita. As amostras serão retiradas da zona de esporulação, de 1cm de diâmetro com um cortador circular e serão conservadas em tubos de ensaio com 2mL de uma solução de conservação (1mL de água destilada e 1mL de 0,04M de CuSO4 / 0,2M de acetato de sódio / pH ácido acético 5,4) (SUASSUNA et al., 2004; OLIVEIRA, 2010), para sua posterior avaliação. A concentração de conídios nessas amostras, servirá para verificar se as diferenças achadas quanto ao tamanho da esporulação e lesão estão correlacionadas com a produção real de conídios nos diferentes genótipos. A determinação da concentração de conídios será feita nos meses de fevereiro e março, depois de finalizadas as colheitas. Os tubos serão agitados durante cerca de 10 segundos para libertar os conídios do tecido. O número de conídios presentes na suspensão será estimado com microscópio óptico e auxílio de câmara de Neubauer. Este procedimento será feito duas vezes para cada suspensão de conídios. A capacidade de esporulação será calculada multiplicando a concentração média de conídios (conídios mL^{-1}) pelo volume da solução de conservação e dividindo-a pela área total da esporulação (cm^2) (KADISH et al., 1990; OLIVEIRA, 2010).

2.5.2.4 Experimento 4: Validação de potenciais marcadores moleculares SNPs, para a característica resistência à podridão-parda em frutos de pessegueiro.

O material vegetal que se utilizará para tentar validar os potencias marcadores moleculares, serão os mesmos genótipos utilizados nos experimentos descritos anteriormente (Tabelas 1 e 2), totalizando 303 *seedlings*, 12 seleções e 11 cultivares. Os protocolos referentes à fenotipagem para testar a resistência do fruto à podridão-parda foram descritos no experimento 4 (ponto 7.2.4.), e os mesmos seguirão os padrões de protocolos já utilizados pelo programa RosBREED e mencionados em várias publicações (CRISOSTO et al., 2007; 2009; BOSTOCK e GRADZIEL, 2009; MARTÍNEZ-GARCÍA et al., 2013; SCARIOTTO, 2016).

Para realizar a genotipagem para a validação dos marcadores, serão coletadas folhas jovens entre os meses de julho e agosto, sendo utilizados entre 0,15 e 0,20g de tecido vegetal por genótipo. As mesmas serão identificadas e armazenadas a -80 °C. As tarefas referentes à genotipagem serão feitas no Laboratório de Biologia Molecular da Embrapa Clima Temperado. A extração do DNA será realizada de acordo com a protocolo CTAB (cationic hexadecyl trimethyl ammonium bromide) com pequenas modificações descritas por Ferreira e Grattapaglia (1998). O DNA será quantificado em gel de agarose 1% e diluído a 20 ng/µl, para as reações de amplificação via PCR.

Os potencias marcadores moleculares (SNPs) que serão analisados para a característica de resistência à podridão-parda nos frutos, serão os marcadores identificados pelo grupo de pesquisa da Embrapa Clima Temperado, por mapeamento associativo de uma população de 144 genótipos do Banco Ativo de Germoplasma e com características contrastantes quanto à resistência à podridão-parda nos frutos. Para a escolha destes SNPs foram considerados as informações de polimorfismo e os padrões de amplificação claros e repetitivos.

Serão desenhados iniciadores (*Primers*), para as potenciais regiões ligadas a característica alvo, com ajuda do programa Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) e será ligado ao "*primer forward*" uma cauda M-13 (M-13.FWD(-29)/Rdye 800 Primer) (LI-COR). Os produtos das reações de amplificação serão separados por eletroforese vertical em gel de poliacrilamida

6,5% com sistema de análise de DNA do sequenciador LI-COR 4300 e o tamanho dos alelos dimensionado com o marcador de DNA de peso molecular de 50-700pb Rdye 800 (LI-COR). As reações primárias de PCR serão diluídas com água milli-q estéril para uma concentração desejável, que permita uma clara visão dos fragmentos amplificados, sendo necessário ajuste para cada *primer*. Posteriormente, 0,8μL da reação diluída será aplicada no gel e a corrida será programada para um tempo de até 3 horas. O software Saga GT (LI-COR) será utilizado para visualização, registro e análise dos fragmentos amplificados.

Para a associação dos dados fenotípicos e genótipos, será calculada a eficiência de seleção (ES) dos marcadores, no qual será baseada na comparação entre avaliações dos dados fenotípicos e genotípicos, calculada de acordo com a fórmula: ES= 100 [(MFMF + mfmf) / (MM + mm), onde MFMF é o número de plantas selecionadas corretamente, com base nos marcadores e na avaliação fenotípica, quando apresentarem a característica (resistência à podridão-parda); mfmf, o número de plantas selecionadas, corretamente, quando não apresentarem a característica (suscetíveis à podridão-parda), com base nos marcadores e na avaliação fenotípica; MM e mm, número total de plantas com e sem a característica, respectivamente, com base apenas nos marcadores.

Além disso, a associação entre os níveis de resistência à podridão-parda e os alelos encontrados serão calculados através de regressão logística, utilizando o procedimento de modelo linear generalizado (GLM) em R (R Core Team de 2013). Os coeficientes dados pelo modelo serão utilizados para calcular a probabilidade de encontrar alelos diferentes para níveis de resistência à doença, usando a fórmula P (marcador alelo / TA) = eβ0-β1*TA / 1+1eβ0-β1*TA, onde β0 e β1 são o coeficiente da regressão estimada.

2.5.3 Resultados esperados

Ao finalizar a execução das avaliações e a interpretação dos resultados obtidos neste projeto, espera-se ter estimativas da herdabilidade das características ciclo do pessegueiro, resistência à podridão-parda na flor e na fruta, assim como constatar a existência ou inexistência de efeito materno nas mesmas características. Buscar novas fontes de resistência à podridão-parda em flor e em frutos. Além de validar ou rejeitar potencias marcadores moleculares SNPs para a característica de resistência à podridão-parda em frutos de pessegueiro.

Estes resultados contribuirão para a tomada de decisões dos melhoristas de pessegueiro em seus programas de melhoramento genético, e acredita-se que contribuirão para um ganho genético mais rápido nestas características, principalmente nas relacionadas com a resistência a *Monilinia fructicola*, objetivo da grande maioria dos programas de melhoramento de pessegueiro no mundo.

Com isso, o programa de melhoramento poderá aumentar sua eficiência e reduzir tempo e recursos para alcançar seus objetivos.

Com base nos resultados obtidos será elaborada uma Tese para a obtenção do título de Doutor. Além disso, os dados obtidos serão divulgados em congressos, simpósios, artigos científicos e/ou boletins informativos, a fim de difundir o conhecimento gerado e favorecer o desenvolvimento de novas pesquisas.

Pessoalmente, o esperado é o crescimento continuo na formação profissional, em uma área muito importante (melhoramento genético em fruticultura) e de minha preferência, trabalhando em um país diferente, com pesquisadores reconhecidos a nível mundial como a Dra. Maria do Carmo Bassols Raseira que além de ser uma ótima pesquisadora é muito melhor pessoa, e com Professores e colegas da Faculdade (UFPel/FAEM/PPGA) e da Embrapa Clima Temperado que me enriquecem em cada conversa.

2.6 Cronograma

Atividades por ano	2016										2017											
	M	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Elaboração do projeto de doutorado	X																					
Aulas na UFPel	X	X	X	X		X	X	X	X	X			X	X	X	X		X	X	X	X	
Revisão bibliográfica		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Elaboração da Dissertação e defesa			X	X	X																	
Preparo do experimento			X	X	X	X	X															
Instalação do experimento				X	X	X		X	X	X	X					X	X	X		X	X	X
Acompanhamento fenológico				X	X	X	X	X	X	X						X	X	X	X	X	X	X
Colheita de flores e avaliações				X	X	X										X	X	X				
Avaliações de resistência em flores				X	X	X	X									X	X	X	X			
Colheita de frutos								X	X	X										X	X	X
Avaliações de resistência em frutos								X	X	X										X	X	X
Coleta de folhas para extração de DNA																	X	X				
Estimativa da concentração de conídios das amostras												X	X									
Acompanhamentos poda e outros manejos				X	X	X	X	X								X	X	X	X	X		
Acompanhamentos fitossanitários e adubações					X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X
Análise prévia dos dados safra 2015-2016 e 2016-2017	X	X	X										X	X	X							
Manutenção do experimento	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Redação de resumos para congressos e artigos científicos				X	X	X										X	X	X				

Atividades por ano	2018												2019									
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
Revisão bibliográfica	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Possível estagio nos EUA			X	X	X	X	X	X	X													
Validação de marcadores SNPs	X	X	X	X	X	X	X	X	X	X	X	X										
Colheita de flores e avaliações						X	X	X														
Avaliações de resistência em flores						X	X	X	X													
Colheita de frutos										X	X	X										
Avaliações de resistência em frutos										X	X	X										
Acompanhamentos fitossanitários e adubações	X	X					X	X	X	X	X	X	X	X	X							
Análise prévia dos dados safra 2017-2018 e 2017-2018			X	X	X										X	X	X					
Manutenção do experimento	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Redação de resumos para congressos e artigos científicos						X	X	X	X	X	X			X	X	X		X	X	X		
Análise dos dados finais															X	X	X					
Elaboração da Tese e defesa															X	X	X	X				

2.7 Recursos necessários

2.7.1 Material de consumo

Descrição	Unid.	Qnt.	Preço unit. (R$)	Total
Baldes	un.	15	15,0	225,0
Fita para identificação e coleta de ramos	un.	5	6,0	30,0
Etiquetas de identificação e outros	-	-	-	200,0
Sacolas plásticas	kg.	5	40,0	200,0
Caixas de colheita de plástico	un.	20	20,0	400,0
Caixas de plástico para a espuma fenólica	un.	20	15,0	300,0
Espuma fenólica (Green-up®)	caixa	12	50,0	600,0
Tesoura de poda (Felco 7)	un.	1	170,0	170,0
Armadilhas mosca da fruta	un.	400	0,5	200,0
Proteína hidrolisada (Cera Trap®)	galão	15	480,0	7.200,0
Embalagens para armazenamento	-	-	-	400,0
Material de laboratório (Fitopatologia)	-	-	-	800,0
Material de laboratório (Biologia Molecular)	-	-	-	25.000,0
Agrotóxicos	-	-	-	1.800,0
Combustível e lubrificantes	-	-	-	2.600,0
			Subtotal	**40.125,0**

2.7.2 Outras despesas

Descrição	Unid.	Qnt.	Preço unit. (R$)	Total
Banner	unid.	8	30,00	320,0
Encadernações	unid.	20	2,00	40,0
Assinatura revista científica	unid.	6	100,00	600,0
Inscrição em eventos	-	-	-	2.500,0
Manutenção de laboratórios e equipamentos	-	-	-	4.000,0
Manutenção de máquinas agrícolas utilizadas nos experimentos	-	-	-	5.000,0
Diárias e passagens	-	-	-	6.000,0
Manutenção de equipamentos de informática	-	-	-	2.000,0
Material bibliográfico	-	-	-	1.500,0
			Subtotal	**21.960,0**

2.7.3 Orçamento total

Descrição	Valor R$
Material de consumo	40.125,0
Outras despesas	21.960,0
Estagio nos Estados Unidos (6-7 meses)	58.000,0
Total Geral	**120.085,0**

2.8 Referências bibliográficas

ADASKAVEG, J.E.; SCHNABEL, G.; FORSTER, H. Diseases of peach caused by fungi and fungal-like organisms: biology, epidimiology and management. In: LAYNE D.R.; BASSI, D. **The peach**: botany, production and uses. Wallingford: CAB International. p. 352-406, 2008.

AGRIOS, G.N. Plant diseases caused by fungi. In: AGRIOS, G.N. **Plant Pathology**. New York: Academic Press, p. 336-339, 1998.

ALLARD, R.W. **Principles of plant breeding**. New York: John Wiley & Sons, Inc., 1960. 485 p.

BOSTOCK, R; GRADZIEL, T. Assessment of brown rot resistance in advanced experimental selections of peach. California Cling Peach Advisory Board. **Annual Report**, California, 13 p., 2009.

CENTELLAS QUEZADA, A. **Herdabilidade da época de floração e estudos sobre a ferrugem da folha [*Tranzchelia dsicolor* Fckl. (Tranz. & Litv.)] em pessegueiro [*Prunus persica* (L.) Batsch]**. 2000. 54p. Tese (Doutorado em Fruticultura de Clima Temperado) Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

CORRÊA, E.R. **Estudo da herdabilidade de alguns carácteres em pessegueiro: ciclo, tonalidade da cor da polpa e compostos fenólicos**. 2007. 52 p. Dissertação (Mestrado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

CRISOSTO, C.; OGUNDIWIN, E.; BOSTOCK, R.; SLAUGHTER, D.; GRADZIEL, T.; MICHAILIDES, T. Development of predictive tools for brown and sour rot resistence in peach and nectarines. California Tree Fruit Agreement. **Annual Research Report**, California, p. 70-78, 2007.

CRISOSTO, C.; GRADZIEL, T.; OGUNDIWIM E.; BOSTOCK, R.; MICHAILIDES, T. Development of predictive tools for brawn and sour rot resistance in peach and nectarines. California tree fruit agreement. **Annual research report**, California, p. 87-93, 2009.

CRUZ, C.D.; REGAZZI, A.J. **Modelos biométricos aplicados ao melhoramento genético.** Universidade Federal de Viçosa, Viçosa: Editora UFV, 1997. 390 p.

ELER, J.P. **Teorias e métodos em melhoramento genético animal**; I- Bases do melhoramento genético animal. Universidade de São Paulo, Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga: USP, 2014. 249 p.

EMBRAPA. **Embrapa Clima Temperado** - Dados meteorológicos de Pelotas em tempo real. Disponível em: <http://agromet.cpact.embrapa.br/online/Current_Monitor.htm/>. Acesso em: ago. 2015.

FABIANE, K.C. **Reação de pessegueiros a *Monilinia fructicola* (Wint.) Honey e sua relação com componentes bioquímicos**. 2011. 130 p. Dissertação (Mestrado em Agronomia) Universidade Tecnológica Federal do Paraná, Pato Branco.

FACHINELLO, J.C.; PASA, M.S.; SCHMTIZ, J.D.; BETEMPS, D.L. 2011. **Situation and perspectives of temperate fruit crops in Brazil**. **Revista Brasileira de Fruticultura**, Jaboticabal/SP, v. 33, n. spe1, p. 109-120, 2011.

FALCONER, D.S.; MACKAY, T.F.C**. Introducción a la genética cuantitativa**. Zaragosa: Acribia S.A, 2001. 490 p.

FAO. FAOSTAT. Food and Agriculture Organization of the United Nations; Statical Division. Roma. Disponível em: <http://faostat3.fao.org/home/E> Acesso em: mar. 2016.

FELICIANO, A.; FELICIANO, A.J.; OGAWA, J.M. *Monilinia fructicola* resistence in peach cultivar Bolinha. **Phytopathology**, St. Paul, v. 77, p. 776-780, 1987.

FERREIRA, F.A.A. **Estudo da segregação de alguns caracteres em pessegueiro (*Prunus persica* (L.) Bastch).** 1976. 46p. Dissertação (Mestrado), Pós-graduação em Ciencias Agrárias da Universidade Federal de Pelotas, Pelotas.

FERREIRA, M.E.; GRATTAPAGLIA, D. **Introdução ao uso de marcadores moleculares em análise genética**. 3a ed. Brasília: EMBRAPA-CENARGEN, 1998. 220 p.

FRANZON, R.C.; RASEIRA, M.C.B. Origem e história do pessegueiro. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, p. 19-23, 2014.

GRIFFITHS, A.J.F.; MILLER, J.H.; SUZUKI, D.T.; LEWONTIN, R.C.; GELBART, W.M. **Genética**, Madrid: McGraw-Hill Interamericana, 2002. 860 p.

IBGE. Produção Agrícola Municipal. **Sistema IBGE de Recuperação Automática (SIDRA)**. Banco de Dados Agregados. 2015. Disponível em: <http://www.sidra.ibge.gov.br/bda/tabela/listabl.asp?c=1613&z=p&o=24>. Acesso em: ago. 2015.

IMAGEJ. **Image Processing and Analysis in Java**. 2015. Disponível em: <http://rsb.info.nih.gov/ij/index.html>. Acesso em: jun. 2015.

KADISH, D., GRINBERGER, M., COHEN, Y. Fitness of metalaxyl-sensitive and metalaxylresistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. **Phytopathology**, Saint Paul, v. 80, p. 200–205, 1990.

LONDERO, P.M.G.; RIBEIRO, N.D.; CERUTTI, T.; MAZEIRO, S.M.; ROSA, D.P.; ROSA, S.S. Efeito materno na expressão dos teores de aminoácidos sulfurados em grãos de feijão. **Ciência Rural**, Santa Maria, v. 39, n. 6, p. 1884-1887, 2009.

LUO, Y.; MORGAN, D.P.; MICHAILIDES, T.J. Risk analysis of brown rot blossom blight of prune caused by *Monilinia fructicola*. **Phytopathology,** Saint Paul, v. 91, n. 8, p. 759-768, 2001.

MAFFIA, L.A.; MIZUBUTI, E.S.G.; ALFENAS, A.C.; MAFIA, R.G. Quantificação de doenças em planta. In: ALFENAS, A.C.; MAFIA, R.G. **Métodos em fitopatologia**. Viçosa: Universidade Federal de Viçosa, p. 161-173, 2007.

MARTÍNEZ-GARCÍA, P.J.; PARFITT, D.E.; BOSTOCK, R.M.; FRESNEDO-RAMÍREZ, J.; VAZQUEZ-LOBO, A.; OGUNDIWIN, E.A.; GRADZIEL, T.M.; CRISOSTO, C.H. Application of genomic and quantitative genetic tools to identify candidate resistance genes for brown rot resistance in peach. **Plos One**, San Francisco, v. 8, n. 11, 12 p., 2013. Disponível em: <journals.plos.org/plosone/article?id=10.1371/journal.pone. 0078634>. Acesso em: mar. 2015.

MAY-DE MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infecção de *Monilinia fructicola* no período da floração e incidência de podridão-parda em frutos de pessegueiro em dois sistemas de produção. **Tropical Plant Pathology**, Viçosa, v. 33, n. 3, p. 227-234, 2008.

MAY-DE MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, p. 355-432, 2014.

MONDINO, P.; ALANIZ, S.; LEONI, C. Manejo integrado de las enfermedades del duraznero en Uruguay. In: SORIA, J. **Manual del duraznero; Manejo integrado de plagas y enfermedades**. Montevideo: INIA. p. 45-76. 2010.

OGAWA, J.M.; ZEHR, E.I.; BIRD, G.W.; RITCHIE, D.F.; URIU, K.; UYEMOTO, J.K. **Compendium of stone fruit diseases**. Saint Paul: The American Phytopathological Society, 1995. 98 p.

OLIVEIRA, S.A.S. **Genetic structure, aggressiveness and fungicide sensitivity of *Phytophthora infestans* populations from the south and southeast regions of Brazil**. 2010. 84 p. Tese, (Doutorado em Fitopatologia), Universidade Federal de Viçosa, Viçosa.

PASCAL, T.; LEVIGNERON, A.; KERVELLA, J.; NGUYEN-THE, C. Evaluation of two screening methods for resistance of apricot, plum and peach to *Monilinia laxa*. **Euphytica**. Netherlands, v. 77, p. 19-23, 1994.

PICOLOTTO, L. **Avaliação bioagronômica de diferentes porta-enxertos para pessegueiro (*Prunus persica* (L.) Batsch)**. 2009. 114 p. Tese (Doutorado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

RASEIRA, M.C.B.; NAKASU, B.H. Cultivares: descrição e recomendação. In: MEDEIROS, C.A.B.; RASEIRA, M.C.B. **A cultura do pessegueiro**. Brasília: Embrapa-SPI, Pelotas: Embrapa – CPACT, p. 29-98, 1998.

RASEIRA, M.C.B.; CENTELLAS-QUEZADA, A. **Pêssego**: Produção. Brasília: Embrapa-SPI, Pelotas: Embrapa Clima Temperado, 2003. 162 p.

RASEIRA, M.C.B.; FRANZON, R.C. Melhoramento genético. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, p. 57-72, 2014.

RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, 2014. 776 p.

ROSBREED. 2016. Disponível em: <www.rosbreed.org>. Acesso em: mar. 2016.

SANTIAGO, M.F. **Impacto da temperatura na intensidade de ocorrência da podridão-parda (*Monilinia fructicola* (Wint.) Honey)**. 2013. 133 p. Dissertação (Mestrado em Fitossanidade), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

SANTOS, J.; RASEIRA, M.C.B.; ZANANDREA, I. Resistência à podridão-parda em pessegueiro. **Bragantia**, Campinas/SP, v. 71, n. 2, p.219-225, 2012.

SCARIOTTO, S. **Variabilidade genética e abordagem bioquímica da reação do fruto de diferentes genótipos de *Prunus persica* à *Monilinia fructicola***. 2016. 133f. Tese (Doutorado em Fitomelhoramento), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

SUASSUNA, N.D., MAFFIA, L.A., MIZUBUTI, E.S.G. Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. **Plant Pathology**, v. 53, p. 405–413, 2004.

UFPEL. **Universidade Federal de Pelotas**. Estação Agroclimatológica de Pelotas. Convênio Embrapa/UFPel/INMET. Disponível em: <http://wp.ufpel.edu.br/agrometeorologia/informacoes/clima-de-pelotas/>. Acesso em: ago. 2015.

WAGNER JÚNIOR, A. **Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey**. 2003. 62f. Dissertação (Mestrado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas.

WAGNER JÚNIOR, A.; FABIANE, K.C.; OLIVEIRA, J.S.M.A.; ZANELA, J.; CITADIN, I. Peaches tree genetic divergence for brown rot reaction. **Revista Brasileira de Fruticultura**, Jaboticabal/SP, v. 33, n. spe1, p. 552-557, 2011.

3 Relatório do trabalho de campo

Todos os trabalhos realizados no marco do doutorado estiveram focados em um objetivo principal: contribuir para o melhor entendimento e busca de resistência genética à podridão-parda no pessegueiro.

Os três primeiros experimentos, que constam no Projeto de Pesquisa, foram realizados com algumas modificações. Essas modificações ocorreram, principalmente, devido à pouca disponibilidade de frutos nos seedlings na safra 2015-2016, causado pelo baixo acúmulo de frio no inverno de 2015 (Apêndice A) e pelo baixo porte das plantas (2 anos de idade). Por esse motivo, nessa safra, não foi possível avaliar 100% dos genótipos quanto à reação de flores e frutos à *Monilinia fructicola*.

Já o quarto experimento proposto não foi realizado. O mesmo consistia na validação de potenciais marcadores moleculares (SNPs) que seriam identificados pelo grupo de pesquisa da Embrapa Clima Temperado, por mapeamento associativo de uma população de 144 genótipos do Banco Ativo de Germoplasma e com características contrastantes quanto à resistência à podridão-parda nos frutos. Esse trabalho, até a presente data, não foi concluído e não se encontram disponíveis esses potenciais SNPs. Como este projeto não foi realizado, foi incluído como parte do doutorado, um estágio de 6 meses no INRA Avignon (França), onde se realizaram mais experimentos associados à resistência da podridão-parda no pessegueiro.

Para melhor entendimento, os experimentos realizados serão separados em experimentos desenvolvidos no Brasil e experimentos desenvolvidos na França.

No **Brasil**, o início dos trabalhos ocorreu em março de 2015, onde se delinearam os experimentos e foram escolhidas as populações a serem estudadas. Todos os trabalhos foram realizados na área experimental da Embrapa Clima Temperado (Sede), Pelotas, RS, localizada na zona Sul do Rio Grande do Sul, sob latitude de 31° 40’ S, longitude 52° 26’ W e altitude de 57 metros, e nos laboratórios de Melhoramento em Fruticultura e de Fitopatologia.

As prioridades na escolha das populações de trabalho foram: número de seedlings disponíveis por progênie, progênies recíprocas e progênies com genitores contrastantes quanto à suscetibilidade à podridão-parda. Foram escolhidas 16 progênies de primeira geração (F1), originárias de hibridações dirigidas (*seedlings*), 10 das progênies utilizadas eram originárias de cruzamentos recíprocos. As progênies contavam com um mínimo de sete, e um máximo de 25 seedlings (303 genótipos *seedlings* em total), que se encontravam plantados em um pomar com espaçamento de 0,5 m entre plantas e 5 m entre linhas. Além destes, os genitores dessas progênies também foram estudados, totalizando 20 genótipos diferentes (cultivares ou seleções), deles estavam disponíveis três plantas por genótipo, as quais foram obtidas por enxertia (clones). As cultivares e as seleções estavam plantadas no campo experimental da Embrapa Clima Temperado (Sede), nos pomares destinados ao Banco Ativo de Germoplasma (BAG), em espaçamento de 2 a 3 m entre plantas e de 5 a 6 m entre linhas, dependendo do pomar. Foram acompanhados, de forma individual, cada *seedling* e cada clone, por três safras (2015-2016, 2016-2017 e 2017-2018). Cabe destacar que nos pomares utilizados não se realizaram aplicações de fungicidas durante a safra, salvo exceções, limitando-se a aplicações de inseticidas e os fungicidas aplicados no inverno.

No experimento relacionado à fenologia, as variáveis utilizadas para realizar as análises foram: plena floração, data de maturação e o período de desenvolvimento do fruto. Na primeira safra de estudo, florações pobres e dessuniformes dificultaram a correta determinação da plena floração, e a baixa produtividade das plantas dificultou ou impossibilitou a determinação da data de maturação dos frutos. Para as duas safras seguintes (2016-2017 e 2017-2018), não existiram grandes dificuldades. Estes caracteres fenológicos foram correlacionados com a porcentagem de podridão-parda presente nos frutos, no campo, na data de colheita.

No experimento de reação de flores à *M. fructicola* (Apêndice B): na safra 2015-2016, alguns genótipos não foram avaliados pela disponibilidade insuficiente de flores. Nas três safras, o inóculo latente proveniente do campo foi muito alto, dificultando os trabalhos no laboratório e aumentando o erro associado às características em estudo. Nas duas primeiras safras, avaliaram-se o máximo possível de genótipos das populações trabalhadas, porém, devido à alta suscetibilidade de todos os genótipos, provavelmente, resultado da alta influência do inóculo do campo, alta concentração de conídios na suspensão usada para as inoculações e/ou condições de incubação favoráveis ao patógeno, decidiu-se por não realizar novamente este experimento, na terceira safra. Em substituição, optouse por realizar um experimento para ajustar um protocolo de fenotipagem mais eficiente. Foram utilizados apenas quatro genótipos (mesma data de floração), quatro concentrações de conídios para realizar as inoculações e dois estadios fenológicos das flores (estado de balão e flor aberta). Além disso, neste último experimento foram quantificados os compostos fenólicos, antocianinas e capacidade antioxidante presentes nas pétalas e estes foram correlacionados com a incidência e severidade da podridão das flores.

No experimento da reação de frutos à *M. fructicola* (Apêndice C): na primeira safra de estudo, devido a problemas climáticos e ao tamanho das plantas, alguns seedlings não foram avaliados, e todos os genótipos, inclusive os genitores, foram avaliados apenas com inoculações feitas por meio da técnica de deposição em gota com ferimento. No entanto, nas duas safras seguintes, as avaliações foram feitas com e sem ferimento. Na última safra (2017-2018), foram tomadas amostras da zona de esporulação de 20 genótipos. Isso foi realizado para ver a correspondência da capacidade real de esporulação com as variáveis mensuradas para a avaliar a esporulação (presença, diâmetro e área da esporulação).

Na **França**, os trabalhos foram realizados no INRA (Institut National de la Recherche Agronomique), mais especificamente no INRA - Provence-Alpes-Côte d'Azur (INRA PACA) de Avignon, dentro da unidade GAFL (Génétique et Amélioration des Fruits et Légumes). O período de estágio foi de maio a novembro de 2018, permitindo concluir os trabalhos da temporada 2017-2018 no Brasil e acompanhar toda uma temporada na França da primavera até o outono. No INRA se realizaram trabalhos com *M. laxa*, principal espécie causadora da podridão-parda na Europa (Apêndice D).

Todas as plantas utilizadas se encontravam a campo em um pomar experimental da estação Saint Paul (Agroparc, Avignon), da qual também foram utilizados os laboratórios e equipamentos para realizar as avaliações dos compostos voláteis. Todas as outras avaliações e trabalhos foram realizados na estação experimental Saint Maurice (Montfavet, Avignon), utilizando diversos laboratórios e equipamentos de fitopatologia, cromatografia liquida (HPLC), pós-colheita, biologia molecular, câmaras de crescimento, assim como a sala de trabalho e reagentes.

Os experimentos foram divididos em três grupos: o primeiro, consistiu no uso de cinco genótipos de nectarineiras, em que foram avaliados frutos inoculados com e sem ferimento, em diferentes estados de desenvolvimento, e acompanhado o progresso da lesão e da esporulação da *M. laxa*. Neste primeiro experimento, foram detectadas reações vermelhas quando os frutos foram inoculados imaturos e com ferimento. Essas reações foram isoladas e submetidas à cromatografia liquida (HPLC-DAD), detectando novos compostos, e verificando a associação destes com a resistência/suscetibilidade à podridão-parda.

O <u>segundo</u> grupo, consistiu em quatro experimentos paralelos realizados com dois genótipos de nectarineiras ('Zephyr' e C216). No primeiro, foram inoculados, com e sem ferimento, frutos imaturos de 'Zephyr' e C216 sendo acompanhado o progresso da lesão e da esporulação da *M. laxa*. No segundo, frutos destes mesmos genótipos, foram submetidos ou não a ferimentos, e amostras foram coletadas 1 e 7 horas depois de realizadas as feridas, para logo realizar a detecção de compostos pela análise de HPLC-DAD. No terceiro experimento, a partir das mesmas amostras do experimento anterior, foi extraído RNA e realizada análise de RNA-seq. Estes dados ainda não se encontram disponíveis, devido a um problema com a plataforma de análise, mas serão adicionados no trabalho final para publicação, logo que os mesmos estejam disponíveis e analisados. No quarto, frutos dessas duas nectarineiras com e sem ferimentos, após de 7 horas, foram coletadas amostras de compostos voláteis para logo realizar cromatografia gasosa (GC-MS) e detectar os compostos que estavam presentes nos mesmos.

O <u>terceiro</u> grupo, foi um conjunto de três experimentos realizados com frutos imaturos de 'Zephyr'. O primeiro foi realizado com o objetivo de testar a existência de uma reação sistêmica da ferida na infecção de *M. laxa*. No segundo, foi avaliada a influência de frutos feridos ou não no crescimento in vitro da *M. laxa*. No terceiro, foi testada a influência de frutos feridos sobre a infecção de *M. laxa.*

A partir dos resultados obtidos, foram gerados nove artigos científicos para serem publicados em revistas científicas, os quais são apresentados a seguir. Os artigos foram formatados de acordo com as normas de cada revista onde foram ou serão submetidos.

4 Artigos desenvolvidos

4.1 Artigo 1. Peach phenological characters: heritability, maternal effect and correlation with brown rot

Artigo submetido na *“Revista Brasileira de Fruticultura – RBF”*

Peach phenological characters: heritability, maternal effect and correlation with brown rot

Maximiliano Dini[1*], Maria do Carmo Bassols Raseira[2], Silvia Scariotto[3],
Priscila Monalisa Marchi[4], Paulo Mello-Farias[5]

Abstract – Peach is a temperate fruit species that has spread to different soil and climatic conditions all over the World. In Brazil, in the early 50s, the species was planted only in São Paulo and in the Southern states, and the harvest period was restricted to a little more than 15 days. Currently, mainly due to peach breeding programs, it is cultivated in subtropical areas and even in high altitude tropical ones, with a harvest period of over 100 days. The knowledge of genetic, phenotypic and environmental parameters that influence characters of economic importance is crucial for guiding breeding programs. The objectives of this study were to estimate the heritability of phenological characters, to evaluate their distribution within populations, to test the possible existence of maternal effect and to evaluate the relationship of these traits with the brown rot incidence (*Monilinia fructicola*). Heritability estimation of characters full bloom, harvest date, and fruit development period were high. The segregation study of these traits suggests the existence of a maternal effect on their heritability, mainly for full bloom and harvest date. The three phenological characters are significantly correlated, and only harvest date has a negative and a significant correlation with brown rot incidence.

Index terms: *Prunus persica* (L.) Batsch, *Monilinia fructicola* (Winter) Honey, progeny segregation.

Caracteres fenológicos do pessegueiro: herdabilidade, efeito materno e correlação com a podridão-parda

Resumo – O pessegueiro é uma frutífera de clima temperado, cujo cultivo se expandiu no mundo, em condições edafoclimáticas muito diferentes. No Brasil, na década de 1950 era cultivado apenas nos estados do Sul e em São Paulo, e a sua colheita estava restrita a 15 dias. Atualmente é cultivado em áreas subtropicais e até mesmo em áreas tropicais de altitude, estando sua colheita estendida a mais de 100 dias. Isto se deve principalmente, ao melhoramento genético. Conhecer os parâmetros genéticos, fenotípicos e ambientais que influenciam nos caracteres de importância econômica em pessegueiros é de grande importância para a orientação de programas de melhoramento. O objetivo deste estudo foi estimar a herdabilidade dos caracteres fenológicos, avaliar sua distribuição nas populações, testar a possível existência de efeito materno e avaliar a relação destes caracteres com a incidência da podridão-parda. A herdabilidade dos caracteres plena floração, data de colheita e

[1] MSc. PhD student. Universidade Federal de Pelotas, Capão do Leão/RS, Brazil, *Email: maxidini@hotmail.com
[2] PhD. Researcher. Embrapa Clima Temperado, Pelotas/RS, Brazil, Email: maria.bassols@embrapa.br
[3] PhD. Postdoc. Universidade Tecnológica Federal do Paraná, Pato Branco/PR, Brazil. Email: silviascariotto@yahoo.com.br
[4] MSc. PhD student. Universidade Federal de Pelotas, Capão do Leão/RS, Brazil, Email: priscilammarchi@yahoo.com.br
[5] PhD. Prof. Universidade Federal de Pelotas, Capão do Leão/RS, Brazil, Email: mellofarias@yahoo.com.br

período de desenvolvimento do fruto foram altas. O estudo da segregação destes caracteres sugere a existência de efeito materno na herdabilidade dos mesmos, principalmente para a plena floração e data de colheita. Os três caracteres fenológicos apresentam correlações significativas entre eles, e apenas a data de colheita apresentou uma correlação negativa e significativa com a incidência de podridão-parda.

Palavras-chave: *Prunus persica* (L.) Batsch, *Monilinia fructicola* (Winter) Honey, segregação na progênie.

Introduction

Peach [*Prunus persica* (L.) Batsch] is a temperate fruit adapted to very different growing conditions. Successful exploitation of this crop depends mostly on its location (HERTER et al., 2014) and the choice of the appropriate cultivar (RASEIRA et al., 2014). In Brazil, until the 1950s, the species was only cultivated in the Southern region and in São Paulo, and in the South, the harvest period was restricted to 15 days, with two commercial cultivars (RASEIRA; NAKASU, 1998). Nowadays, the planting has expanded to subtropical areas and even to altitude tropical areas (CITADIN et al., 2014). The harvest period has been extended to more than 100 days, and there are, approximately, 100 peach cultivars destinated for table, industry or dual purpose, besides nectarine cultivars (RASEIRA et al., 2014). This is, in great part, due to national peach breeding.

Flowering and harvest date and fruit development are important phenological traits for peach production, especially to determine the adequate cultivar for growers’ purpose, and for cultural practices decision-making.

The flowering date, mainly the full bloom (FB), is very important, especially in regions of frost occurrences, because flowers and young fruits are very sensitives to frost damage, and later bloom can be an alternative to avoid such damages (DIRLEWANGER et al., 2012; CITADIN et al., 2014; RASEIRA; FRANZON, 2014). Flowering date is a complex character, and the genetic components have not yet been clearly identified. Besides the accumulation influence of chilling hours to complete dormancy, heat requirement has to be achieved for a normal and uniform flowering. At the same location, dates may differ along the years, but flowering sequence remains relatively constant among the cultivars year after year (RASEIRA; FRANZON, 2014; SCORZA; SHERMAN, 1996).

Harvest date (HD) is another goal on most breeding programs due to its importance on the productive system, either due to the demand to substitute some commercial cultivars for new ones with higher production and quality aspects, or for the necessity to extend the harvest period. Ripening date is considered a polygenic inheritance character, involving major genes and other minor ones (BYRNE et al., 2012; RASEIRA; FRANZON, 2014).

The importance of the fruit development period (FDP) is due to the possibility of having early ripening cultivars, with late bloom, which may escape from frost occurrence. Short FDP brings benefits to growers, since the commercial value is improved, and the production costs and risks are reduced due to the shorter period that fruits remain in the tree. The risks to which the fruit yield is subjected (wind, hail, pests, diseases, among others) are higher in long-cycle cultivars. Besides reducing risks and costs, when peach trees are cultivated in humid and rainy regions, brown rot (*Monilinia* spp.) incidence is higher (MAY-DE MIO et al., 2008; MAY-DE MIO et al., 2014; MONDINO et al., 2010), requiring frequent fungicide pulverizations, almost every week. This increases grower's and consumer's concern about health and environmental contamination risks (BARÓ-MONTEL et al., 2019; ELSHAFIE et al., 2015), as well as the generation of fungus strains resistant to fungicides (LUO et al., 2010; HILY et al., 2011; ZHU et al., 2012; CHEN et al., 2017; FU et al., 2017).

The knowledge of genetic, phenotypic, and environmental parameters, which directly or indirectly, influences economic important crop traits is fundamental for breeding programs design (RAMALHO et al., 2012). Considering these, the aim of this research was to estimate FB, HD and FDP heritability, as well as to evaluate their distribution in the populations, testing the existence of maternal effect and the relationship of these characters with brown rot incidence.

Materials and methods

The experiment was performed at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil (Latitude 31º 40 'S, Longitude 52º 26' W, altitude 57m) during the 2015-2016, 2016-2017 and 2017- 2018 growing seasons. Sixteen first generation (F_1) progenies controlled crosses were evaluated, being 10 out of them reciprocal crosses. The parents, corresponding to different cultivars and advanced selections, were also evaluated (Table 1).

F_1 progenies were planted in an experimental orchard with trees spaced at 0.5 m within rows and 5 m between rows, where each tree corresponded to a different individual (seedling). Parents had been planted before the trial at working collections orchards on close areas, and trees were spaced at 2 m within rows and 5 m between rows. Each parent has three trees, propagated by budding (clones).

Observations were made in each tree in order to obtain the phenological characters. Data about plants phenology were recorded through the observation of beginning date and full bloom (10% and > 50% of opened flowers, respectively); and ripening dates and harvesting time. Beginning of harvest was considered when at least 10 fruits reached the commercial maturity, and FDP was calculated by the interval (in days) between the date of FB and HD.

The variance observed between the three clones of each parent gave the environmental effect estimation, and the parents' variances average was used as the average environmental variance (σ_e^2). The observed variance among plants of the same progeny was used as the total phenotypic variance (σ_p^2 - genetic plus environmental effects). The genetic variance (σ_g^2) was calculated by subtracting the environmental variance from the total variance of each progeny (CENTELLAS-QUEZADA, 2000; WAGNER JÚNIOR, 2003; CÔRREA, 2007). Broad-sense heritability (H^2) was estimated dividing the genetic variance of each population by the total variance, as shown in the following calculation formula (ALLARD, 1960; DIRLEWANGER et al., 2012; GRIFFITHS et al., 2015):

$$H^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{\hat{\sigma}_e^2}{e}}$$

Where: $\hat{\sigma}_g^2$ = estimated genetic variance; $\hat{\sigma}_e^2$ = estimated environmental variance; e = number of environments (seasons of experiment).

Narrow-sense heritability (h^2) estimates were obtained by linear regression between the average parent and progenies values. The estimated value of h^2 corresponds to the slope of the regression line, which means, the regression coefficient "b" of the line equation $Y = a + bx$ (GRIFFITHS et al., 2015).

Frequency distribution and their histograms were built with FDP data, measured in days between FB and HD, from June 1st.

The maternal effect was evaluated by comparing the population of each cross with its reciprocal, testing the three phenological characters studied (FB, HD and FDP) by the Mann-Whitney test at 5% of probability. This non-parametric test was used due to the nature of the variables (days), being discrete quantitative variables; with two independent samples; without a normal distribution; and presenting populations with different number of individuals. Using the median, as the central distribution parameter, the following contrasts were tested: F_1 versus (vs.) F_1 reciprocal, P_f vs. P_m, $P_f + P_m$ vs. F_1, P_f vs. F_1, P_m vs. F_1, $P_f + P_m$ vs. F_1 reciprocal, P_f vs. F_1 reciprocal, P_m vs. F_1 reciprocal. Where P_f is the female parent, P_m the male parent and F_1 the progeny of hybrid individuals (LONDERO et al., 2009; WU et al., 2012).

Brown rot incidence (BRI) was estimated by the percentage of fruits with symptoms in relation to the total number of fruits. BRI and the phenological characters (FB, HD and FDP) were submitted to Spearman correlation.

Results and discussion

High variability associated to FB and HD characters was observed on the studied populations, evidenced by FB and HD intervals, 36 to 87 days and 138 to 230 days, respectively, both from June 1st. These intervals were closely aligned to the average values of the parents, 42 to 80 and 141 to 215, for FB and HD, respectively. The variability observed on these characters has already been mentioned and studied in several other studies, and among the most recent studies are Hartmann (2013) and Frett (2016).

High variability was also observed for FDP character, indicated by the interval of 72 to 178 days among the individuals of the evaluated progenies. This result was expected, since the same character among the parents ranged from 81 to 157 days, and due to the fact that additive action genes determine this character (SOUZA et al., 1998; VILEILA-MORALES et al., 1981). The present study values are similar to those found by Hartmann (2013), who evaluated nine progenies and eight parents for FDP, and observed high variability for this character, with intervals of 50 to 159 days and 66 to 133 days, for the progenies and the parents, respectively.

For FB, HD and FDP characters, the H^2 estimated values were very high (Table 2). These results are similar to those found in the literature, where the authors agree that broad-sense heritability for these characters is high to very high. Thus, values as 98% (CENTELLAS-QUEZADA, 2000), 67 to 89% (DIRLEWANGER et al., 2012), and 82% (HARTMANN, 2013) are mentioned for FB. Furthermore, for HD, H^2 was estimated ranging from 76 to 99% (DIRLEWANGER et al., 2012), 92% (HARTMANN, 2013), and 58-80% (FRETT, 2016). For FDP, Hartmann (2013) estimated H^2 = 91%, and Corrêa (2007) from 90 to 92%.

The estimation of H^2 is not the most useful for plant breeders, being h^2 more important, and it is calculated by dividing the additive genetic variance by the total phenotypic variance. The selection effect does not depend on the total genetic variance, but on the magnitude of the additive genetic variance. Consequently, h^2 is more relevant to predict the selection response (GRIFFITHS et al., 2015).

The h^2 estimates for FB were from 59.29 to 85.04%, according to the evaluated growing season, with an average of 68.92% (Table 2). The h^2 mean value for FB is similar and intermediate in comparison to those estimated by Hansche (1990) who found 60%, by Hartmann (2013) with 62%, and by Souza et al. (1998) with 78%; and it is almost twice the estimated value found in Hansche et al. (1972), which was 39%.

h^2 for HD was estimated among 66.51 and 81.55%, with an average of 72.23% (Table 2). This value is similar to other estimations for this character, 72% (FRETT, 2016), 79% (HANSCHE et al., 1972), and lower than the estimates observed by Hansche (1986) and Souza et al. (1998), which were 84% and 94%, respectively. Another estimate found byHarman (2013) was 7%, different from the previous ones. The same author mentioned that it was not expected such a low estimate for this character and justified it by stating that the additive genetic variance only explained 4% of the phenotypic variance, while the non-additive genetic component was responsible for 51% of the total phenotypic variance, indicating the presence of major genes associated with this characteristic. In our study, on average, there was a slight tendency towards the early parents.

In addition, h^2 for FDP was estimated between 52.78 and 71.27%, with an average of 65.60% (Table 2). This value was lower than that estimated by Souza et al. (1998), which obtained 91%, but was similar to the one found by Corrêa (2007), 65%, for the same character. These authors conclude that h^2 for FDP in peach trees was very high and high, respectively, in contrast with the estimated values (6%) by Hartmann (2013). According to Hartmann (2013), this low value was due to the estimated additive genetic component which explained only 3% of the total phenotypic variation, while the non-additive genetic component was responsible for 44% of the total phenotypic variation, indicating the association of major genes with this characteristic. It is interesting to point out that the populations studied by Hartmann (2013) were different than the ones available in Brazil.

In most cases, studies that approach phenological characters such as the ones tested in this study indicated that they are considered highly transmissible characters. In other words, they are from an easy inheritance, since narrow-sense heritability is high to very high, and, therefore, selection based on phenotype is effective (ALLARD, 1960; FALCONER; MACKAY, 2001).

The evaluated characters were strongly affected by environmental effects, mainly FB. The 2015-2016 and 2017-2018 growing seasons showed insufficient accumulation of chilling hours below 7.2ºC (74 and 77 hours, respectively) and higher temperatures than the historical averages in the winter and spring. This leads to an earlier and irregular flowering, influencing the other two characters (HD and FDP). In the 2016-2017 growing season, the three phenological characters had the highest h^2 estimates, due to the lower environmental influence (smaller $\widehat{\sigma}_e^2$). During the winter of 2016, according to the data obtained from the weather station of Embrapa Temperate Agriculture, the accumulation of chilling hours was 172, from May to September, positively affecting all genotypes that presented later and more concentrated flowering (AGROMET-EMBRAPA, 2019).

The relative frequency distribution showed that, in general, the progenies were similar do their parents (Figures 1, 2 and 3). The existence of transgressive segregation (individuals in the progenies with values below or above their parents') was generally verified for each one of the three characters and for most progenies, as also observed in previous studies (CORRÊA, 2000; FRETT, 2016; HANSCHE et al., 1972; HANSCHE, 1986; HARTMANN, 2013; SOUZA et al., 1998).

According to the histograms, it was observed differences when using a female or male parent, since the progenies distribution is not the same when compared to their respective reciprocal progenies (Figures 1B to 1F, 2B to 2F and 3B to 3F). Furthermore, it was noted a tendency to group a larger number of individuals within or near the female parent class; although, this has not happened in all the progenies. This trend, observed in the histograms for the three phenological characters evaluated could suggest some kind of maternal effect. When this occurs, heritability estimates that not considering this maternal effect could lead to a bias in the heritability coefficients estimates (ELER, 2014). Reciprocal crosses are the simplest evidence of maternal effect, since they produce individuals genetically similar but phenotypically different (ELER, 2014; RAMALHO et al., 2012), if there is indeed a significant maternal effect. This tendency of individuals' concentration (seedlings) in the class corresponding to the female parent was previously mentioned by Corrêa (2007), for the FDP character, and by Frett (2016) in a HD study.

This possible effect was tested comparing contrasts for the three phenological characters studied by the Mann-Whitney test, and Figure 4 synthesizes the results. This scheme contains the five F_1 progenies with their reciprocal progenies, indicating the parents and progenies median values, and whether the contrast was significant or not.

Firstly, when parents were compared (female vs. male parent), in all cases and for the three characters, the difference between them was significant. On the other hand, for the contrasts between the reciprocal progenies (F_1 vs. reciprocal F_1), only one of the reciprocal crosses was significant for the FB character (2008.159 vs. 2009.38). This indicates differences when using Conserva 1526 and 'Cerrito', as female or male parents, since the progenies generated by them were statistically different. In the other reciprocal crosses and in all the HD and FDP characters contrasts, there were no significant differences between the reciprocal progenies, indicating that there is no difference for this character when using one of the parents as female or male.

When the two parents were confronted with their progenies, $P_f + P_m$ vs. F_1 e $P_f + P_m$ vs. F_1 reciprocal contrast, only three out of 30 tested contrasts showed significant differences. One cross for each character was used; for FB, it was 'Chimarrita' + Cascata 1055 vs. 2012.43, and for HD and FDP, it was Conserva 1662 + 'Maciel' vs. 2012.68. For the first crossing, the progenies were closer to the female parent ('Chimarrita'), which had earlier flowering. In the other two significant crosses, the progeny was also closer to the female parent (Conserva 1662), with later HD and longer FDP. In the remaining contrasts, there was no significant difference, indicating that the median of FB, HD and FDP of the parent is equal to their progenies' median.

For FB character (Figure 4A), when P_f vs. F_1, P_m vs. F_1, P_f vs. F_1 reciprocal e P_m vs. F_1 reciprocal were confronted, i.e., the male and female parents were separately against their own progenies. It was observed only five crosses with significant differences out of the 20 tested contrasts, and four of these correspond to the male parent against their progeny. Thereby, there is a trend towards the female parent for the FB character, since nine out of ten contrasts among the female parents against their own progenies showed no significant differences. However, no major differences were observed between male parents against their own progenies, where four of them showed significant differences and six did not.

When studying the P_f vs. F_1, P_m vs. F_1, P_f vs. F_1 reciprocal and P_m vs. F_1 reciprocal contrasts analysis in the HD character (Figure 4B), it was observed that five out of 20 tested contrasts were significant, and four of these correspond to the male parent against its progeny. In the same way as observed for FB, a trend towards the female parent was identified.

For the FDP character (Figure 4C), in these same contrasts, it was observed that seven out of 20 tested contrasts were significant. Four of these correspond to the same cross and their reciprocal (Conserva 1526 x 'Cerrito' e 'Cerrito' x Conserva 1526), and these parents were highly contrasting for FDP. In relation to the 16 remaining contrasts, only three were significant; two of the male parents against his own progeny and one of the female parents with his respective progeny. Thus, it can be highlighted again a slight tendency towards the female parent, although most of the contrasts were not significant, considering the parent medians to be equal to their F_1 progenies medians.

In summary, after analyzing the individual progenies distribution for the three studied phenological characters and due to their high heritability estimates, it may be inferred that there is a predominantly genes additive action (ALLARD, 1960). Deviations could be attributed to genes of major effect or to a possible maternal effect (ROACH; WULFF, 1987).

When the phenological characters were correlated between each other, all of them showed highly significant correlations (Table 3). A very high positive correlation was found between HD and FDP, indicating that the later the genotypes harvest, the larger is the FDP. Souza et al. (1998) and Hartmann (2013) also observed high correlations between these two characters. This could be expected since selection for low chill generally leads to early blooming, even in genotypes selected for harvest season.

Regarding BRI, the variability was very high and strongly affected by the rainfall occurrence during the harvest season. The percentage of BRI ranged from 0 to 100%, with an average of 39.59% among all genotypes, on the three growing seasons evaluated. The correlations between BRI and phenological characters only showed a low negative correlation with HD (Table 3), indicating that the later the HD, the lower is the BRI, and vice-versa. This last correlation can be explained because the late harvesting was originated by parents with certain degree of resistance to *Monilinia fructicola* infection; hypothesis that should be confirmed with controlled conditions trials.

Conclusions

The heritability of phenological characters (full bloom, harvest date, and fruit development period), in peach, is medium to high.

The inheritance of the studied phenological characters is predominantly additive, and deviations can be attributed to the maternal effect or major genes.

The phenological characters are correlated to each other and, in the studied progenies, brown rot incidence is negatively correlated with the harvest date.

References

AGROMET-EMBRAPA, Laboratório de Agrometeorologia, Embrapa Clima Temperado, Relatório: Horas de frio, Embrapa Sede - Pelotas (Monte Bonito). Pelotas: Embrapa, 2019. Disponível em <http://agromet.cpact.embrapa.br/>. Acesso em: 28 mar. 2019.

ALLARD, R.W. **Principles of plant breeding**. New York: John Wiley & Sons, Inc., 1960. 485 p.

BARÓ-MONTEL, N.; EDUARDO, I.; USALL, J.; CASALS, C.; ARÚS, P.; TEIXIDÓ, N.; TORRES, R. Exploring sources of resistance to brown rot in an interspecific almond × peach population. **Journal of the Science of Food and Agriculture**, v. 99, n. 8, p. 4105-4113, 2019. DOI: 10.1002/jsfa.9640.

BYRNE, D.H.; RASEIRA, M.B.; BASSI, D.; PIAGNANI, M.C.; GASIC, K.; REIGHARD, G.L.; MORENO, M.A.; PÉREZ, S. Peach. In: BADENES, M.L.; BYRNE, D.H. **Fruit breeding**. New York: Springer p. 505-569, 2012.

CENTELLAS-QUEZADA, A. **Herdabilidade da época de floração e estudos sobre a ferrugem da folha [*Tranzchelia dsicolor* Fckl. (Tranz. & Litv.)] em pessegueiro [*Prunus persica* (L.) Batsch]**. 2000. 54f. Tese (Doutorado em Fruticultura de Clima Temperado) Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2000.

CHEN, S.; YUAN, N.; SCHNABEL, G.; LUO, C. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. **Molecular Plant Pathology**, v. 18, n. 1, p. 90-97, 2017. DOI: 10.1111/mpp.12387.

CITADIN, I.; SCARIOTTO, S.; SACHET, M.R.; ROSA, F.J.; RASEIRA, M.C.B.; WAGNER JUNIOR, A. Adaptability and stability of fruit set and production of peach trees in a subtropical climate. **Scientia Agricola**, Piracicaba, v. 71, n. 2, p. 133-138, 2014. DOI: 10.1590/S0103-90162014000200007.

CORRÊA, E.R. **Estudo da herdabilidade de alguns carácteres em pessegueiro: ciclo, tonalidade da cor da polpa e compostos fenólicos**. 2007. 52f. Dissertação (Mestrado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2007.

DIRLEWANGER, E.; QUERO-GARCÍA, J.; LE DANTEC, L.; LAMBERT, P.; RUIZ, D.; DONDINI, L.; ILLA, E.; QUILOT-TURION, B.; AUDERGON, J.M.; TARTARINI, S.; LETOURMY, P.; ARÚS, P. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. Oxford: **Heredity**, v. 109, p. 280-292, 2012. DOI: 10.1038/hdy.2012.38.

ELER, J.P. **Teorias e métodos em melhoramento genético animal**; I- Bases do melhoramento genético animal. Universidade de São Paulo, Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga: USP, 2014. 249 p.

ELSHAFIE, H.S.; MANCINI. E.; CAMELE, I.; DE MARTINO, L.; DE FEO, V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. **Industrial Crops and Products**, v. 66, p.11-15, 2015. DOI: 10.1016/j.indcrop.2014.12.031.

FALCONER, D.S.; MACKAY, T.F.C**. Introducción a la genética cuantitativa**. Zaragosa: Acribia S.A, 2001. 490 p.

FRETT, T.J. **Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (*Xap*) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted aelection (MAS) in the University of Arkansas peach and nectarine breeding program**, 2016. 718p. Thesis (Doctor of Philosophy in Plant Science), Clemson University, Arkansas, 2016.

FU, W.; TIAN, G.; PEI, Q.; GE, X.; TIAN, P. Evaluation of berberine as a natural compound to inhibit peach brown rot pathogen *Monilinia fructicola*. **Crop Protection**, v. 91, p. 20-26, 2017. DOI: 10.1016/j.cropro.2016.09.008.

GRIFFITHS, A. J. F.; WESSLER, S. R.; CARROLL, S. B.; DOEBLEY, J. **Introduction to Genetical Analysis** (10th ed.), New York: W. H. Freeman and Company, 2015. 896 p.

HANSCHE, P.E. Heritability of fruit quality traits in peach and nectarine breeding stocks dwarfed by the *dw* gene. **HortScience**, v. 21, n. 5, p. 1193-1195, 1986.

HANSCHE, P. E. Heritability of spring bloom and fall leaf abscission dates in *Prunus persica*. **HortScience**, v. 25, n. 12, p. 1639-1641, 1990.

HANSCHE, P. E.; HESSE, C. O.; BERES, V. Estimates of genetic and environmental effects on several traits in peach. **Journal of the American Society for Horticultural Science**, Alexandria, v. 97, n. 1, p. 76-79, 1972.

HARTMANN, T.P. **Heritability and phenotypic correlations in peach [*Prunus persica* (L.) Batsch]**, 2013. 136p. Thesis (Master of Science), Texas A&M University, Texas, 2013.

HILY, J.M.; SINGER, S.D.; VILLANI, S.M.; COX, K.D. 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**, v. 67, p. 385-396, 2011. DOI: 10.1002/ps.2074.

HERTER, F.G.; WREGE, M.S.; TONIETTO, J.; FLORES, C.A. Adaptação edafoclimática. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 45-56, 2014.

LONDERO, P.M.G.; RIBEIRO, N.D.; CERUTTI, T.; MAZEIRO, S.M.; ROSA, D.P.; ROSA, S.S. Maternal effect in sulfur amino acids content expression in common bean grains. **Ciência Rural**, Santa Maria, v. 39, n. 6, p. 1884-1887, 2009. DOI: 10.1590/S0103-84782009005000129.

LUO, C.X.; HU, M.J.; JIN, X.; YIN, L.F.; BRYSON, P.K.; SCHNABEL, G. An intron in the cytochrome *b* gene of *Monilinia fructicola* mitigates the risk of resistance development to Qol fungicides. **Pest Management Science**, v. 66, p. 1308-1315, 2010. DOI: 10.1002/ps.2016.

MAY-DE MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. **Tropical Plant Pathology**, Viçosa, v. 33, n. 3, p. 227-234, 2008. DOI: 10.1590/S1982-56762008000300008.

MAY-DE MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 355-432, 2014.

MONDINO, P.; ALANIZ, S.; LEONI, C. Manejo integrado de las enfermedades del duraznero en Uruguay. In: SORIA, J. Manual del duraznero; Manejo integrado de plagas y enfermedades. Montevideo: INIA. p. 45-76, 2010

RAMALHO, M.A.P.; SANTOS, J.B.; PINTO, C.B.P.; SOUZA, E.A.; GONÇALVES, F.M.A.; SOUZA, J.C. **Genética na agropecuária**. Lavras: UFLA, 2012. 565 p.

ROACH, D.A.; WULFF, R.D. Maternal effects in plants. **Annual Reviews Ecology and Systematics**, Palo Alto, California, v. 18, p. 209-235, 1987.

RASEIRA, M.C.B.; FRANZON, R.C. Melhoramento genético. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, p. 57-72, 2014.

RASEIRA, M.C.B.; NAKASU, B.H. Cultivares: descrição e recomendação. In: MEDEIROS, C.A.B.; RASEIRA, M.C.B. A cultura do pessegueiro. Brasília: Embrapa-SPI, p. 29-98, 1998.

SCORZA, R.; SHERMAN, W.B. Peaches. In: JANICK, J.; MOORE, J.N. **Fruit breeding, tree and tropical fruit: temperature fruits**. New York: J. Wiley & Sons, Inc., p. 325-440, 1996.

SOUZA, V.A.B.; BYRNE, D.H. E TAYLOR, J.F. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruits traits. **Journal of the American Society for Horticultural Science.** v. 123, n. 4, p. 604-611, 1998. DOI: 10.21273/JASHS.123.4.604

VILEILA-MORALES, E.A.; SHERMAN, W.B.; WILCOX, C.L.; ANDREWS. C.P. Inheritance of short fruit development period in peach. **Journal of the American Society for Horticultural Science.** v. 106, p. 399-401, 1981

WAGNER JÚNIOR, A. **Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey**. 2003. 62f. Dissertação (Mestrado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2003.

WU, B. H.; ZHAO, J. B.; CHEN, J.; XI, H. F.; JIANG, Q.; LI, S. H. Maternal inheritance of sugars and acids in peach (*P. persica* (L.) Batsch) fruit. **Euphytica**, v. 188, p. 333–345, 2012. DOI: 10.1007/s10681-012-0668-2

ZHU, F.; BRYSON, P.K.; SCHNABEL, G. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. **Pest Management Science**, v. 68, n. 7, p. 1003-1009, 2012. DOI: 10.1002/ps.3255.

Table 1 – Parents of F_1 progenies and number of seedlings of each progeny, in the Peach Breeding Program at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

	F_1 progeny identification	**Parents** ♀	x	♂	**N° of seedlings**
Reciprocal crosses	2008.159	Conserva 1526		'Cerrito'	7
	2009.38	'Cerrito'		Conserva 1526	23
	2012.26	Cascata 1055		'Chimarrita'	18
	2012.43	'Chimarrita'		Cascata 1055	25
	2012.49	Conserva 672		Conserva 1526	18
	2012.61	Conserva 1526		Conserva 672	7
	2012.52	Conserva 947		Conserva 1600	17
	2012.66	Conserva 1600		Conserva 947	12
	2012.68	Conserva 1662		'Maciel'	24
	2012.88	'Maciel'		Conserva 1662	17
	2012.31	Cascata 1359		Cascata 1577	31
	2012.46	Chorão		'Maciel'	25
	2012.99	Necta 506		'Sunmist'	20
	2012.107	Necta 532		Necta 480	25
	2012.111	Necta 540		'Morena'	25
	2012.114	'BRS Rubimel'		TX2D163	21

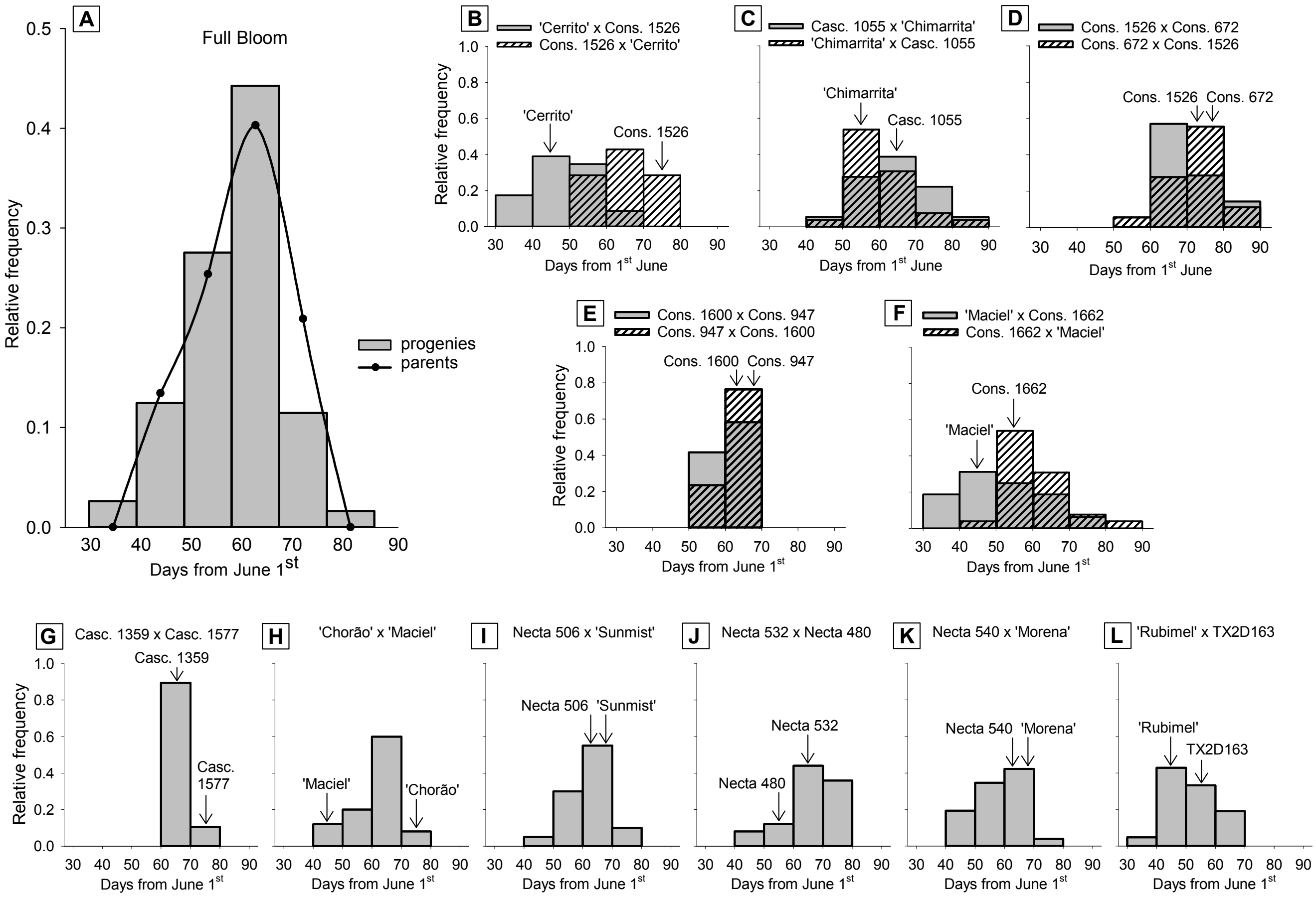


Figure 1 – Full bloom histograms (expressed in days from June 1st). Frequency of seedlings for all progenies and all parents used on the study (A), individual F_1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Clima Temperado, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

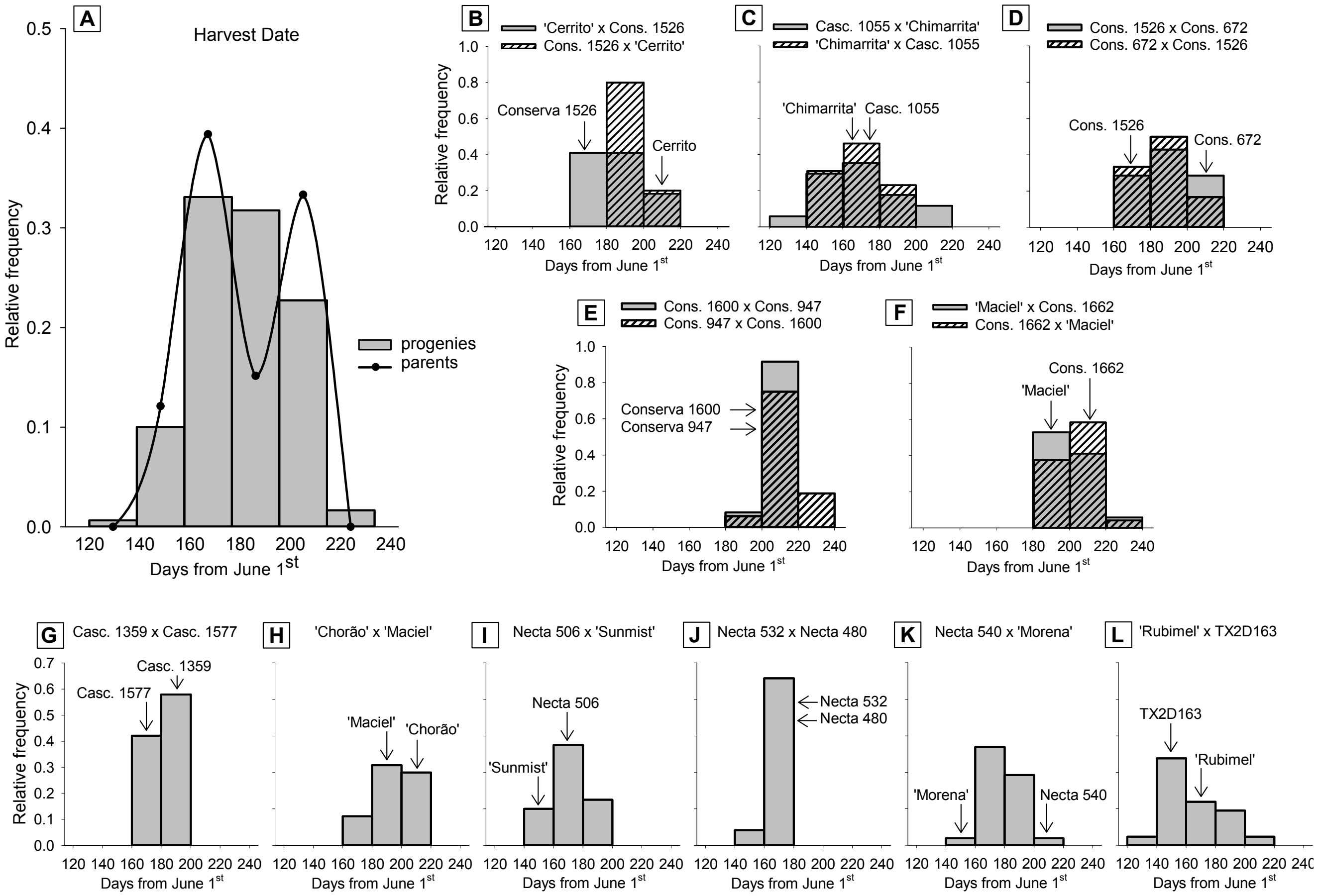


Figure 2 – Harvest date histograms (expressed in days from June 1st). Frequency of seedlings for all progenies and all parents used on the study (A), individual F_1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Clima Temperado, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

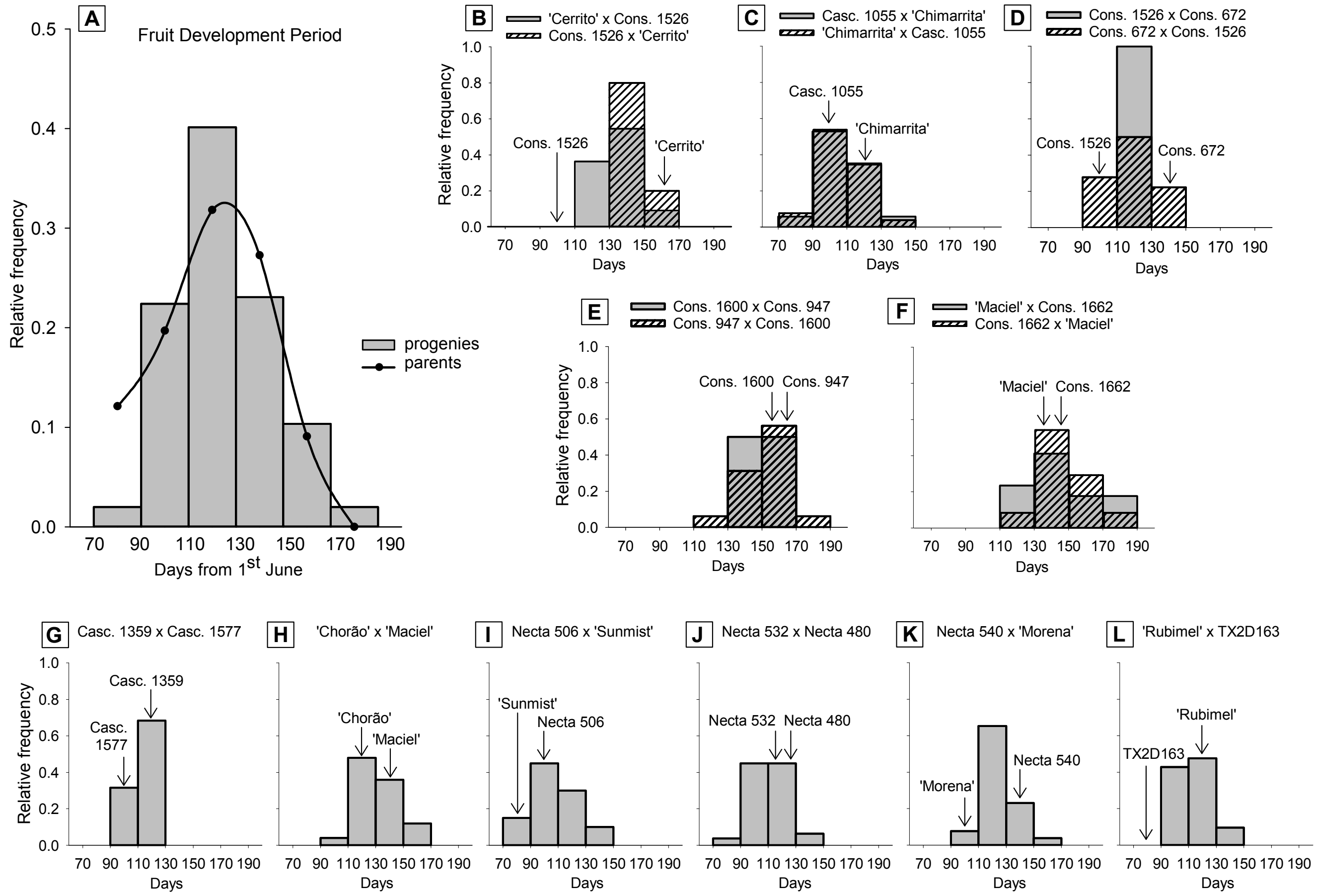


Figure 3 – Fruit development period histograms (expressed in days). Frequency of seedlings for all progenies and all parents used on the study (A), individual F_1 progenies (B to L), being B to F reciprocal crosses. Arrows represent parents. Peach Breeding Program at Embrapa Clima Temperado, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

Table 2 – Broad-sense (H^2) and narrow-sense (h^2) estimated heritability for full bloom (FB), harvest date (HD) and fruit development period (FDP) in peach progenies of the Peach Breeding Program at Embrapa Clima Temperado, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

	H^2	h^2			
		2015/16	2016/17	2017/18	Mean
FB	96.21%	59.29%	85.04%	62.42%	68.92%
HD	98.43%	66.51%	81.55%	68.64%	72.23%
FDP	95.29%	71.27%	72.75%	52.78%	65.60%

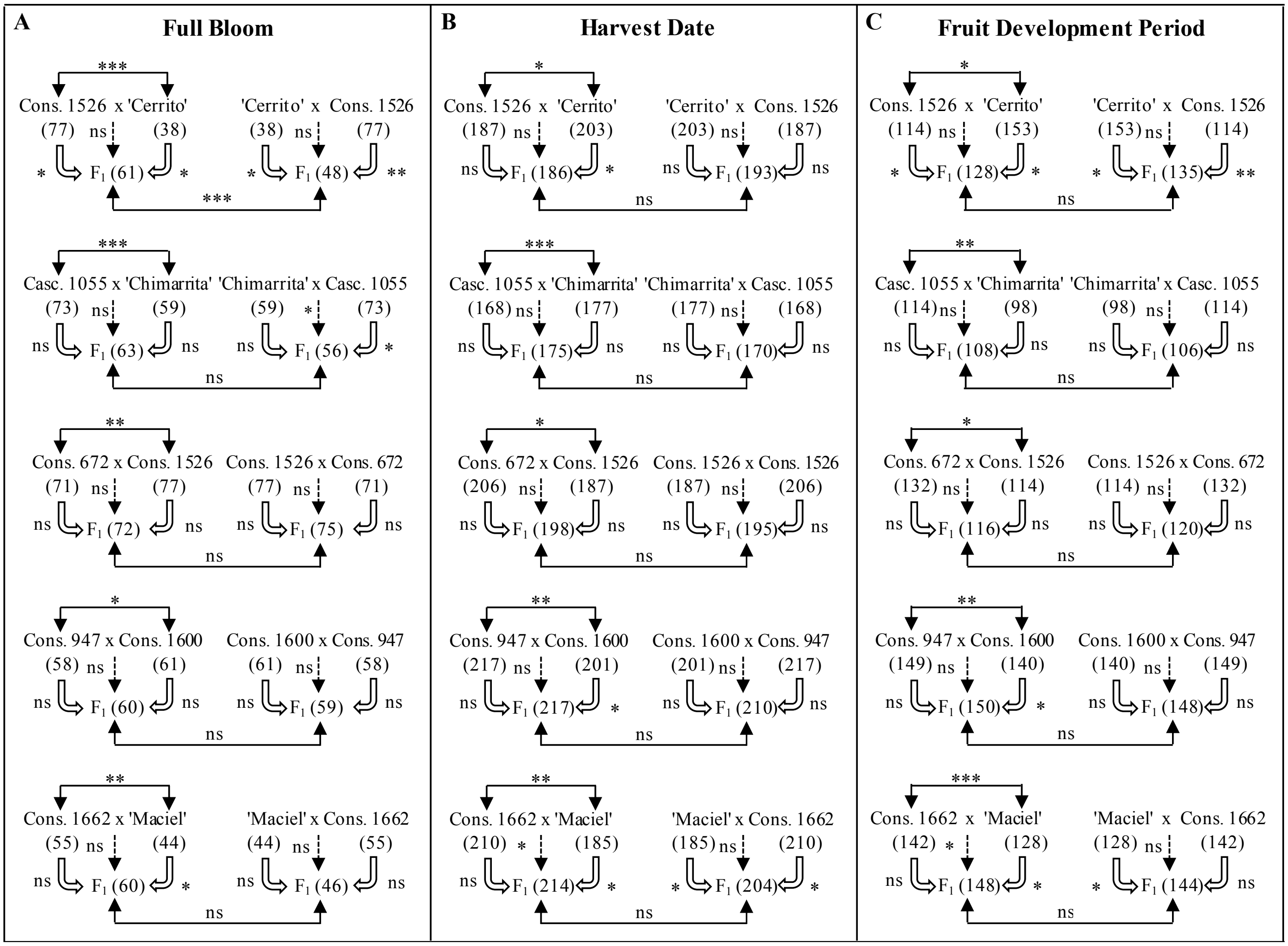


Figure 4 – Parental and F_1 progenies and F_1 reciprocal, with their respective median values (parentheses) for the full bloom (A), harvest date (B) (expressed in days from June 1st) and fruit development period (C) (expressed in days). Significance was tested with the Mann-Whitney test in the contrasts between F_1 vs. F_1 reciprocal, female parental (P_f) vs. male parental (P_m), $P_f + P_m$ vs. F1, P_f vs. F_1, P_m vs. F_1, $P_f + P_m$ vs. F_1 reciprocal, P_f vs. F_1 reciprocal, and P_m vs. F_1 reciprocal.
^{ns}, *, ** and ***, nonsignificant and significant at $P \leq 0.05$, ≤ 0.01, and ≤ 0.001, respectively.
Median values were calculated first between the three different growing seasons (2015-2016, 2016-2017 and 2017-2018). Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

Table 3 – Spearman´s correlation between phenological characters (full bloom, harvest date and fruit development period) and percentage of brown rot incidence in peach progenies orchard of the Peach Breeding Program at Embrapa Clima Temperado, in 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

	FB	HD	FDP	BRI
FB [(1)]	-	**	***	ns
HD [(2)]	0.144	-	***	*
FDP [(3)]	-0.270	0.887	-	ns
BRI [(4)]	-0.056	-0.121	-0.062	-

[(1)] full bloom; [(2)] harvest date; [(3)] fruit development period; [(4)] brown rot incidence in the orchard (%).

In the lower diagonal the Spearman´s correlation value. In the upper diagonal: ns, *, **, ***; nonsignificant and significant at $P \leq 0.05$, ≤ 0.01, and ≤ 0.001, respectively.

4.2 Artigo 2. Blossom blight resistance in peach: heritability and progeny segregation

Artigo a ser submetido na revista *"Ceres"*

12 - Plant breeding applied to agriculture
12.05 - Plant Breeding
12.05.05 - Breeding of fruit trees

Blossom blight resistance in peach: heritability and progeny segregation

Maximiliano Dini[3]*, Maria do Carmo Bassols Raseira[4], Bernardo Ueno[5]

ABSTRACT

This study aimed to identify genotypes with higher levels of resistance to blossom blight; to estimate the heritability of this character; study the frequency distribution in populations; and the existence of maternal effect over it. The studied populations present low phenotypic variability regarding the resistance/susceptibility to *Monilinia fructicola*, being most of them susceptible or very susceptible to the disease. Among the tested genotypes, Maciel and Cerrito cultivars showed less susceptibility to the disease, transmitting this character to their progenies. Heritability estimates of the blossom blight resistance were medium to low. The low heritability and its distribution in the progenies suggest that the character has additive inheritance, without detecting deviations associated with maternal effects. Further studies aiming to adjust a phenotype protocol for this character are needed in order to accurately differentiate genotypes with different levels of genetic resistance. Such studies should be mainly related with the conidia concentration to be inoculated, as well as the elaboration of a more detailed scale of severity.

Key words: *Prunus persica* (L.) Batsch, *Monilinia fructicola* (Winter) Honey, genetic resistance, heritability, progeny segregation.

[2] Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Programa de Pós-Graduação em Agronomia, Pelotas, Rio Grande do Sul, Brazil. maxidini@hotmail.com.br
[3] Fruit Breeding, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. maria.bassols@embrapa.br
[4] Plant Pathology, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. bernardo.ueno@embrapa.br
*Corresponding author: maxidini@hotmail.com

INTRODUCTION

The fungus *Monilinia fructicola* (Winter) Honey, is the most important pathogen of the peach culture in Brazil, as well as in other producing regions of the world due to the large losses that may cause, when its control is incorrectly done (ADASKAVEG *et al.*, 2008; AGRIOS, 1998; FORTES & MARTINS, 2003; MAY-DE MIO *et al.*, 2008, 2014; OGAWA *et al.*, 1995). This pathogen may attack the peach during the whole cycle, but especially during flowering and fruit ripening, which are the most susceptible phases (BLEICHER, 1997; MAY-DE MIO *et al.*, 2014). The cycle of the disease begins during flowering, causing blossom blight. Normally necrotic flowers remain attached to the branch, which may be infected by the fungus, resulting in cancers and twig blight (MAY-DE MIO *et al.*, 2008, 2014; MONDINO *et al.*, 2010).

The blossom blight is the primary infection of the disease and has great epidemiological importance, since it is a inoculum source for secondary infections in the fruits, directly, by the production of conidia or in the form of latent infections in the fruit in formation, developing only during the ripening stage of the fruit (GARCIA-BENITEZ *et al.*, 2016; 2017; MAY-DE MIO *et al.*, 2014; MONDINO *et al.*, 2010; THOMIDIS, 2017).

Under mild, humid and rainy weather conditions this disease can cause total crop loss and, in an attempt to reduce these losses, growers may apply fungicides weekly. Nowadays, with the increasing worry about the environment and the health of growers and consumers (BARÓ-MONTEL *et al.*, 2019; ELSHAFIE *et al.*, 2015), as well as the occurrence of fungus strains resistant to the main fungicide molecules used (LUO *et al.*, 2010; HILY *et al.*, 2011; ZHU *et al.*, 2012; CHEN *et al.*, 2017; FU *et al.*, 2017), emphasize the importance of other control strategies such as genetic resistance, in order to reduce the usage of these substances. This is the most efficient way to control the disease, reducing production costs and environmental impact.

The selection of resistant genotypes is still very limited due to the lack of knowledge of good of resistance or immunity sources (RASEIRA & FRANZON, 2014). The peach resistance to *Monilinia* is a quantitative and polygenic trait, considered as a character of difficult transmission from the parents to the progenies and highly influenced by the environment (WAGNER JÚNIOR *et al.* 2003, 2005; RASEIRA & FRANZON, 2014). However, there are significant differences in susceptibility among the available genotypes (ADASKAVEG *et al.* 2008; SANTOS & UENO, 2014).

There are evidences that there is no correlation between flower and fruit resistance (FABIANE, 2011; SANTOS *et al.*, 2012; WAGNER JUNIOR *et al.*, 2003). In cv. Bolinha for example, that has been widely studied as a pattern of resistance to this disease, there was low level of resistance in flowers, unlike to the reaction in fruits (SANTOS *et al.*, 2012). Therefore, the selection of resistant genotypes must be done independently, for the blossom blight and brown rot in fruits (RASEIRA & FRANZON, 2014; WAGNER JUNIOR *et al.*, 2003), however there are few studies focused on resistance in flowers. Thus, the aims of this work were: to identify genotypes with higher levels of resistance; to estimate the heritability; to study their distribution in populations; and to test the existence of a maternal effect.

MATERIAL and METHODS

The study was performed at Embrapa Clima Temperado, in Pelotas, RS, Brazil (Latitude 31º 40' S, Longitude 52º 26' W, altitude 57 m), in the years 2015 and 2016. It was tested the susceptibility to blossom blight in peaches from reciprocal hybridizations

(F_1 progenies), as well as their parents. The reciprocal F_1 progenies used were: 2008.159 (Conserva 1526 x 'Cerrito') and 2009.38 ('Cerrito' x Conserva 1526); 2012.26 (Cascata 1055 x 'Chimarrita') and 2012.43 ('Chimarrita' x Cascata 1055); 2012.49 (Conserva 672 x Conserva 1526) and 2012.61 (Conserva 1526 x Conserva 672); 2012.52 (Conserva 947 x Conserva 1600) and 2012.66 (Conserva 1600 x Conserva 947); 2012.68 (Conserva 1662 x 'Maciel') and 2012.88 ('Maciel' x Conserva 1662). The progenies had a minimum of seven and a maximum of 25 seedlings evaluated.

For testing the reaction to blossom blight, the technique of detached flowers cited by Fabiane (2011) was used as the most efficient technique to this purpose. The experiment was arranged in a randomized complete block design, considering each genotype as one treatment (seedlings and parents). For the seedling 12 flowers were inoculated, divided into three replicates of four flowers. In the case of the parents, there were three plants obtained by grafting (clones) in which were evaluated three replicates of four flowers per clone. Four more flowers per genotype (or clone) without inoculation (control), were also observed to estimate the proportion of latent inoculum coming from the field. The selections Conserva 655 and Cascata 1055 were used as standard of high and low susceptibility to blossom blight, respectively (FABIANE, 2011). The cultivar Bolinha, standard of resistance to *M. fructicola* in fruits was also included.

The fungus isolate was obtained from mummified fruits, infected by *M. fructicola*, collected at four different sites of Embrapa Clima Temperado peach orchards (Pelotas, RS, Brazil). From these, fragments of approximately 5mm were collected and transferred to Petri dishes containing Potato Dextrose Agar (PDA) culture medium and incubated in a growth chamber at 25 ± 2°C for seven to ten days, with 12 hours light. Contamination with other fungi or bacteria was eliminated by successive passages until the pure culture was obtained. The fungal isolate was stored in test tubes with PDA culture medium in a cold chamber (4 ± 1°C). Whenever necessary, the fungus was passaged on ripe peach fruits to re-insulate it in 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 a Neubauer chamber. The concentration was adjusted to 1 x 10^5 conidia mL^{-1} (FABIANE, 2011; SANTOS et al., 2012; WAGNER JÚNIOR et al. 2005).

Productive branches containing flower buds at half inch green and pink stages, stages 3 and 4 according to Chapman and Catlin (1976) were collected from the individuals to be tested. The branches were prepared by removing open or damaged flowers and were kept in buckets with water, inside a cold room during 48 hours at 4 ± 1°C, in order to standardize flowering (SANTOS *et al.*, 2012), and also to avoid or reduce the contamination with pathogens (LUO *et al.*, 2001; MAY-DE MIO *et al.*, 2008). After 48 hours in the chamber the branches were left for another 24 hours at room temperature for the opening of flowers (anthesis). After this period, 16 open flowers with no symptoms were selected from each plant. It was used plastic boxes (50 × 35 × 10 cm) with phenolic foam with cells (2.5 × 2.5 × 3.8 cm) (Green-up®) previously washed in running water for 30 minutes. In each cell of the foam one flower with a small portion of the twig was fixed.

The inoculation was done by spraying, using a fine droplet spray, with approximately 0.8 mL of the *M. fructicola* conidial suspension, per box (FABIANE, 2011; SANTOS *et al.*, 2012) containing between 140 and 200 flowers. Adjustment of the spray volume was done using water-sensitive cards, aiming at the correct coverage of flowers.

After inoculation, the boxes with the flowers, were covered with a plastic bag and placed in a growth chamber (Fitotron), with 23°C±1°C temperature, 75% humidity and 12h light. After 72 and 120 hours, the incidence and severity of the blossom blight were evaluated. Those flowers with petals with necrotic spots were considered infected (FABIANE, 2011; SANTOS *et al.*, 2012; WAGNER JÚNIOR 2003). Severity was assessed on a grading scale from 0 to 4 (Table 1 and Figure 1).

Infections in anthers and or pistils were not evaluated because these organs are the most sensitive to fungi (MAY-DE MIO *et al.*, 2014; OGAWA *et al.* 1995) and also present growth of other pathogens such as *Cladosporium*, *Penicillium*, *Alternaria* and *Botrytis* (MAY-DE MIO *et al.*, 2008; OGAWA *et al.*, 1995). Furthermore the work was developed without previous flower disinfection, having influence of the natural inoculum from the field.

The grading scale used (Table 1) was elaborated aiming at its ease of use during evaluation. The scale limits that determine the scores correspond to the percentage area of a flower with the presence of necrotic spots.

Table 1: Grading scale for assessing the severity of the blossom blight in peach.

Score	Description
0	Without infection
1	Necrotic spots on the petals covering > 1% ≤ 20%
2	Necrotic spots on the petals > 20% ≤ 40%
3	Necrotic spots on the petals > 40% ≤ 60%
4	Necrotic spots on the petals > 60%

The limits described in the grading scale were transformed into a scale of figures (Figure 1), with the ImageJ program and photographs of flowers evaluated 72 and 120 hours after inoculation (hai) with *M. fructicola* under the same conditions of the experiment. Therefore, the standard of this scale was specific for artificially inoculated flowers and under the conditions of this experiment.

Scale

without infection 20% of necrotic spots 40% of necrotic spots 60% of necrotic spots

Figure 1: Severity scale used to evaluate the blossom blight in peach flowers artificially inoculated by spraying.

To evaluate the segregation of the progenies and to test for the existence of maternal effect, relative frequency histograms were constructed with the severity data. The maternal effect comparing the progeny of one of the crosses with the reciprocal progeny was also tested using the Mann-Whitney test at 5% significance Dini *et al.*, 2019b fenologia RBF).

The broad sense heritability (H^2) was estimated according to Dini *et al.* (2019b fenologia RBF) for the character of resistance to the blossom blight.

The calculation was based on the data obtained in the two evaluation seasons, the estimated environmental variance ($\hat{\sigma}^2_e$) divided by two (number of environments) (DIRLEWANGER et al., 2012; GRIFFITHS et al., 2015).

RESULTS and DISCUSSION

It was possible to evaluate the reaction to *M. fructicola* in the flowers in 129 e 148 *seedlings* in the years of 2015 and 2016, respectively. Nine parents and the genotypes

Conserva 655 and 'Bolinha' were evaluated in both years. The lack of data for all individuals available is due to the fact that many of them did not bloom or the flowers were not in adequate condition during the experiment period (either by age or plant size, or even by adverse climatic factors). The vegetative cycle of 2015 and 2016 was characterized by the occurrence of higher temperatures than normal during flowering (July to August), and high rainfall (Figure 2), conditions that favored the attack of *M. fructicola* on flowers. There were cases in which the samples had to be discarded due to the high infection of the flower buds.

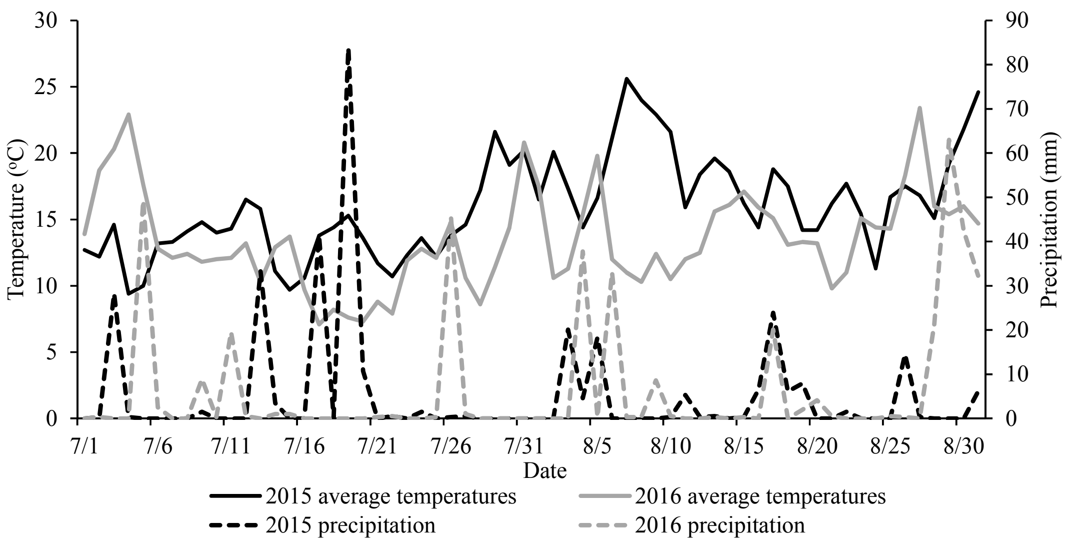


Figure 2: Average temperatures and daily rains in July and August of 2015 and 2016, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. Source: AGROMET/CPACT/EMBRAPA (Pelotas, Rio Grande do Sul, Brazil).

It should be noted that the phytosanitary management of peach seedlings, in the Embrapa Clima Temperado breeding program, is restricted to fungicide applications in the winter period. During spring and summer only insecticides are applied to pest control. This management is performed, because one of the aims of the program of Embrapa is to obtain cultivars that present good performance regarding to the main pathogens, as the *M. fructicola*.

A high variability in the incidence of blossom blight in non-inoculated flowers (field infestation) was detected. This fact was indicated by the high phenotypic variance observed, mainly, in the evaluation after 72 hours, among the individuals of the progenies and parents evaluated. Which was 1015.07 and 610.53 for progenies, and 1144.94 and 729.66 for parents, in the years of 2015 and 2016, respectively (Table 2). This shows the presence of inoculum in the orchard during blossom. The variability can be explained by the different levels of susceptibility to the disease associated to the genotype, by differences in blossom time (temporal) and geographic location within of the Embrapa orchard (spatial), among studied genotypes (SANTOS *et al.*, 2012). The high incidence of blossom blight, even without a previous inoculation has already been reported (KESKE *et al.*, 2010; SANTOS *et al.*, 2012) and is due to the high pressure from the natural inoculum present in peach orchards in southern Brazil (FORTES & MARTINS, 1998; MAY-DE MIO *et al.*, 2009; 2014) and favorable climatic conditions for the disease at flowering season (Figure 2).

The score range for the disease incidence was the maximum possible (0 to 100% incidence) in flowers without inoculation, in the evaluations performed in the 72h and in the two years of evaluation, for both progenies and parents. When the flowers were inoculated, after 72 hours the score range was also maximum in the year 2015, but in 2016, it was between 50.00 and 100% and 44.44 to100%, for the progenies and parents respectively (Table 2).

Table 2: Descriptive statistics of the variables of incidence and severity of blossom blight in 10 reciprocal peach progenies and their parents, evaluated after 72 and 120 hours, with and without artificial inoculation, in the years 2015 and 2016, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

		Non-inoculated				Inoculated			
		72 hours		120 hours		72 hours		120 hours	
	Progenies[1]	Inc. (%)	Sev.[3] (0 to 4)	Inc. (%)	Sev. (0 to 4)	Inc. (%)	Sev. (0 to 4)	Inc. (%)	Sev. (0 to 4)
2015	Means	69.93	0.81	84.15	1.27	93.08	2.24	97.14	3.48
	Median	75.00	0.75	100	1.00	100	2.46	100	4.00
	PV	1015.07	0.25	565.88	0.55	321.35	1.08	88.69	0.69
	SD	31.86	0.50	23.79	0.74	17.93	1.04	9.42	0.83
	CV (%)	45.56	62.19	28.27	58.72	19.26	46.51	9.69	23.90
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	41.67	0.75
	Maximum	100	2.75	100	3.75	100	3.91	100	4.00
	Parents[2]								
	Means	57.41	0.66	72.22	1.15	86.94	1.95	91.71	3.27
	Median	50.00	0.50	75.00	1.00	100	2.08	100	3.75
	PV	1144.94	0.24	1169.87	0.73	798.29	1.04	380.88	1.29
	SD	33.84	0.49	34.20	0.86	28.25	1.02	19.52	1.14
	CV (%)	58.94	74.69	47.36	74.52	32.50	52.33	21.28	34.77
	Minimum	0.00	0.00	0.00	0.00	0.00	0.00	30.00	0.58
	Maximum	100	2.00	100	3.25	100	3.83	100	4.00
2016	Progenies								
	Means	85.81	0.97	96.29	1.64	96.94	1.66	99.24	3.12
	Median	100	1.00	100	1.50	100	1.50	100	3.25
	PV	610.63	0.16	138.68	0.49	55.13	0.48	13.70	0.67
	SD	24.71	0.40	11.78	0.70	7.42	0.69	3.70	0.82
	CV (%)	28.80	40.57	12.23	42.57	7.66	41.56	3.73	26.16
	Minimum	0.00	0.00	25.00	0.25	50.00	0.63	75.00	0.88
	Maximum	100	2.50	100	3.50	100	4.00	100	4.00
	Parents								
	Means	81.17	0.86	95.06	1.49	94.96	1.36	99.28	2.56
	Median	100	1.00	100	1.25	100	1.33	100	2.50
	PV	729.66	0.08	161.64	0.49	157.90	0.17	6.88	0.65
	SD	27.01	0.29	12.71	0.70	12.57	0.41	2.62	0.81
	CV (%)	33.28	33.30	13.37	46.88	13.23	30.29	2.64	31.45
	Minimum	0.00	0.00	50.00	0.50	44.44	0.44	88.89	1.43
	Maximum	100	1.25	100	3.50	100	2.50	100	4.00

[1] Results of 129 e 148 different genotypes belonging to F_1 progenies, in the 2015 and 2016 seasons, respectively; [2] 9 parental genotypes were evaluated (advanced selections and cultivars); [3] Scale of 0 to 4 as being 0 petals without lesions and 4 petals with 60% or more of the area with lesions. PV = phenotypic variance; SD = standard deviation; CV = coefficient of variation.

However, when the flowers were tested with inoculation, the phenotypic variability regarding resistance/susceptibility to *M. fructicola* was low, with most genotypes being classified as susceptible or very susceptible to the disease. These presented overall averages of incidence between 86.94 and 96.94% (72 hai), and between 91.71 and 99.28% (120 hai). The overall averages of severity degree were between 1.36 and 2.24 (72 hai), and between 2.54 and 3.48 (120 hai) (Tabela 2).

Analyzing the percentage of lesions incidence in the flowers of the selections and cultivars tested, when no inoculation was performed, the genotypes with the lowest incidence were 'Maciel', Conserva 672, Conserva 1526, 'Cerrito' and Cascata 1055 with less than 65% when evaluated at 72 hours, and less than 80% when evaluated at 120 hours (Figure 3).

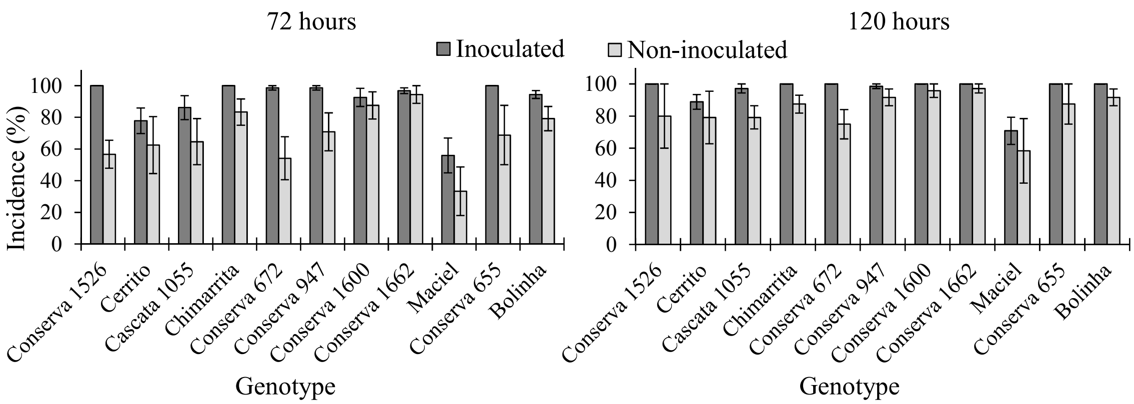


Figure 3: Blossom blight incidence in peach genotypes submitted or not to *M. fructicola* inoculation, evaluated after 72 and 120 hours. The columns correspond to the average of two harvest seasons (2015 and 2016) and the vertical bars in each column refer to the standard error. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

When inoculated, the genotypes that presented the lowest susceptibility were 'Maciel' and 'Cerrito', with 55.88 e 77.78% average incidence, respectively, in the evaluation performed 72 hai (Figure 3). The selection Cascata 1055, used as a control of low susceptibility, showed an incidence of 86.11%, therefore, higher than the two cultivars mentioned above and above the incidence reported by Fabiane (2011), which was

30.10%. The cultivar Bolinha, presented a high incidence (94.44%), similar to that obtained by Santos *et al.* (2012). The high incidence observed suggests that all the studied genotypes present high susceptibility to the disease under the tested conditions.

In addition to the genetic component, this may occur due to the influence of several factors, such as: very high conidia concentration (1 x 10^5 conidia mL^{-1}), susceptible phenological state (open flower), incubation conditions favorable to the disease, and high presence of inoculum in the field.

In the evaluation performed at 120 hai, only the cultivars Maciel and Cerrito had an incidence lower than 90%, while most of the genotypes showed around 100% incidence. This indicated that the evaluation at 120 hai, under the tested conditions, did not differentiate the genotypes (Figure 3).

The 'Cerrito,' Cascata 1055 and 'Maciel' parents presented the highest percentage of flowers (considering the two years of evaluation) within categories 0 and 1 (severity scale), with 64.89, 80.95 and 93.55%, respectively, in the evaluation performed 72 hai (Figure 4). When evaluated 120 hai, only the cultivars 'Cerrito' and 'Maciel' presented a considerable percentage within these categories, 28.17 and 44.44%, respectively.

The progenies 2008.159, 2009.38, 2012.68 and 2012.88 presented the lowest incidence (<85%) and severity (Figure 4). This indicates that the less susceptible 'Cerrito' and 'Maciel', parents transmitted this trait to their progeny. This can be verified, since in the first two progenies 'Cerrito' is one of the parents, as well as 'Maciel' for the last two.

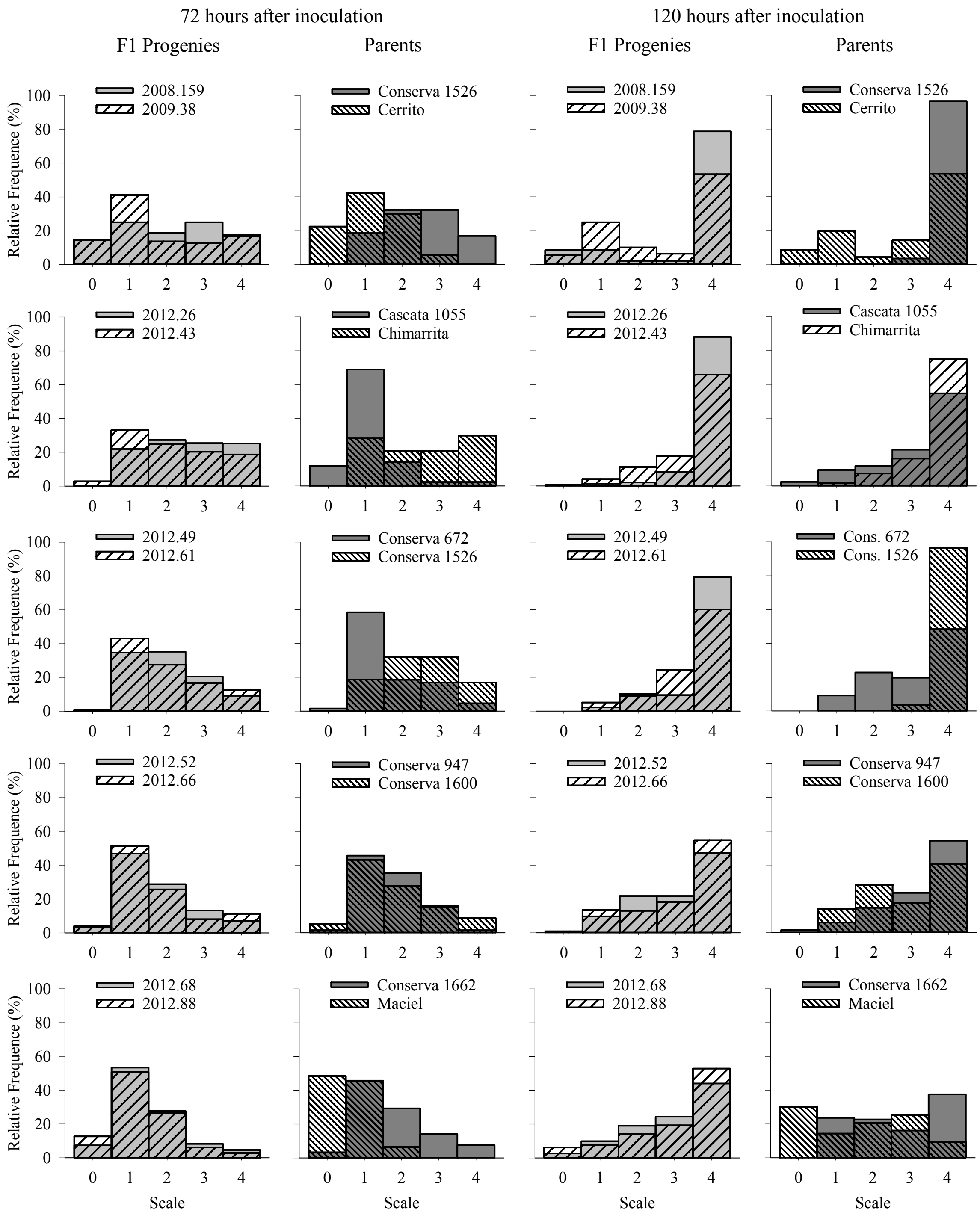


Figure 4: Severity of blossom blight in F1 progenies and their parents inoculated with *M. fructicola* and evaluated 72 and 120 hours after inoculation, years 2015 and 2016. The severity scale used (0 to 4) is detailed in Table 1 and in Figure 1. Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

The severity scale of blossom blight was not fully efficient to quantify susceptibility to *M. fructicola*. This was evidenced by the lack of normal distribution in the histograms of relative frequencies (Figure 4). When evaluated 72 hai most of the genotypes remained in category 1 (necrotic spots covering between 1% and 20% of the petals) (Table 1). Similarly, when evaluations were performed 120 hai, most genotypes were located in the category 4 (necrotic spots covering more than 60% of the petals) (Table 1). This suggests that there should be more categories between these percentages to segregate more levels of susceptibility to the disease. Although this have not great practical importance, it is interesting in the epidemiology of the disease since genotypes that develop infections and sporulations more rapidly, increase the rate of dissemination of the disease (MAY-DE MIO *et al.*, 2014; MONDINO *et al.*, 2010; RIOS & DEBONA, 2018).

In the histograms, a different behavior was not evident when a genitor was used as female or male parent, leading to the conclusion that there is no maternal effect. (Dini *et al.*, 2019 a cor da polpa). Through the Mann-Whitney test, the hypothesis of maternal effect in the five reciprocal crosses studied was tested. The tested contrast, in all cases, was F1 progeny versus its reciprocal F1 progeny for the studied parameters (incidence and severity). In none of the cases the test was significant ($p > 0.05$), that is, there were no significant differences between the reciprocal progenies, indicating that there is no maternal effect on the transmission of this trait (DINI *et al.* 2019a; 2019b; LONDERO *et al.*; 2009).

The estimated H^2 values were medium to low, varying between 11 and 43%, depending on the studied population, with an average of 24% for incidence and 17% for severity.

In the study of Wagner Junior (2003) in which the H^2 of the blossom blight was estimated through the incidence, the values ranged between 6 and 66%, depending on the studied population, with averages between 30 and 42%. This same author also concluded that the H^2 for this trait is low and highly variable among populations.

Further studies aiming at adjusting a phenotype protocol for this character become necessary to be able to correctly differentiate genotypes with different levels of genetic resistance. Mainly, studies related to the concentration of conidia to be used, phenological state of the flower, as well as the elaboration of a scale of severity more efficient to differentiate the genotype

CONCLUSIONS

The studied populations presented low phenotypic variability regarding to resistance/susceptibility to *Monilinia fructicola*, with most genotypes being susceptible or very susceptible.

The cultivars Maciel and Cerrito are less susceptible to blossom blight, transmitting this character to their progenies.

The heritability of the resistance to blossom blight in peach is medium to low.

No maternal effect on the transmission of susceptibility to blossom blight was detected.

REFERENCES

ADASKAVEG, J.E.; SCHNABEL, G.; FORSTER, H. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: LAYNE D.R.; BASSI, D. The peach: botany, production and uses. Wallingford: CAB International. p. 352-406, 2008.

AGRIOS, G.N. Plant diseases caused by fungi. In: AGRIOS, G.N. Plant Pathology. New York: Academic Press, p. 336-339, 1998.

BARÓ-MONTEL, N.; EDUARDO, I.; USALL, J.; CASALS, C.; ARÚS, P.; TEIXIDÓ, N.; TORRES, R. Exploring sources of resistance to brown rot in an interspecific almond × peach population. Journal of the Science of Food and Agriculture, v. 99, n. 8, p. 4105-4113, 2019. DOI: 10.1002/jsfa.9640.

BLEICHER, J. Doenças de rosáceas de caroço. In: KIMATI, H.; AMORIM, L.; BERGAMIN FILHO, A.; CAMARGO, L.E.A.; REZENDE, J.A.M. Manual de fitopatologia. São Paulo: CERES, v. 2, p. 621-627, 1997.

CHAPMAN, P.J.; CATLIN, G.A. Growth stages in fruit trees - from dormant to fruit set. New York's Food and Life Sciences Bulletin, n. 58, 11p. 1976. https://ecommons.cornell.edu/handle/1813/5062.

CHEN, S.; YUAN, N.; SCHNABEL, G.; LUO, C. Function of the genetic element ‘Mona’ associated with fungicide resistance in *Monilinia fructicola*. Molecular Plant Pathology, v. 18, n. 1, p. 90-97, 2017. DOI: 10.1111/mpp.12387.

DINI, M.; RASEIRA, M.C.B.; SCARIOTTO, S.; CARRA, B.; ABREU, E.S.; MELLO-FARIAS, P.; CANTILLANO, R.F.F. Color shade heritability of peach flesh. Journal of Agricultural Science, v. 11, n. 8, p. - . 2019a. DOI:

DINI, M.; RASEIRA, M.C.B.; SCARIOTTO, S.; MARCHI, P.M.; MELLO-FARIAS, P. Peach phenological characters: heritability, maternal effect and correlation with brown rot. 2019b.

DIRLEWANGER, E.; QUERO-GARCÍA, J.; LE DANTEC, L.; LAMBERT, P.; RUIZ, D.; DONDINI, L.; ILLA, E.; QUILOT-TURION, B.; AUDERGON, J.M.; TARTARINI, S.; LETOURMY, P.; ARÚS, P. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three Prunus species: peach, apricot and sweet cherry. Heredity, v. 109, p. 280-292, 2012. DOI: 10.1038/hdy.2012.38.

ELSHAFIE, H.S.; MANCINI. E.; CAMELE, I.; DE MARTINO, L.; DE FEO, V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. Industrial Crops and Products, v. 66, p.11-15, 2015. DOI: 10.1016/j.indcrop.2014.12.031.

FABIANE, K.C. Reação de pessegueiros a *Monilinia fructicola* (Wint.) Honey e sua relação com componentes bioquímicos. 2011. 130f. Dissertação (Mestrado) Universidade Tecnológica Federal do Paraná, Pato Branco, 2011.

http://repositorio.utfpr.edu.br/jspui/bitstream/1/264/1/PB_PPGAG_M_Fabiane%2C%20Keli%20Cristina_2011.pdf

FORTES, J.F.; MARTINS, O.M. Sintomatologia e controle das principais doenças. In: MEDEIROS, C.A.B.; RASEIRA, M.C.B. A cultura do pessegueiro. Brasília: Embrapa-SPI, Pelotas: Embrapa – CPACT, p. 243-260, 1998.

FU, W.; TIAN, G.; PEI, Q.; GE, X.; TIAN, P. Evaluation of berberine as a natural compound to inhibit peach brown rot pathogen *Monilinia fructicola*. Crop Protection, v. 91, p. 20-26, 2017. DOI: 10.1016/j.cropro.2016.09.008.

GARCIA-BENITEZ, C.; MELGAREJO, P.; DE CAL, A.; FONTANIELLA, B. Microscopic analyses of latent and visible *Monilinia fructicola* infections in nectarines. PLoS ONE, v. 11, n. 8, 2016. DOI: 10.1371/journal.pone.0160675.

GARCIA-BENITEZ, C.; MELGAREJO, P.; DE CAL, A. detection of latent *Monilinia* infections in nectarine flowers and fruit by qPCR. Plant Disease, v. 101, p. 1002-1008, 2017. DOI: 10.1094/PDIS-11-16-1682-RE.

GRIFFITHS, A. J. F.; WESSLER, S. R.; CARROLL, S. B.; DOEBLEY, J. Introduction to Genetical Analysis (10th ed.), New York: W. H. Freeman and Company, 2015. 896 p.

HILY, J.M.; SINGER, S.D.; VILLANI, S.M.; COX, K.D. 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, v. 67, p. 385-396, 2011. DOI: 10.1002/ps.2074.

KESKE, C.; AMORIM, L.; BIASI, L.A.; MAY-DE MIO, L.L. Blossom blight and brown rot on organic peach production system. Ciência Rural, Santa Maria, v. 40, n. 8, p. 1682-1688, 2010. DOI: 10.1590/S0103-84782010000800002.

AGROMET/CPACT/EMBRAPA, Laboratório de Agrometeorologia, Embrapa Clima Temperado. Disponivél em: <http://agromet.cpact.embrapa.br/>. Acesso 12 Dez. 2017.

LONDERO, P.M.G.; RIBEIRO, N.D.; CERUTTI, T.; MAZEIRO, S.M.; ROSA, D.P.; ROSA, S.S. Maternal effect in sulfur amino acids content expression in common bean grains. Ciência Rural, v. 39, n. 6, p. 1884-1887, 2009. DOI: 10.1590/S0103-84782009005000129.

LUO, C.X.; HU, M.J.; JIN, X.; YIN, L.F.; BRYSON, P.K.; SCHNABEL, G. An intron in the cytochrome *b* gene of *Monilinia fructicola* mitigates the risk of resistance development to QoI fungicides. Pest Management Science, v. 66, p. 1308-1315, 2010. DOI: 10.1002/ps.2016.

MAY-DE MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. Tropical Plant Pathology, Viçosa, v. 33, n. 3, p. 227-234, 2008. DOI: 10.1590/S1982-56762008000300008

MAY-DE MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 355-432, 2014.

MONDINO, P.; ALANIZ, S.; LEONI, C. Manejo integrado de las enfermedades del duraznero en Uruguay. In: SORIA, J. Manual del duraznero; Manejo integrado de plagas y enfermedades. Montevideo: INIA. p. 45-76, 2010. DOI: 10.13140/2.1.3283.8085.

OGAWA, J.M.; ZEHR, E.I.; BIRD, G.W.; RITCHIE, D.F.; URIU, K.; UYEMOTO, J.K. Compendium of stone fruit diseases. Saint Paul: The American Phytopathological Society, 1995. 98 p.

RIOS, J.A.; DEBONA, D. Efeito epidemiológico da resistência de hospdeiro. In: DALLAGNOL, L.J. Resistência genética de plantas a patógenos. Pelotas: UFPel, p. 126-149, 2018. http://guaiaca.ufpel.edu.br:8080/handle/prefix/4207

RASEIRA, M.C.B.; FRANZON, R.C. Melhoramento genético. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 57-72, 2014.

SANTOS, J.; RASEIRA, M.C.B.; ZANANDREA, I. Resistência à podridão-parda em pessegueiro. Bragantia, Campinas/SP, v. 71, n. 2, p. 219-225, 2012. DOI: 10.1590/S0006-87052012005000022.

SANTOS, J.; UENO, B. Controle da podridão-parda no Brasil. In: MITIDIERI, M.; CASTILLO, J.A. Manejo de la podredumbre morena (*Monilinia fructicola* y *M. laxa*) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina. Santiago de Chile, p. 73-77, 2014.

THOMIDIS, T. Influence of relative virulence and latent infections on the development of *Monilinia* to Greek peach orchards. Crop Protection, v. 94, p. 159-165, 2017. DOI: 10.1016/j.cropro.2016.12.001.

WAGNER JÚNIOR, A. Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey. 2003. 62f. Dissertação (Mestrado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2003.

WAGNER JÚNIOR, A.; RASEIRA, M.C.B.; FORTES, J.F.; PIEROBOM, C.R.; SILVA, J.B. Non-correlation of flower and fruit resistance to brown rot (*Monilinia fructicola* (Wint.) Honey) among 27 peach cultivars and selections. Journal of the American Pomological Society, v. 59, n.3, p. 148-152, 2005. https://search.proquest.com/openview/ee43cc92b705e843d027edc95ed4c4fd/1?pq-origsite=gscholar&cbl=46215

ZHU, F.; BRYSON, P.K.; SCHNABEL, G. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. Pest Management Science, v. 68, n. 7, p. 1003-1009, 2012. DOI: 10.1002/ps.3255.

AKNOWLEDGE MENTS, FINANCIAL SUPPORT and FULL DISCLOSURE

This study was partially funded by the Agencia Nacional de Investigación e Innovación-Uruguay (ANII) in the first year and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES) in the second year. The authors thank Embrapa Clima Temperado and all its staff, especially Everton Pederzolli and Gilberto Kuhn for their assistance in the experiments. The authors have no conflicts of interest regarding the manuscript.

4.3 Artigo 3. Blossom blight resistance in peach: phenotyping and antioxidants content in petals

Artigo submetido na Revista *"Brazilian Archives of Biology and Technology – BABT"*

Blossom blight resistance in peach: phenotyping and antioxidants content in petals

Running title: Blossom blight resistance in peach

Maximiliano Dini, Maria do Carmo Bassols Raseira, Bernardo Ueno, Marcia Vizzotto

Abstract

Brown rot and blossom blight caused by fungi of the genus Monilinia are the most important peach diseases. The increased concern with the environment and the health of workers and consumers, as well as the emergence of fungus isolates resistant to the main fungicide molecules favor control strategies such as genetic resistance. The objective of this study was to adjust a phenotyping protocol for the susceptibility to blossom blight in peach, as well as to quantify the antioxidant compounds present in the petals of these flowers and their correlation with the incidence and severity of the disease. The experiment was arranged in a randomized complete block split-plot design, the plot being four concentrations of Monilinia fructicola conidia; the subplot two phenological flower stage; and the sub-subplot four peach genotypes. The quantification of antioxidant compounds and their correlation with susceptibility to blossom blight was performed in the four genotypes analyzed. Phenotyping was more efficient when concentrations between 400 and 4,000 conidia mL^{-1} were used, regardless of phenological flower stage. The phenolic compounds, anthocyanins and antioxidant activity are positively correlated among them, and negatively correlated with the blossom blight incidence and severity. In order to blossom blight susceptibility phenotyping, it is recommended to use flowers in the pink or bloom stage, inoculum equivalent to 20-200 conidia per flower, and perform the evaluation at 96 hours after inoculation. This study suggests that more intense pink flowers have a higher content of antioxidant compounds and less blossom blight susceptibility.

Key words: *Prunus persica* (L.) Batsch, *Monilinia fructicola* (Winter) Honey, phenolic compounds, anthocyanins, antioxidant activity.

INTRODUCTION

Brown rot is considered one of the most important diseases of the peach tree culture and it is caused by three species of the genus *Monilinia* - *M. laxa* (Aderh. & Ruhl.) Honey, *M. fructigena* Honey, and *M. fructicola* (Winter) Honey – the latter being responsible for the disease in Brazil and in much of the world[1,2,3,4,5,6].

In the Americas, the fungus *M. fructicola* is important during the whole peach cycle, being the blossom and fruit ripening the most susceptible stages[5,7]. The main symptoms the disease are, the blossom blight, twig cankers and fruit rot[5,6,8] whicht, under conditions of high humidity and mild temperatures, such as in Brazil, may be visible 48 hours after infection[6].

Symptoms begin from the first blossoms, causing necrosis and flowers death. Normally, the flowers remain attached to the twig, which can be infected by the fungus, resulting in cankers and blight of one year old twigs[5,8]. The blossom blight is considered a primary infection, and has a great epidemiological importance, since it is an inoculum source for secondary fruit infections, directly by the production of conidia, or in the form of quiescent infections in developing fruits, manifesting only at the fruit ripening[5,6,8].

The brown rot may cause up of 60% in fruit losses, under hot and humid weather conditions; reduction in yield due to flower damage; loss of plant vigor by the death of buds and branches, from sprouting to harvesting; besides the high costs for its control (cultural and chemical) [3,6,8].

The increasing concern over environmental protection and consumers and workers health[9,10] together with the emergence of fungal isolates resistant to the main fungicide molecules used[11,12,13,14,15,] emphasized control strategies such as genetic resistance, seeking to reduce the use of pesticides. That is the most effective way to control the disease, reducing production costs and environmental impact. However, despite all efforts, the selection of resistant genotypes is still limited due to the scarcity or lack of knowledge of good resistance or immunity sources[16]. In addition, there are few studies in the literature focused on flower resistance, and these indicate that there is no correlation between flower and fruit resistance to *M. fructicola*[16,20,21,22,23].

The plant resistance to pathogens may be due to structural and/or biochemical mechanisms, either constitutive or induced. The structural mechanisms are physical barriers against pathogen penetration and/or colonization, whereas the biochemical mechanisms include substances capable of inhibiting (preforming) or producing (post formed) adverse conditions for its survival in host tissues, in response to its presence[17,18,19].

Thus, the aim of this work was to adjust a protocol to perform the phenotyping of the susceptibility to blossom blight in the peach tree, as well as to quantify the phenolic compounds content, anthocyanins and antioxidant activity in the petals of the flowers, and to test their correlation with the incidence and severity to blossom blight.

MATERIAL AND METHODS

The work was conducted in the laboratories of Fruit Breeding, Phytopathology and in the Food Nucleus of Embrapa Clima Temperado, in Pelotas, RS, Brazil (Latitude 31º40'S, Longitude 52º26'W, altitude 57m), in the year 2017. The study was divided in two parts, the first one refers to the adjustment of the phenotyping technique, and the second one refers to the quantification of phenolic compounds, anthocyanins and antioxidant activity and their correlation with the susceptibility to blossom blight.

Phenotyping

The experiment was arranged in a randomized complete block split-plot design, with the four conidia concentrations of *M. fructicola* (CC) (0, 400, 4.000 e 40.000 conidia mL^{-1}) as the plot; two phenological flower stages (PFS) (balloon and open flower stages), the subplot; and the four peach genotypes with showy flowers (Gen) ('Bolinha', 'Eragil', 'Ônix' and Conserva 1526), the sub-subplot.

For testing the reaction to blossom blight, it was used the technique of detached flowers cited by Fabiane[20]), considered as the most efficient technique to this purpose. In the same day, twigs containing flower buds at half-inch green and pink stages, stages 3 and 4 according to Chapman and Catlin[25] were collected from the four genotypes. Plants kept in the working collection of the Embrapa Clima Temperado Peach Breeding Program were used, and the four genotypes were under the same chemical and cultural management. The branches were prepared by removing the open or damaged flowers and then kept in buckets with water, inside a cold room (4±1°C), in order to standardize flowering[21], and also to reduce the contamination with pathogens[8,26]. After 48 hours in the cold room, the branches were left for another 24 hours at room temperature (20±5°C) in the laboratory. Later, the flowers with no damage and pathogen-associated symptoms were then chosen at the balloon and open stages, stages 4 and 5, respectively (Chapman and Catlin25).

Plastic boxes (50 × 35 × 10 cm) with phenolic foam (Green-up®) previously washed in running water during 30 minutes were used, and in each cell of the foam (2.5 × 2.5 × 3.8 cm), one flower with a small portion of the twig was fixed.

The inoculation was done by spraying using a fine droplet sprayer; the volume of sprayed suspension was adjusted using water-sensitive cards, aiming at the correct flowers coverage. The four CCs were chosen for the average number of conidia that would reach each flower. The boxes contained four blocks (replicates) of 10 flowers of the four genotypes 160 flowers per box. The volume of the conidial suspension used was 0.8 mL per box, considering that 400 conidia per mL correspond to approximately 2 conidia per flower, 4,000 conidia per mL to 20 conidia per flower, and 40,000 conidia per mL to 200 conidia per flower.

The fungus isolates, inoculum preparation, inoculation form and incubation conditions were described by Dini et al.[27] but the *M. fructicola* suspension was adjusted for the different concentrations of conidia used.

The incidence and severity of blossom blight were evaluated at 72, 96 and 120 hours after inoculation (hai), and the flowers with necrotic spots in the petals were considered as infected. The severity was evaluated according to the scale (Fig. 1), modified in relation to that proposed by Dini et al.[27], using the ImageJ program and photographs of flowers evaluated 72, 96 and 120 hai with *M. fructicola* under the same conditions of the experiment. The original scale had only five classes[27], and the authors mentioned the difficulty in identifying genotypes with greater or lesser susceptibility. Thus, the evaluation scale was modified to nine classes, being the first class (0) referring to the absence of symptoms of blossom blight, the following four (1 to 4) referring to necrotic spots between 1% and 20% of flower surface, and the remaining four classes (5 to 8) referring to necrotic spots > 20% of the flower surface (Fig. 1).

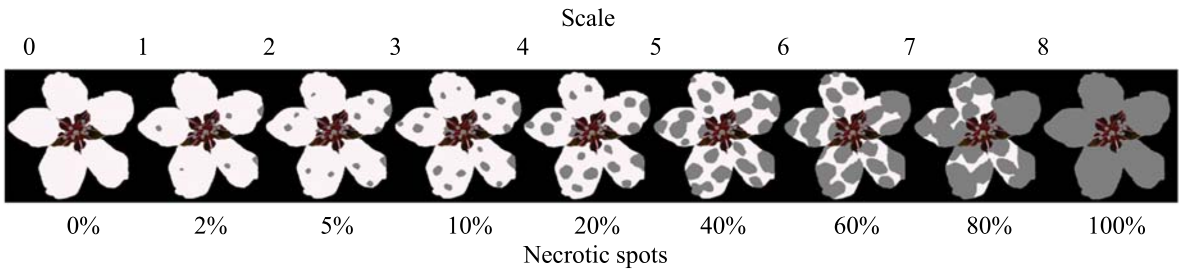


Figure 1. Scale used for the evaluation of infection and severity of blossom blight on artificially inoculated peach flowers with spray.

Infections in the anthers and/or pistil were not evaluated, because these organs are more sensitive to fungi[5,6] and present growth of other genera such as *Cladosporium*, *Penicillium*, *Alternaria* and *Botrytis*[4,6]. Furthermore the flowers were not previously disinfected, having influence of the natural inoculum from the field.

The blossom blight incidence and severity data were transformed using arcsin $\sqrt{x}$ and $\sqrt{x}$, respectively, for the residuals to fit to the normal distribution, according to the Shapiro-Wilk test. They were then subjected to analysis of variance (ANOVA) using a sub-subdivided plots model. The means were compared with Tukey test ($p \leq 0.05$).

Determination of total concentrations of phenolic compounds, anthocyanins and antioxidant activity in petals of the peach blossom

The experiment was in a completely randomized design, where each of the four peach genotypes ('Bolinha', 'Eragil', 'Ônix' e Conserva 1526) was considered as a treatment. The flower petals were collected in the same week as for the previous experiment. Three replicates of approximately 1 g of petals (fresh weight) from each genotype were used and for each of the quantifications.

For the phenolic compounds extraction and antioxidant activity determination, in each sample was added 14 mL methanol, and 13 mL of ethanol acidified with hydrochloric acid (85:15, 95% ethanol: 1.5N HCl), for the anthocyanin extraction.

Total phenolic compounds were quantified based on the method adapted by Swain and Hillis[28]. The absorbance was measured at 725 nm wavelength with a spectrophotometer. Chlorogenic acid was used as the standard for the calibration curve. The concentration of total phenolic compounds was calculated and expressed in mg Chlorogenic acid equivalent per 100 g of tissue (mg CAE 100 g^{-1}).

The total anthocyanins content was quantified by the method adapted from Fuleki and Francis[29]. The reading was performed in a spectrophotometer at an absorbance of 535 nm. Cyanidin 3-glucoside was used as the standard for the calibration curve and the results were expressed as mg Cyanidin 3-glucoside equivalent per 100g of tissue (mg C3GE 100g^{-1}).

The total antioxidant activity was estimated by the technique adapted by Brand-Williams et al.[30] using the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). The absorbance was measured

at a 515 nm wavelength in a spectrophotometer. Trolox was used as standard for the calibration curve and the results were expressed in µg Trolox equivalent per g of tissue (µg TE g^{-1}).
The quantified values for phenolic compounds, anthocyanins and antioxidant activity were submitted to ANOVA and the means were compared using Tukey's test ($p \leq 0.05$). These values were also submitted to the Spearman's correlation analysis, with the values referring to the incidence and severity of *M. fructicola* from the previous experiment.

RESULTS AND DISCUSSION

Phenotyping

There was no interaction between the three studied factors (CC, PFS and Gen) for incidence and severity of brown rot in any of the evaluations performed (72, 96 and 120 hai). Concerning the double interactions, it is emphasized that the CC $^{\times}$ Gen interaction was highly significant ($p \leq 0.001$) for the two parameters and in the three evaluations (Table 1)

Table 1. ANOVA summary: Effects of conidial concentration of *Monilinia fructicola* (plot), phenological flower stage (subplot), genotype (sub-subplot), doubles interactions and triple interaction for incidence and severity of blossom blight in peach, evaluated at 72, 96 and 120 hours after inoculation (hai).

Parameters	hai	*p*-values						
		CC	PFS	Gen	CC x PFS	CC x Gen	PFS x Gen	CC x PFS x Gen
Incidence	72	***	***	***	**	***	ns	ns
	96	***	ns	***	ns	***	ns	ns
	120	***	*	***	ns	***	*	ns
Severity	72	***	***	***	ns	***	*	ns
	96	***	ns	***	ns	***	**	ns
	120	***	ns	***	*	***	**	ns

Note. Main factors: Conidial Concentration of *M. fructicola* (CC), Phenological Flower Stage (PFS) and Genotype (Gen); double interaction: CC*PFS, CC*Gen and PFS*Gen; triple interaction: CC*PFS*Gen; ^{ns}, *, **, *** nonsignificant, significant at 0.05, 0.01 and < 0.001 probability by the F-test, respectively.

Regarding to the averages for each genotype within each CC (Table 2), it was observed that in the evaluation performed at 72 hai, even when no inoculum was used (0 conidia mL^{-1}), high values were obtained for the average incidence of blossom blight (54.45 to 72.38%), no significant differences between the genotypes were found. This high incidence of blossom blight even without previous inoculation has already been reported[21,31] and is due to the high pressure of the inoculum naturally present in the peach orchards in southern Brazil at the flowering time[3,4,5].
Blossom blight varying between 0 and 100%, evaluated at 72 hai and without previous inoculation, was observed by Santos et al[21]. These differences were attributed, besides the resistance/susceptibility, to the temporal (flowering date) and spatial (geographical position within the orchard) differences of the different genotypes. In the same study[21], the cultivar Bolinha it was used, with a 30 to 50% incidence of blossom blight when no inoculum was used, similar incidence values to those obtained in the present study (between 30 to 70%) when the evaluation was at 72 hai.
This suggests that for a more detailed study about genetic resistance to this disease, would be necessary to keep the plants under controlled conditions, free from the presence of the natural inoculum, or to use a more efficient technique for twig disinfection before flowering and to cover them until its use. Thus, it might be possible to decrease the environmental effect and more accurately assess the genetic portion of the phenotypic expression[32,33].

Table 2. Incidence and severity according to conidial concentration of *Monilinia fructicola* (CC) and genotype, evaluated at 72, 96 and 120 hours after inoculation (hai).

Parameters	hai	Genotype	CC (conidia mL^{-1})			
			0	400	4,000	40,000
Incidence (%)	72	'Bolinha'	54.45 aA[1]	68.75 aA	72.23 aA	92.50 aB
		'Eragil'	60.63 aA	64.46 aA	77.81 aA	98.44 aB
		'Ônix'	72.38 aA	95.14 bB	100 bB	100 aB
		Conserva 1526	72.10 aA	93.16 bB	97.23 bB	100 aB
	96	'Bolinha'	69.45 aA	80.00 aA	83.48 aA	97.50 aB
		'Eragil'	70.00 aA	81.88 aAB	91.56 abBC	100 aC
		'Ônix'	76.19 aA	96.53 bB	100 bB	100 aB
		Conserva 1526	79.09 aA	96.11 bB	100 bB	100 aB
	120	'Bolinha'	74.18 aA	80.00 aA	84.73 aA	100 aB
		'Eragil'	79.69 aA	85.63 aA	91.43 aA	100 aB
		'Ônix'	80.48 aA	97.36 bB	100 bB	100 aB
		Conserva 1526	82.90 aA	97.5 bB	100 bB	100 aB
Severity (0 to 8)[2]	72	'Bolinha'	0.86 aA	0.93 aA	1.25 aA	1.89 aB
		'Eragil'	1.00 aA	1.13 aA	1.36 aA	3.03 bB
		'Ônix'	1.23 abA	1.83 bB	2.25 bBC	2.99 bC
		Conserva 1526	1.53 bA	2.08 bAB	2.40 bBC	2.84 bC
	96	'Bolinha'	1.46 aA	1.53 aA	1.74 aA	3.28 aB
		'Eragil'	1.56 aA	1.78 abA	2.08 aA	4.01 abB
		'Ônix'	1.91 aA	2.34 bcAB	3.05 bB	4.30 bcC
		Conserva 1526	1.84 aA	2.81 cB	3.90 cC	4.98 cD
	120	'Bolinha'	1.91 aA	1.88 aA	2.05 aA	3.93 aB
		'Eragil'	2.38 abA	2.65 bA	2.70 bA	4.63 abB
		'Ônix'	2.51 bA	2.83 bA	3.93 cB	4.89 bC
		Conserva 1526	2.75 bA	3.50 cB	4.39 cC	5.19 bC

Note. [1] Averages followed by the same lowercase letter in the column and followed by the same capital letter in the row do not differ by the Tukey test ($p < 0.05$); [2] Scale of 0 to 8 used is shown in Figure 1.

It was not possible to differentiate the genotypes regarding the incidence of the disease when a CC of 40,000 conidia mL^{-1} was used, observing extremely high averages, between 92.5 and 100% of blossom blight incidence, indicating that the CC used was very high.

When the concentration of 400 and 4,000 conidia mL^{-1} were used, it was possible to significantly differentiate two groups between the tested genotypes, a group of lower susceptibility ('Bolinha' and 'Eragil'), and another group with higher susceptibility to blossom blight ('Onix' and Conserva 1526), in the three evaluations (72, 96 and 120 hai) (Table 2).

The cultivar Bolinha is mentioned in several studies presenting a high blossom blight incidence (between 62 and 100%) when inoculated with *M. fructicola*[4,5,21]. In the present study, mean values of incidence in this cultivar were similar in any of the three CCs tested (68.75, 72.23, 92.50%, CC of 400, 4,000 and 40,000, respectively). However, this cultivar was less susceptibility when compared to the other three genotypes (Table 2). Possibly, with the use of a greater number of genotypes, Bolinha would not be among the most resistant genotypes, since the averages were very high. Previous studies identified genotypes with much lower average incidence, even though they were inoculated, as 'Jubileu' and Conserva 930[21], Conserva 1070 and Conserva 1055[20], 'Magno,' 'Leonense' and another four selections from the Embrapa Peach Breeding Program[23], all with averages less than 35% of blossom blight incidence.

Analyzing the behavior of the severity of blossom blight, the results were different from the incidence. In most cases, there were significant differences between genotypes regarding the severity at the four inoculum concentrations, at the three evaluation times. Significant differences were not detected only when no inoculum (0 conidia mL^{-1}) was used, in the evaluation performed at 96 hai (Table 2). This shows that the scale used was efficient to quantify the severity of blossom blight and differentiate genotypes.

When the concentrations of 400 and 4,000 conidia mL^{-1} were used, it was possible to significantly differentiate up to three levels among the tested genotypes in the evaluations performed at 96 and 120 hai. With 4,000 conidia mL^{-1} and, in the evaluation at 96 hai, 'Bolinha' and 'Eragil' presented less severity of blossom blight, followed by 'Onyx 'and finally Conserva 1526. In the evaluation at 120 hai, 'Bolinha' presented lower severity, followed by 'Eragil', and finally 'Onix' and Conserva 1526, the latter two being significantly different from 'Bolinha' but not from 'Eragil'.

When the CC x PFS interaction was analyzed, it was observed that it was significant in only two cases; the first was for incidence when evaluated at 72 hai, and the second for severity when evaluated at 120 hai (Table 1).

In the first case, for the concentrations of 0, 400 and 4000 conidia mL^{-1} were observed significant differences between the two phenological flower stages, with the balloon presenting a lower incidence than the open flower (Table 3). When the concentration of 40,000 conidia mL^{-1} was used and the evaluation performed 96 and 120 hai, there was no significant difference. However, the difference observed may be due to the larger surface of the open flower, since they received a higher proportion of conidia per flower when compared to the ballons.

Table 3. Incidence and severity according to conidial concentration of *Monilinia fructicola* (CC) and phenological flower stage (PFS), evaluated at 72, 96 and 120 hours after inoculation (hai).

Parameters	hai	PFS[1]	CC (conidia mL^{-1})			
			0	400	4,000	40,000
Incidence (%)	72	Pink (4)	53.94 aA[2]	70.13 aB	81.27 aC	96.09 aC
		Bloom (5)	75.84 bA	90.63 bB	92.36 bB	99.38 aC
	96	Pink (4)	71.53	86.63	93.91	98.75
		Bloom (5)	75.84	90.63	93.61	100
	120	Pink (4)	77.02	87.74	93.21	100
		Bloom (5)	81.6	92.5	94.86	100
Severity (0 to 8)[3]	72	Pink (4)	0.86	1.14	1.48	2.44
		Bloom (5)	1.44	1.84	2.15	2.93
	96	Pink (4)	1.59	2.03	2.60	4.11
		Bloom (5)	1.79	2.19	2.78	4.18
	120	Pink (4)	2.22 aA	2.84 aB	3.30 aB	4.75 aC
		Bloom (5)	2.56 aA	2.58 aA	3.23 aB	4.56 aC

Note. [1] Phenological flower stage: pink (4) and bloom (5) stages of the phenological classification of Chapman and Catlin[25]; [2] Averages followed by the same lowercase letter in the column and followed by the same capital letter in the row do not differ by the Tukey test ($p < 0.05$); [3] Scale of 0 to 8 used is shown in Figure 1.

Comparing the CCs tested in each PFS, it is observed that the treatments in which inoculum was used were always different from the treatment without the use of inoculum, and that when 40,000 conidia mL^{-1} were used, the average incidence of blossom blight was very high (between 96.09 and 100%).

The severity of the disease presented significant differences when evaluated at 120 hai, due to the CC factor (Table 3). There were no significant differences between the two PFS within each CC level. Comparing the severities between the CCs tested in flowers at balloon and bloom stages, it was observed that in both PFS, the severity for 40,000 conidia mL^{-1} was higher than all other CCs. Regarding the flower stage, there were no differences between the uninoculated flowers and those with 400 mL^{-1} conidia.

Analyzing the interaction PFS x Gen it is observed that the incidence was significant when evaluated at 120 hai. Comparing the two PFS, it was only significant in the Eragil cultivar, presenting a higher incidence in open flower PFS (93.13%) compared to the floral bud (85.24%) (Table 4).

In the severity parameter, the three evaluations showed significant differences (Table 1). Mainly due to the effect of the genotype not to the PFS (Table 4). In the most cases the cultivar Bolinha presented the lowest severities and the selection Conserva 1526 the highest.

Table 4. Incidence and severity according to suspension genotype and phenological flower stage (PFS), evaluated at 72, 96 and 120 hours after inoculation (hai).

Parameters	hai	Flower stage[1]	Genotype			
			'Bolinha'	'Eragil'	'Ônix'	Conserva 1526
Incidence (%)	72	Pink (4)	64.38	63.17	87.51	86.38
		Bloom (5)	79.59	87.5	96.25	94.86
	96	Pink (4)	84.38	83.59	90.11	92.74
		Bloom (5)	80.84	88.13	96.25	94.86
	120	Pink (4)	85.42 aA[2]	85.24 aA	92.67 aB	94.64 aB
		Bloom (5)	84.03 aA	93.13 bB	96.25 aB	95.56 aB
Severity (0 to 8)[3]	72	Pink (4)	1.11 aA	1.23 aA	1.74 aB	1.84 aB
		Bloom (5)	1.35 aA	2.03 bB	2.41 bBC	2.58 bC
	96	Pink (4)	2.16 aA	2.22 aA	2.71 aB	3.24 aC
		Bloom (5)	1.84 aA	2.49 aB	3.09 aC	3.53 aC
	120	Pink (4)	2.71 bA	3.06 aB	3.43 aBC	3.91 aC
		Bloom (5)	2.17 aA	3.11 aB	3.64 aC	4.01 aC

Note. [1] Phenological flower stage: bloom (5) and pink (4) of the phenological classification of Chapman & Catlin[25]; [2] Averages followed by the same lowercase letter in the column and followed by the same capital letter in the row do not differ by the Tukey test ($p < 0.05$); [3] Scale of 0 to 8 used is shown in Figure 1.

This absence of significant effects due to the effect of the PFS, justifies the use of flowers in any of the two tested phenological stages and validates the previous works that used flowers at balloon stage[20,21,23] and those that used open flowers[0,24,27,31].

In summary, after analyzing all the possible combinations, and the factors that present greater effects regarding the blossom blight susceptibility in the peach tree, it is suggested as a protocol for the phenotyping of this character, the use between 20 and 200 conidia per flower (depending on whether the phenotyping is done in the absence of natural inoculum or not), with flowers in the balloon stage and/or open flowers, and make the evaluations preferably at 96 hai.

Phenolic compounds, anthocyanins and antioxidante activity

The four tested genotypes presented variability regarding the total content of phenolic compounds, anthocyanins and antioxidant activity in its flower petals. The phenolic compounds content was higher in the cultivar Bolinha, with an average of 1356.0 mg CAE 100 g^{-1}, without presenting significant differences with 'Eragil' (1299.5 mg CAE 100 g^{-1}). 'Ônix' e Conserva 1526 presented the lowest mean values, with 1171.1 and 1155.9 mg CAE 100 g^{-1} respectively. These last two genotypes also did not present significant differences with 'Eragil' (Figure 2A).

Studies report a concentration of phenolic compounds in peach fruits of cultivar Maciel[36] between 119.6 and 333.6 mg CAE 100 g^{-1}, that is, up to 10 times lower than those observed in this study. Although the cultivar Maciel was not used in the present study, this suggests that, in peach trees, the concentration of these compounds is higher in the petals than in the fruits.

For the anthocyanin content, the cultivars Bolinha and Eragil presented the highest averages, 77.7 e 68.3 mg C3GE 100g^{-1}, respectively (Figure 2B). The four genotypes have showy flowers but differ in the intensity of the pink color of their petals, being 'Bolinha' and 'Eragil' darker than 'Onyx' and Conserva 1526, which was confirmed by the difference in the anthocyanins content found. Studying the pulp composition and fruit skin of numerous peach and nectarine genotypes, Scariotto[37] reported great variability in the anthocyanin content from 0 to 18.0 and 0 to 525.1 µg de Cyanidin 3-glucoside per 100g^{-1} of dry weight in the flesh and skin, respectively. These values are up to a thousand times smaller than those found in the present study, indicating that the concentration of anthocyanins present in the petals is much higher than the concentration in the fruits, as mentioned for phenolic compounds.

The antioxidant activity estimates were higher in Eragil Bolinha and Onyx cultivars (3775.0, 3729.1 and 3180.3 µg TE g^{-1}, respectively), however, 'Onix' did not differ in relation to Conserva 1526 (Figure 2C). Studying extracts of peach blossoms, Li and Wang[35] reported high antioxidant activity, from 25 to 100 µg mL^{-1}. While Ramm et al.[36] obtained averages between

3284.7 and 8101.0 μg TE g^{-1} in peach fruits. This suggests that the total antioxidant activity present in the fruits is higher than that in the petals. This can be explained by the existence of several other compounds in the fruit that also have antioxidant activity, such as vitamin C and carotenoids.

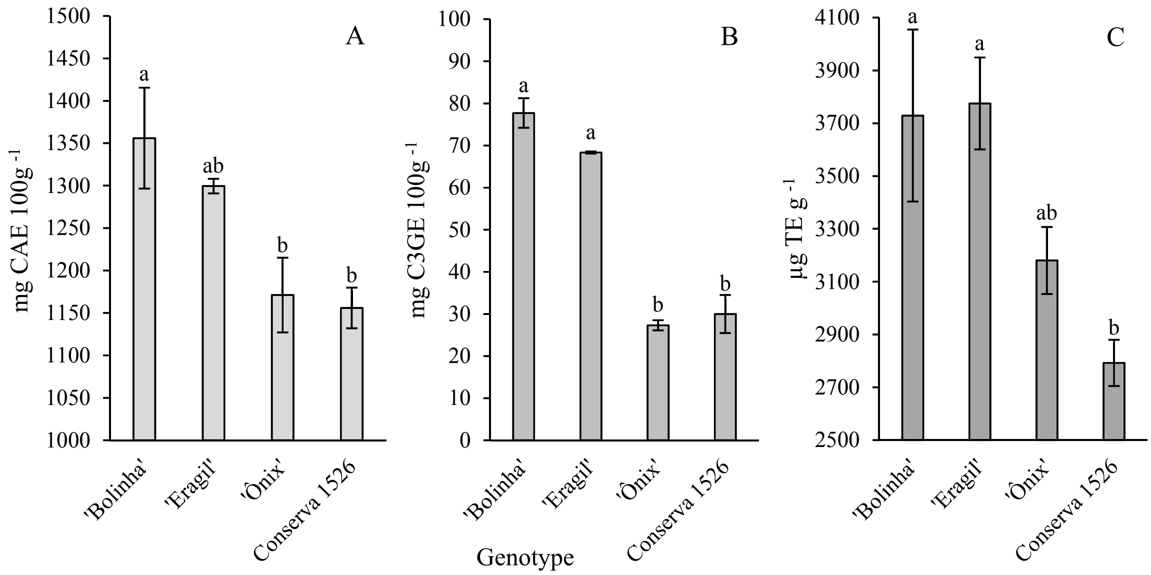


Figure 2. Means of total phenolic compounds (A) expressed as mg Chlorogenic acid equivalent per 100 g of tissue (mg CAE 100 g^{-1}), total anthocyanins (B) expressed as mg Cyanidin 3-glucoside equivalent per 100 g of tissue (mg C3GE 100g^{-1}), and total antioxidant activity (C) expressed as μg Trolox equivalent per g of tissue (μg TE g^{-1}) of the flower petals of four peach genotypes. Columns with the same letter do not differ by the Tukey test ($p < 0.05$); the bars represent the standard error of the mean.

When the correlations between the three compounds quantified in the petals were tested, high positive correlations were observed, 0.87 between phenolic compounds and anthocyanins, 0.83 between phenolic compounds and antioxidant activity, and 0.70 between anthocyanins and antioxidant activity (Table 5). The total content of phenolic compounds has been associated with antioxidant activity in flowers of several plants[34]. Studying the distribution of anthocyanins in different tissues of the peach tree, Chaparro et al.[38] observed four times more cyanidin-3-glycoside (anthocyanin) in a red flower genotype when compared to pink flower genotypes. This suggests that the darker the intensity of the pink color of the petals, the higher the content of phenolic compounds and the antioxidant activity, since the accumulation of anthocyanins is responsible for the color of the flowers in the peach tree[39].

In the present study, the PFS was not significant to quantify the blossom blight susceptibility. Thus, PFS was not considered when the correlations were tested. Spearman correlations and their significance (*p*-value) for the incidence and severity data, evaluated at 96 hai (Table 5), are presented. Evaluations at 72 and 120 ha had similar values, however, the absolute values in the correlations were lower (data not shown).

All correlations between incidence and severity of blossom blight and antioxidant compounds in the petals were negative (Table 5). When the concentrations of 400 and 4,000 mL^{-1} conidia (concentrations more effective to test the susceptibility of this disease, according to the previous experiment) were used, the correlations ranged between -0.65 and -0.89, and were all significant. These results indicate that the higher the content of phenolic compounds, anthocyanins and the antioxidant activity in the peach blossom petals, the lower blossom blight susceptibility. Phenolic compounds present in the flesh and epidermis of peach fruits were negatively associated with brown rot[37], especially with sporulation, emphasizing that they were more effective when present in fruit epidermis than in flesh. Likewise, negative correlations were found between the anthocyanins content present in the peach skin with the severity of the lesion (-0.48) and sporulation (-0.49) [40].

Table 5. Spearman´s correlation between phenolic compounds (PC), anthocyanins (Ant), antioxidant activity (AA), incidence (Inc) and severity (Sev) of blossom blight.

		PC	Ant	AA	0		400		4,000		40,000	
					Inc	Sev	Inc	Sev	Inc	Sev	Inc	Sev
PC		-	***	**	ns	ns	***	***	***	***	ns	*
Ant		0.87	-	**	*	*	***	***	***	***	ns	*
AA		0.83	0.70	-	ns	ns	**	**	**	***	ns	*
0	Inc	-0.49	-0.52	-0.41	-	ns	ns	*	*	*	ns	*
	Sev	-0.36	-0.52	-0.24	0.56	-	ns	ns	*	*	ns	*
400	In	-0.83	-0.74	-0.70	0.28	0.36		***	***	***	ns	*
	Sev	-0.85	-0.75	-0.65	0.59	0.39	0.81	-	***	***	ns	**
4,000	Inc	-0.82	-0.89	-0.68	0.61	0.64	0.77	0.83	-	***	ns	***
	Sev	-0.82	-0.83	-0.77	0.62	0.54	0.75	0.80	0.91	-	ns	***
40,0000	Inc	-0.36	-0.36	-0.20	0.34	-0.14	0.11	0.23	0.21	0.28	-	ns
	Sev	-0.69	-0.72	-0.65	0.6	0.52	0.57	0.67	0.78	0.85	0.42	-

Note. 0, 400, 4.000 and 40.000 = conidial concentration of *Monilinia fructicola* of inoculation into conidia mL^{-1}; Incidence and severity of blossom blight correspond to the evaluation 96 hours after inoculation for 'Bolinha', 'Eragil', 'Ônix' and Conserva 1526 peach genotypes; In the lower diagonal the Spearman´s correlation value; In the upper diagonal: ^{ns}, *, **, ***; nonsignificant and significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

Similarly, the expression of genes responsible for Reactive oxygen species (ROS) and hydrogen peroxide production was studied in peach blossom petals[41], in response to host (*M. fructicola*) and non-host (*Penicillium digitatum)* fungal pathogens. The exogenous application of antioxidants in the blossom significantly reduced their blight, and the accumulation of hydrogen peroxide was higher in response to *M. fructicola*. In the present study, there was no exogenous application of antioxidants, but when the content of these substances was higher the blossom blight was lower.

These results suggest that an indirect selection to the sensibility to blossom bight can be made. Selecting for high levels of phenolic compounds, anthocyanins and/or antioxidant activity in flower petals, the susceptibility to blossom blight can be reduced. This is of great importance for peach breeding programs, especially where blossom blight is a problem and genetic resistance is the best alternative to face the disease. A much faster phenotyping may also be suggested, based on the intensity of the pink color of the petals, but this hypothesis has to be proven in studies with a higher number of genotypes and evaluation in several harvest seasons.

CONCLUSIONS

Phenotyping for blossom blight was more efficient when a concentration between 400 and 4,000 conidia mL^{-1} was used, regardless of the phenological state of the flower, whether balloon or open flower.

The scale used was efficient to quantify the severity of blossom blight.

For phenotyping the blossom blight susceptibility, it is recommended to use flowers at balloon and/or open stages, and a fungus suspension equivalent to 20-200 conidia of *M. fructicola* per flower, performing the evaluation at 96 hours after inoculation.

The content of phenolic compounds, anthocyanins and antioxidant activity are positively correlated among them, and these are negatively correlated with the incidence and severity of blossom blight.

ACKNOWLEDGMENTS

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES) for the financial support, through the first author's doctoral scholarship. The authors thank Embrapa Clima Temperado staff, especially the laboratory assistant Everton Pederzolli for the support in this experiment.

REFERENCES

1. ADASKAVEG, J.E.; SCHNABEL, G.; FORSTER, H. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: LAYNE D.R.; BASSI, D. The peach: botany, production and uses. Wallingford: CAB International. p. 352-406, 2008.

2. AGRIOS, G.N. Plant diseases caused by fungi. In: AGRIOS, G.N. Plant Pathology. New York: Academic Press, p. 336-339, 1998.

3. FORTES, J.F.; MARTINS, O.M. Sintomatologia e controle das principais doenças. In: MEDEIROS, C.A.B.; RASEIRA, M.C.B. A cultura do pessegueiro. Brasília: Embrapa-SPI, Pelotas: Embrapa – CPACT, p. 243-260, 1998.

4. MAY-DE MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. Tropical Plant Pathology, Viçosa, v. 33, n. 3, p. 227-234, 2008. DOI: 10.1590/S1982-56762008000300008

5. MAY-DE MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 355-432, 2014.

6. OGAWA, J.M.; ZEHR, E.I.; BIRD, G.W.; RITCHIE, D.F.; URIU, K.; UYEMOTO, J.K. Compendium of stone fruit diseases. Saint Paul: The American Phytopathological Society, 1995. 98 p.

7. BLEICHER, J. Doenças de rosáceas de caroço. In: KIMATI, H.; AMORIM, L.; BERGAMIN FILHO, A.; CAMARGO, L.E.A.; REZENDE, J.A.M. Manual de fitopatologia. São Paulo: CERES, v. 2, p. 621-627, 1997.

8. MONDINO, P.; ALANIZ, S.; LEONI, C. Manejo integrado de las enfermedades del duraznero en Uruguay. In: SORIA, J. Manual del duraznero; Manejo integrado de plagas y enfermedades. Montevideo: INIA. p. 45-76, 2010. DOI: 10.13140/2.1.3283.8085.

9. BARÓ-MONTEL, N.; EDUARDO, I.; USALL, J.; CASALS, C.; ARÚS, P.; TEIXIDÓ, N.; TORRES, R. Exploring sources of resistance to brown rot in an interspecific almond × peach population. Journal of the Science of Food and Agriculture, v. 99, n.8, p.4105-4113, 2019. DOI: 10.1002/jsfa.9640.

10. ELSHAFIE, H.S.; MANCINI. E.; CAMELE, I.; DE MARTINO, L.; DE FEO, V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. Industrial Crops and Products, v. 66, p.11-15, 2015. DOI: 10.1016/j.indcrop.2014.12.031.

11. LUO, C.X.; HU, M.J.; JIN, X.; YIN, L.F.; BRYSON, P.K.; SCHNABEL, G. An intron in the cytochrome b gene of *Monilinia fructicola* mitigates the risk of resistance development to Qol fungicides. Pest Management Science, v. 66, p. 1308-1315, 2010. DOI: 10.1002/ps.2016.

12. HILY, J.M.; SINGER, S.D.; VILLANI, S.M.; COX, K.D. 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, v. 67, p. 385-396, 2011. DOI: 10.1002/ps.2074.

13. ZHU, F.; BRYSON, P.K.; SCHNABEL, G. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. Pest Management Science, v. 68, n. 7, p. 1003-1009, 2012. DOI: 10.1002/ps.3255.

14. CHEN, S.; YUAN, N.; SCHNABEL, G.; LUO, C. Function of the genetic element ‘Mona’ associated with fungicide resistance in *Monilinia fructicola*. Molecular Plant Pathology, v. 18, n. 1, p. 90-97, 2017. DOI: 10.1111/mpp.12387.

15. FU, W.; TIAN, G.; PEI, Q.; GE, X.; TIAN, P. Evaluation of berberine as a natural compound to inhibit peach brown rot pathogen *Monilinia fructicola*. Crop Protection, v. 91, p. 20-26, 2017. DOI: 10.1016/j.cropro.2016.09.008.

16. RASEIRA, M.C.B.; FRANZON, R.C. Melhoramento genético. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. Pessegueiro. Brasília: Embrapa, p. 57-72, 2014.

17. DALLAGNOL, L.J.; ARAUJO FILHO, J.V. Uma visão geral da resistência genética da planta a microrganismos. In: DALLAGNOL, L.J. Resistência genética de plantas a patógenos. Pelotas: UFPel, p. 14-64, 2018. http://guaiaca.ufpel.edu.br:8080/bitstream/prefix/4207/1/RESIST%C3%8ANCIA%20GEN%C3%89TICA%20DE%20PLANTAS%20A%20PAT%C3%93GENOS_EBOOK.pdf

18. PASCHOLATI, S.F. Fisiologia do parasitismo: como as plantas se defendem dos patógenos. In: AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A. Manual de Fitopatologia, Volume I - Princípios e Conceitos. São Paulo: Agronômica Ceres. v.1, 2011. p. 593-636.

19. SCHWAN-ESTRADA, K.R.F.; STANGARLIN, J.R.; PASCHOLATI, S.F. Mecanismos bioquímicos de defesa vegetal. In: PASCHOLATI, S.F.; LEITE, B.; STANGARLIN, J.R.; CIA, P. Interação planta patógeno – Fisiologia, bioquímica e biologia molecular. Piracicaba: FEALQ, v. 13, p. 227-248, 2008.

20. FABIANE, K.C. Reação de pessegueiros a *Monilinia fructicola* (Wint.) Honey e sua relação com componentes bioquímicos. 2011. 130f. Dissertação (Mestrado) Universidade Tecnológica Federal do Paraná, Pato Branco, 2011. http://repositorio.utfpr.edu.br/jspui/bitstream/1/264/1/PB_PPGAG_M_Fabiane%2C%20Keli%20Cristina_2011.pdf

21. SANTOS, J.; RASEIRA, M.C.B.; ZANANDREA, I. Resistência à podridão-parda em pessegueiro. Bragantia, Campinas/SP, v. 71, n. 2, p. 219-225, 2012. DOI: 10.1590/S0006-87052012005000022.

22. WAGNER JÚNIOR, A. Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey. 2003. 62f. Dissertação (Mestrado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2003.

23. WAGNER JÚNIOR, A.; RASEIRA, M.C.B.; FORTES, J.F.; PIEROBOM, C.R.; SILVA, J.B. Non-correlation of flower and fruit resistance to brown rot (*Monilinia fructicola* (Wint.) Honey) among 27 peach cultivars and selections. Journal of the American Pomological Society, v. 59, n.3, p. 148-152, 2005. https://search.proquest.com/openview/ee43cc92b705e843d027edc95ed4c4fd/1?pq-origsite=gscholar&cbl=46215.

24. SANTIAGO, MF. Michele Freitas. Impact of temperature increase on the intensity of occurrence of brown rot (*Monilinia fructicola* (Wint) Honey) in peach in Brazil. 2013, 154f. Dissertation (Master of Science) – Programa de Pós-Graduação em Fitossanidade. Federal University of Pelotas. Pelotas, RS, Brazil.

25. CHAPMAN, P.J.; CATLIN, G.A. Growth stages in fruit trees - from dormant to fruit set. New York's Food and Life Sciences Bulletin, n. 58, 11p. 1976. https://ecommons.cornell.edu/handle/1813/5062.

26. LUO, Y.; MORGAN, D.P.; MICHAILIDES, T.J. Risk analysis of brown rot blossom blight of prune caused by *Monilinia fructicola*. Phytopathology, Saint Paul, v. 91, n. 8, p. 759-768, 2001.

27. DINI 2019 ou 2016 (artigo ou a dissertação)

28. SWAIN, T.; HILLIS, W.E. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. Journal Science of Food Agriculture, v. 10, n. 1, p. 63-68, 1959. DOI: 10.1002/jsfa.2740100110.

29. FULEKI, T.; FRANCIS, F.J. Quantitative methods for anthocyanins. Extraction and determination of total anthocyanin in cranberries. Journal Food Science, v. 33, p. 72-77, 1968. DOI: 10.1111/j.1365-2621.1968.tb00887.x.

30. BRAND-WILLIAMS, W.; CUVELIER, M.E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie, v.28, p.25-30, 1995. DOI: 10.1016/S0023-6438(95)80008-5.

31. KESKE, C.; AMORIM, L.; BIASI, L.A.; MAY-DE MIO, L.L. Blossom blight and brown rot on organic peach production system. Ciência Rural, Santa Maria, v. 40, n. 8, p. 1682-1688, 2010. DOI: 10.1590/S0103-84782010000800002.

32. FALCONER, D.S.; MACKAY, T.F.C. Introducción a la genética cuantitativa. Zaragosa: Acribia S.A, 2001. 490 p.

33. GRIFFITHS, A. J. F.; WESSLER, S. R.; CARROLL, S. B.; DOEBLEY, J. Introduction to Genetical Analysis (10th ed.), New York: W. H. Freeman and Company, 2015. 896 p.

34. KAISOON, O.; SIRIAMORNPUN, S.; WEERAPREEYAKUL, N.; MEESO, N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of Functional Foods, v. 3, p. 88-99, 2011. DOI: 10.1016/j.jff.2011.03.002.

35. LI, C.; WANG, M.H. Antioxidant activity of peach blossom extracts. Journal of the Korean Society for Applied Biological Chemistry, v. 54, n. 1, p. 46-53, 2011. DOI: 10.3839/jksabc.2011.006.

36. RAMM, A.; SOUZA, A.L.K.; MANICA-BERTO, R.; TIMM, C.R.F.; SCHUCH, M.W.; VIZZOTTO, M. Development of 'Maciel' peach trees on different propagation systems. Revista Brasileira de Fruticultura, Jaboticabal, v. 40, n. 4., p. 1-10, 2018. DOI: /10.1590/0100-29452018477.

37. SCARIOTTO, S. Variabilidade genética e abordagem bioquímica da reação do fruto de diferentes genótipos de *Prunus persica* à *Monilinia fructicola*. 2015. 133f. Tese (Doutorado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2015.

38. CHAPARRO, J.X.; WERNER, D.J.; WHETTEN, R.W.; O'MALLEY, D.M. Inheritance, genetic interaction, and biochemical characterization of anthocyanin phenotypes in peach. Journal of Heredity, v. 86, n. 1, p. 32-38, 1995. DOI: 10.1093/oxfordjournals.jhered.a111522.

39. Zhou, H.; Peng, P.; Zhao, J.; Owiti, A.; Ren, F.; Liao, L.; Wang, L.; Deng, X.; Jiang, Q.; Han, Y. Multiple R2R3-MYB transcription factors involved in the regulation of anthocyanin accumulation in peach flower. Frontiers in Plant Science, v. 7, n. 1557, p. 1-11, 2016. DOI: 10.3389/fpls.2016.01557.

40. Liu, J.; Macarisin, D.; Wisniewski, M.; Sui, Y.; Droby, S.; Norelli J.; Hershkovitz, V. Production of hydrogen peroxide and expression of ROS-generating genes in peach flower petals in response to host and non-host fungal pathogens. Plant Pathology, v. 62, p. 820–828, 2013. DOI: 10.1111/j.1365-3059.2012.02683.x.

4.4 Artigo 4. Heritability and segregation of resistance to brown rot in peach fruits

Artigo submetido na Revista *"Acta Horticulturae"*
IX International Peach Symposium ISHS, București, Romênia, julho de 2017

Heritability and segregation of resistance to brown rot in peach fruits

M. Dini[1,*], S. Scariotto[2], M.C.B. Raseira[2] and B. Ueno[2]

[1]Programa de Pós-Graduação em Agronomia (PPGA), Universidade Federal de Pelotas (UFPel), Pelotas-RS, Brazil; [2]Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Embrapa Clima Temperado, Pelotas-RS, Brazil.

Abstract

Brown rot caused by the fungus *Monilinia fructicola* is the most important disease in peach production areas of Brazil. The increased concern with the environment, consumers and workers` health, emphasizes control strategies other than fungicide applications. Among them genetic resistance is the most efficient. However, availability of resistant genotypes is still limited. Thus the main objective of this work was to seek sources of brown rot resistance, as well as to study the segregation, estimate the heritability and verify the possible existence of maternal effect. Heritability of brown rot resistance was investigated in peach fruits of several genotypes from the Embrapa peach breeding program. Sixteen progenies and 20 parents were evaluated. Disinfested fruits were wounded with a microsyringe and inoculated by deposition of a 10µL drop of a 2.5 x 10^4 spores mL^{-1} of *M. fructicola*. The fungus inoculum was obtained from a culture originated from peach mummies of four different sites. After inoculation, the fruits were incubated under controlled conditions for 72 hours, previous evaluation of lesion size and sporulation. High phenotypic variability and transgressive segregation were observed for brown rot resistance in fruits. Several genotypes showed similar resistance as cv. Bolinha, the standard Brazilian cultivar for resistance. The heritability of brown rot resistance in fruits (diameter of the lesion and sporulation), is medium. Parental selection based on phenotype, enables a medium genetic advance for brown rot resistance. The selections Conserva 947 and Conserva 1600 were the parents with higher brown rot resistance (similar to 'Bolinha'), passing this trait to their offspring.

Keywords: *Prunus persica*; *Monilinia fructicola*; genetic resistance; genetic variability; Brazilian genotypes.

INTRODUCTION

Brown rot (BR) is considered one of the most important diseases of peach (*Prunus persica* (L.) Batsch). It can be caused by three species of the genus *Monilinia*, *M. laxa* (Aderh. & Ruhl.) Honey, *M. fructigena* (Aderh. & Ruhl.), and *M. fructicola* (Winter) Honey. The latter is the cause of the disease in Brazil and in most of the world. These pathogens infect blooms, twigs and fruit in the field with a variety of symptoms, including blossom blight, cankers on woody tissues and fruit rot, with significant economic losses (Adaskaveg et al., 2008; May-de Mio et al., 2014; Grzegorczyk et al., 2017).

In the fruits, the first symptoms are tan-brown, small and circular patches that develop into brown spots, with colonization of the tissues by the fungus (May-de Mio et al., 2014). Under optimal environmental conditions for the disease (high humidity and mild temperatures), these symptoms may be visible after 48 hours of infection (Ogawa et al., 1995). At the maturation stage, infected fruits develop a firm rot, brown lesion that advances rapidly, taking all the fruit. On the lesion, we can see the fungus sporulation with a powdery appearance and a grayish color (Mondino et al., 2010).

The increased concern with the environment and the health of workers and consumers, emphasizes control strategies such as genetic resistance, seeking to reduce the

* E-mail: maxidini@hotmail.com

use of pesticides. This is the most effective way to prevent the disease, reducing production costs and environmental impact. However, the selection of resistant genotypes is still limited, despite being among the objectives of many peach breeding programs in the world, due to the scarcity or lack of knowledge of good sources of high resistance or immunity (Raseira and Franzon, 2014).

The use of genetic resistance in commercial peach orchards has been limited because there are not commercial cultivars with high resistance or immunity to *Monilinia* spp. and good fruit quality. However, there are significant differences on level of susceptibility among the available genotypes (Adaskaveg et al., 2008; Santos and Ueno, 2014).

The Brazilian cultivar Bolinha is considered to have some degree of horizontal resistance to BR in fruits (Feliciano et al., 1987), and has been used up to now, as a standard, by several research groups around the world (Byrne et al. 2012, Raseira and Franzon, 2014). However, this cultivar presents some problems that make it unfeasible for its use in commercial production, such as low quality, reduced size and premature fruit drop (Feliciano et al., 1987; Gradziel and Wang, 1993; Santos and Ueno, 2014).

The main objective of this work was to seek sources of brown rot resistant genotypes, as well as study the segregation, estimate the heritability and verify the possibility of any maternal effect.

MATERIALS AND METHODS

Fruits of peach and nectarine, used in this study, were collected from Embrapa Clima Temperado peach breeding program, Pelotas, Rio Grande do Sul, Brazil, in 2015-2016 and 2016-2017 growing season. The evaluations of the reaction to BR infection were realized in Embrapa´s laboratories of Fruit Breeding and Plant Pathology.

The reaction of fruits to BR was investigated in peach fruits of sixteen populations F1 (seedlings) and 20 parents (cultivars or selections). The first ten populations originate from reciprocal crosses (Table 1).

Table 1. Progeny F1, parents and number of seedlings of the Embrapa peach breeding program, Pelotas, Rio Grande do Sul, Brazil.

Progeny F_1	Parents[1]			Number of seedlings
	♀		♂	
2008.159	Conserva 1526	×	‘Cerrito’	7
2009.38	‘Cerrito’	×	Conserva 1526	23
2012.26	Cascata 1055	×	‘Chimarrita’	18
2012.43	‘Chimarrita’	×	Cascata 1055	25
2012.49	Conserva 672	×	Conserva 1526	18
2012.61	Conserva 1526	×	Conserva 672	7
2012.52	Conserva 947	×	Conserva 1600	17
2012.66	Conserva 1600	×	Conserva 947	12
2012.68	Conserva 1662	×	‘Maciel’	24
2012.88	‘Maciel’	×	Conserva 1662	17
2012.31	Cascata 1359	×	Cascata 1577	19
2012.46	Chorão	×	‘Maciel’	25
2012.99	Necta 506	×	‘Sunmist’	20
2012.107	Necta 532	×	Necta 480	25
2012.111	Necta 540	×	‘Morena’	25
2012.114	‘BRS Rubimel’	×	TX2D163	21

[1]Genotypes proceeded by the word Conserva, Cascata or Necta, correspond to selections of the Embrapa breeding program for fresh market, canning or are nectarine selections respectively.

Fruits, at firm ripening stage, were selected for absence of apparent damage and infection. For inoculation, fruit surface was disinfested by dipping for 1 minute in 70% alcohol solution, followed by a 3 minutes immersion in a 0.5% solution of NaClO. Ten minutes later, fruits were rinsed, twice, by dipping them in separate buckets of distilled water, and then allowed to dry on paper towels. They were then placed in transparent plastic boxes (24.0 x 23.0 x 10.0cm) on rings of plastic over moistened paper at the base.

The experimental design was completely randomized, considering each genotype as a treatment. Samples of five fruits of each individual seedling were evaluated. Each fruit was considered as a repetition. There were three clones of each parental genotype and five fruits per clone were evaluated, totalizing 15 fruits per parent. Cultivar cv. Bolinha was used as a standard of low susceptibility (Feliciano et al., 1987; Santos et al. 2012) and cv. Atenas as standard of highly susceptible (Fabiane, 2011; Wagner Jr. et al., 2011).

The *M. fructicola* inoculum was obtained from a culture originated from peach mummies of four different sites. The inoculation was made by deposition of a 10µL drop of a 2.5 x10^4 spores mL^{-1} suspension, using a microsyringe with a metal tip, which was also used to cause a wound in the fruits. After inoculation, the fruits were incubated at 25±1°C and 75% relative humidity for 72 hours (Crisosto et al., 2009; Scariotto, 2016).

At the end of this period, the fruits were evaluated for disease incidence and severity. For the brown rot incidence (BRI), it was considered the number of fruits that present lesion, transformed into percentage of the total number of inoculated fruit.

The severity was evaluated by measuring the lesion diameter (LD), using a digital caliper, and using the average of two perpendicular measurements (Martínez-García et al., 2013; Santos et al., 2012). Sporulation (SPO) was also evaluated, with presence or absence observed, and if so, the sporulation diameter (SPD), similar to the lesion measurement procedure was measured (Scariotto, 2016).

The lesion area (LA) and sporulation area (SPA), in mm^2, was calculated by the formula: A= (π x C x L)/4, C being the length and L the width (Maffia et al., 2007; Pazolini et al., 2016). In the same way, with the measures of fruit diameter and height the area of a face of the fruit (half of the fruit) was calculated. From these measurements the percentage of the total of one face of the fruit that was affected by the lesion (%LA) and sporulation (%SPA) was calculated. Thus the fruit size was considered in each measurement making them comparable, which was important since fruit size was very variable among genotypes.

Broad-sense heritability was estimated for resistance to BR in fruits. For these estimates, the mean variance observed among clones was considered as the mean environmental variance ($\hat{\sigma}_E^2$). The variance observed among individuals belonging to the same progeny was used as the total phenotypic variance ($\hat{\sigma}_P^2$) (genetic effect plus environmental). The genetic variance ($\hat{\sigma}_G^2$) was obtained by the difference between the $\hat{\sigma}_E^2$ and the $\hat{\sigma}_P^2$ (Wagner Jr., 2003). Finally, the calculation of the broad-sense heritability (H^2) was estimated by dividing the genetic variance by the total variance: H^2= $\hat{\sigma}_G^2/(\hat{\sigma}_G^2+\hat{\sigma}_E^2)$.

Narrow-sense heritability (h^2) was estimated from the regression of the average of parental phenotypic values vs. offspring phenotypic values (Griffiths et al., 2002; Visscher et al., 2008).

The maternal effect was evaluated by comparing the progeny of one of the crosses with the progeny of their reciprocal crossing (F1 vs. reciprocal F1), by the t-test at 5% significance (Londero et al., 2009) for the studied parameters.

RESULTS AND DISCUSSION

High variability and transgressive segregation was observed for all evaluated parameters, related to the incidence and severity of *M. fructicola* in the inoculated fruits. The phenotypic variance and the interval between maximum and minimum of these variables show this high variability, among the progenies as well as among the parents. Greater variability was detected for SPO, with maximum ranges of 0 to 100% presence of sporulation in the fruits, and $\hat{\sigma}_P^2$ between 1010.4 and 1453.5, in the two years of evaluation (Table 2).

Table 2. Descriptive statistics of incidence and severity of brown rot in 16 peach progenies and 20 parental genotypes evaluated in the 2015-2016 and 2016-2017 cycles, in Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

		2015-2016						2016-2017					
		BRI[1] (%)	LD (mm)	%LA (%)	SP (%)	SPD (mm)	%SPA (%)	BRI (%)	LD (mm)	%LA (%)	SP (%)	SPD (mm)	%SPA (%)
Progenies	Mean	97.1	29.9	37.7	63.2	16.2	17.5	96.5	29.3	41.7	62.7	14.9	17.2
	SD[2]	9.0	9.1	20.3	38.1	12.0	18.7	11.1	8.6	20.2	37.2	10.7	16.4
	$\hat{\sigma}^2_P$	80.1	82.4	411.5	1453.5	143.5	347.7	122.6	74.6	406.1	1380.6	115.0	268.0
	CV (%)	9.2	30.4	53.8	60.4	73.9	106.8	11.5	29.5	48.4	52.3	72.2	95.2
	Min	50.0	4.7	1.2	0.0	0.0	0.0	20.0	2.8	1.5	0.0	0.0	0.0
	Max	100.0	55.7	100.0	100.0	55.7	100.0	100.0	47.7	98.2	100.0	51.2	98.2
Parents	Mean	100.0	29.6	30.1	74.3	16.2	12.8	99.7	31.2	41.9	62.2	16.3	19.1
	SD	0.0	7.6	16.0	31.8	9.2	11.4	1.5	7.5	22.2	33.2	11.6	20.8
	$\hat{\sigma}^2_P$	0.0	57.1	255.3	1010.4	85.4	130.3	2.2	55.8	490.9	1103.3	133.7	433.3
	CV (%)	0.0	25.6	53.2	42.8	56.9	89.2	1.5	23.9	52.8	53.4	71.0	109.1
	Min	100.0	16.3	8.3	0.0	0.0	0.0	93.3	19.5	13.3	0.0	0.0	0.0
	Max	100.0	45.0	65.8	100.0	29.2	39.8	100.0	46.5	91.7	100.0	45.2	86.8

[1]BRI= brown rot incidence; LD= lesion diameter; %LA= percentage of the one face of the fruit affected by the lesion; SP= percentage of fruits with presence of sporulation; SPD= sporulation diameter; %SPA= percentage of the one face of the fruit affected by sporulation. [2]SD= standard deviation; $\hat{\sigma}^2_P$= phenotypic variance; CV= coefficient of variation; Min= minimum; Max= maximum.

The mean values observed for BRI in fruits were between 96.5 and 100%, higher than those found in previous studies (Wagner Jr., 2003; Fabiane, 2011). These differences may be due to the technique used, since these authors sprayed the inoculum on non-wounded fruits. Evaluating peach cultivars and selections by inoculation methods non-wound fruit, Santos et al. (2012) reported averages of LD ranging from 0.8 to 34.9mm, 72 hours after inoculation. These values were lower than those found in the present experiment, probably due to the inoculation method utilized. The fruit skin is the main structural barrier against fungus infection, and any injury serves as the gateway to the pathogen (Gradziel and Wang, 1993). On the other hand, when inoculation technique was the same as here, (Scariotto, 2016), that is, inoculation by drop deposition on wounded fruits, results for BRI, ID and SPD were similar to the ones obtained in the present study.

For example, the cv. Bolinha, considered with good level of horizontal resistance to BR, in the work of Santos et al. (2012) was reported with a mean LD of 2.9mm. However, on wounded fruits, Scariotto (2016) observed a mean LD of 9.1mm, and in the present work, LD 18.4 and 19.6mm in diameter were found for the first and second evaluation cycles, respectively. The resistance of this cultivar is mainly due to the skin characteristics, such as the compaction of epidermal cells and the thickness of the cuticle (Feliciano et al., 1987; Gradziel and Wang, 1993; Santos et al., 2012). Also, by the higher production of phenolic compounds in the skin, when compared to other cultivars (Gradziel and Wang, 1993; Gradziel et al., 1998; Santos et al., 2012; Scariotto, 2016). However, the injury breaks this physical-chemical barrier.

For the characters lesion and sporulation diameter to BR (Table 3), among the parents used, the selections Conserva 947, Conserva 1662, Conserva 1600 e Conserva 672 were the least susceptible among all the parents used, with lower values in general, similar to cv. Bolinha. The selection Conserva 672 and Conserva 1600 have previously been cited as moderately resistance to BR in fruit (Wagner Jr., 2003; Scariotto, 2016).

Among the progenies, the best performance, for all the variables studied for BR in the fruit, were the progenies 2012.52 and 2012.66 with lower values for all the evaluated parameters. These two progenies are reciprocal, having as parents the selections Conserva

947 and Conserva 1600, already mentioned among the least susceptible to BR in fruits, evidencing that they are good parents for this purpose.

Table 3. Averages of incidence and severity of lesion and sporulation of brown rot in peach, evaluated for 16 progenies and 20 parents, for two years at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

Progenies	N°	FA[1] (mm²)	BRI (%)	LD (mm)	LA (mm²)	%LA (%)	SP (%)	SPD (mm)	SPA (mm²)	%SPA (%)
2008.159	5	2302.4	80.0	28.3	622.0	41.4	80.0	19.4	304.6	31.5
2009.38	11	2338.6	100.0	35.5	808.2	33.3	92.6	12.7	154.8	30.1
2012.26	16	1742.3	98.6	28.3	732.7	45.1	69.6	16.7	341.5	21.0
2012.43	24	2048.4	93.8	31.6	879.0	48.0	76.8	19.7	443.6	24.5
2012.49	14	2212.1	98.3	30.3	789.7	38.1	56.9	13.4	273.0	12.5
2012.61	6	2170.5	92.0	33.6	973.7	47.2	84.0	22.3	470.0	22.8
2012.52	13	2355.1	88.9	24.0	574.0	26.7	24.4	4.6	71.9	3.2
2012.66	9	2273.1	92.5	25.2	555.7	25.6	10.0	2.1	34.9	1.4
2012.68	17	2272.6	98.6	27.8	659.2	32.8	34.3	11.7	146.4	7.3
2012.88	16	2532.6	100.0	32.5	895.4	38.1	49.2	16.4	264.5	10.5
2012.31	18	1723.8	94.0	31.2	709.5	38.1	67.9	14.1	250.9	15.5
2012.46	25	1898.2	99.2	32.4	883.7	51.0	73.4	16.9	337.0	19.5
2012.99	15	1643.2	98.2	34.8	964.9	65.0	81.8	23.2	541.8	36.4
2012.107	16	1680.5	100.0	35.9	955.2	60.2	91.7	21.0	424.0	29.2
2012.111	15	2093.2	95.2	29.2	771.0	43.5	80.6	19.6	412.6	24.0
2012.114	20	2009.7	97.9	32.1	831.4	39.4	82.6	20.9	457.9	21.8
Parents										
Conserva 1526	3	2921.9	100.0	36.0	1107.6	38.8	81.7	21.9	487.6	17.1
'Cerrito'	3	2379.1	100.0	26.8	580.1	24.3	58.4	11.3	179.2	7.3
Cascata 1055	2	2788.1	100.0	32.0	837.6	30.2	74.5	17.1	332.7	11.6
'Chimarrita'	3	2727.5	100.0	38.6	1201.3	44.5	89.0	23.6	515.8	19.1
Conserva 672	2	2786.9	100.0	26.1	570.9	21.0	50.0	9.0	131.0	4.8
Conserva 947	3	3122.4	96.7	18.4	290.9	11.8	3.9	0.7	8.5	0.4
Conserva 1600	3	2703.5	96.7	24.2	485.1	19.5	32.6	5.2	65.5	2.6
Conserva 1662	3	2850.9	100.0	23.4	455.9	16.2	28.4	3.7	42.9	1.6
'Maciel'	3	3006.8	100.0	32.8	916.8	28.7	60.0	16.2	346.5	10.3
Cascata 1359[2]	2	1858.5	100.0	28.6	642.5	36.3	100.0	17.0	232.6	12.7
Cascata 1577	2	2169.1	100.0	32.3	950.2	44.9	100.0	22.5	464.5	22.1
Chorão	3	1440.9	100.0	35.6	1017.0	69.7	86.7	24.7	582.6	39.0
Necta 506	2	1857.9	100.0	37.9	1150.2	62.5	93.8	28.6	687.2	37.6
'Sunmist'	2	1525.9	100.0	31.7	805.2	56.5	100.0	24.2	509.5	37.0
Necta 532[2]	2	1853.2	100.0	46.5	1697.0	91.7	100.0	45.2	1601.7	86.8
Necta 480[2]	3	2180.0	100.0	27.8	657.0	30.6	54.5	12.3	219.0	9.8
Necta 540[2]	3	1829.9	100.0	34.3	937.0	52.1	80.0	18.6	365.8	20.4
'Morena'[2]	3	1868.9	100.0	22.4	482.9	25.5	55.6	11.8	202.5	10.5
'BRS Rubimel'	3	2600.4	96.7	32.6	893.3	34.5	91.4	15.9	292.0	10.8
TX2D163[2]	3	2620.4	100.0	27.0	582.5	22.2	46.7	8.8	136.3	5.2
Controls										
'Atenas'	3	2505.2	100.0	37.8	1159.3	47.1	82.9	23.1	515.7	21.1
'Bolinha'	3	2415.5	86.1	18.4	326.4	14.1	13.2	2.1	25.8	0.7

[1]FA= fruit area; BRI= brown rot incidence; LD= lesion diameter; LA= lesion area; %LA= percentage of the one face of the fruit affected by the lesion; SP= percentage of fruits with presence of sporulation; SPD= sporulation diameter; SPA= sporulation area; %SPA= percentage of the one face of the fruit affected by sporulation. [2]Avrages only of the 2016-2017 cycle.

No evidence was found of the occurrence of maternal effect for the characters incidence and severity of BR in fruits, since the t test was not significant for the five populations and their reciprocals ($P \geq 0.05$). These characters must have quantitative inheritance, predominantly additive, being preferable to use both parents with some level of resistance when the objective is the obtaining of resistant progenies.

The H^2 for LD and SPD were estimated as 0.57 and 0.46, respectively, among all families evaluated in the first cycle. For the second cycle, H^2 for LD and SPD were estimated as 0.46 and 0.42, respectively. The H^2 of the LD and SPD were higher than the estimates of Scariotto (2016), which were 0.50 and 0.13, respectively. These differences can be attributed to the different methods of estimating the parameters and the use of different genotypes. Scariotto (2016) used a mixed model procedure, where the components of variance are estimated using restricted maximum likelihood/best linear unbiased prediction (REML/BLUP). Wagner Jr. (2003), using the same methodology as this work to estimate the heritability of resistance to BR, but using the incidence, estimated H^2 at 0.64, a value close to that found in this work for the LD.

The h^2 was estimated only for the second evaluation cycle, since some parents did not have evaluation data in the first cycle, h^2 for LD was estimated to be 0.42 and 0.39 for SPD (Figure 1). The estimated value of h^2 corresponds to the regression coefficient "*b*" of the equation of the line $Y = a + bx$, the slope (Griffiths et al., 2002; Visscher et al., 2008).

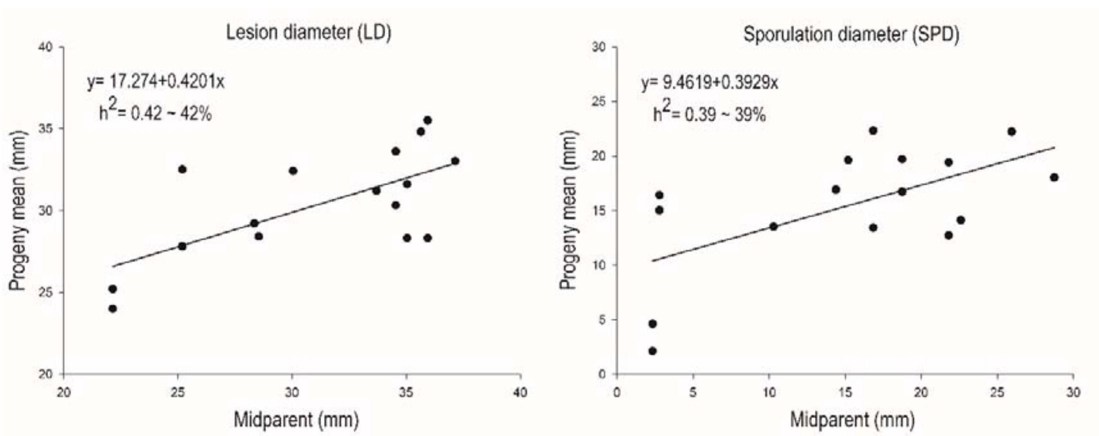


Figure 1. Narrow-sense heritability (h^2) estimated by regression midparent and the mean of all the progenies, for lesion diameter (LD) and sporulation diameter (SPD). The slope coefficient is the value of the h^2 estimating. In the cycle 2016-2017, Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

The H^2 can be considered medium for LD and SPD. In any case, this estimate is of little use to the breeders, being h^2 of major importance. The effect of selection depends on the magnitude of the additive genetic variance and not on the total genetic variance. Thus, narrow-sense heritability is the relevant for predicting the selection response (Griffiths et al., 2002). The estimated h^2 can also be considered as medium, indicating that the selection of the parents based on the phenotype, may be fairly effective, and a moderate genetic advance for these BR resistance characters is expected.

CONCLUSIONS

- The selections Conserva 947 and Conserva 1600 are interesting to be used in crosses aiming brown rot resistance in fruits.
- The heritability of brown rot resistance in fruits (diameter of the lesion and sporulation) is medium. Parental selection, based on phenotype, enables a medium genetic advance for brown rot resistance.

- The studied populations present genetic variability and transgressive segregation regarding the resistance to *Monilinia fructicola* in fruits
- No evidence of maternal effect was observed.

ACKNOWLEDGEMENTS

The ANII (Agencia Nacional de Investigación e Innovación), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and Embrapa Clima Temperado.

Literature cited

Adaskaveg, J.E., Schnabel, G., and Forster, H. (2008). Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In The peach: botany, production and uses, D.R. Layne, and D. Bassi, eds. (Wallingford, Oxfordshire, UK: CABI Publishing), p.352-406.

Byrne, D.H., Raseira, M.B., Bassi, D., Piagnani, M.C., Gasic, K., Reighard, G.L., Moreno, M.A., and Pérez, S. (2012). Peach. In Fruit breeding, M.L. Badenes, and D.H. Byrne, eds. (New York, USA: Springer) p.505-569.

Crisosto, C.; Gradziel, T. and Ogundiwin, E. (2009). Development of predictive tools for brown and sour rot resistance in peach and nectarines. Annual research report, California tree fruit agreement, 87-93.

Fabiane, K.C. (2011). Reação de pessegueiros a *Monilinia fructicola* (Wint.) Honey e sua relação com componentes bioquímicos. MSc thesis (Pato Branco, PR, Brazil: UTFPR).

Feliciano, A., Feliciano, A.J., and Ogawa, J.M. (1987). *Monilinia fructicola* resistance in peach cultivar Bolinha. Phytopathology *77*, 776-780.

Gradziel, T.M., Thorpe, M.A., Bostock, R.M., Wilcox, S., and Monetl, R. (1998). Breeding for brown rot (*Monilinia fructicola*) resistance in cling stone peach with emphasis on the role of fruit phenolics. Acta Horticulturae *465*, 161-170.

Gradziel, T.M., and Wang, D. (1993). Evaluation of brown rot resistance and its relation to enzymatic browning in cling stone peach germplasm. J. Am. Soc. Hortic. Sci., *118*(5), 675-679.

Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R.C., and Gelbart, W.M., eds. (2002). Genética (Madrid, Spain: McGraw-Hill Interamericana), pp.860.

Grzegorczyk, M., Zarowska, B., Restuccia, C., and Cirvilleri, G. (2017). Postharvest biocontrol ability of killer yeasts against *Monilinia fructigena* and *Monilinia fructicola* on stone fruit. Food Microbiol. *61*, 93–101 http://doi.org/10.1016/j.fm.2016.09.005.

Londero, P.M.G., Ribeiro, N.D., Cerutti, T., Mazeiro, S.M., Rosa, D.P., and Rosa, S.S. (2009). Efeito materno na expressão dos teores de aminoácidos sulfurados em grãos de feijão. Ciência Rural *39*(6), 1884-1887 http://dx.doi.org/10.1590/S0103-84782009005000129.

Maffia, L.A., Mizubuti, E.S.G., Alfenas, A.C., and Mafia, R.G. (2007). Quantificação de doenças em planta. In Métodos em fitopatologia, A.C. Alfenas, and R.G. Mafia, eds. (Viçosa, MG, Brazil: UFV), p.161-173.

Martínez-García, P.J., Parfitt, D.E., Bostock, R.M., Fresnedo-Ramírez, J., Vazquez-Lobo, A., Ogundiwin, E.A., Gradziel, T.M., and 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), e78634 http://doi.org/10.1371/journal.pone.0078634.

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

Mondino, P.; Alaniz, S.; Leoni, C. (2010). Manejo integrado de las enfermedades del duraznero en Uruguay. In Manual del duraznero; Manejo integrado de plagas y enfermedades, J. Soria, ed. (Montevideo, Uruguay: INIA), p.45-76.

Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., and Uyemoto, J.K., eds. (1995). Compendium of stone fruit diseases (Saint Paul, USA: The American Phytopathological Society), 98p.

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

Raseira, M.C.B., and Franzon, R.C. (2014). Melhoramento genético. In Pessegueiro, M.C.B. Raseira, J.F.M. Pereira, and F.L.C. Carvalho, (Brasília, DF, Brazil: Embrapa), p.57-72.

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

Santos, J., and Ueno, B. (2014). Controle da podridão-parda no Brasil. In Manejo de la podredumbre morena (*Monilinia fructicola* y *M. laxa*) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina, M. Mitidieri, and Castillo, J.A., eds. (Santiago de Chile, Chile: CYTED/RedFrut-San), p.73-77.

Scariotto, S. (2016). Variabilidade genética e abordagem bioquímica da resistência do fruto à podridão-parda em diferentes genótipos de *Prunus persica*. PhD thesis (Pelotas, RS, Brazil: UFPel).

Visscher, P.M., Hill, W.G., and Wray, N.R. (2008). Heritability in the genomics era - concepts and misconceptions. Nat. Rev. Genet. 9, 255–266 http://dx.doi.org/10.1038/nrg2322.

Wagner Júnior, A. (2003). Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey. MSc thesis (Pelotas, RS, Brazil: UFPel).

Wagner Júnior, A., Fabiane, K.C., Oliveira, J.S.M.A., Zanela, J., and Citadin, I. (2011). Peaches tree genetic divergence for brown rot reaction. Revista Brasileira de Fruticultura *33*, 552-557 http://dx.doi.org/10.1590/S0100-29452011000500075.

4.6 Artigo 5. *Monilinia fructicola* lesion and sporulation in peach and nectarine fruits

Artigo a ser submetido na Revista *“Pesquisa Agropecuária Brasileira - PAB”*

Monilinia fructicola lesion and sporulation in peach and nectarine fruits

Maximiliano Dini[(1)], Maria do Carmo Bassols Raseira[(2)], Silvia Scariotto[(3)], Silvia Carpenedo[(2)], Bernardo Ueno[(2)]

[(1)] Universidade Federal de Pelotas, Faculdade de Agronomia Eliseu Maciel, Programa de Pós-Graduação em Agronomia, Caixa Postal 354, CEP 96010-900, Capão do Leão, RS, Brazil. E-mail: maxidini@hotmail.com.

[(2)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Rodovia BR 392, km 78, Caixa Postal 403, CEP 96010-971, Pelotas, RS, Brazil. E-mail: maria.bassols@embrapa.br, bernardo.ueno@embrapa.br.

[(3)] Universidade Tecnológica Federal do Paraná, Campus Pato Branco, Via do Conhecimento, km 1, CEP 85503-390, Pato Branco, PR, Brazil. E-mail: silviascariotto@yahoo.com.br.

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 their use, favors adoption of other control strategies, being genetic resistance the most important one. This study was carried out aiming to evaluate the susceptibility/resistance of several peach and nectarine genotypes to brown rot. Both wounded and non-wounded fruits harvest from 20 genotypes were inoculated with 10 µL of *M. fructicola* suspension (2.5×10^4 conidia mL^{-1}). Wounded fruits showed susceptibility to *M. fructicola*, since 92 to 100% of them had brown rot symptoms. The disease incidence was between 18 and 100% when non-wounded fruits were inoculated. High variability was detected for sporulation presence in both wounded and non-wounded fruits, with ranges between 16 to 96% and 0 to 94%, respectively. The sporulation capacity of the fungus was very variable among the genotypes (between 0.1 to 96.0 conidia mm^2) and as expected, it is positively correlated with the presence, diameter and area of sporulation. The genotypes Conserva 947, Conserva 1662, Conserva 672, Conserva 1600 and 'Bolinha', were the most brown rot resistant.

Index terms: *Prunus persica*, brown rot, genetic resistance.

Lesão e esporulação da _Monilinia fructicola_ em frutos de pessegueiro e nectarina

Resumo – O fungo *Monilinia fructicola* causador da podridão-parda, é um dos principais patógenos do pessegueiro. O surgimento de isolados do fungo resistentes a fungicidas, assim como a preocupação em reduzir o uso destes, favorecem a adoção de outras estratégias de controle, sendo a resistência genética a mais importante. Os objetivos deste estudo foram avaliar a suscetibilidade/resistência de diversos genótipos de pessegueiros e nectarineiras à podridão-parda. Frutos com e sem ferimento colhidos de 20 diferentes genótipos foram inoculados, com 10 µL de suspensão a $2{,}5x10^4$ conídios mL^{-1}. Os frutos com ferimento mostraram-se suscetíveis *M. fructicola*, com incidência entre 92 e 100% de podridão-parda. Porém sem ferimento a incidência foi entre 18 e 100% dos frutos inoculados. Para a presença de esporulação foi verificada alta variabilidade, com resultados entre 16 e 96% para a inoculação com ferimento e 0 e 94% para frutos inoculados sem ferimento. A capacidade de esporulação do fungo foi muito variável entre os genótipos (entre 0,1 a 96,0 conídios mm^2) e está positivamente correlacionada com a presença, diâmetro e área da esporulação. Os genótipos Conserva 947, Conserva 1662, Conserva 672, Conserva 1600 e 'Bolinha', de modo geral, foram os mais resistentes à podridão-parda.

Termos para indexação: *Prunus persica*, podridão-parda, resistência genética.

Introduction

The fungus *Monilinia fructicola* (Winter) Honey, the causing agent of brown rot is one of the main pathogen of the peach culture (*Prunus persica* (L.) Batsch), in Brazil as well as in most part of the world. The damages may occur at any time of the peach cycle, starting at blooming until postharvest. The main disease symptoms are blossom blight, twig cankers and 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 (OGAWA et al., 1995; ADASKAVEG et al., 2008; MAY-DE-MIO et al., 2008, 2014).

Brown root is mainly controlled through fungicides sprays (THOMIDIS et al., 2009). However, the increasing concern about the environment preservation and workers and consumers health (BARÓ-MONTEL et al., 2019; ELSHAFIE et al., 2015), as well as the emergence of fungicide resistant pathogen populations (LUO et al., 2010; HILY et al., 2011; ZHU et al., 2012; CHEN et al., 2017; FU et al., 2017), result in the demand for others control strategies such as genetic resistance. However, the use of resistance has been limited in commercial orchards because, although there are significant differences in susceptibility among available genotypes, commercial cultivars that are resistant or immune to brown rot are not yet available (ADASKAVEG et al. 2008; SANTOS & UENO, 2014). The Brazilian peach cultivar Bolinha, presents a superior level of resistance in fruits than other already evaluated cultivars (FELICIANO et al., 1987, SANTOS et al., 2012; FU et al., 2018). However, poor fruit quality and productivity problems discouraged its cultivation.

The resistance of 'Bolinha' is associated to a greater compaction of the epidermal cells and cuticle thickness (structural resistance), that is the major barrier against the infection of the pathogen (FELICIANO et al., 1987; GRADZIEL & WANG, 1993; SANTOS et al., 2012). Another factor that may contribute to the resistance presented by this cultivar is the production of phenolic compounds (biochemical resistance), in greater quantity than most cultivars (GRADZIEL & WANG, 1993; SANTOS et al., 2012), resulting in a longer fungus incubation period, when compared to more susceptible cultivars (OGAWA et al., 1995).

The fruit resistance to brown rot is a quantitative and polygenic trait (MARTINEZ-GARCIA et al., 2013; PACHECO et al., 2014; BARÓ-MONTEL et al., 2019). This type of resistance delays the epidemic development in the field, 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).

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

Materials and Methods

The study was performed at Embrapa Clima Temperado, in the laboratories of fruit breeding and phytopathology, in Pelotas, Rio Grande do Sul, Brazil (31º40' S and 52º26' W; 57 m altitude). Fruit reaction to brown rot of 20 different genotypes, among cultivars and selections of the Embrapa peach collection was tested. The genotypes were as follows: four nectarines 'Sunmist', Necta 506, Necta 532 and Necta 540 and, 16 peach '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 whipping type selection (Chorão). Except for 'Sunmist' that was released by (University of Florida) and TX2D163 that is from Texas A&M University, all genotypes are from the Embrapa Peach Breeding Program.

The experimental design was completely randomized, and each genotype was considered as one treatment, with four replications of five fruits. The inoculation was made using the technique of drop deposition, on wounded 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 strain was obtained from mummified fruits infected by *M. fructicola* collected in peach orchards of Embrapa Clima Temperado. From these, fragments of approximately 5 mm were transferred to Petri dishes containing Potato Dextrose Agar (PDA) culture meda 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 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

Fruits at commercial ripening 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. The fruits were arranged into transparent plastic boxes (24 x 23 x 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 distilled and sterilized water.

The inoculation was made by dropping the inoculum suspension on fruits with and without wounding. For the inoculation on wounded fruits (penetration of 1 mm into the fruits), it was used an inoculation syringe of 100 µL coupled to a repeating dispenser 50x (Hamilton®). In both cases 10 µL suspention of *M. fructicola* (2.5×10^{4} conidia mL^{-1}) and Tween-80® (0.1 g L^{-1}) was used in each fruit (CRISOSTO et al., 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. The presence of sporulation (SPP) was also evaluated, and if so, sporulation diameter (SPD) was measured, in the same way as the LD. The LD and SPD in wounded fruits, were subjected to Pearson correlation

The lesion (LA) and sporulation (SPA) areas were calculated, considering as circular shape, by the formula: $A = (\pi \times D1 \times D2) / 4$, being D1, the diameter of the first measurement and D2, the diameter of its perpendicular measurement (PAZOLINI et al., 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 samples of 5 mm diameter were collected from five fruits inoculated with wounds on the sporulation zone of each fruit, 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. Sporulation capacity (SPc) 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) (KADISH et al., 1990). The SPc 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 ($p \leq 0.05$). For the statistical analysis, the data expressed as a 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 averages of the genotypes of all analyzed variables regarding lesion and sporulation, grouping the genotypes for resistance to rot- brown 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 the incidence of brown rot (BRI), ranging from 92 to 100% (Figure 1). When the fruits were not wounded at the inoculation, the variability was very large, 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). Similar results were reported by Baró-Montel et al. (2019) in which approximately 100% of wounded fruits of different genotypes developed the disease, and the non-wounded fruits presented wide variability (between 0 and 80%). On other studies with non-wounded fruits, results had averages between 60 and 100% (OBI et al., 2017) and between 50 and 100% (OBI et al., 2019). It should be noted that in these three studies the evaluations were performed 120 hai and in the present study the evaluations were performed 72 hai.

Regarding to SPP, high variability was detected for both inoculation in wounded and non-wounded fruit, with intervals between 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. When the fruits were not wounded at inoculation, it was observed that besides these four, two more genotypes showed less than 3% of fruits with SPP (Conserva 947, Conserva 1662, 'Bolinha', Conserva 672, Conserva 1600 and 'Maciel'). Working with different genotype evaluated five days after inoculation with *M. laxa*, Obi et al. (2019) found averages of 84.8 and 80.2% fruits colonized (sporulated), probably due to the longer incubation time plus genetic differences.

All ANOVAs performed for LD and LA were highly significant ($p < 0.0001$) for both wounded and non-wounded fruits, and for all harvest seasons. In fruits inoculated with injury, 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 verified in the first harvest season and four in the second and third. The genotypes identified in the lower 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). Working with inoculations of *M. fructicola* in fruits and evaluating at 72 hai, 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 incidence of the disease.

It is important to note that the variable LA was more efficient than LD to differentiate the levels of susceptibility. Highlighting that some genotypes, such 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 permitted to allocate these genotypes in groups of less susceptibility in comparison to when LD was used as a variable of severity. This is in accordance with Walter et al. (2004), who performed a screening for brown rot resistance in apricot fruits (*Prunus armeniaca*), observed that the LA, measure after 72 hai, was the variable that best discriminated the genotypes.

When the inoculation was made in non-wounded fruits, the variability was even higher among genotypes, with values of LD and LA ranging from 0.0 to 41.6 mm and from 0.0 to 79.5%, respectively. But, the accuracy of the experiment was lower, with coefficients of variation between 62.1 and 84.1%. In this case, 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 was already mentioned as 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). Testing the susceptibility of non-wounded fruits of several peach genotypes to *M. laxa*, Obi et al. (2017) obtained LD averages between 38.0 to 62.5 mm in one experiment, and 56.5 to 48.9 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 (120 hai), higher pathogen virulence (*M. laxa*) and/or the high susceptibility of the genotypes when compared to the Embrapa genotypes.

All ANOVAs performed for SPD, SPA and SPcapacity, were highly significant ($p < 0.0001$) for both wounded and non-wounded fruits, and for all harvest seasons (Table 2). When the inoculation was made without wounded the fruits, the SPD and SPA averages were between 0.0 to 36.8 mm and 0.0 to 69.5%, respectively.

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. The genotypes better rated for SPA, in the three harvests, were the same as for SPD plus the selection Conserva 672, all with less than 7.3% (Table 2).

On fruits non-wounded at the inoculation, 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 lower susceptibility group, with lower 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). Working with non-wounded fruits inoculated with *M. laxa* (evaluated five days after inoculation), Obi et al. (2017) obtained SPD averages between 0 to 51.5 mm among the tested genotypes, and Obi et al. (2019) obtained SPD averages of 52.5 and 45.3 mm during two years of evaluation.

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 consequently, SPA will also be smaller. It should be pointed out that in the case of non-wound inoculation, although the fruits are visually unharmed, 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. 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 conidium would have to be coincident with the place where the micro-wound is located, making few conidia to be successful in infection (OGAWA et al., 1995; 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), but they only used fruits with LD and SPD greater than 0 mm. However, in the present study, the correlation was not higher due to the great presence of fruits that presented lesions, but not sporulation of the fungus (concentration of points on the Y axis) (Figure 2).

In the case of SPc, the variability among genotypes was also very large, 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, the lowest SPc group was composed by genotypes that presented less than 27.1 conidia mm^2. The group of higher SPc was formed by the genotypes Necta 506, Conserva 1526 and Chorão, with 59.8, 78.9 and 96.0 conidia mm^2, respectively (Tabela 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 and, suggesting that *M. fructicola* shows more pathogenicity.

The genotypes Cascata 1359, Necta 540 and Necta 532 (3.2, 5.2 and 20.6 conidia 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 SPc 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). The contrary, it was observed in fruits of 'Cerrito', whose values for LD, SPD, LA and SPA were generally low, but their SPc was high (57.5 conidia mm^2) (Table 2).

The four variables studied in the 2017-2018 cycle related to sporulation were positively correlated among them ($p < 0.001$) (Tabela 3). SPc presented a correlation of 0.66, 0.67 and 0.74 with SPA, SPD and SPP respectively being the later considered as medium to high, suggesting that if the SPP of *M. fructicola* in fruits is high, the SPc will also be high. Correlations from 0.41 to 0.70, between LA and SPc, had been reported by Walter et al. (2004). 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 impossible to do.

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).

The genotypes Conserva 947, Conserva 1662, Conserva 672 and Conserva 1600 compose the group that presented the 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, because it presented the lowest average (90%) 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. In turn, 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 SPc 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 variables. 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. The 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 SPc (5.2 and 3.2 conidia mm^2, respectively). It suggests that even though these genotypes are very susceptible to brown rot their SPc to propagate the disease is low. The last four genotypes, 'Sunmist', Necta 532, Cascata 1577 and Chorão were separated into three groups and presented the worst results against brown rot, being considered the most susceptible.

The use of more than one variable and different inoculation techniques (with and without wound), may be important for the evaluation of brown rot resistance in peach. The best results in relation to brown rot resistance were noted in the genotypes Conserva 947, Conserva 1662, Conserva 672, Conserva 1600 and 'Bolinha'. The four selections have fruit quality far superior to 'Bolinha', what shows the progress regarding this by the Embrapa Peach Breeding Program.

Conclusion

1. The lesion diameter is positively correlated to the sporulation.
2. The sporulation capacity is positively correlated to the sporulation diameter, sporulation area and mainly to the sporulation presence.
3. Conserva 947, Conserva 1662, Conserva 672, Conserva 1600 genotypes presented better results regarding brown rot resistance, similar to 'Bolinha'.

Acknowledgments

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

References

ADASKAVEG, J.E.; SCHNABEL, G.; FORSTER, H. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: LAYNE D.R.; BASSI, D. (Ed.). **The peach: botany, production and uses**. Wallingford: CAB International, 2008. p.352-406.

BARÓ-MONTEL, N.; EDUARDO, I.; USALL, J.; CASALS, C.; ARÚS, P.; TEIXIDÓ, N.; TORRES, R. Exploring sources of resistance to brown rot in an interspecific almond × peach population. **Journal of the Science of Food and Agriculture**, v.99, n.8, p.4105-4113, 2019. DOI: 10.1002/jsfa.9640.

CHEN, S.; YUAN, N.; SCHNABEL, G.; LUO, C. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. **Molecular Plant Pathology**, v.18, n.1, p.90-97, 2017. DOI: 10.1111/mpp.12387.

CRISOSTO, C.; GRADZIEL, T. OGUNDIWIN, E. Development of predictive tools for brown and sour rot resistance in peach and nectarines. **California tree fruit agreement**, Annual Research Report 2009, p.87-93, 2009.

DALLAGNOL, L.J.; ARAUJO ILHO, J.V. Uma visão geral da resistência genética da planta a microrganismos. In: DALLAGNOL, L.J. (Ed.). **Resistência genética de plantas a patógenos**. Pelotas: UFPel, 2018. p.13-64.

ELSHAFIE, H.S.; MANCINI. E.; CAMELE, I.; DE MARTINO, L.; DE FEO, V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. **Industrial Crops and Products**, v.66, p.11-15, 2015. DOI: 10.1016/j.indcrop.2014.12.031.

FELICIANO, A.; FELICIANO, A.J.; OGAWA, J.M. *Monilinia fructicola* resistance in peach cultivar Bolinha. **Phytopathology**, v.77, n.6, p.776-780, 1987. DOI: 10.1094/Phyto-77-776.

FU, W.; BURRELL, R.; LINGE, C.S., SCHNABEL, G.; GASIC, K. Breeding for brown rot (*Monilinia* spp.) tolerance in Clemson University Peach Breeding Program. **Journal of the American Pomological Society**, v.72, n.2, p.94-100, 2018.

FU, W.; TIAN, G.; PEI, Q.; GE, X.; TIAN, P. Evaluation of berberine as a natural compound to inhibit peach brown rot pathogen *Monilinia fructicola*. **Crop Protection**, v.91, p.20-26, 2017. DOI: 10.1016/j.cropro.2016.09.008.

GRADZIEL, T.M.; WANG, D. Evaluation of brown rot resistance and its relation to enzymatic browning in cling stone peach germplasm. **Journal of the American Society of Horticultural Science**, v.118, n.5, p.675-679, 1993. DOI: 10.21273/JASHS.118.5.675.

KADISH, D., GRINBERGER, M., COHEN, Y. Fitness of metalaxyl-sensitive and metalaxyl resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. **Phytopathology**, v.80, n.2, p.200-205, 1990. DOI: 10.1094/Phyto-80-200.

KUSHALAPPA, A.C.; GUNNAIAH, R. Metabolo-proteomics to discover plant biotic stress resistance genes. **Trends in Plant Science**, v.18, p.522-531, 2013. DOI: 10.1016/j.tplants.2013.05.002.

HILY, J.M.; SINGER, S.D.; VILLANI, S.M.; COX, K.D. 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**, v.67, p.385-396, 2011. DOI: 10.1002/ps.2074.

LUO, C.X.; HU, M.J.; JIN, X.; YIN, L.F.; BRYSON, P.K.; SCHNABEL, G. An intron in the cytochrome b gene of *Monilinia fructicola* mitigates the risk of resistance development to QoI fungicides. **Pest Management Science**, v.66, p.1308-1315, 2010. DOI: 10.1002/ps.2016.

MARTÍNEZ-GARCÍA, P.J.; PARFITT, D.E.; BOSTOCK, R.M.; FRESNEDO-RAMÍREZ, J.; VAZQUEZ-LOBO, A.; OGUNDIWIN, E.A.; GRADZIEL, T.M.; CRISOSTO, C.H. Application of genomic and quantitative genetic tools to identify candidate resistance genes for brown rot resistance in peach. **PloS One**, v.8, n.11, e78634, 2013. DOI: 10.1371/journal.pone.0078634.

MAY-DE MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. **Tropical Plant Pathology**, v.33, n.3, p.227-234, 2008. DOI: 10.1590/S1982-56762008000300008

MAY-DE MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. (Ed.). **Pessegueiro**. Brasília: Embrapa, 2014. p.355-432.

MICHAILIDES, T.J.; MORGAN, D.P. Influence of fruit-to-fruit contact on the susceptibility of French prune to infection by *Monilinia fructicola*. **Plant Disease**, v.81, n.12, p.1416-1424, 1997. DOI: 10.1094/PDIS.1997.81.12.1416.

OBI, V.I.; BARRIUSO, J.J.; MORENO, M.A.; GIMÉNEZ, R.; GOGORCENA, Y. Optimizing protocols to evaluate brown rot (*Monilinia laxa*) susceptibility in peach and nectarine fruits. **Australasian Plant Pathology**. V.46, n.2, p.183-189, 2017. DOI: 10.1007/s13313-017-0475-2.

OBI, V.I.; BARRIUSO, J.J.; USALL, J.; GOGORCENA, Y. Breeding strategies for identifying superior peach genotypes resistant to brown rot. **Scientia Horticulturae**, v.246, p.1028-1036, 2019. DOI: 10.1016/j.scienta.2018.10.027.

OGAWA, J.M.; ZEHR, E.I.; BIRD, G.W.; RITCHIE, D.F.; URIU, K.; UYEMOTO, J.K. **Compendium of stone fruit diseases**. Saint Paul: The American Phytopathological Society, 1995. 98p.

PACHECO, I.; BASSI, D.; EDUARDO, I.; CIACCIULLI, A.; PIRONA, R.; ROSSINI, L.; VECCHIETTI, A. QTL mapping for brown rot (*Monilinia fructigena*) resistance in an intraspecific peach (*Prunus persica* L. Batsch) F1 progeny. **Tree Genetics & Genomes**, v.10, n.5, p.1223-1242, 2014. DOI: 10.1007/s11295-014-0756-7.

PAZOLINI, K., SANTOS, I., CITADIN, I., STORCK, L., FLORES, M.F. Sampling plan for assessing brown rot severity in peaches subjected to different plant extracts. **Revista Caatinga**, v.29, n.3, p.519-527, 2016. DOI: 10.1590/1983-21252016v29n301rc.

RIOS, J.A.; DEBONA, D. Efeito epidemiológico da resistência de hospedeiro. In: DALLAGNOL, L.J. (Ed.). **Resistência genética de plantas a patógenos**. Pelotas: UFPel, 2018. p.126-149.

SANTOS, J.; RASEIRA, M.C.B.; ZANANDREA, I. Resistência à podridão-parda em pessegueiro. **Bragantia**, v.71, n.2, p.219-225, 2012. DOI: 10.1590/S0006-87052012005000022.

SANTOS, J.; UENO, B. Controle da podridão-parda no Brasil. In: MITIDIERI, M.; CASTILLO, J.A. (Ed.). **Manejo de la podredumbre morena (*Monilinia fructicola* y *M. laxa*) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina**. Santiago de Chile, 2014. p.73-77.

SCARIOTTO, S.; SANTOS, J.; RASEIRA, M.C.B. Search for resistance sources to brown rot in Brazilian peach genotypes. **Acta Horticulturae**, v.1084, p.211-216, 2015. DOI: 10.17660/ActaHortic.2015.1084.29.

THOMIDIS, T.; MICHAILIDES, T.; EXADAKTYLOU, E. Contribution of pathogens to peach fruit rot in northern Greece and their sensitivity to iprodione, carbendazim, thiophanate-methyl and tebuconazole fungicides. **Journal of Phytopathology**, v.157, p.194–200, 2009. DOI: 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. Methods of screening apricot fruit for resistance to brown rot caused by *Monilinia* spp. **Australasian Plant Pathology**, v.33, p.541-547, 2004. DOI: 10.1071/AP04062.

ZHU, F.; BRYSON, P.K.; SCHNABEL, G. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. **Pest Management Science**, v.68, n.7, p.1003-1009, 2012. DOI: 10.1002/ps.3255.

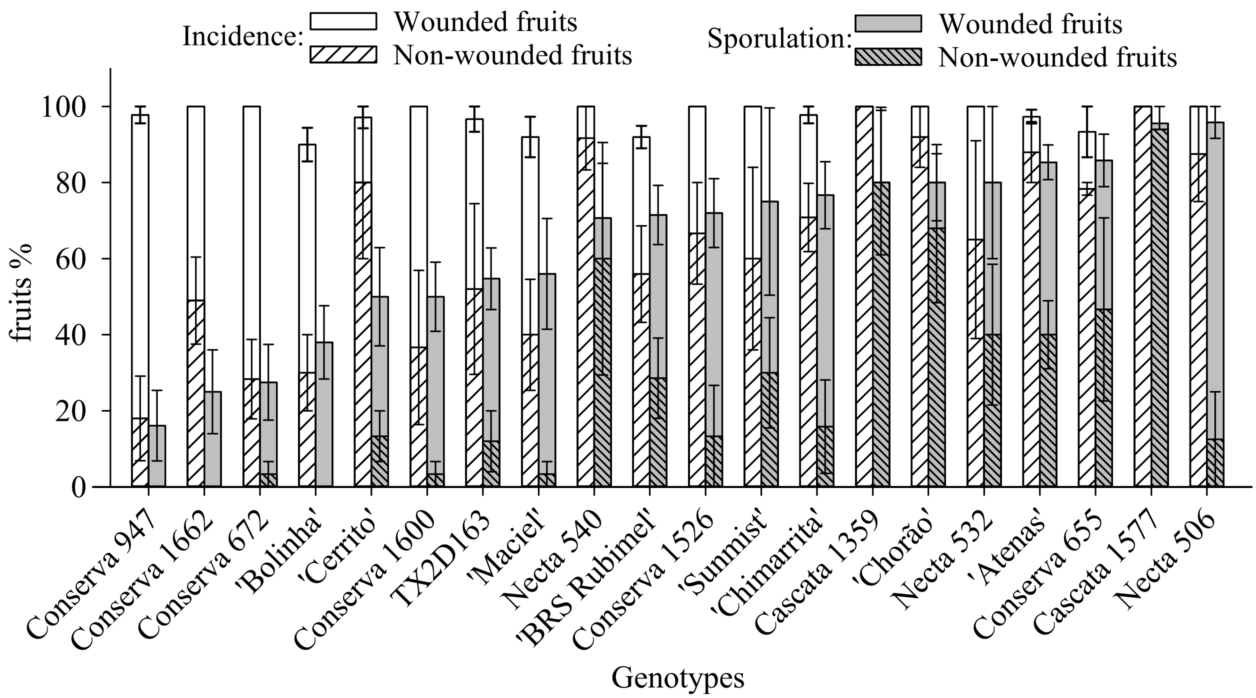


Figure 1. Brown rot incidence and sporulation presence in 20 peach genotypes inoculated with *Monilinia fructicola* in wounded and non-wounded fruits, evaluated at 72 hours after inoculation. The columns correspond to the average values of three harvest seasons (2015-2016, 2016-2017 and 2017-2018) for 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, Brazil.

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, 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 (%)
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
'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
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
'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
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
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 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
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
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
'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
'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
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
'Chimarrita'	37.1 c	42.4 c	14.2 b	9.5 b	40.0 c	46.5 c	32.0 b	41.1	35.6 c	44.0 c
'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
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
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
'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 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
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 $p \leq 0.05$. [(2)]LD = evaluated in two perpendicular measures by fruits. [(3)]LA = percentage of the one face of the fruit lesioned by *Monilinia fructicola*.

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, 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 (%)	SPc[4] (conidia mm^2)	SPD (mm)	SPA (%)
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
'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
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
'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
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
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 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
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
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
'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
'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
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
'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
'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
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
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
'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 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
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]SPc = Sporulation capacity, evaluated in five fruits per genotype in three samples of 5 mm of diameter per fruit, expressed in conidia per mm^2.

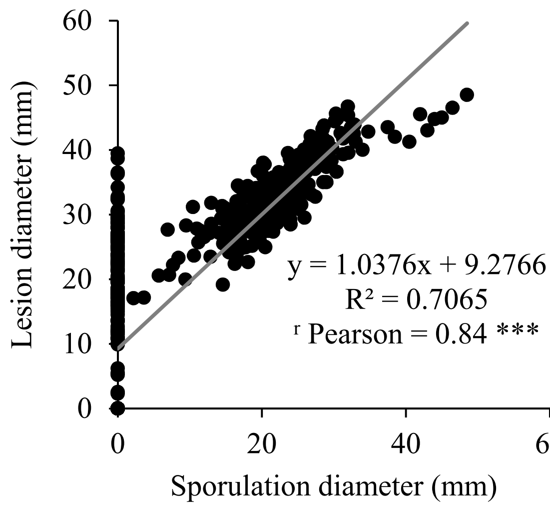


Figure 2. Correlation between sporulation and lesion diameter in wounded fruits of 20 peaches genotypes inoculated with *Monilinia fructicola* evaluated over three seasons (2015-2016, 2016-2017 and 2017-2018), Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. *** = significant at $p \leq 0.001$.

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

Sporulation variables	Sporulation capacity	Sporulation presence	Sporulation diameter	Area covered by sporulation
Sporulation capacity	-	2.00^{-04}	1.20^{-03}	1.60^{-03}
Sporulation presence	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)] In the lower diagonal the Spearman's correlation value and in the upper diagonal p-value.

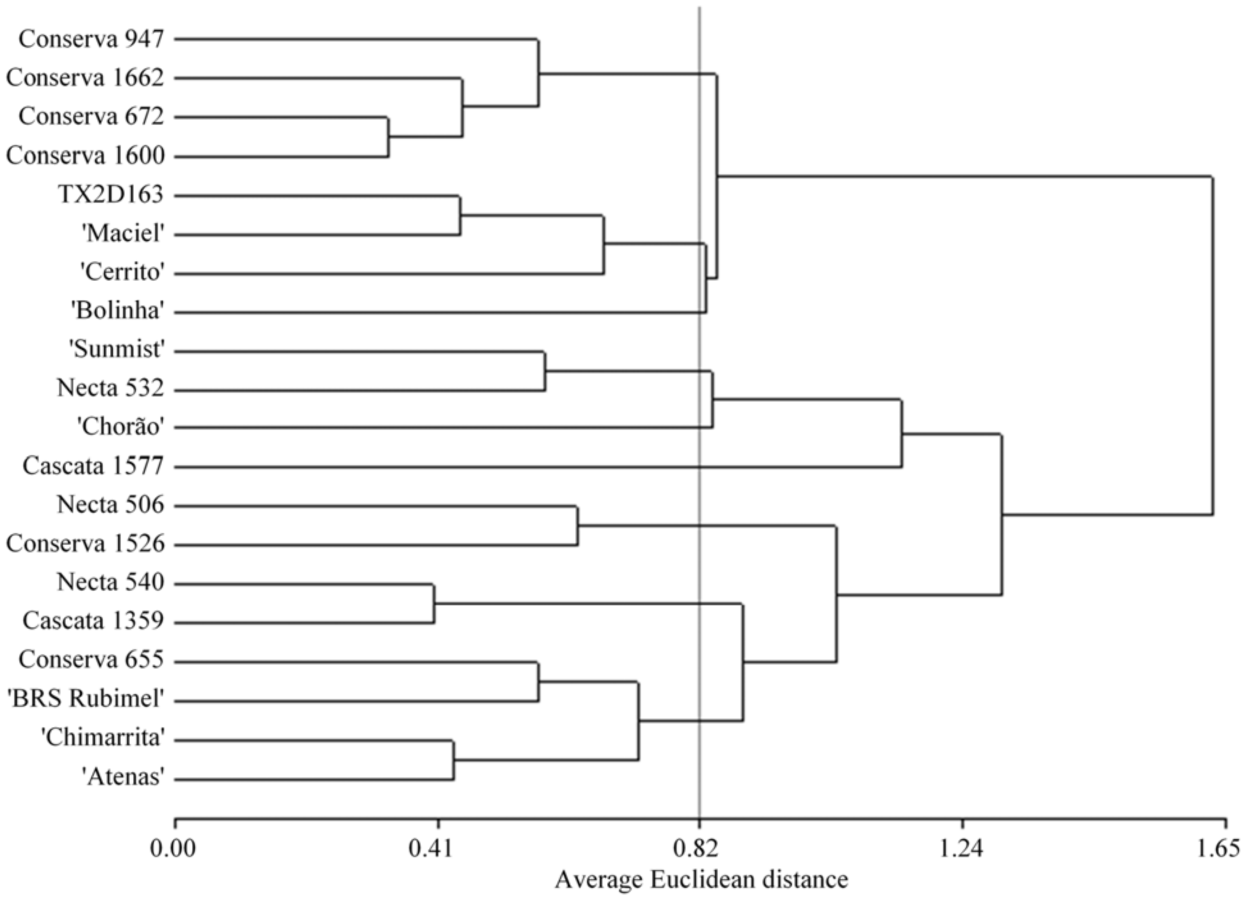


Figure 3. Dendrogram representing analysis of conglomerates among 20 evaluated genotypes, obtained by Unweighted Pair-Group Method using Arithmetic averages (UPMGA). Average Euclidean distance based on the means of incidence and severity of the lesion and sporulation of brown rot in fruits. Cut-off point 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, Brazil.

4.5 Artigo 6. Breeding peaches for brown rot resistance in Embrapa

Artigo a ser submetido na Revista *“Scientia Horticulturae”*

Breeding peaches for brown rot resistance in Embrapa

Maximiliano Dini[a,*]; Maria do Carmo Bassols Raseira[b]; Silvia Scariotto[c]; Bernardo Ueno[b]

[a] Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.

[b] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Rodovia BR 392, km 78, Caixa Postal 403, CEP 96010-971, Pelotas, Rio Grande do Sul, Brazil.

[c] Universidade Tecnológica Federal do Paraná, Campus Pato Branco, Via do Conhecimento, km 1, CEP 85503-390, Pato Branco, Paraná, Brazil.

* Corresponding author at: Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.

E-mail addresses: maxidini@hotmail.com, mdini@inia.org.uy (M. Dini).

Breeding peaches for brown rot resistance in Embrapa

Keywords: *Prunus persica* (L.) Batsch, *Monilinia fructicola* (Winter) Honey, genetic resistance, progeny segregation, genetic advance, lesion, sporulation.

Abstract. Brown rot, caused by *Monilinia* spp., is the main stone fruit disease. Major efforts to detect sources of resistance is being applied by several breeding programs worldwide. The main objective of this study was to seek sources of brown rot resistance, as well as to study the segregation, estimate the heritability, verify the possible existence of maternal effect, and estimate the genetic advances of the Embrapa Peach Breeding Program. For this purpose, 20 parents (cultivars or advanced selections) and 303 seedlings, representing 16 breeding families (progenies), and 'Bolinha' (control) have been phenotyped for fruit reaction to brown rot using wounded and non-wounded inoculation procedures in 2015-2016, 2016-2017 and 2017-2018 growing seasons. Wounded fruits were very susceptible to brown rot incidence, however, on non-wounded fruits the incidence and severity showed high variability among the evaluated genotypes. Conserva 947 and Conserva 1600 and their progenies (2012.52 and 2012.66), presented lower disease incidence and severity than most of the evaluated genotypes. Genetic advance estimated was -5.2 to -30.2% (wounded fruits) and between -15.0 to -25.0% (non-wounded fruits) for brown rot resistance. Selected genotypes presented equal or better results than 'Bolinha' in relation to brown rot resistance, being several of them, far superior fruit quality than 'Bolinha', which demonstrates the progress of the Embrapa Peach Breeding Program, not only in terms of resistance to *M. fructicola*.

1. Introduction

Peach (*Prunus persica* L. Batsch) breeding programs started in Brazil, in early 1950`s, in the Instituto Agronomico de Campinas, São Paulo. A few years later a similar program started in Rio Grande do Sul State. The latter was first coordinate by the Agriculture Secretary of Rio Grande do Sul. However, in the late 50`s - early 1960`s, the program was moved to Pelotas (Rio Grande do Sul), and them coordinate by a federal institution. With the advent of Embrapa in 1973, the program was not only preserved but also increased (Raseira et al., 2008).

Rio Grande do Sul State is the main producer, with about 69% of the Brazilian production, occupying more than 12.5 thousand hectares, in the year 2017. Pelotas, where the Embrapa Peach Breeding is located, stands out as the largest national producer of peaches, accounting for more than 14% of the country's total production (IBGE, 2019). The area is characterized by a very humid weather and mild temperatures, so very favorable to fungus diseases. Among them, brown rot, caused by *Monilinia fructicola* (Winter) Honey, is far the most important and for that reason, the Embrapa Peach Breeding Program has the search for *M. fructicola* resistance as one of the priorities (Raseira and Franzon, 2014). Under these conditions the disease symptoms may be visible 48 hours after infection. Brown rot damages can occur from flowering to post-harvest. The main symptoms are the blossom blight, cankers in branches and brown rot in fruits (Ogawa et al., 1995; Adaskaveg et al., 2008; May-de-Mio et al., 2008, 2014).

The use of genetic resistance has been limited in commercial orchards, since commercial peach cultivars immune to brown rot are still unavailable. However, there are significant differences in susceptibility among available genotypes (Adaskaveg et al. 2008; Santos and Ueno, 2014). Brown rot control is mainly done by fungicide sprays (Thomidis et al., 2009). Thus, genetic resistance is a priority in many breeding programs worldwide, mainly due to the emergence of fungus isolates resistant to fungicides (Luo et al., 2010; Hily et al., 2011; Zhu et al., 2012; Chen et al., 2017; Fu et al., 2017) and the increase on environment concern and workers and consumers health (Raseira and Franzon, et al. 2014; Baró-Montel et al., 2019; Elshafie et al., 2015).

The Brazilian cultivar Bolinha shows higher levels of resistance in fruits than most cultivars, however, its poor fruit quality, together with problems of premature fall, discourage its commercial cultivation (Feliciano et al., 1987, Santos et al., 2012; Scariotto et al., 2015; Scariotto, 2016; Dini et al., 2018, 2019a; Fu et al., 2018).

The type of fruit resistance to brown rot is quantitative and polygenic (Martínez-García et al., 2013; Pacheco et al., 2014; Baró-Montel et al., 2019). This type of resistance slows the development of the epidemic in the orchard, in spite of the genotype being susceptible to the disease (Kushalappa and Gunnaiah, 2013; Dallagnol and Araujo Filho, 2018).

Knowledge of the genetic, phenotypic and environmental parameters that influence directly or indirectly the characters of economic importance in a culture is fundamental for the breeding programs guidance (Ramalho et al., 2012). Given the limited information on peach brown rot resistance and with the intention of contributing to the genetic improvement of this crop, the objectives of this study were: evaluate the distribution of brown rot resistance in fruits of different populations; verify the possible existence of maternal effect for this character;

estimate the heritability; identify genotypes with higher levels of resistance; and estimate the genetic advance for this character in the Embrapa Peach Breeding Program.

2. Materials and methods

2.1. Plant material

The study was developed at Embrapa Clima Temperado, Fruit Breeding and Phytopathology Laboratories, in Pelotas, Rio Grande do Sul, Brazil (Lat. 31º40'S, Long. 52º26'W, alt. 57 m asl.) in the 2015-2016, 2016-2017 and 2017-2018 seasons. Fruit reaction to brown rot was tested in 16 F1 progenies of the Embrapa Peach Breeding Program, including ten progenies from five reciprocal crosses. Fruits of the individual seedlings and their parents were evaluated (Table 1).

Table 1. Progeny identification, parents and number of seedlings of each progeny, in the Peach Breeding Program at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.

Parents	♀	×	♂	N° seedlings
2008.159	Conserva 1526		'Cerrito'	7
2009.38	'Cerrito'		Conserva 1526	23
2012.26	Cascata 1055		'Chimarrita'	18
2012.43	'Chimarrita'		Cascata 1055	25
2012.49	Conserva 672		Conserva 1526	18
2012.61	Conserva 1526		Conserva 672	7
2012.52	Conserva 947		Conserva 1600	17
2012.66	Conserva 1600		Conserva 947	12
2012.68	Conserva 1662		'Maciel'	24
2012.88	'Maciel'		Conserva 1662	17
2012.31	Cascata 1359		Cascata 1577	31
2012.46	Chorão		'Maciel'	25
2012.99	Necta 506		'Sunmist'	20
2012.107	Necta 532		Necta 480	25
2012.111	Necta 540		'Morena'	25
2012.114	'Rubimel'		TX2D163	21

Seedlings were planted in an experimental orchard spaced of 0.5 m apart on the row and 5 m between rows. The parent trees were planted in work collection of Embrapa, spaced 2 m between trees and 5 m between rows. Each parent has three trees obtained by asexual propagation (clones).

2.2. Experimental design and treatments

The experimental design was completely randomized, and each genotype was considered as one treatment. Five wounded fruits (in each of the three seasons) and five non-wounded fruits (in two seasons 2016-2017 and 2017-2018) per seedling were used. Three clones of each parent and ten fruits per clone, five wounded and five non-wounded, were evaluated in the same seasons. The Bolinha cultivar was used as a standard for low brown rot susceptibility (Feliciano et al., 1987; Santos et al. 2012).

2.3. Pathogen culture, conidia production, and inoculation

The procedure adopted for inoculations was the same described by Dini et al. (2019b). Briefly, the fungus isolate was obtained from four peach orchards in Embrapa Clima Temperado. For the inoculation with wounded (penetration of 1 mm into the fruits), it was used an inoculation syringe of 100 µL coupled to a repeating dispenser 50x (Hamilton®). In both cases 10 µL suspention of *M. fructicola* (2.5×10^4 conidia mL^{-1}) and Tween-80® (0.1 g L^{-1}) was used in each fruit. Incubation was at 23±1°C and 12 hours photoperiod for three days (Crisosto et al., 2009; Martínez-García et al., 2013; Scariotto et al., 2015; Fu et al., 2018; Dini et al., 2019a, 2019b; Obi et al., 2017, 2019).

2.4. Brown rot evaluation

Incidence and severity were evaluated according to Dini et al. (2019b). Briefly, fruits inoculated with and without wound were evaluated, according to the disease incidence and severity at 72 hours after inoculation (HAI). Brown rot incidence (BRI) and sporulation presence (SPP) were calculated as the percentage of fruits that presented symptoms and sporulation of *M. fructicola*, respectively; lesion diameter (LD) and sporulation diameter (SPD) were evaluated through two perpendicular measurements per fruit in the equatorial region; lesion area (LA) and lesion sporulation (SPA) were calculated and expressed as the percentage of fruit that was affected by the lesion and sporulation, respectively. Thus, fruit size was considered in each measurement making them comparable, which was important since fruit size was very variable among genotypes.

2.5. Statistical and genetic analysis

To evaluate the progenies segregation and to test for the possibility of maternal effect, relative frequency histograms were constructed with the severity data. The maternal effect was tested comparing the F1 progeny with the F1 reciprocal progeny, by the t-test ($p < 0.05$) (Londero et al., 2009).

Broad-sense heritability (H^2) was estimated according Dini et al. (2019a Acta) for resistance to brown rot in fruits. The calculation was based on the data obtained in all seasons evaluated for wounded and non-wounded fruits, and the estimated environmental variance ($\hat{\sigma}_e^2$) divided by three and two, respectively (environments number = seasons of evaluation). Narrow-sense heritability (h^2) was estimated from the regression of the average of parental phenotypic values vs. offspring phenotypic values (Falconer and Mackay, 1996; Dirlewanger et al., 2012; Griffiths et al., 2015).

Genetic advance (GA%), also called response to selection or genetic progress was estimated, and expressed in percentage of the population mean, with the formula:

$$GA\% = \frac{(\overline{X}_s - \overline{X}_o)\, h^2}{\overline{X}_o} * 100$$

Where, $\overline{X}_s$, mean of the selected genotypes; $\overline{X}_o$, original mean (base population); $\overline{X}_s - \overline{X}_o$ = SD, selection differential; h^2, narrow-sense heritability (Falconer and Mackay, 1996). 'Bolinha' was then used as reference for the selection of the best genotypes.

3. Results

A total of 303 seedlings, 20 parents (cultivars and advanced selections) and the cultivar Bolinha were evaluated for their reaction to brown rot on wounded and non-wounded fruits. When the fruits were inoculated, after being wounded, all genotypes were susceptible to BRI, varying between 92 to 100% in the parents and between 83 to 99% in the progenies (Fig. 1A). However, 22 genotypes showed a BRI of less than 80%, most of them being on progenies 2012.52 (4 seedlings), 2012.66 (5 seedlings) and 2012.68 (5 seedlings).

When inoculation was made on non-wounded fruits, them was a wade variability, between 18 to 100% in among parent plants and between 14 to 69% in the progenies (Fig. 1B). The progenies with the lowest means were 2012.52 (20%), 2012.66 (14%), 2012.68 (34%) and 2012.88 (30%). A total of 43 seedlings presented less than 20% BRI, and most of them are part of the aforementioned progenies (5, 5, 6 and 4 seedlings, respectively). It should be noted that the progenies 2012.52 and 2012.66 are a product of a controlled cross between Conserva 947 and Conserva 1600, and in turn the progenies 2012.68 and 2012.88 are a product of the cross between Conserva 1662 and 'Maciel', parents who presented, together with Conserva 672, the lowest means. In addition, the progeny 2012.114 stood out with six seedlings with less than 20% of BRI and all seedlings produced fruits with a marked pilosity.

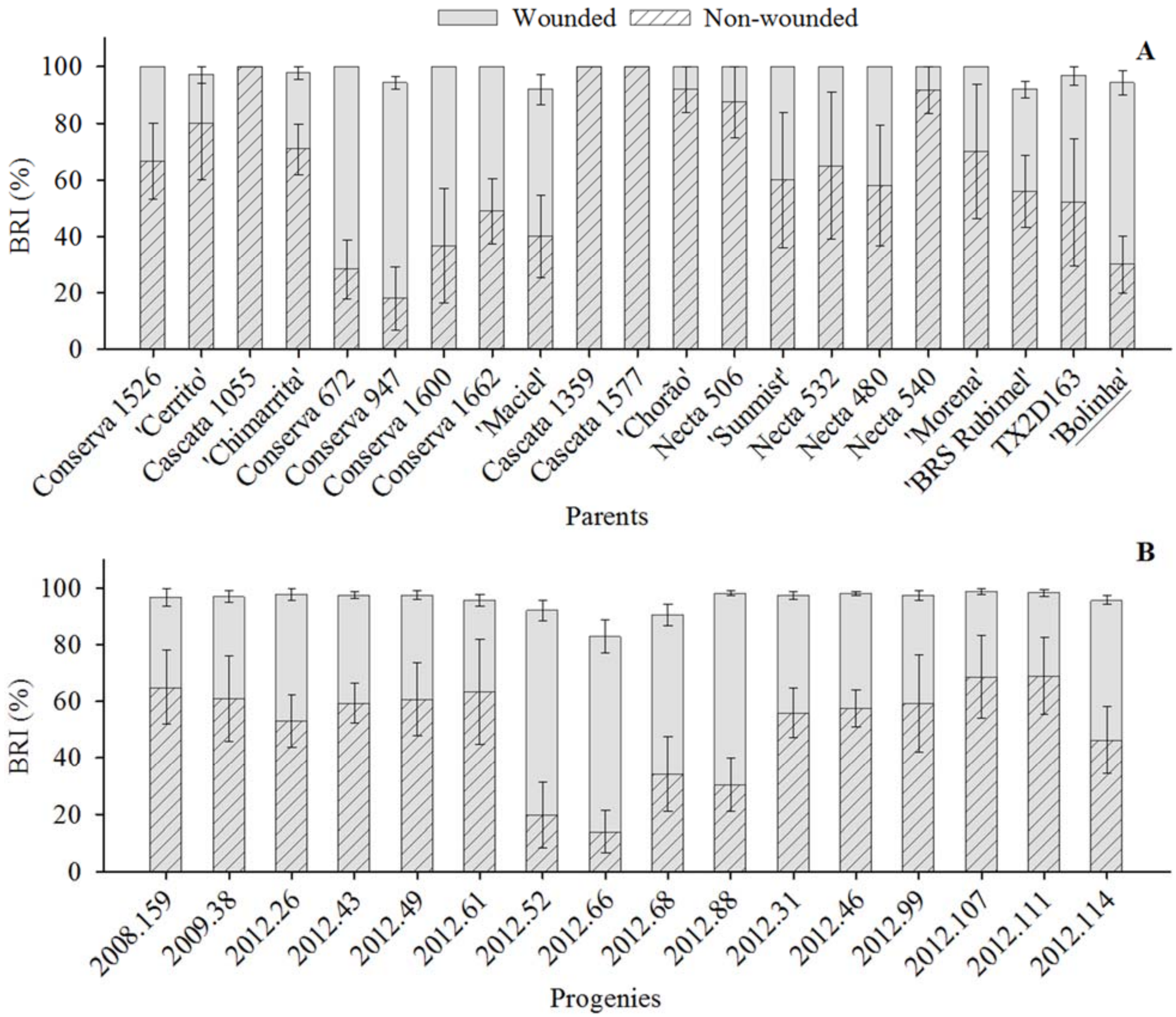


Fig. 1. Brown rot incidence (BRI) on wounded and non-wounded fruits of parents (A) and progenies (B) average for three and two seasons (2015-16; 2016-17; 2017-18 and 2016-17; 2017-18, respectively). 'Bolinha' was included as control. Embrapa Peach Breeding Program, Pelotas, Rio Grande do Sul, Brazil.

According to their LA, when the fruits were inoculated with wound, the seedlings followed approximately a normal distribution. On the other hand, on non-wounded fruits, the seedlings presented a distribution like Lognormal or Weibull, concentrated in the classes of less LA (0 to 10 and 10 to 20% of the area affected by lesion) (Fig. 2).

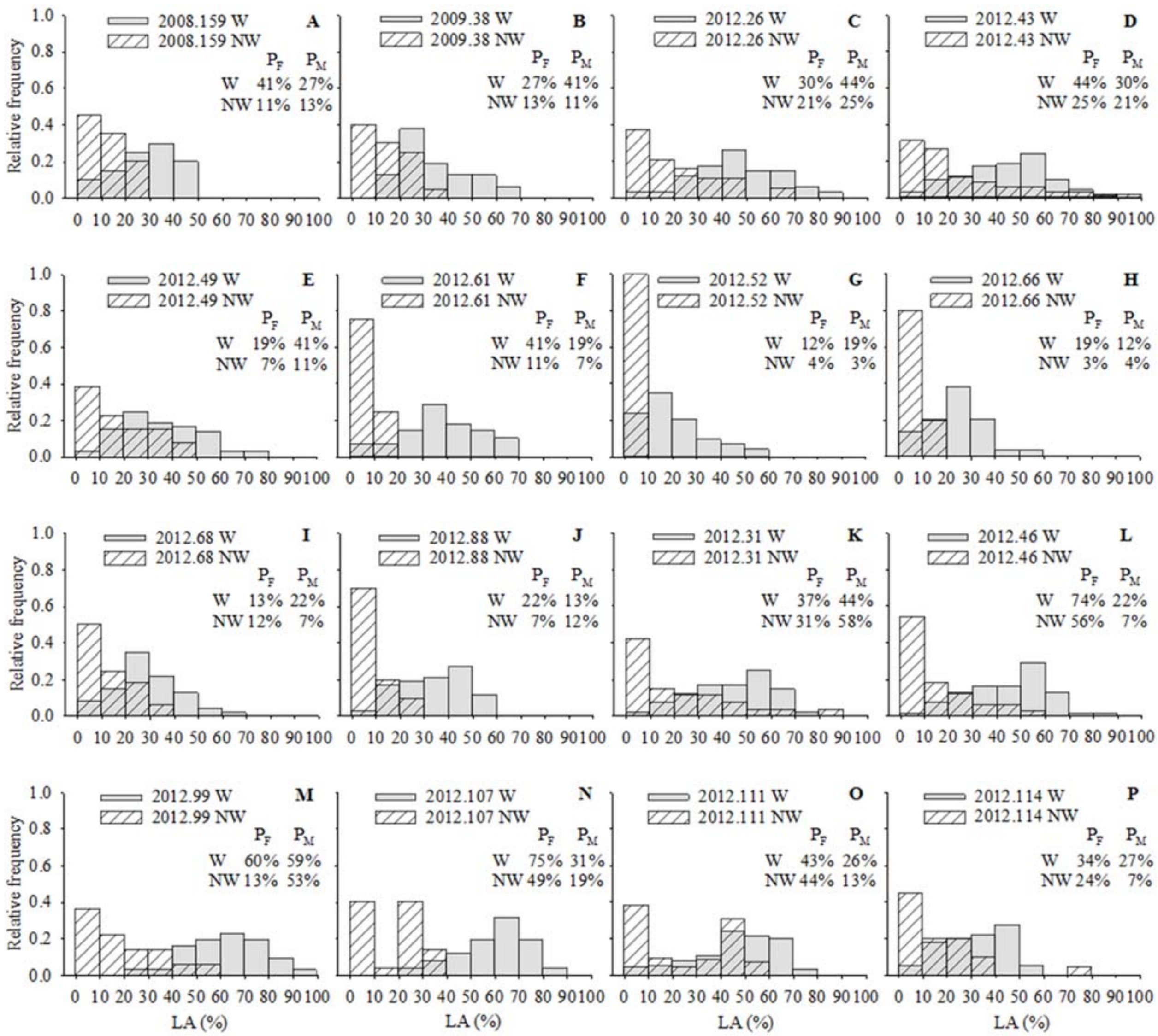


Fig. 2. Relative frequency histograms of 16 progenies classified by lesion area (LA) expressed as percentage of the fruit that was affected by the brown rot lesion, on wounded (W) and non-wounded (NW) fruits. The mean values of female (P_f) and male (P_m) parents for each population are indicated. Embrapa Peach Breeding Program, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

The 2012.52 and 2012.66 progenies (Fig. 2G and 2H), grouped the highest percentage of seedlings in the first two categories of LA (58 and 35%, respectively) when the fruits were evaluated with wound, and without wound all the genotypes of these progenies, together with the 2012.61 progeny (Fig. 2F), were within the first two categories (0 to 10 and 10 to 20% of the area affected by lesion). The parents of 2012.52 and 2012.66 progenies (Conserva 947 and

Conserva 1600) presented the lowest means of LA for both wounded fruits (12 and 19% respectively) and non-wounded fruits (4 and 3%, respectively). Conserva 672 selection, one of the parents of 2012.61 progeny, also presented low LA value (19 and 7%, for wounded and non-wounded fruits, respectively), as well as the parents of 2012.68 and 2012.88 progenies (Conserva 1662 and 'Maciel') of LA 13 and 22% (wounded fruits) and 12 and 7% (non-wounded fruits), respectively (Fig. 2I and 2J).

The 2012.26 and 2012.43 progenies (Fig. 2C and 2D) and the 2012.99, 2012.107 and 2012.111 nectarines progenies (Fig. 2L, 2M and 2N, respectively) were the most susceptible to brown rot on wounded fruits, with a high concentration of seedlings in the categories of 40 to 70% of the area affected by lesion.

In relation to SPP, high variability was detected for both wounded and non-wounded fruits, with intervals for the parents between 16 to 96% and 0 to 94%, respectively (Fig. 3A) and range of 14 to 69% and 0 to 35% for wounded and non-wounded fruits, respectively (Fig. 3B). Highlight the 2012.52 and 2012.66 progenies, with 20 and 14% of SPP, respectively, on wounded fruits, while both progenies had no seedlings with SPP on non-wounded fruits. Secondly, highlight the 2012.68 and 2012.88 progenies with less than 35% of SPP on wounded fruits and, with 2012.61 progeny, they stand out with less than 11% of SPP on non-wounded fruits. Among the parents, those who presented lower SPP mean for wounded and non-wounded fruits were Conserva 947 and Conserva 1600 (parents of 2012.52 and 2012.66), Conserva 672 (parent of 2012.49 and 2012.61) and Conserva 1662 (parent of 2012.68 and 2012.88) (Fig. 3A).

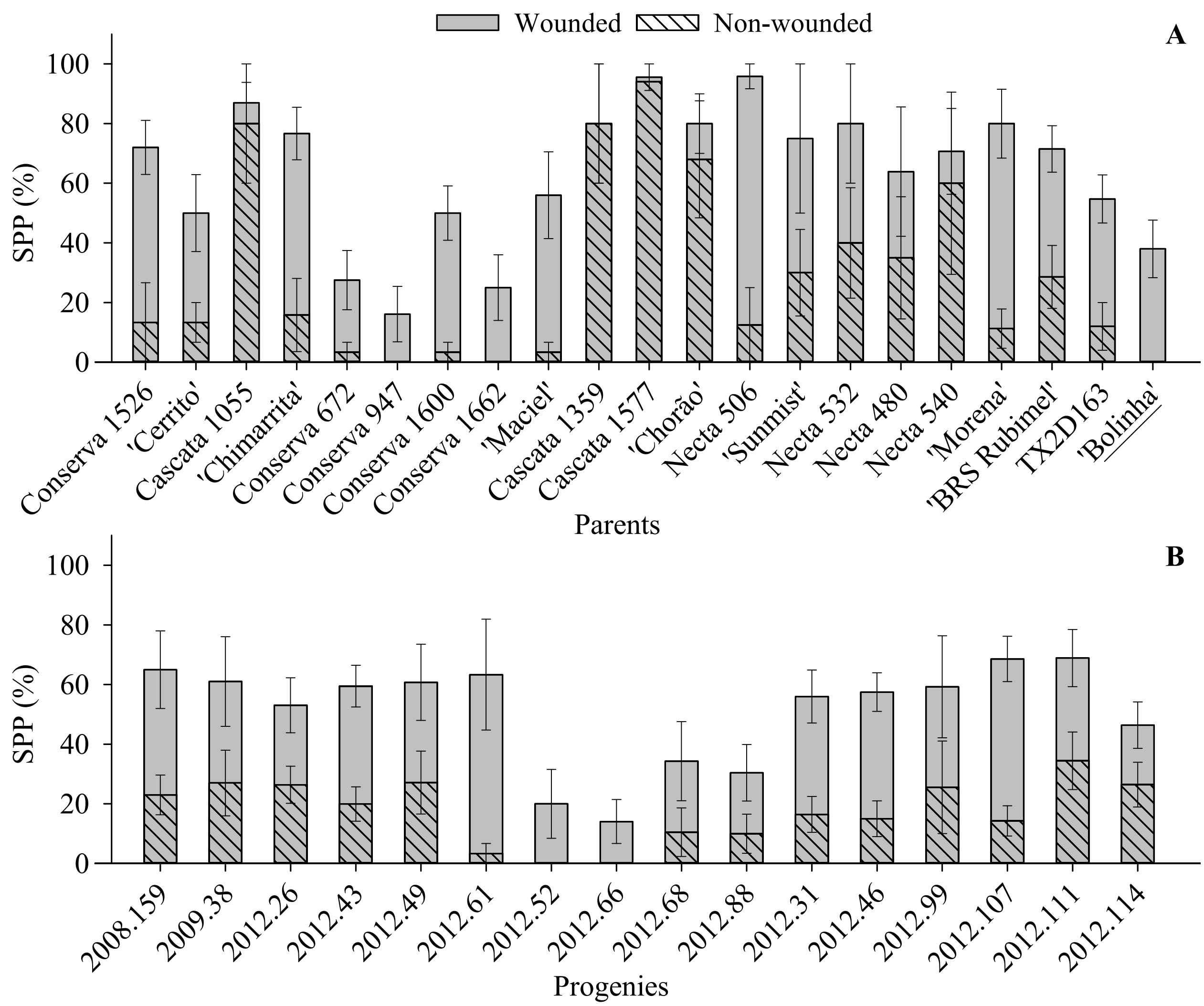


Fig. 3. Sporulation presence (SPP) on wounded and non-wounded fruits of parents (A) and progenies (B) average for three and two seasons (2015-16; 2016-17; 2017-18 and 2016-17; 2017-18, respectively). 'Bolinha' was included as control. Embrapa Peach Breeding Program, Pelotas, Rio Grande do Sul, Brazil.

For SPA, on wounded and non-wounded fruits, the seedlings followed approximately a Lognormal or Weibull distribution, with a high concentration in the lower categories, mainly in the first one (0 to 10% of the area affected by sporulation) (Fig. 4). The only progenies that presented an approximately normal distribution were those of nectarines, on wounded fruits (Fig. 4M, 4N and 4O).

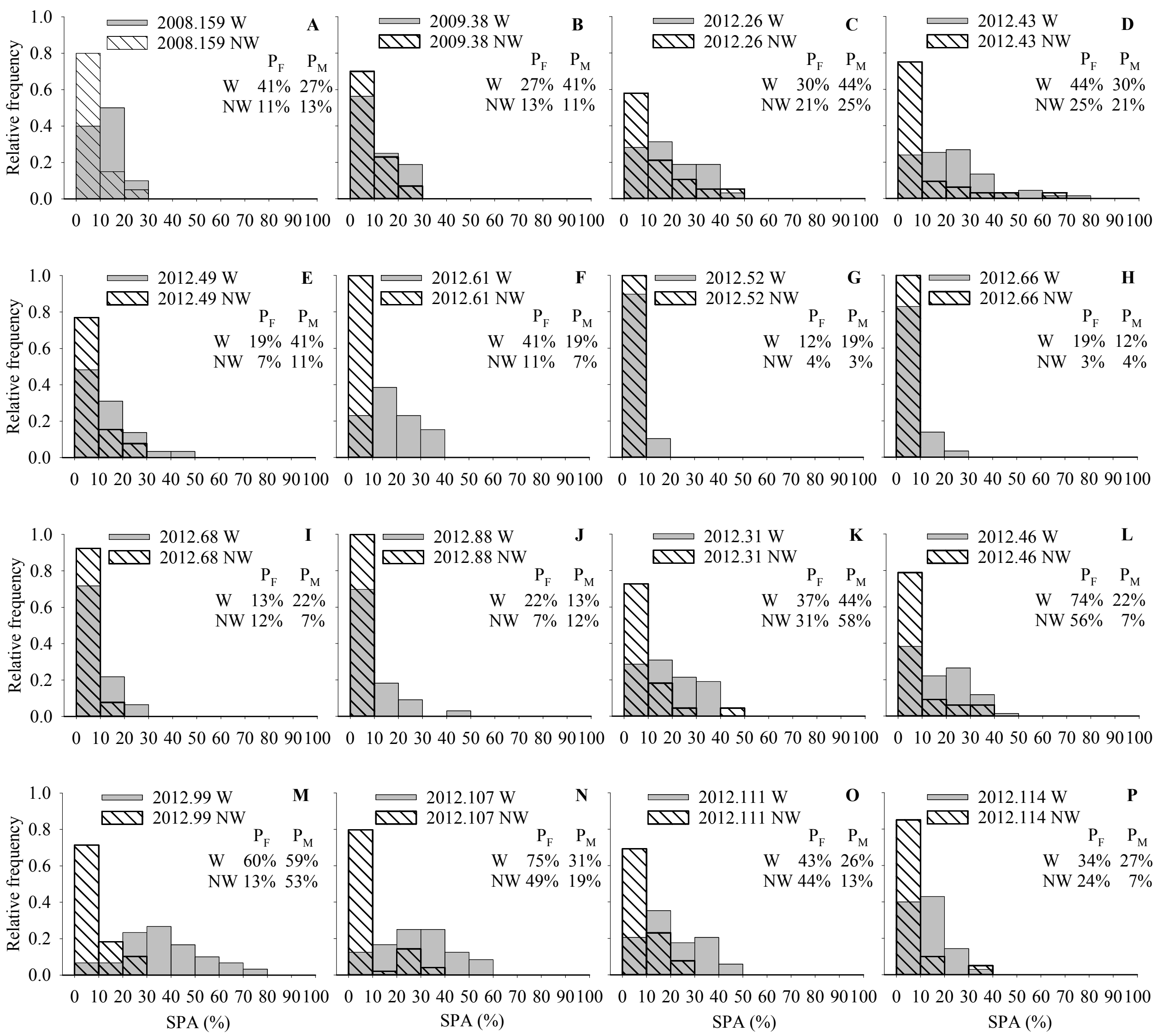


Fig. 4. Relative frequency histograms of 16 progenies classified by sporulation area (SPA) expressed as a percentage of the fruit that was affected by the brown rot sporulation, on wounded (W) and non-wounded (NW) fruits. The mean values of female (P_f) and male (P_m) parents for each population are indicated. Embrapa Peach Breeding Program, 2015-2016, 2016-2017 and 2017-2018 growing seasons, Pelotas, Rio Grande do Sul, Brazil.

Through the *t*-test, the hypothesis of maternal effect in the five studied reciprocal crosses was tested. The tested contrasts were F1 progeny versus their reciprocal, for the studied parameters (BRI, LD, LA, SPP, SPD and SPA), in all cases, the differences were not significant ($p > 0,05$), indicating that there is no maternal effect involved on the transmission of this trait.

H^2 estimates were from medium to low, for the different parameters, ranging between 30.1 to 51.7% on wounded fruits and between 25.0 to 40.5% on non-wounded fruits. The h^2 estimates, varied depending on the year of evaluation, but the obtained means were between 18.3 to 41.6% for wounded fruits and between 18.7 to 33.9% for non-wounded fruits (Table 2).

Table 2. Broad-sense (H^2) and narrow-sense (h^2) estimated heritability for incidence and severity of *M. fructicola* on wounded and non-wounded peach fruits, Embrapa Peach Breeding Program, Pelotas, Rio Grande do Sul, Brazil.

Fruits	Traits*	H^2 (%)	h^2 (%)			
			2015-2016	2016-2017	2017-2018	Mean
Wounded	BRI	42.5	35.8	37.6	31.1	34.8
	LD	51.7	47.1	42.1	35.5	41.6
	LA	44.7	33.5	41.1	25.5	33.4
	SPP	27.5	23.2	18.0	13.8	18.3
	SPD	41.2	33.0	39.3	35.9	36.1
	SPA	30.1	21.0	28.9	18.5	22.8
Non-wounded	BRI	26.7	-	20.2	17.1	18.7
	LD	40.5	-	34.6	30.1	32.4
	LA	29.8	-	28.6	20.2	24.4
	SPP	25.0	-	20.1	11.8	16.0
	SPD	31.1	-	29.3	19.9	24.6
	SPA	23.6	-	21.8	16.9	19.4

*BRI, brown rot incidence; LD, lesion diameter; LA, lesion area; SPP, sporulation presence; SPD, sporulation diameter; SPA, sporulation area.

Using the 'Bolinha' as the cut-off point for resistance selection (Supplementary Fig. S1), the genetic advances obtained by the Embrapa Peach Breeding Program were estimated. The selection differential (SD) is the subtraction of the selected genotypes mean ($\overline{X}_s$) minus the original mean ($\overline{X}_o$).

The GA% associated with each variable was estimated for wounded and non-wounded fruits, separately (Table 3). The selected genotypes number (SGN) was between 11 to 76, and GA% estimates were between -5.2 to -30.2% on wounded fruits. On the other hand, SGN on non-wounded fruits was between 53 to 93, and GA% estimates were between -15.0 to -27.3%.

Using the cultivar Bolinha as control, four seedlings (2012.52.17, 2012.52.2, 2012.66.11 and 2012.68.24) and a selection (Conserva 947) were identified, with better results in all variables of incidence and severity to brown rot (BRI, LD, LA, SPP, SPD and SPA) on wounded fruits. In the case of non-wounded fruits, 40 seedlings and three selections (Conserva 947, Conserva 1600, and Conserva 672) had results equal or better than 'Bolinha.

Table 3. Genetic advancement by selection of genotypes better than or equal to 'Bolinha'.

	Wounded						Non-wounded					
	BRI	LD	LA	SPP	SPD	SPA	BRI	LD	LA	SPP*	SPD*	SPA*
$\bar{X}_o$	96.3	29.5	39.7	60.6	14.6	15.9	54.0	12.8	16.6	19.4	4.5	5.4
'Bolinha'	94.3	20.2	13.3	37.1	5.5	2.1	30.0	6.5	4.0	0	0	0
$\bar{X}_s$	81.8	14.5	8.1	15.8	2.4	0.5	11.0	1.9	0.9	0	0	0
SD	-14.5	-15.0	-31.6	-44.8	-12.2	-15.5	-43.0	-10.9	-15.7	-19.4	-4.5	-5.4
SGN	58	21	11	76	63	41	53	59	56	93	93	93
h^2	0.35	0.42	0.33	0.18	0.36	0.23	0.19	0.32	0.24	0.16	0.25	0.19
GA (direct)	-5.0	-6.2	-10.5	-8.2	-4.4	-3.5	-8.1	-3.5	-3.8	-3.2	-1.1	-1.0
GA%	-5.2	-21.1	-26.5	-13.5	-30.2	-22.1	-15.0	-27.3	-22.8	-16.4	-25.0	-18.6

BRI, brown rot incidence; SPP, sporulation presence; LD, lesion diameter; SPD, sporulation diameter; LA, lesion area; SPA, sporulation area; h^2, mean of narrow-sense heritability; SD, selection differential; SGN, selected genotypes number; $\bar{X}_s$, mean of the selected genotypes; $\bar{X}_o$, original mean (base population); GA (direct), direct response; GA%, genetic advance (selection response in percentage of the population mean); *'Bolinha' value = 0.

4. Discussion

Exploring sources of brown rot resistance in an interspecific population of almond × peach, Baró-Montel et al. (2019) reported similar results to those of the present study regarding BRI, where close to 100% of wounded fruits developed the disease, and non-wounded fruits varied between 0 to 80% of BRI. On the other hand, working with non-wounded fruits, BRI averages were reported between 60 to 100% (Obi et al., 2017) and between 50 to 100% (Obi et al., 2019). These three studies performed the evaluation at 120 HAI and in the present study were made at 72 HAI.

In inoculation without wound, the lower development of the disease may be due to the delay in fungus infection and/or in the lower probability of conidia having a successful penetration of fruit skin. Although visually the fruits appear to be undamaged, there may be microcracks where the fungus can infect. In the case of *M. fructicola*, it has been reported that the wound is necessary and is the main gateway for infection (Michailides and Morgan, 1997; Gradziel et al., 2003; Gibert et al., 2007; Fu et al., 2018). Thus, the conidia number that would have success in the infection, in the case of inoculation with wound would be much greater in relation to inoculation without wound. For this reason, the standard error bars are much smaller in the evaluations with injury when compared to the uninjured evaluations, both for BRI in the parents and in the progenies (Fig. 1). This indicates that the sample means were more reliable when the wound was used, and this methodology is recommended, since microcracks of diverse origin can exist and are very associated to the environmental conditions and not to the genotype, presenting a disparity between the evaluated seasons (Kappel and Sholberg, 2008; Measham et al., 2009; Baró-Montel et al., 2019; Obi et al., 2019).

The 2012.114 progeny, with 46% BRI on non-wounded fruits, presented six seedlings with less than 20% of BRI (Fig. 1B). This progeny was characterized by producing fruits with a great pilosity, and this characteristic associated to the inoculation without wound can be considered a structural barrier, hindering the development and infection of *M. fructicola* conidia in the fruit (Oliveira Lino et al., 2016; Debona and Rodrigues, 2018). However, in stone fruits the subject is controversial, since there are reports where the trichomes favor the infection of *Monilinia* spp. (Hall, 1971; Fernández et al 2011; Garcia-Benitez et al., 2016). These studies mention that microcracks at the base of the trichomes may result in entry points of the fungus. In addition, the high density and length of the trichomes in some genotypes of the progeny 2012.114 can have caused a failure in the technique of inoculation, since the drop with conidial suspension of *M. fructicola* may have dried before coming into contact with the fruit skin.

In the 2012.107 and 2012.111 nectarine progenies, an erratic behavior was observed when the fruits were evaluated without wound (Fig. 2N e 2L). Basides a large number of fruits that did not present BRI, but it was not associated with a specific genotype. This can be explained by the presence of waxes associated with the nectarine’s cuticle, and this may be considered a structural barrier to fungal infection (Gradziel et al., 2003; Kappel and Sholberg, 2008; Oliveira Lino et al., 2016). However, it can also be considered only an error in the inoculation technique, because a more waxy surface can cause the drop containing the conidial suspension of *M. fructicola* to simply drain from the fruit or dry without having sufficient time of contact with the fruit skin, so that the fungus did not infect.

High variability related to sporulation variables such as SPP (Fig. 3) and SPA (Fig. 4), was detected both for wounded and non-wounded fruits. In a study in which non-wunded fruits were inoculated with *M. laxa*, and evaluated 120 HAI, Obi et al. (2019) obtained averages higher than in the present study (above 80%) of fruits with fungus sporulation (colonized), probably due to the longer incubation time and/or genetic differences. Genotypes that present rot, but do not result in 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 and Debona, 2018). Among the more than 300 genotypes evaluated in this study, 23 (wounded fruits) and 93 (non-wounded fruits) genotypes did not present sporulation in any of their fruits, evaluated for three and two years, on wounded and non-wounded fruits, respectively.

No maternal significant effect was detected in the inheritance of the characters associated with brown rot resistance. The use of reciprocal crosses are the simplest evidence of the maternal effect, since they produce genetically similar but phenotypically different individuals (Ramalho et al., 2012, Griffiths et al., 2015), if there is indeed a significant maternal effect. In stone fruits, there are rare cases where a possible maternal effect has been mentioned (Corrêa, 2005, Dini et al., 2019c, Wu et al., 2012) or maternal inheritance (Bielsa et al., 2014, Bouhadida et al., 2007, Panda et al., 2003), and has never been associated with resistance to pests or diseases.

All H^2 and h^2 estimates were from medium to low and, considering that the peach resistance to brown rot is quantitative (Martínez-García et al., 2013; Pacheco et al., 2014; Baró-Montel et al., 2019), the parents selection based on their phenotype may be from reasonable to not very effective. Studying the reaction to *M. fructicola* on wounded peach fruits from of the Embrapa Peach Breeding Program's work collection, Scariotto (2016), estimated the H^2 of LD and SPD in 50 and 13%, respectively. The first estimate was like that of the present study (51.7%), but the second estimate was considerably lower (41.2%). On the other hand, Wagner Júnior (2003), testing the response on non-wounded peach fruits of populations, also from Embrapa Peach Breeding Program, estimated the H^2 of BRI in 64%, differing with this study where for this variable and in that condition, it was 26.7%. Although the site where these works were done was the same, there were differences in methods, climatic conditions and genotypes. Considering these heritability estimates (low to medium values), moderate genetic advance due to selection can be expected, for the characters associated with brown rot resistance and with great environmental influence (Falconer and Mackay, 1996).

The lowest GA% was associated with BRI on wounded fruits (-5.2%), due to the low variability associated with this character on the studied populations. GA% of -13.5 to -30.2%, were estimated for the other variables on wounded fruits. On the other hand, on non-wounded fruits, GA% estimates were between -15.0 to -27.3%, and in the case of the selected genotypes with respect to sporulation there were those that presented 0 (SPP, SPD and SPA) equal to 'Bolinha'.

It was not found references, in the literature, to genetic advances (genetic gains or response to selection) in relation to diseases resistance and/or pests in *Prunus* spp. Estimating the heritability and predicted selection response of quantitative traits in peach, Souza et al. (1998), obtained predictions from 4.61 to 61.25% on fruit quality characteristics (fruit mass, soluble solids, acidity, fruit length and others) and between 20.44 to 95.00% for phenological characters (full bloom, ripening and fruit development period). In the same way, Chandrababu and Sharma (1999) studying the heritability and genetic gain in several characteristics of almond (*Prunus dulcis*), obtained values of genetic gain between 37.98 to 187.27% for growth and yield characters, 33.77 to 132.79% for flowering and fruiting characters, and 23.08 to 74.36% for fruit and kernel characters. Polygenic traits have low heritability, high environmental influence and the genetic gain expected by selection based on the phenotype is generally moderate to low (Falconer and Mackay, 1996, Williams and Brown, 1956).

Among the five genotypes selected with the best results on wounded fruits, there were three seedlings and one selection related directly to 'Bolinha'. The advanced selection Conserva 947 was selected from a cross between 'Bolinha' × P60-22 (Mexican polen) and, the seedlings (2012.52.17, 2012.52.2 and 2012.66.11) originated from the crossing between Conserva 947 × Conserva 1600 (2012.52), and its reciprocal (2012.66). Among the 43 selected genotypes with the best results on non-wounded fruits, there were 11 genotypes related to 'Bolinha', five seedlings of 2012.52 progeny and five of 2012.66 progeny, in addition to selection Conserva 947. Besides these genotypes, the other progenies that participated with higher number of selected genotypes were 2012.68 (one and six, on wounded and and non-wounded fruits, respectively) and 2012.88 (four, on non-wounded fruits), progenies originated from the crossing between Conserva 1662 × 'Maciel' and their reciprocal cross, respectively. Another of selected genotypes, Conserva 672 advanced selection, had previously been reported as being one of the genotypes that presented the best results on non-wounded fruits (Wagner Júnior, 2003).

All selected genotypes with and without wound, presented equal or better results than 'Bolinha' for brown rot resistance in fruits and, several of them, mainly the selections (Conserva 947, Conserva 1600, and Conserva 672) are of superior fruit quality to 'Bolinha', which demonstrates the progress of the Embrapa Peach Breeding Program, not only regarding resistance to *M. fructicola*.

5. Conclusions

The studied populations present variability regarding the brown rot resistance, without any evidence of maternal effect. The heritability of brown rot resistance in fruits is medium to low. Selection of genotypes based on phenotypes better or equal to 'Bolinha', allowed to estimate moderate to low genetic advances for brown rot resistance. The Embrapa Peach Breeding Program is achieving genetic advances in fruit resistance to *M. fructicola*, currently counting with several genotypes comparable to 'Bolinha' but with better fruit quality.

Acknowledgements

To the National Agency for Research and Innovation (ANII-Uruguay) for providing MSc scholarship founds and the Coordination of Improvement of Higher Education Personnel (CAPES-Brazil) for providing PhD scholarship founds, and Embrapa staff, especially Everton Pederzolli, Maicon Bönemann and Gilberto Kuhn.

References

Adaskaveg, J.E., Schnabel, G., Forster, H., 2008. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: Layne D.R., Bassi, D. (Eds.), The Peach: Botany, Production and Uses. CAB International, Wallingford, UK, pp. 352-406.

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. J. Sci. Food Agric. 99, 8, 4105-4113. http://dx.doi.org/10.1002/jsfa.9640.

Bielsa, B., Jiwan, D., Marti, A.I.F., Dhingra, A., Rubio-Cabetas, M.J., 2014. Detection of SNP and validation of a SFP InDel (deletion) in inverted repeat region of the *Prunus* species chloroplast genome. Sci. Hort. (Amsterdam) 168, 108-112. http://dx.doi.org/10.1016/j.scienta.2014.01.028.

Bouhadida, M., Martín, J.P., Eremin, G., Pinochet, J., Moreno, M.Á., Gogorcena, Y., 2007. Chloroplast DNA diversity in *Prunus* and its implication on genetic relationships. J. Amer. Soc. Hort. Sci. 132, 5, 670-679. http://dx.doi.org/10.21273/JASHS.132.5.670.

Chandrababu, R.J., Sharma, R.K., 1999. Heritability estimates in almond [*Prunus dulcis* (Miller) D.A. Webb]. Sci. Hort. (Amsterdam) 79, 237-243. http://dx.doi.org/10.1016/S0304-4238(98)00216-7.

Chen, S., Yuan, N., Schnabel, G., Luo, C., 2017. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. Mol. Plant. Pathol. 18, 1, 90-97. http://dx.doi.org/10.1111/mpp.12387.

Crisosto, C., Gradziel, T., Ogundiwin, E., 2009. Development of predictive tools for brown and sour rot resistance in peach and nectarines. California tree fruit agreement, Annual Research Report 2009, 87-93.

Corrêa, E.R., 2007. Estudo da herdabilidade de alguns carácteres em pessegueiro: ciclo, tonalidade da cor da polpa e compostos fenólicos. 52p. Dissertation (MSc.), Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS, Brazil.

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

Debona, D., Rodrigues, F.Á., 2018. Alterações bioquímicas e estruturais em plantas induzidas após a detecção do patógeno. In: Dallagnol, L.J. (Ed.), Resistência genética de plantas a patógenos. UFPel, Pelotas, RS, Brazil, pp. 150-193.

Dini, M., Scariotto, S., Raseira, M.C.B., Ueno, B., Camargo, R., 2018. Herdabilidade e segregação da resistência à podridão-parda em pessegueiro. Boletim de Pesquisa e Desenvolvimento 298. Embrapa Clima Temperado, Pelotas, RS, Brazil.

Dini, M., Scariotto, S., Raseira, M.C.B., Ueno, B., 2019a. Heritability and segregation of resistance to brown rot in peach fruits. Acta Hort. (under review).

Dini, M., Raseira, M.C.B., Scariotto, S., Carpenedo, S., Ueno, B., 2019b. *Monilinia fructicola* lesion and sporulation in peach and nectarine fruits. Pesqui. Agropecu. Bras. (under review).

Dini, M., Raseira, M.C.B., Scariotto, S., Marchi, P.M., Mello-Farias, P., 2019c. Peach phenological characters: heritability, maternal effect and correlation with brown rot. Rev. Bras. Frutic. (under review).

Dirlewanger, E., Quero-García, J., Le Dantec, L., Lambert, P., Ruiz, D., Dondini, L., Illa, E., Quilot-Turion, B., Audergon, J.M., Tartarini, S., Letourmy, P., Arús, P., 2012. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. Heredity, 109, 280-292. http://dx.doi.org/10.1038/hdy.2012.38.

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. Ind. Crops Prod. 66, 11-15. http://dx.doi.org/10.1016/j.indcrop.2014.12.031.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, Fourth ed. Longman, Harlow, UK.

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

Fernandez, V., Khayet, M., Montero-Prado, P., Heredia-Guerrero, J. A., Liakopoulos, G., Karabourniotis, G., del Río, V., Domínguez, E., Tacchini, I., Nerín, C., Val, J., Heredia, A., 2011. New insights into the properties of pubescent surfaces: peach fruit as a model. Plant Physiol. 156, 2098−2108. http://dx.doi.org/10.1104/pp.111.176305.

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. J. Am. Pom. Soc. 72, 2, 94-100.

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 Prot. 91, 20-26. http://dx.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, e0160675. http://dx.doi.org/10.1371/journal.pone.0160675.

Gibert, C., Chadœuf, J., Vercambre, G., Génard, M., Lescourret, F., 2007. Cuticular cracking on nectarine fruit surface: spatial distribution and development in relation to irrigation and thinning. J. Amer. Soc. Hort. Sci. 132, 5, 583-591. http://dx.doi.org/10.21273/JASHS.132.5.583.

Gradziel, T.M., Bostock, R.M., Adaskaveg, J.E., 2003. Resistance to brown rot disease in peach is determined by multiple structural and biochemical components. Acta Hort. 622, 347-352. http://dx.doi.org/10.17660/ActaHortic.2003.622.34.

Griffiths, A.J.F., Wessler, S.R., Carroll, S.B., Doebley, J., 2015. Introduction to Genetic Analysis, tenth ed. W. H. Freeman and Company, New York, NY.

Hall, R., 1971. Pathogenicity of *Monilinia fructicola*. Part II. Penetration of peach leaf and fruit. J. Phytopathol. 72, 281-290. http://dx.doi.org/10.1111/j.1439-0434.1971.tb03200.x.

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 Manag. Sci. 67, 385-396. http://dx.doi.org/10.1002/ps.2074.

Kappel, F., Sholberg, P.L., 2008. Screening sweet cherry cultivars from the Pacific Agri-Food Research Centre Summerland breeding program for resistance to brown rot (*Monilinia fructicola*). Can. J. Plant Sci., 88, 4, 747-752. https://dx.doi.org/10.4141/CJPS07137.

Kushalappa, A.C., Gunnaiah, R., 2013. Metabolo-proteomics to discover plant biotic stress resistance genes. Trends Plant Sci. 18, 522-531. http://dx.doi.org/10.1016/j.tplants.2013.05.002.

Londero, P.M.G., Ribeiro, N.D., Cerutti, T., Mazeiro, S.M., Rosa, D.P., Rosa, S.S., 2009. Maternal effect in sulfur amino acids content expression in common bean grains. Ciência Rural 39, 6, 1884-1887. http://dx.doi.org/10.1590/S0103-364 84782009005000129.

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 Qol fungicides. Pest Manag. Sci. 66, 1308-1315. http://dx.doi.org/10.1002/ps.2016.

Martínez-García, P.J., Parfitt, D.E., Bostock, R.M., Fresnedo-Ramírez, J., Vazquez-Lobo, A., Ogundiwin, E.A., Gradziel, T.M., 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, e78634. http://dx.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. Trop. Plant Pathol. 33, 3, 227-234. http://dx.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: Raseira, M.C.B., Pereira, J.F.M., Carvalho, F.L.C. (Eds.), Pessegueiro. Embrapa, Brasília, DF, Brazil, pp. 355-432.

Measham, P.F., Bound, S.A., Gracie, A.J., Wilson, S.J., 2009. Incidence and type of cracking in sweet cherry (*Prunus avium* L.) are affected by genotype and season. Crop Pasture Sci. 60, 10, 1002-1008. http://dx.doi.org/ 10.1071/CP08410.

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. http://dx.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. Australas. Plant Path. 46, 2, 183-189. http://dx.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. Sci. Hort. (Amsterdam) 246, 1028-1036. http://dx.doi.org/10.1016/j.scienta.2018.10.027.

Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., Uyemoto, J.K., 1995. Compendium of Stone Fruit Diseases, first ed. The American Phytopathological Society, Saint Paul, MN.

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. J. Agric. Food Chem. 64, 20, 4029-4047. http://dx.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 Genet. Genomes 10, 5, 1223-1242. http://dx.doi.org/10.1007/s11295-014-0756-7.

Panda, S., Martín, J.P., Aguinagalde, I., 2003. Chloroplast DNA study in sweet cherry cultivars (*Prunus avium* L.) using PCR-RFLP method. Genet. Resour. Crop. Ev. 50, 489-495. http://dx.doi.org/10.1023/A:1023986416037.

Ramalho, M.A.P., Santos, J.B., Pinto, C.B.P., Souza, E.A., Gonçalves, F.M.A., Souza, J.C., 2012. Genética na agropecuária. UFLA, Lavras, MG, Brazil.

Raseira, M.C.B., Barbosa, W., Nakasu, B.H., Pereira, J.F.M., 2008. Pêssego. In: Albuquerque, A.C.S., Silva, A.G. (Eds.), Agricultura Tropical; Quatro décadas de inovações tecnológicas, instituicionais e políticas. V.1 Produção e produtividade agrícola. Embrapa, Brasília, DF, Brazil, pp. 519-529.

Raseira, M.C.B., Franzon, R., 2014. Melhoramento genético. In: Raseira, M.C.B., Pereira, J.F.M., Carvalho, F.L.C. (Eds.), Pessegueiro. Embrapa, Brasília, DF, Brazil, pp. 57-72.

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

Santos, J., Raseira, M.C.B., Zanandrea, I., 2012. Resistance to brown rot in peach plants. Bragantia 71, 2, 219-225. http://dx.doi.org/10.1590/S0006-87052012005000022.

Santos, J., Ueno, B. Controle da podridão-parda no Brasil, 2014. In: Mitidieri, M., Castillo, J.A. (Eds.), Manejo de la podredumbre morena (*Monilinia fructicola* y *M. laxa*) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina. Santiago de Chile, Chile, pp. 73-77.

Scariotto, S., Santos, J., Raseira, M.C.B., 2015. Search for resistance sources to brown rot in Brazilian peach genotypes. Acta Hort. 1084, 211-216. http://dx.doi.org/10.17660/ActaHortic.2015.1084.29.

Scariotto, S., 2016. Variabilidade genética e abordagem bioquímica da resistência do fruto à podridão-parda em diferentes genótipos de *Prunus persica*. 130p. Thesis (PhD.), Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS, Brazil.

Souza, V.A.B., Byrne, D.H., Taylor, J.F., 1998. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruits traits. J. Am. Soc. Hortic. Sci. 123, 4, 604-611. http://dx.doi.org/10.21273/JASHS.123.4.604.

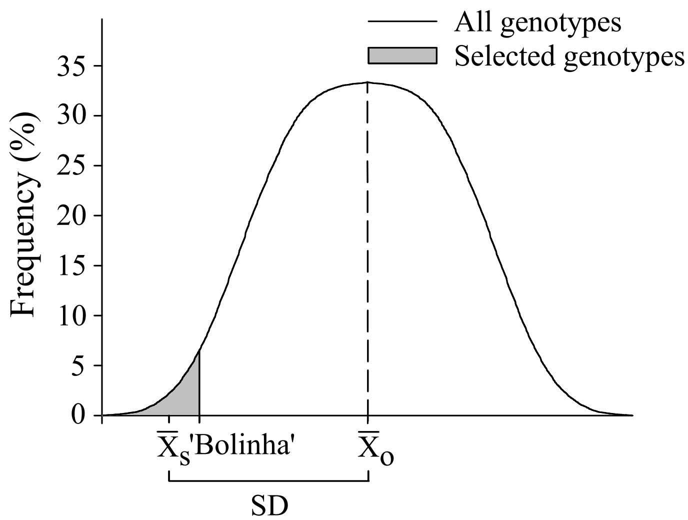
Thomidis, T., Michailides, T., Exadaktylou, E., 2009. Contribution of pathogens to peach fruit rot in northern Greece and their sensitivity to iprodione, carbendazim, thiophanate-methyl and tebuconazole fungicides. J. Phytopathol. 157, 194–200. http://dx.doi.org/10.1111/j.1439-0434.2008.01469.x.

Wagner Júnior, A., 2003. Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey. 62p. Dissertation (MSc.), Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS, Brazil.

Wu, B.H., Zhao, J.B., Chen, J., Xi, H.F., Jiang, Q., Li, S.H., 2012. Maternal inheritance of sugars and acids in peach (*P. persica* (L.) Batsch) fruit. Euphytica 188, 333-345. http://dx.doi.org/10.1007/s10681-012-0668-2.

Willimas, W., Brown, A.G., 1956. Genetic response to selection in cultivated plants: gene frequencies in varieties of *Prunus persica*. Proc. R. Soc. Lond. B 145, 337-347. http://dx.doi.org/10.1098/rspb.1956.0046

Zhu, F., Bryson, P.K., Schnabel, G., 2012. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. Pest Manag. Sci. 68, 7, 1003-1009. http://dx.doi.org/10.1002/ps.3255.



Supplementary Fig. S1. Hypothetical distribution of all genotypes evaluated from the 16 populations, the selection was made based on the incidence and severity means of 'Bolinha'. The selection differential (SD) is the subtraction of the selected genotypes mean ($\overline{X}_s$) minus the original mean ($\overline{X}_o$).

4.7 Artigo 7. Fruit reaction to brown rot at different development stages and inoculation time after wounded

Artigo a ser submetido na Revista *“Scientia Horticulturae”*

Fruit reaction to brown rot at different development stages and inoculation time after wounded

Maximiliano Dini[a,*]; Bénédicte Quilot-Turion[b]; Maria do Carmo Bassols Raseira[c]

[a] Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.

[b] GAFL, INRA, 84143, Montfavet, France.

[c] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Rodovia BR 392, km 78, Caixa Postal 403, CEP 96010-971, Pelotas, Rio Grande do Sul, Brazil.

* Corresponding author at: Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.
E-mail addresses: maxidini@hotmail.com, mdini@inia.org.uy (M. Dini).

Fruit reaction to brown rot at different development stages and inoculation time after wounded

Keywords: *Prunus persica* (L.) Batsch, *Monilinia laxa* (Aderh. & Ruhl.) Honey, HPLC analysis, phenolic compounds, terpenoid.

Abstract. Brown rot is the economically most important peach disease. The mechanisms of resistance are not yet clear, as well as the influence of fruit wounds. The objective of this study was to evaluate this latter topic on fruits inoculated with *Monilinia laxa* at different development stages. For this purpose, five nectarine genotypes (cv. Zephyr and four advanced selections of GAFL-INRA) have been phenotyped for fruit reaction to brown rot. Fruit at three stages of development were inoculated at two different times, immediately after wounding and seven hours after wounding. At pit hardening stage (first inoculation date), fruit were slightly susceptible to *M. laxa* whereas ripe fruit were very susceptible (last inoculation date). In both case, no effect of inoculation time was observed. In the second inoculation date (15 to 19 weeks after full bloom), the inoculation done immediately after wounding resulted in larger lesion and sporulation diameters, greater infection and sporulation probability, and less infection and sporulation delay, than the inoculation done 7 hours after wounding. A red reaction was detected, associated with inoculation immediately after fruit were wounded (11 to 15 weeks after full bloom). These red areas were isolated and subjected to HPLC analysis. Among the detected compounds, nine phenolic were only present in the samples extracted from the red zones, and six other compounds were presented in greater proportion when compared with samples that did not present this type of reaction. Among the latter, the two major phenolic compounds were identified as Eriodictyol-7-glucoside and Naringenin-7-glucoside (syn.: Prunin). These compounds may be actively involved in plant-pathogen reactions and/or activation of metabolic pathways involved in the susceptibility/resistance of peach to *M. laxa*.

1. Introduction

Peach (*Prunus persica* L. Batsch) is the most important fruit worldwide in the stone fruit group and the third most important within the economically important Rosaceae family, after apple and pear (FAOSTAT, 2019). Brown rot (BR), caused by any of three species of the genus *Monilinia* is the economically most important disease in peach. *M. laxa* (Aderh. & Ruhl.) Honey,

Abbreviations: BR, brown rot; HAI, hour after inoculation; HPLC, high performance liquid chromatography; LD, lesion diameter; SpoD, sporulation diameter; Tri, Triterpenoid; Fla, Flavan-3-ol; Fle, Flavanone; HCD, Hydroxycinnamic derivatives; Trid, Triterpenoid derivatives; Flo, Flavonol; Ant, Anthocyanin; E7glu, Eriodictyol-7-glucoside; N7glu, Naringenine-7-glucoside (syn.: Prunin).

is predominant in Europe, China, South Africa, Chile, North America, and the Middle East; *M. fructicola* (Wint.) Honey, is important in South and North America, and more recently present in Europe and Asia; whereas *M. fructigena* (Aderh. & Ruhl.) Honey, present in Europe and China, is of narrow distribution and less importance (Ogawa et al., 1995; Adaskaveg et al., 2008; Hu et al., 2011). The period of the disease incidence extends from bloom to postharvest, and the main symptoms are the blossom blight, branch canker, and lesions on immature and mature fruits, the latter being the most sensitive host phenological phase (Ogawa et al., 1995; Adaskaveg et al., 2008; May-de-Mio et al., 2008, 2014).

The frequent application of fungicides required for BR control remains a major obstacle to sustainable production. Consequently, genetic resistance BR is highly desirable, but its adoption is still very limited in commercial orchards, since commercial peach cultivars resistant or immune to BR are unavailable even though it is a priority of several breeding programs worldwide (Adaskaveg et al. 2008; Raseira and Franzon, et al. 2014; Fresnedo-Ramírez et al., 2017). It is also a target of research institutions groups to search for molecular markers for the resistance, FruitBreedomics (Europe) and the RosBREED (North American) projects were the most important collective initiatives (Oliveira Lino et al., 2016; Fu et al., 2018).

The BR resistance is of quantitative inheritance (Martínez-García et al., 2013; Pacheco et al., 2014; Baró-Montel et al., 2019), and therefore its heritability is medium to low with great environmental influence (Scariotto, 2016, Fresnedo-Ramírez et al., 2017, Dini et al., 2019a, 2019b). The mechanisms of resistance are not yet clear, and great uncertainties still exist regarding the influence of fruit micro-cracks or wounds as a gateway to the fungus and as possible activator of reactions that result in induced resistance. Similarly, the role of phenolic compounds and other compounds in the susceptibility/resistance to *Monilinia* spp is not clear. The objective of this study was to evaluate the effect of wounding fruit before inoculation with *Monilinia laxa* at different development stages.

2. Materials and methods

2.1. Plant material

The nectarine cultivar Zephyr and four selections (C207, C216, F115, and H165) of the ‘Génétique et Amélioration des Fruits et Légumes’ (GAFL) research unit of INRA Avignon germplasm collection were used. The plants were located at the experimental orchard at Saint

Paul station (INRA Avignon). Full bloom was registered on February 10th for Zephyr and C207, February 23th for C216, February 27th for F115 and March 8th for H165, 2018 season. Fruit were collected and taken to the laboratory of Saint Maurice station (INRA Avignon), where they were selected for absence of apparent lesions or infections. Measurements of individual fruit weight, height, equatorial diameter on the suture and perpendicular to suture were taken for all fruit. Fruit disinfection was done with hot water at 60°C for 30 seconds. Then 10 fruits were placed on metal rings inside clear plastic boxes (40 × 28 × 18 cm), previously disinfected with 75% alcohol.

2.2. Experimental design and treatments

The experimental design was completely randomized, with a tri-factorial arrangement, being five nectarine genotypes, two inoculation times (immediately after wounding the fruits or seven hours after wounding, further called IAW and I7hAW, respectively) and three stages of fruit development (three inoculation dates further called date1, date2, date3). Ten fruit were used, for each genotype, inoculation time and harvest date, considering each fruit as a repetition. The first inoculation began on May 28th, 2018, corresponding to 11 to 15 weeks after full bloom (WAFB). Fruit were in the pit hardening stage. The second date began on June 20th, 2018, 15 to 19 WAFB. The third evaluation corresponded to the date of commercial maturation of each genotype used. For the early maturing nectarine C216, it was on July 4th, 2018 (21 WAFB); for the nectarines 'Zephyr', H165 and C207 on July 23th, 2018 (23, 21 and 20 WAFB, respectively); and for the late nectarine F115, it was on August 28th, 2018 (26 WAFB).

2.3. Pathogen culture, conidia production, and inoculation

The inoculum used to perform the inoculations was prepared under aseptic conditions on the day of inoculation, using the same strain of *M. laxa* (Ml3) preserved in Petri dishes with PDA media. *M. laxa* suspension was adjusted to 1.0×10^5 conidia mL^{-1} using a Mallassez chamber and an optical microscope. Distilled water with a drop of Tween-80® was used to break the surface tension and improve the homogeneity of the suspension.

The fruit were wounded with a razor blade making a longitudinal cut from the apex to the peduncle, with a depth not exceeding 3 mm. Inoculation was carried out with a micropipette, depositing 10 µL of inoculum on the wound of each fruit. Inoculations were either immediately after wounding or seven hours after. The boxes containing the fruit were placed in a growth chamber at 25±1°C, relative humidity 80% and a photoperiod of 12 hours.

2.4. Brown rot evaluation

Evaluations were performed on each fruit every 24 hours, for a period of ten days post-inoculation, starting 24 hours after inoculation (HAI). The presence or absence of infection was recorded, according to the appearance of the necrotic lesion, characteristic of BR. The lesion measurement was taken perpendicular to the wound, in the largest lesion zone, using a digital caliper. The presence or absence of *M. laxa* sporulation was also recorded, and when present, the sporulation diameter was taken in the same way described for the lesion.

2.5. Exploration of red reaction

At the end of the first evaluation (June 7th, 2018), skin samples of all genotypes were taken to perform high performance liquid chromatography (HPLC) analyses. Samples were pooled of ten fruits of same genotype either previously inoculated immediately after wounding (sample 1) or after seven hours after wounding (sample 2), to obtain enough material for the HPLC analyzes. In the presence of red reaction areas, the samples were taken from these regions. In case where the red reaction did not occur, samples were taken in similar amount and position to the referred ones. After collected, skin samples were immediately frozen in liquid nitrogen, kept at -80°C. Then they were ground to a fine powder and lyophilized for three days. The HPLC analyses were made in INRA´s laboratories (INRA Avignon, Saint Maurice, GAFL).

The phenolic compounds were extracted from 50 mg of the lyophilized-dry powder. The material was homogenized in an Ultra-turrax homogenizer (IKA labortechnik, JK Jank and Kunkel) for 1 minute with 8 mL of extraction solution (ethanol 95%) and placed in rotary shaker (Rotator SB3, Stuart), under controlled environment at 4°C for 4 hours, followed by centrifugation (Sigma 4K15, 5000 rpm, 5 minutes, 4°C). The volume of the supernatant was carefully recovered to prevent contamination with the pellet. The recovered supernatant was placed in pyrex tube (12 mL) which was then, placed in a Speed Vac Concentrator (SC210A, Thermo Electron Corporation) for the solvent evaporation. The resultant residue was dissolved in 1000 µL methanol 100% (Prolabo Hypersolv Chromanorm), filtered (membrane PTFE 0.45 µm), and collected into an autosampler vial 1.5 mL for HPLC analysis and kept at -20°C until analysis.

Extracts were analyzed using an HPLC system (Shimadzu - Prominence) equipped with a reversed phase C18 column (Merck Superspher RP18 end capped) coupled with a photodiode array detector, operated by Shimadzu software (LC Solutions). The extract content was measured by a method, developed for quantification of phenolic compounds in peach fruits. Acidified water pH 2.6 (with H3PO4) and methanol 100% (Prolabo Hypersolv Chromanorm) were used as solvents for the mobile phases. The HPLC solvents programming was dependent on the polarity of the molecules that were extracted, starting with 97% water and 3% methanol, to extract the most polar molecules and ending with 0% water and 100% methanol, extracting the most apolar molecules. A 10 μL aliquot of the filtered extract was injected into a HPLC. The column was maintained at 30°C.

The methanol extracts were analyzed simultaneously for free forms of triterpenoids (210nm), flavan-3-ols and flavanones (280 nm), hydroxycinnamic acids and derivate forms of triterpenoids (315 nm), flavonols (350 nm), and anthocyanidin (520 nm). The phenolic compounds were characterized according to their UV lambda maximum, retention times, and co-chromatography with known standards when available.

2.6. Statistical analysis

In order to estimate the disease progress, probability, delay and growth rate lesion and sporulation, scored data were plotted in graphs. Probability of infection was estimated by the percentage of fruit with lesion, in each inoculation date. Delay was calculated by the time at which the infection was first observed (in hours), and the progression rate was calculated by maximum progression of infection between two observation dates. For the sporulation data, the same graphs and parameters were calculated.

Analyses of variance (ANOVA) were performed by F-test ($p \leq 0.01$) and means were compared by Tukey's multiple range test. For the identified compounds the peaks area from HPLC analyses were used to perform the statistical analyses. Five replications of red area five without red were used for comparing each compound.

Statistical analyses and graphical representations (ggplot2 package) were performed using R software (R Core Team, 2018).

3. Results

In the first inoculation date (May 28), with small immature fruit (26.7 g of average mass, 11 to 15 weeks after full bloom) 14% of them were infected (Fig. 1A) and 9% displayed sporulation (Fig. 1B) of *M. laxa* at the end of the evaluation period (240 HAI). On the second inoculation date (June 20), with fruit of intermediate size but still immature (46.7 g of average mass, 15 to 19 weeks after full bloom), 100% of C216 fruit presented *M. laxa* infection and sporulation, regardless inoculation time. On the contrary, 'Zephyr', H165 and C207 genotypes had a higher number of fruits with presence of lesion and sporulation when inoculated immediately after wounding. F115 genotype showed only one fruit with BR lesion and fungus sporulation. On the third inoculation date, corresponding to the harvest date of each genotype, 100% of the fruits presented *M. laxa* infection and sporulation.

Infection probability on the first inoculation date, ranged between 0 to 0.40 and 0 to 0.10 (Fig. 2A), for IAW and I7hAW inoculation conditions, respectively. On the second inoculation date, the highest variability was observed, with infection probability ranging between 0.10 and 1.00 and between 0 and 1.00, for IAW and I7hAW, respectively. When mature fruits were inoculated, the probability of infection was 1.00, for all genotypes and in the two inoculation conditions.

In the case of inoculations made immediately after wounding, the infection delay was between 200 and 240 hours, between 24 and 200 hours and between 24 and 120 hours, for the first, second and third inoculation dates (Fig. 2B), except for F115 genotype. For fruit inoculations done seven hours after wounding, the delay was between 216 to 240 hours, between 24 and 240 hours and between 24 and 144 hours, for the first, second and third inoculations, in C216, 'Zephyr' and H165 genotypes. In the case of F115 genotype, no fruit was completely infected on the first inoculation date, and in the second, only one fruit of the inoculation immediately after wounding had disease symptoms.

Progression rate was between 0.10 to 0.40 on the first inoculation date, between 0.10 to 0.70 on the second inoculation date, and between 0.40 to 1.10 on the inoculation at mature stage (Fig. 2C).

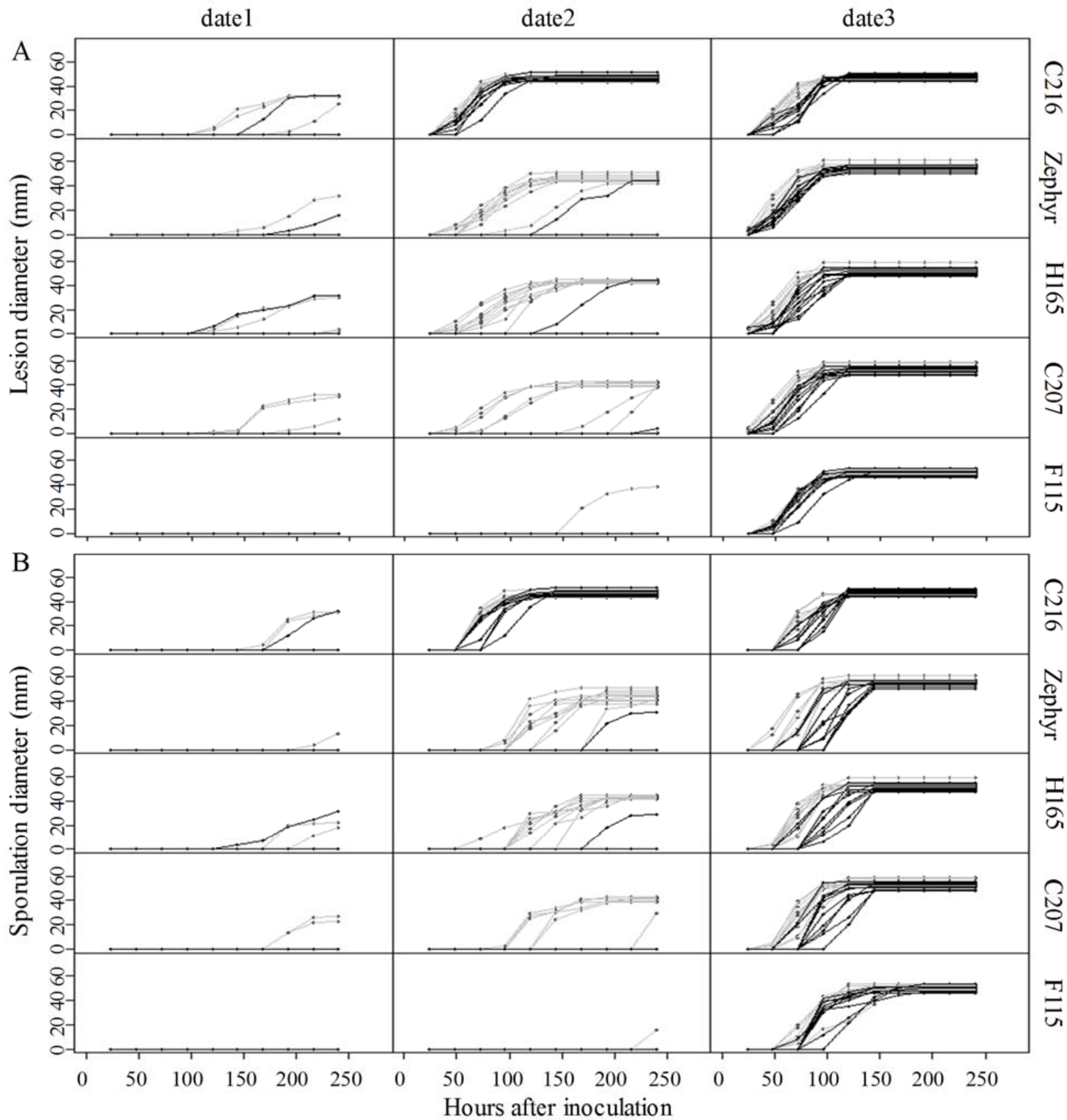


Fig. 1. Lesion (A) and sporulation (B) diameter in five nectarine genotypes inoculated with *M. laxa* immediately after wounded (gray line) and inoculated 7 hours after wounded (black line), in three stages of fruit development. date1 = May 28th, 11 to 15 weeks after full bloom (WAFB); date2 = June 20th, 15 to 19 WAFB; date3 = corresponds to the ripening date of each genotype (20 to 26 WAFB). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

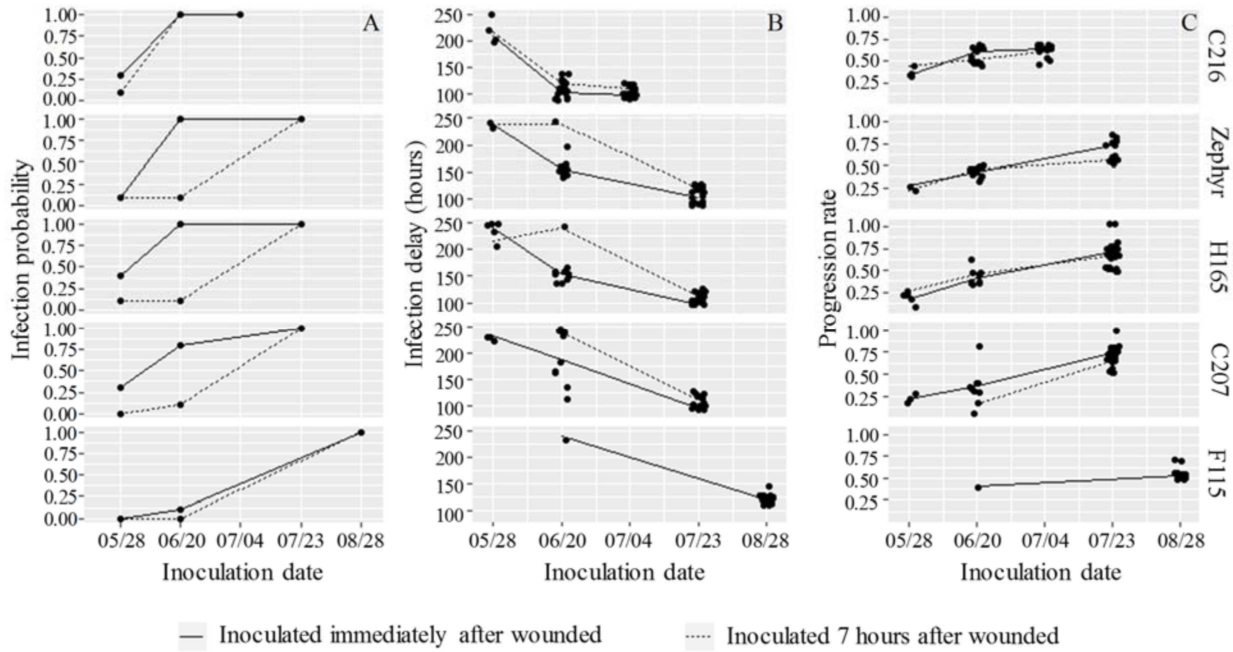


Fig. 2. *Monilinia laxa* infection probability (A), infection delay (B) and progression rate (C) in five nectarine genotypes inoculated with *M. laxa* immediately after wounded (plain lines) or inoculated seven hours after wounded (dashed lines), in three stages of fruit development. First date (05/28), 11 to 15 weeks after full bloom (WAFB); second date (06/20), 15 to 19 WAFB; third its ripening date of each genotype (20 to 26 WAFB). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Sporulation probability, delay and rate had a similar tendency as lesion data and are provided in Supplementary Fig. S1.

The ANOVA analysis for the delay, progression rate, lesion (LD) and sporulation (SpoD) diameters were performed to test the influence of the different times of inoculation procedure (immediately and seven hours after wounded). All observations (every 24 hours) were tested for LD and SpoD. The results for all of them were similar, therefore, only the analysis of the latest evaluation (240 HAI) is presented. The triple interaction (Genotype × Inoculation time × Development stage) was significant only for the sporulation delay (*p*-value = 0.0008), and for all others it was not significant (*p*-value > 0.01). Among the double interactions, Genotype × Inoculation time was not significant for any of the variables tested (*p*-value> 0.01); Genotype × Development stage was significant for all variables (*p*-value < 0.01), except sporulation rate (*p*-value = 0.1491); Inoculation time x Development stage was significant for infection delay, LD and SpoD. Analyzing the main factors, it was observed that the Inoculation time was not significant (*p*-value > 0.01) for any of the variables tested, since its effect depends largely on the Development stage. Genotype was significant for all variables except for lesion rate, and the Development stage was highly significant (*p*-value < 0.0001) for all variables (Table 1).

Table 1. ANOVA summary: Effects of genotype (Gen), inoculation time (InocTime), development stage (DevStage) and interactions for *Monilinia laxa* lesion and sporulation in INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

p-value[1]	Lesion Delay	Progression Rate	LD[2]	Sporulation Delay	Sporulation Rate	SpoD[2]
Genotype	0.0025**	0.2822^{ns}	<0.0001***	<0.0001***	0.0003***	<0.0001***
Inoculation Time	0.0190^{ns}	0.0244^{ns}	0.5113^{ns}	0.3180^{ns}	0.6580^{ns}	0.3148^{ns}
Development Stage	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
Gen×InocTime	0.0561^{ns}	0.4073^{ns}	0.0396^{ns}	0.1293^{ns}	0.0955^{ns}	0.0446^{ns}
Gen×DevStage	0.0026**	<0.0001***	<0.0001***	0.0006***	0.1491^{ns}	<0.0001***
InocTime×DevStage	<0.0001***	0.6532^{ns}	<0.0001***	0.0127^{ns}	0.5323^{ns}	<0.0001***
Gen×InocTime×DevStage	0.02403^{ns}	0.5149^{ns}	0.3433^{ns}	0.0008***	0.8843^{ns}	0.5836^{ns}

(1) F-tests (*p*-value): *** = $p < 0.001$, ** = $p < 0.01$, ^{ns} = non-significant ($p > 0.01$). (2) LD and SpoD, lesion diameter and sporulation, respectively, data from the evaluation done 240 hours after inoculation (HAI).

Significant difference (*p*-value > 0.01) was not observed between the two different inoculation times on the first and last inoculation dates. On the contrary, on the second date the LD and SpoD from fruit inoculated immediately after wounding were larger (*p*-value < 0.01) than for fruit inoculated 7 hours after wounding. These differences can be attributed to Zephyr, C207 and H165 genotypes. The same way, lesion delay decreased as the fruit grew, and on the second date it showed significant differences between Inoculation Time (*p*-value < 0.01), presenting a greater delay in the inoculations carried out seven hours after wounding (Fig. 2B).

Using Zephyr cultivar as an example, the two inoculation times at the three development stages are presented in Fig. 3. It is noteworthy that in the first date, red reactions were observed.. In the particular case of 'Zephyr' (Fig. 3), the red spots were associated only with the inoculation immediately after wounding and were located around the wound and at some scattered points near it. The same was observed in the selections H165 and C216, whereas the genotype C207 presented red spots in fruit inoculated at both inoculation times. However, the genotype F115 did not present fruits with red reactions in either of the two conditions, and it should be emphasized that this genotype did not present any infected fruit with BR, in this first inoculation date and very few in the second date. More photos and details of all the observed conditions at the first date can be seen on the Supplementary Fig. S2.

Fig. 3. Brown rot in 'Zephyr' fruit at three development stages, inoculated with *Monilinia laxa* immediately after wounding and 7 hours after wounding, at three fruit development stages: date1 = May 28th 15 weeks after full bloom (WAFB); date2 = June 20th (19 WAFB); date3 = July 23th, ripening stage (21 WAFB). Photos were taken 240 hours after inoculation. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Sample from red reaction zones were isolated and submitted to HPLC analyses, as described in the methodology. A total of 44 phenolic and terpenoid compounds were present in the samples. Four of them belong to the family triterpenoid (Tri), five are flavon-3-ol (Fla), six falvanone (Fle), 10 hydroxycinnamic derivatives (HCD), 11 triterpenoid derivatives (Trid), six flavonol (Flo) and two anthocyanin (Ant) (Table 2).

It should be pointed out that some compounds were only observed in the red reaction samples, including the six compounds of the flavonone family, and three hydroxycinnamic derivatives (HCD1, HCD3 and HCD4). In addition, other six compounds showed significant differences (p-value <0.01), being found in a greater proportion in the samples that presented the red reaction. These compounds were four flavan-3-ol (Fla1, Fla2, Fla3 and Catechin), a hydroxycinnamic derivative (HCD1) and an anthocyanin (Cyanidin-3-glucoside). On the other hand, a hydroxycinnamic derivative (cis-Neochlorogenic acid) was significantly (p-value = 0.00919) lower in the red reactions samples (Table 2). An HPLC chromatography spectra performed on the red-reaction and non-reaction samples is available in Supplementary Fig. S3, using 'Zephyr' as an example. Compounds showing significant differences between the two conditions are specified.

Table 2. Compounds identified by HPLC, wavelength (λ), retention time (RT), lambda maximum (λ max), abbreviation used and analysis of variance using the peaks area and contrast between red reaction and reactionless, in immature wounded fruits (11 to 15 weeks after full bloom). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Family[1]	Compound	λ (nm)	RT (min)	λ max (nm)	Abbrev.	*p*-value[3] Compound	*p*-value[3] Family
Tri	trihydroxy-urs-12-en-28-oic acid (1)[2]	210	124.3	198	thu1	0.030^{ns}	0.482^{ns}
	trihydroxy-urs-12-en-28-oic acid (2)[2]	210	124.7	198	thu2	0.049^{ns}	
	Oleanolic acid	210	131.9	198	Ole	0.496^{ns}	
	Ursolic acid	210	132.6	198	Urs	0.676^{ns}	
Fla	Flavan-3-ol (1)	280	25.3	253/278	Fla1	1.54^{-4} ***	1.20^{-3} **
	Procyanidin B1	280	27.7	254/278	ProcyaB1	0.254^{ns}	
	Flavan-3-ol (2)	280	28.9	256/278	Fla2	5.16^{-3} **	
	Flavan-3-ol (3)	280	30.2	255/278	Fla3	9.08^{-3} **	
	Catechin	280	34.1	251/278	Cat	8.95^{-4} ***	
Fle	Flavanone (1)	280	32.0	259/285	Fle1	Red reaction	Red reaction
	Flavanone (2)	280	47.2	253/282	Fle2	Red reaction	
	Eriodictyol-7-glucoside	280	66.0	283	E7Glu	Red reaction	
	Flavanone (3)	280	66.5	218/283	Fle3	Red reaction	
	Naringenine-7-glucoside (syn.: Prunin)	280	76.5	212/282	N7Glu	Red reaction	
	Flavanone (4)	280	99.7	284	Fle4	Red reaction	
HCD	cis-Neochlorogenic acid	315	20.2	266/316	c3CQ	9.19^{-3} **	0.644^{ns}
	Neochlorogenic acid	315	23.2	217/324	t3CQ	0.012^{ns}	
	Hydroxycinnamic derivative (1)	315	39.0	253/314	HCD1	Red reaction	
	Chlorogenic acid	315	43.1	217/326	t5CQ	0.739^{ns}	
	cis-Chlorogenic acid	315	54.2	284/317	c5CQ	0.733^{ns}	
	Hydroxycinnamic derivative (2)	315	56.6	280/311	HCD2	0.437^{ns}	
	5-*p*-Coumaroylquinic acid	315	57.4	253/311	pCQ	9.90^{-3} **	
	3,5-Dicaffeoylquinic acid	315	79.1	263/328	x3.5diCQ	0.334^{ns}	
	Hydroxycinnamic derivative (3)	315	99.2	265/330	HCD3	Red reaction	
	Hydroxycinnamic derivative (4)	315	100.6	221/329	HCD4	Red reaction	
Trid	*p*-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (1)[2]	315	125.9	252/289	cdhu1	0.245^{ns}	0.193^{ns}
	p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (2)[2]	315	126.3	295/319	cdhu2	0.058^{ns}	
	p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (3)[2]	315	126.5	250/311	cdhu3	0.133^{ns}	
	p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (4)[2]	315	126.8	251/285	cdhu4	0.098^{ns}	
	p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (5)[2]	315	127.6	298/308	cdhu5	0.150^{ns}	
	p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid (6)[2]	315	128.4	249/308	cdhu6	0.103^{ns}	
	feruloyl-2,3-dihydroxy-urs-12-en-28-oic acid[2]	315	129.0	282/322	fdhu	0.681^{ns}	
	3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid (1)[2]	315	135.2	253/286	cou1	0.856^{ns}	
	3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid (2)[2]	315	135.4	250/307	cou2	0.953^{ns}	
	3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid (3)[2]	315	135.7	257/314	cou3	0.852^{ns}	
	3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid (4)[2]	315	135.9	255/313	cou4	0.818^{ns}	
Flo	Quercetin-3-galactoside	350	84.4	255/354	Q3Gal	0.228^{ns}	0.239^{ns}
	Quercetin-3-glucoside	350	86.2	255/354	Q3Glu	0.234^{ns}	
	Quercetin-3-rutinoside	350	86.8	256/354	Q3Rut	0.251^{ns}	
	Kaempferol-3-galactoside	350	92.9	265/347	K3Gal	0.273^{ns}	
	Kaempferol-3-glucoside	350	95.1	265/347	K3Glu	0.436^{ns}	
	Kaempferol-3-rutinoside	350	95.6	266/345	K3Rut	0.331^{ns}	
Ant	Cyanidin-3-glucoside	520	62.8	279/518	Cya3Glu	8.34^{-5} ***	1.41^{-4} ***
	Cyanidin-3-rutinoside	520	64.8	276/525	Cya3Rut	0.135^{ns}	

[(1)] Compounds family: Tri = Triterpenoid; Fla = Flavan-3-ol; Fle = Flavanone; HCD = Hydroxycinnamic derivatives; Trid = Triterpenoid derivatives; Flo = Flavonol; Ant = Anthocyanin. [(2)] Ursolic acid or Oleanolic acid as principal structure. [(3)] F-tests (*p*-value): *** = $p < 0.001$, ** = $p < 0.01$, ^{ns} = non-significant ($p > 0.01$).

Summing up the compounds detected according to their family, the flavan.3.ol and anthocyanin, stand up as being in greater proportion in the red reactions, besides the flavonone which were only present in this condition (Table 2 and Fig. 4). Boxplot-type graphs comparing the content for each particular compound between the samples for the red reaction and reactionless are available in Supplementary Fig. S4.

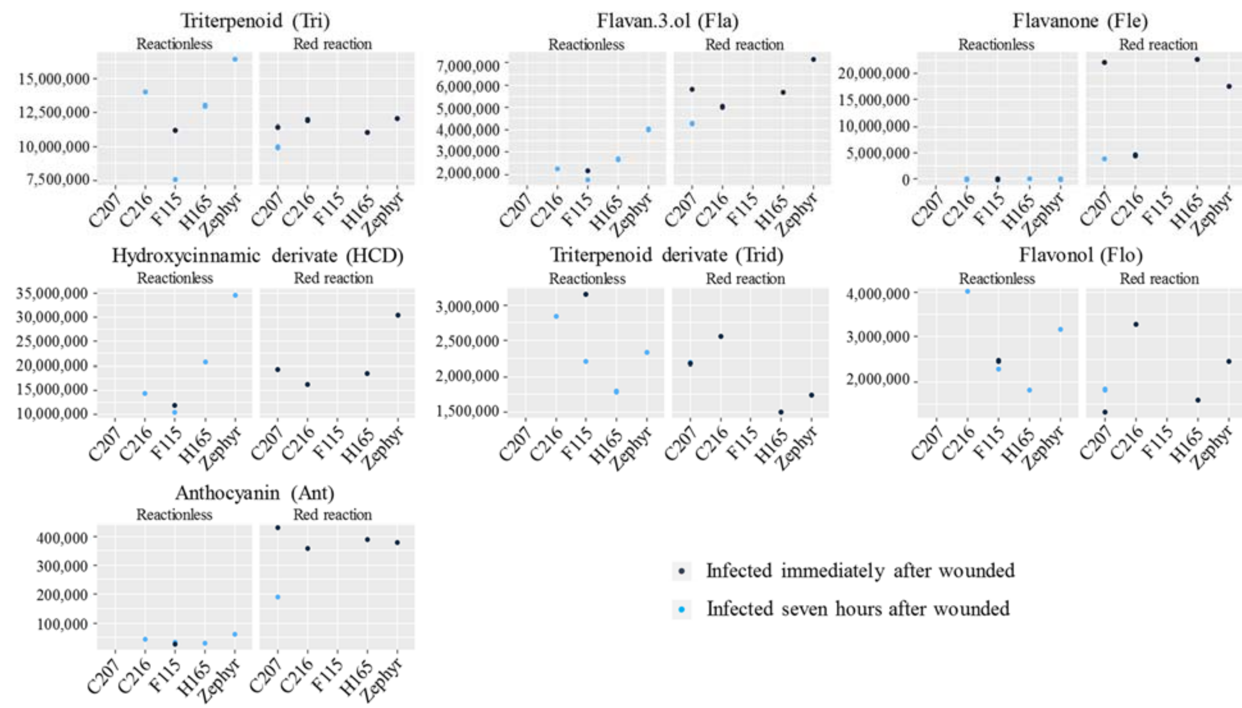


Fig. 4. HPLC-identified compounds in immature fruit (11 to 15 weeks after full bloom) skin samples from five nectarine genotypes (C207, C116, F115, H165 and 'Zephyr'), with reactionless and red reaction, after wounding and inoculation with *Monilinia laxa*, either immediately and seven hours after. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Among the compounds identified only in the samples from red reaction zones, two compounds of the flavonone family were found in higher proportion (Supplementary Fig. S3). The corresponding compounds showed typical UV-absorbance characteristics of flavanones and using the pure compounds in a joint analysis with the samples previously analyzed, they were identified as Eriodictyol-7-glucoside (Fig. 5A) Naringenin-7-glucoside (syn.: Prunin) (Fig. 5B).

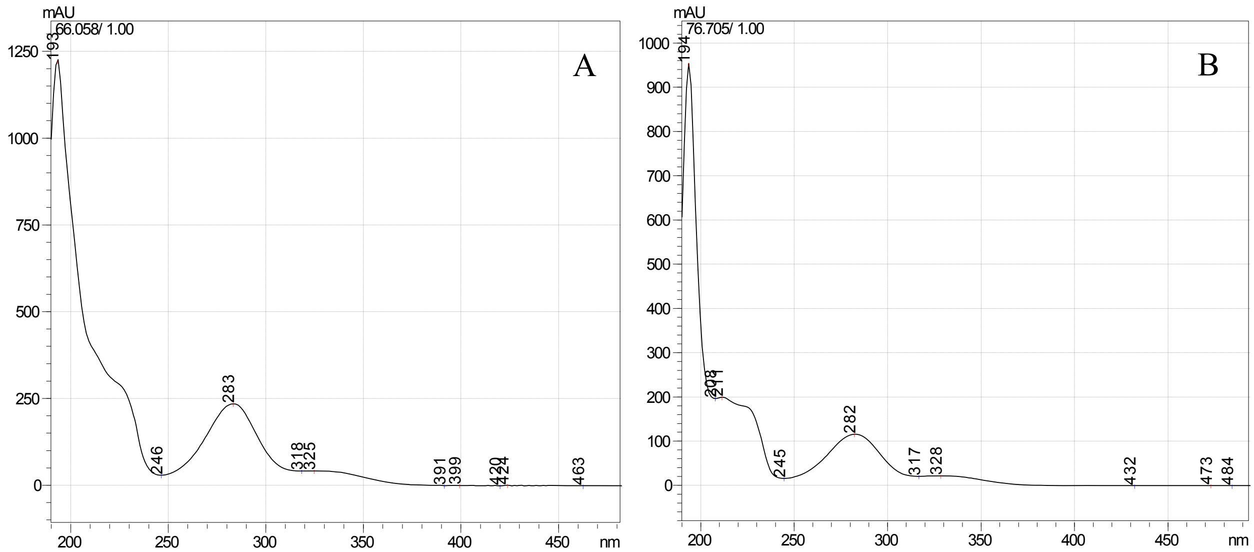


Fig. 5. HPLC spectrum of the two main compounds, Eriodictyol-7-glucoside (A) and Naringenin-7-glucoside syn.: Prunin (B), present in the red reaction area, detected in wounded immature fruits (11 to 15 weeks after full bloom) infected immediately after wounding. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

4. Discussion

Infection susceptibility to *Monilinia* spp. is high during the early stages of fruit development, decreases during green fruit stages (pit hardening) and then increases again as fruit matures, being most susceptible to infection during the preharvest ripening stage (Biggs and Northover, 1988; Gradziel, 1994; Emery et al., 2000; Mari et al., 2003; Adaskaveg et al., 2008). Making weekly inoculations with *M. laxa* in apricot and peach, wounded and without wounds fruit, Mari et al. (2003) observed three different susceptibility stages, a first stage, where susceptibility was high, a second stage (rest period) when susceptibility decrease (10 to 15 WAFB) and a third stage (1 to 2 weeks before total maturation) when fruit increase their susceptibility to *M. laxa*. Similar results were reported by Guidarelli et al. (2014) inoculating weekly non-wounded peach fruit with *M. laxa*. In the present study, the first inoculation coincided with the green fruit stage (fruits in pit hardening), where susceptibility to *M. laxa* was low. On the second inoculation date, C216 genotype (early harvest) probably had already entered the ripening period of the fruits thus increasing its susceptibility, on the contrary, genotype F115 (late harvest) was still in the stage of green fruits. The Zephyr, H165 and C207 genotypes, were intermediate between the two previous ones (Fig. 1 and Fig. 3).

During the stone hardening stage, green fruit show a low susceptibility to BR. At this stage, wound healing might be faster and more efficient than later. Indeed, it is the stage when we observed a significant difference in infection parameters between the two inoculation times (IAW and I7hAW) that can be associated with a rapid and efficient response of defense of the fruit 7 hours after wounding.

Micro-cracks and wounds can heal in a period of minutes to several hours, depending on the lesion degree, the fruit physiological stage and the environmental conditions (Dean and Kolattukudy, 1976; Spotts et al, 1998). It has already been shown, in other species, that healing can induce defense pathways (Chistopher et al., 2004, Smith et al., 2004, Janisiewicz et al., 2016, Han et al., 2017). This may also explain the appearance of red reactions only in inoculated fruit immediately after wounding, on the first evaluation date (Fig. 3 and Fig. S2). In the inoculation done seven hours after wounding, the injure may have begun to heal, preventing the conidia penetration in the fruit or triggering not visible resistance mechanisms.

The red reaction seems to be associated with double stress (wounding and immediate infection) and may be associated with active resistance mechanisms of the fruit to the pathogen, trying to avoid or reduce the negative effects of the infection. When red reaction samples were submitted to HPLC analysis, changes in the phenolic compounds were detected, including six compounds from the flavonone family (Fle1, Fle2, Fle3, Fle4, E7glu and N7glu) and three hydroxycinnamic derivatives (HCD1, HCD3 and HCD4) which were not observed in the samples that did not present the red reaction, or were in such small amount, impossible to measure (Fig. S3). In addition to these compounds, four other flavan-3-ol (Fla1, Fla2, Fla3 and Catechin), a hydroxycinnamic derivative (HCD1) and an anthocyanin (Cyanidin-3-glucoside) were present in a larger proportion in samples isolated from red spots (Table 2 and Fig. S4).

Guidarelli et al. (2014) performed a microarray-based on transcriptome analysis to compare the expression of genes between susceptible (ripening phase) and resistant fruits (green fruits, at the pit hardening stage). They observed that genes related to flavonoids and phenylpropanoids are differentially expressed between the two stages, supporting the role of these metabolites in the fruit response to *M. laxa*. It is worth noting the ad equation with the results from the present study, in which four flavonoid subgroups (flavonone, flavan.3.ol, flavonol and anthocyanin) and a subgroup of phenylpropanoids (hydroxycinnamic derivates) were detected on the first evaluation date in green fruits (Fig. 4) and several of these compounds were present in greater proportion or only associated with red reactions (Fig. S4).

Studying the effect of dioxygenase inhibitors on the resistance-related flavonoid metabolism of apple and pears, Roemmelt et al. (2003) reported changes of the pattern of flavonoids and phenylpropanoids of apple leaves treated with prohexadione-Ca, more specifically they observed an increase in the concentration of luteoliflavan, E7glu, N7glu and HCD. Such induced changes in the flavonoid composition of the leaves correlate with a reduced susceptibility against the pathogenic bacterium *Erwinia amylovora*. The authors postulated that these changes in flavonoid composition are responsible for the observed pathogen resistance induced by prohexadione-Ca treatment. Among the compounds mentioned by the authors, E7glu, N7glu and HCD, coincide with the compounds detected only or in greater proportion in the red reactions. This suggests a resistance-induced response to *M. laxa*, when inoculated simultaneously with the injury. On the other hand, working with the silencing of flavanone-3-hydroxylase in apple trees Flachowsky et al. (2012), were able to increase the accumulation of flavanones, but failed to reduce susceptibility to bacterial fire caused by *E. amylovora*.

E7glu and N7glu (= Prunin) (Fig. 5), which were the majority compounds in the red reactions (Fig. S3) had already been reported in several works related to fruit species, such as apple and pear leaves (Halbwirth et al., 2003; Roemmelt et al., 2003; Flachowsky et al., 2012), of *Prunus davidiana* stems (Choi et al., 1991; Jung et al., 2016), tomato fruit cuticles (Domínguez et al., 2009), in several *Citrus* species (Castillo et al., 1993; Del Río et al., 1995; Frydoonfar et al., 2003; Mir and Tiku, 2014, Orallo et al., 2005), and even in peach buds (Erez and Lavee, 1969). However, no record of them was found on peach fruits, this is probably the first time they are reported.

The results suggest that flavonoid and phenylpropanoid compounds may have an influence on to the response of fruit to *M. laxa* infection. Future studies should be carried on evaluating the changes in the fruit metabolic composition, as well as their volatile compounds and the genetic expression of wounded or non-wounded fruits, inoculated at different times after the injury, during different fruit development stages. Also, a further exploration of the red reaction and its association with brown rot susceptibility/resistance is of interest.

Acknowledgements

To the Coordination of Improvement of Higher Education Personnel (CAPES-Brazil) for providing PhD scholarship founds, and GAFL - INRA Avignon staff, especially Marie-Noëlle Corre and Véronique Signoret

References

Adaskaveg, J.E., Schnabel, G., Forster, H., 2008. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: Layne D.R., Bassi, D. (Eds.), The Peach: Botany, Production and Uses. CAB International, Wallingford, UK, pp. 352-406.

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. J. Sci. Food Agric. 99, 8, 4105-4113. http://dx.doi.org/10.1002/jsfa.9640.

Castillo, J., Benavente, O., Del Rio, J.A., 1993. Hesperetin 7-0-Glucoside and Prunin in *Citrus* Species (*C. aurantium* and *C. paradisi*). A Study of their quantitative distribution in immature fruits and as immediate precursors of Neohesperidin and Naringin in *C. aurantium*. J. Agric. Food Chem. 41, 1920-1924. http://dx.doi.org/10.1021/jf00035a021.

Christopher, M.E., Miranda, M., Major, I.T., Constabel, C.P., 2004. Gene expression profiling of systemically wound-induced defenses in hybrid poplar. Planta 219, 936-947. http://dx.doi.org/10.1007/s00425-004-1297-3.

Choi, J.S., Yokozawa, T., Oura, H., 1990. Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of *Prunus davidiana* stems and its main component, Prunin. Planta Med. 57, 208-211. http://dx.doi.org/10.1055/s-2006-960075.

Dean, B.B., Kolattukudy, P.E., 1976.Synthesis of suberin during wound-healing in jade leaves, tomato fruit, and bean pods. Plant Physiol. 58, 3, 411-416. http://dx.doi.org/10.1104/pp.58.3.411.

Del Río, J.A., Fuster, M.D., Sabater, F., Porras, I., García-Lidón, A., Ortuño, A., 1995. Effect of Benzylaminopurine on the Flavanones Hesperidin, Hesperetin 7-O-Glucoside, and Prunin in Tangelo nova fruits. J. Agric. Food Chem. 43, 2030-2034. http://dx.doi.org/10.1021/jf00056a014.

Dini, M., Scariotto, S., Raseira, M.C.B., Ueno, B., 2019a. Heritability and segregation of resistance to brown rot in peach fruits. Acta Hort. (under review).

Dini, M., Raseira, M.C.B., Scariotto, S., Carpenedo, S., Ueno, B., 2019b. *Monilinia fructicola* lesion and sporulation in peach and nectarine fruits. Pesqui. Agropecu. Bras. (under review).

Domínguez, E., Luque, P., Heredia, A., 2009. Sorption and interaction of the flavonoid naringenin on tomato fruit cuticles. J. Agric. Food Chem. 57, 16, 7560-7564. http://dx.doi.org/10.1021/jf9011455.

FAOSTAT, 2019. Crop production. Food and Agriculture Organization of the United Nations. FAOSTAT (database, Accessed 02.06.2019). http://www.fao.org/faostat/en/#data/QC.

Erez, A., Lavee, S., 1969. Prunin identification, biological activity and quantitative change in comparison to Naringenin in dormant peach buds. Plant Physiol. 44, 342-346. http://dx.doi.org/10.1104/pp.44.3.342.

Flachowsky, H., Halbwirth, H., Treutter, D., Richter, K., Hanke, M.V., Szankowski, I., Gosch, C., Stich, K., Fischer, T.C., 2012. Silencing of flavanone-3-hydroxylase in apple (*Malus* × *domestica* Borkh.) leads to accumulation of flavanones, but not to reduced fire blight susceptibility. Plant Physiol. Biochem. 51, 18-25. http://dx.doi.org/10.1016/j.plaphy.2011.10.004.

Fresnedo-Ramírez, J., Famula, T.R., Gradziel, T.M., 2017. Application of a Bayesian ordinal animal model for the estimation of breeding values for the resistance to *Monilinia fructicola* (G.Winter) Honey in progenies of peach [*Prunus persica* (L.) Batsch]. Breeding Sci. 67, 2, 110-122. http://dx.doi.org/10.1270/jsbbs.16027.

Frydoonfar, H.R., McGrath, D.R., Spigelman, A.D., 2003. The variable effect on proliferation of a colon cancer cell line by the citrus fruit flavonoid Naringenin. Colorectal Dis. 5, 2, 149-152. http://dx.doi.org/10.1046/j.1463-1318.2003.00444.x.

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. J. Am. Pom. Soc. 72, 2, 94-100.

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., Baraldi, E. 2014. Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. Eur. J. Plant. Pathol. 140, 3, 503-513. http://dx.doi.org/10.1007/s10658-014-0484-8.

Halbwirth, H., Fischer, T.C., Roemmelt, S., Spinellid, F., Schlangena, K., Peterekc, S., Sabatini, E., Messinae, C., Speakman, J.B., Andreotti, C., Rademacher, W., Bazzie, C., Costa, G., Treutter, D., Forkmann, G., Stich, K., 2003. Induction of antimicrobial 3-deoxyflavonoids in pome fruit trees controls fire blight. Z. Naturforsch. 58, 765-770. http://dx.doi.org/10.1515/znc-2003-11-1201.

Han, C., Jin, P., Li, M., Wang, L., Zheng, Y., 2017. Physiological and transcriptomic analysis validates previous findings of changes in primary metabolism for the production of phenolic antioxidants in wounded carrots. J. Agric. Food Chem. 65, 7159-7167. http://dx.doi.org/10.1021/acs.jafc.7b01137.

Hu, M.J., Cox, K.D., Schnabel, G., Luo, C.X., 2011. *Monilinia* species causing brown rot of peach in China. PLoS ONE 6, 9, e24990. http://dx.doi.org/10.1371/journal.pone.0024990.

Janisiewicz, W.J., Nichols, B., Bauchan, G., Chao, T.C., Jurick II, W.M., 2016. Wound responses of wild apples suggest multiple resistance mechanism against blue mold decay. Postharvest Biol. Technol. 117, 132-140. http://dx.doi.org/10.1016/j.postharvbio.2015.12.004.

Jung, H.A., Ali, M.Y., Bhakta, H.K., Min, B.S., Choi, J.S., 2016. Prunin is a highly potent flavonoid from *Prunus davidiana* stems that inhibits protein tyrosine phosphatase 1B and stimulates glucose uptake in insulin-resistant HepG2 cells. Arch. Pharm. Res. 40, 1, 37-48. http://dx.doi.org/10.1007/s12272-016-0852-3.

Mari, M., Casalini, L., Baraldi, E., Bertolini, P., Pratella, G.C., 2003. Susceptibility of apricot and peach fruit to *Monilinia laxa* during phenological stages. Postharvest Biol. Technol. 30, 1, 105-109. http://dx.doi.org/10.1016/S0925-5214(03)00138-8.

Martínez-García, P.J., Parfitt, D.E., Bostock, R.M., Fresnedo-Ramírez, J., Vazquez-Lobo, A., Ogundiwin, E.A., Gradziel, T.M., 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, e78634. http://dx.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. Trop. Plant Pathol. 33, 3, 227-234. http://dx.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: Raseira, M.C.B., Pereira, J.F.M., Carvalho, F.L.C. (Eds.), Pessegueiro. Embrapa, Brasília, DF, Brazil, pp. 355-432.

Measham, P.F., Bound, S.A., Gracie, A.J., Wilson, S.J., 2009. Incidence and type of cracking in sweet cherry (*Prunus avium* L.) are affected by genotype and season. Crop Pasture Sci. 60, 10, 1002-1008. http://dx.doi.org/10.1071/CP08410.

Mir, I.A., Tiku, A.B., 2014. Chemopreventive and therapeutic potential of "Naringenin," a flavanone present in *Citrus* fruits. Nutr. Cancer 67, 1, 27-42. http://dx.doi.org/10.1080/01635581.2015.976320.

Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., Uyemoto, J.K., 1995. Compendium of Stone Fruit Diseases, first ed. The American Phytopathological Society, Saint Paul, MN.

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. J. Agric. Food Chem. 64, 20, 4029-4047. http://dx.doi.org/10.1021/acs.jafc.6b00104.

Orallo, F., Camiña, M., Álvarez, E., Basaran, H., Lugnier, C., 2005. Implication of cyclic nucleotide phosphodiesterase inhibition in the vasorelaxant activity of the citrus-fruits flavonoid (+/-)-naringenin. Planta Med. 71, 2, 99-107. http://dx.doi.org/10.1055/s-2005-837774.

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 Genet. Genomes 10, 5, 1223-1242. http://dx.doi.org/10.1007/s11295-014-0756-7.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R Core Team, Vienna, Austria (URL). http://www.R-project.org/.

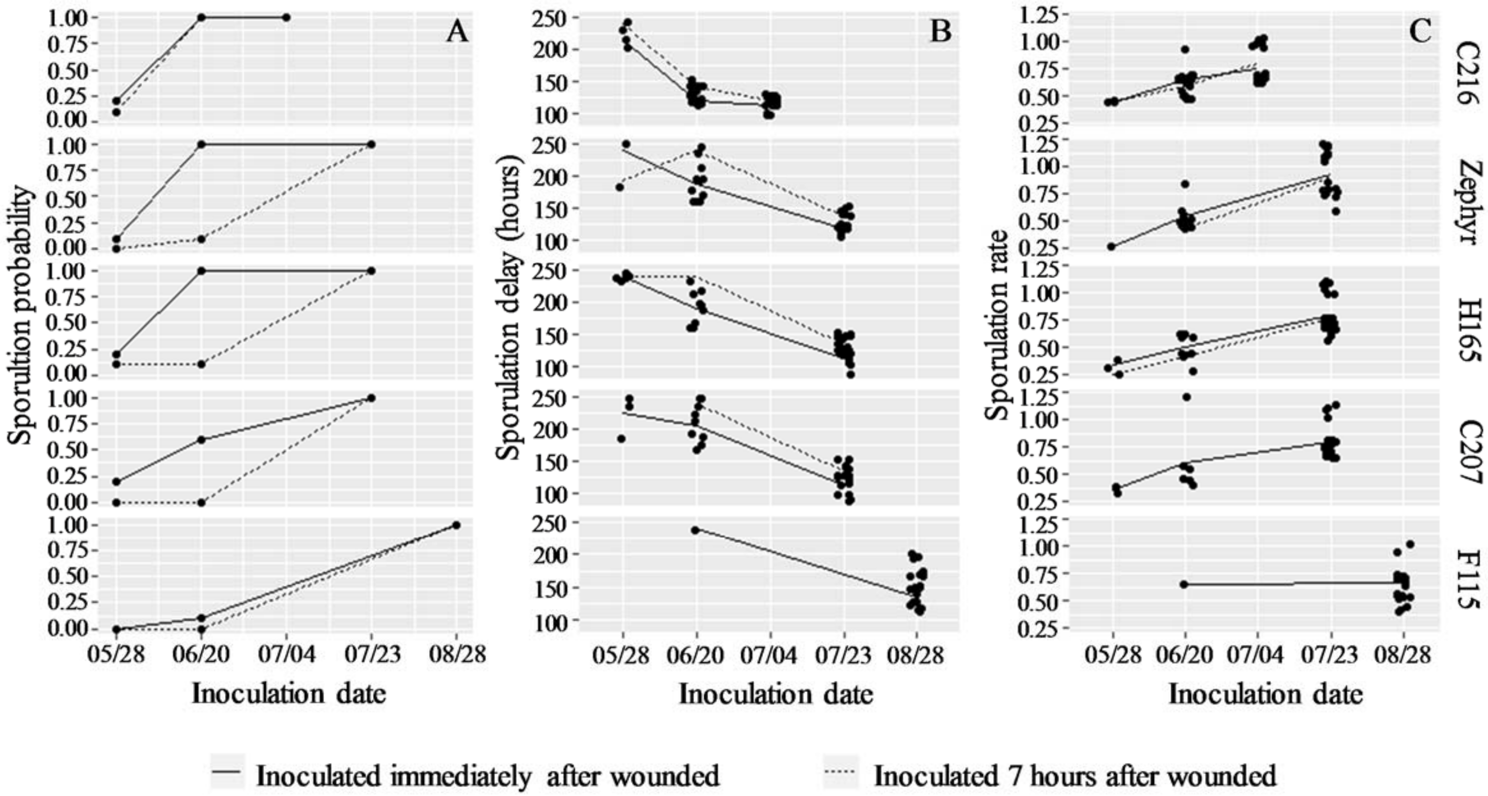
Raseira, M.C.B., Franzon, R., 2014. Melhoramento genético. In: Raseira, M.C.B., Pereira, J.F.M., Carvalho, F.L.C. (Eds.), Pessegueiro. Embrapa, Brasília, DF, Brazil, pp. 57-72.

Roemmelt, S., Fischer, T.C., Halbwirth, H., Peterek, S., Schlangen, K., Speakman, J.B., Treutter, D., Forkmann, G., Stich, K., 2003. Effect of dioxygenase inhibitors on the resistance-related flavonoid metabolism of apple and pears: chemical, biochemical and molecular biological aspects. Europ. J. Hort. Sci. 68, 3, 129-136.

Scariotto, S., 2016. Variabilidade genética e abordagem bioquímica da resistência do fruto à podridão-parda em diferentes genótipos de *Prunus persica*. 130p. Thesis (PhD.), Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS, Brazil.

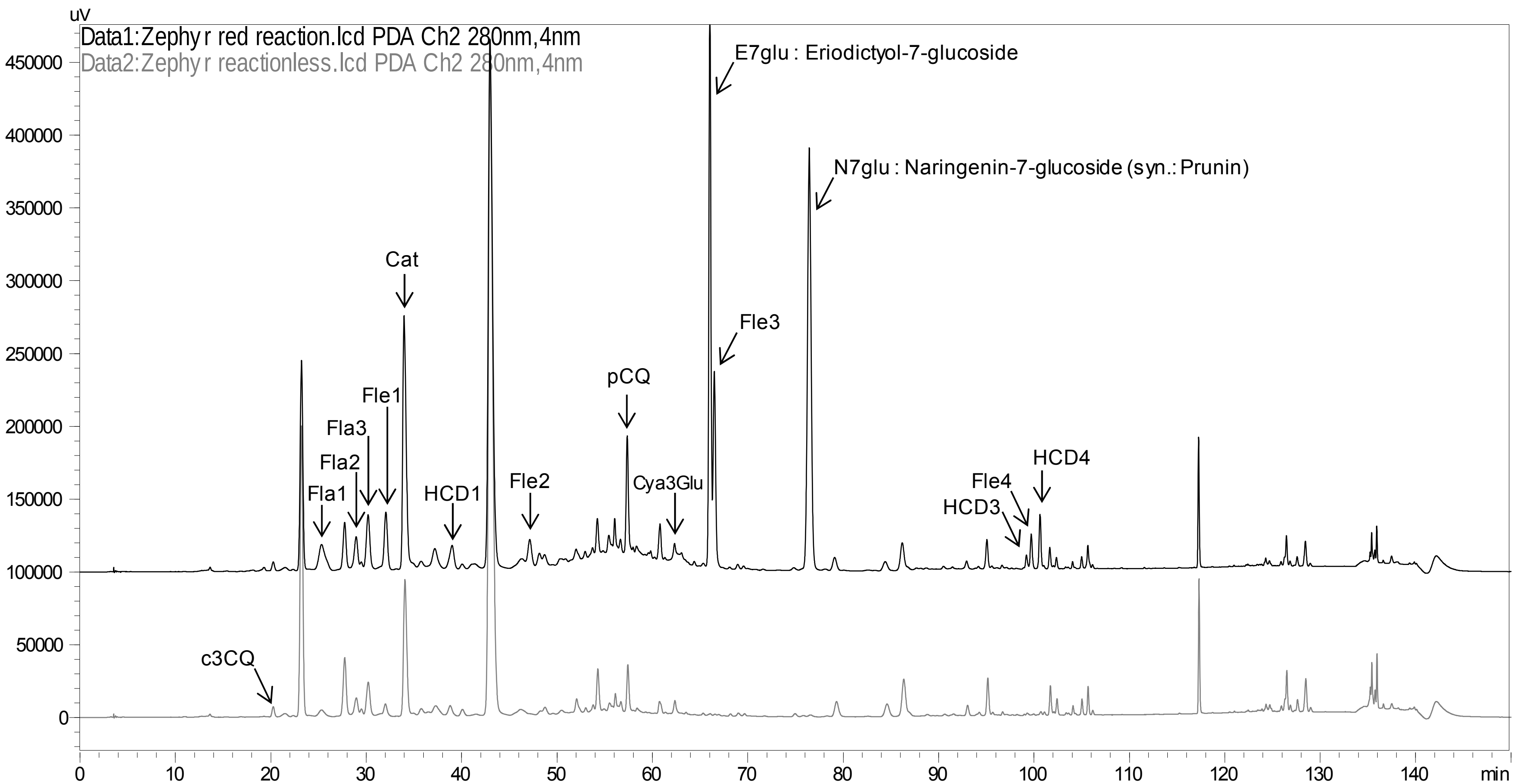
Smith, C.M., Rodriguez-Buey, M., Karlsson, J., Campbell, M.M., 2004. The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. New Phytol. 164, 123-136. http://dx.doi.org/10.1111/j.1469-8137.2004.01151.x.

Spotts, R.A., Sanderson, P.G., Lennox, C.L., Sugar, D., Cervantes, L.A., 1998. Wounding, wound healing and staining of mature pear fruit. Postharvest Biol. Tec. 13, 27-36. http://dx.doi.org/10.1080/00221589.1981.11514980.

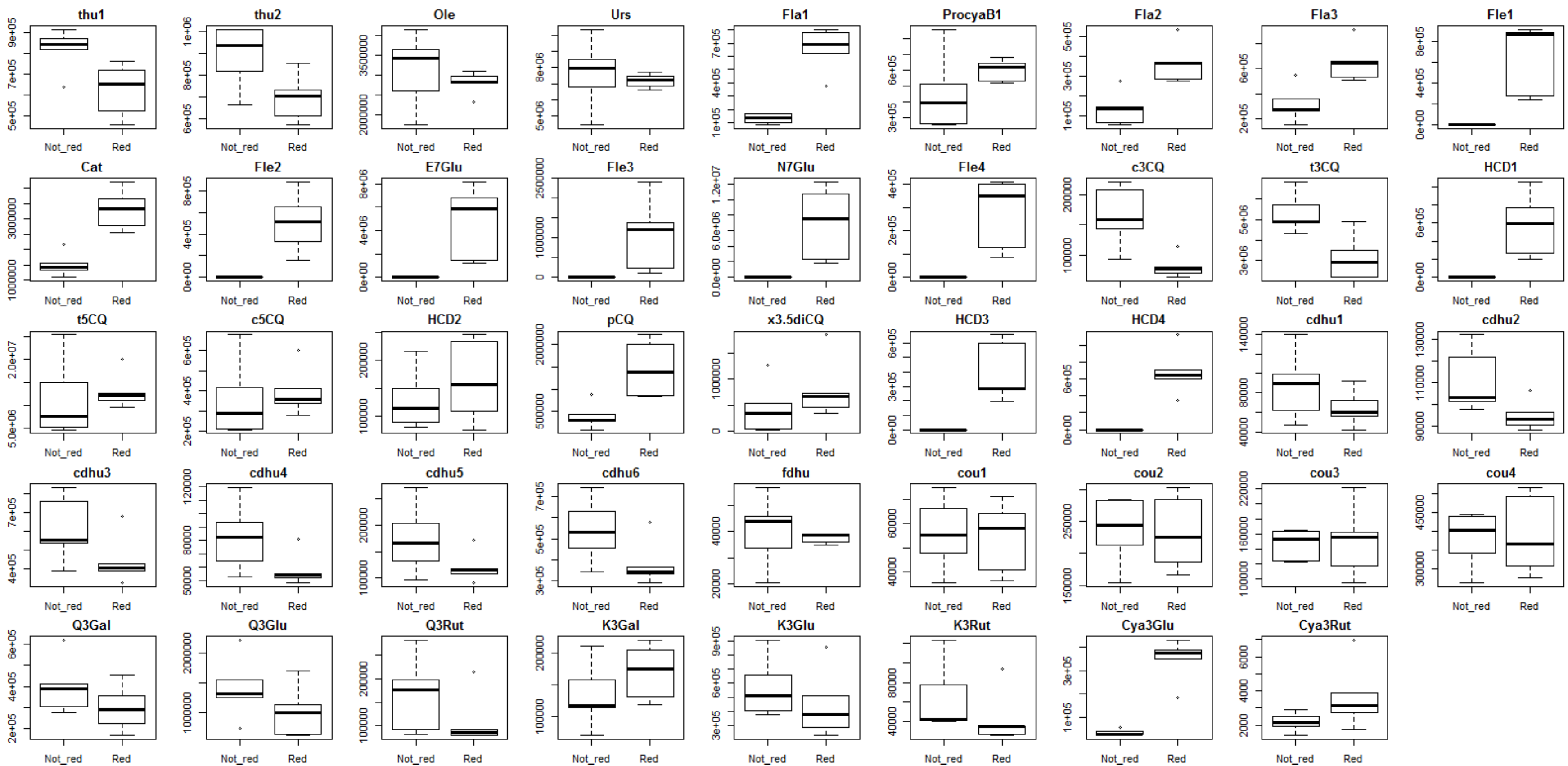


Supplementary Fig. S1. *Monilinia laxa* sporulation probability (A), sporulation delay (B) and sporulation rate (C) in five nectarine genotypes inoculated immediately after wounding and inoculated seven hours after wounding, in three stages of fruit development. First date (05/28), 11 to 15 weeks after full bloom (WAFB); second date (06/20), 15 to 19 WAFB; third its ripening date of each genotype (20 to 26 WAFB). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Supplementary Fig. S2. All conditions found on wounded fruits inoculated with *Monilinia laxa*, in the first inoculation date (May 28th), 11 to 15 weeks after full bloom. A) Inoculation immediately after woundind (from left to right): red spots scattered near the scar (mainly 'Zephyr' and C207); red reaction on the scar (H165, C216, 'Zephyr' and C207); infection above the red reaction (H165, C216, 'Zephyr' and C207). B) Inoculation 7 hours after wounding (from left to right): reactionless (F115, H165, C216 and 'Zephyr'); some red reaction on the scar (mainly C207); red reaction on the scar and small scattered red spots (C207). Photos of evaluation done 240 hours after inoculation. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.



Supplementary Fig. S3. HPLC chromatography (280 nm) of 'Zephyr', immature fruits (15 weeks after full bloom) with red reaction (wounded + *Monilinia laxa* immediate inoculation) and fruits with reactionless (wounded + *M. laxa* inoculation seven hours after wounding). INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France. Abbreviations: Fla, Flavan-3-ol; Cat, Catechin; Fle, Flavanone; HCD, Hydroxycinnamic derivative; pCQ, 5-*p*-Coumaroylquinic acid; c3CQ, cis-Neochlorogenic acid.



Supplementary Fig. S4. Boxplot of the HPLC peaks area of compounds identified to reactionless (Not_red) and red reaction (Red), in immature fruits (11 to 15 weeks after full bloom), year 2018. Abbreviations of compounds in Table 2. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

4.8 Artigo 8. Reaction of wounded nectarine fruits: brown rot infection, phenolic and volatile compounds

Artigo a ser submetido na Revista *“Scientia Horticulturae”*

Reaction of wounded nectarine fruits: brown rot infection, phenolic and volatile compounds

Maximiliano Dini[a,*]; Bénédicte Quilot-Turion[b]; Maria do Carmo Bassols Raseira[c]

[a] Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.

[b] GAFL, INRA, 84143, Montfavet, France.

[c] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Rodovia BR 392, km 78, Caixa Postal 403, CEP 96010-971, Pelotas, Rio Grande do Sul, Brazil.

* Corresponding author at: Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil.

E-mail addresses: maxidini@hotmail.com, mdini@inia.org.uy (M. Dini).

Reaction of wounded nectarine fruits: brown rot infection, phenolic and volatile compounds

Keywords: *Prunus persica* (L.) Batsch, *Monilinia laxa* (Aderh. & Ruhl.) Honey, HPLC, gas chromatography.

Abstract. Resistance to brown rot, the most important disease of stone fruit, is sought worldwide. Chemical factors are the most exploited, because they are potential passive or active mechanisms in defense of the plant to pathogens. The aim of this study was to analyze the effect of wounding nectarine fruits on the changes in phenolic compounds and volatile compounds, and on the development of *Monilinia laxa* infection. The objective was to explore mechanisms that could expain the fact that inoculation done 7 hours after wounding fruit results in slowed and reduced brown rot infection compared to inoculation done immediately after wounding. The hypothesis underlying this work is that the processes carried out by the fruit after an injury, such as the synthesis of new compounds, can impact the development of the infection. For this, fruit of 'Zephyr' and C216 nectarines, infected 130 days after full bloom with *M. laxa*, immediately and seven hours after wounded, have been phenotyped for fruit reaction to brown rot. Phenolic and triterpenoid compounds were analyzed by HPLC-DAD analysis and volatile compounds by GC-MS analysis, studying the difference between wounded and non-wounded fruits. The cultivar Zephyr showed higher lesion and sporulation probabilities caused by *M. laxa* when inoculated immediately after wounded than after seven hours. A total of 30 phenolic and terpenoid compounds were identified in the HPLC analysis performed. In general, within each genotype, the treatment with wounds presented less proportion of the compounds than the treatment without wound and 'Zephyr' showed a higher proportion of compounds than C216. 70 volatile compounds were detected and 13 were associated with the two genotypes in wounded fruits. Only the compounds *β*-Ocimene, trans-α-Bergamo and cis-*β*-Farnesene displayed significantly higher values in 'Zephyr' samples compared to C216. Although this study does not allow the association between the compounds and susceptibility to *M. laxa*, the parallel results presented open up prospects to explore possible ways of increasing fruit defense against brown rot.

1. Introduction

Brown rot mainly caused by *Monilinia laxa* (Aderh. & Ruhl.) Honey and *M. fructicola* (Winter) Honey is the main fungal disease of peaches and nectarines (*Prunus persica* L. Batsch).

Abbreviations: DAFB, days after full bloom; HAI, hour after inoculation; HPLC-DAD, high-performance liquid chromatography with diode-array detection; GC-MS, gas chromatograph with mass spectrometer; ANOVA, analysis of variance; PCA, principal component analysis.

This disease causes the blossom blight, branches cankers, twig blight, and pre and post-harvest rot in fruits, causing economically important losses, which can reach more than 80% in years when the climatic conditions are favorable for the disease development (Ogawa et al., 1995; Adaskaveg et al., 2008). The fruit development stage is related to brown rot susceptibility, a susceptible early stage, followed by a phase that coincides with the pit hardening of less susceptible, and soon the susceptibility increases again until be maximum in ripe fruit (Mari et al., 2003; Gell et al., 2008; Guidarelli et al., 2014).

Chemical and physical factors are considered responsible for the differences in brown rot resistance as reported in each of the fruit development stages (Gradziel et al., 2003; Villarino et al., 2011). Among the physical factors, the compaction and arrangement of the epidermal and subepidermal cells of some peach genotypes, such as found in the Brazilian cultivar Bolinha, which has a high level of brown rot resistance, have been reported. In addition, the Bolinha fruit have less trichomes and a thicker cuticle with higher phenolic content than other fruit of similar maturity, more susceptible to brown rot (Feliciano et al., 1987; Gradziel, 1994; Ogawa et al., 1995; Lee and Bostock et al., 2007). Among the chemical factors, the potential role of phenolic and triterpenoid compounds in this resistance has been reported in several studies because their concentrations are particularly high in peach genotypes with higher resistance to fungus (Feliciano et al., 1987; Lee et al., 1990; Tomas-Barberán et al. 1990; Bostock et al., 1999; Villarino et al., 2011). In general, the concentrations of these compounds are initially high in immature fruits, decreasing during ripening. However, high amounts of some of these compounds persist during maturation stages and are attributed to greater or lesser resistance to brown rot (Lee et al., 1990; Senter and Callahan, 1990; Villarino et al., 2011).

Several studies have also showed antifungal properties of volatile compunds from fruit and vegetables (Cowan, 1999; Isaman, 2000; Tripathi and Dubey, 2004; Neri et al., 2007). Some of these compounds may play an interesting role in the control of defense gene expression, probably as a signal mediator of plant-pathogenic signals (Farmer, 2001). Some studies on the antifungal activity of aromatic compounds of plants against *Monilinia* spp. have been reported with variable but promising results (Wilson et al., 1987; Caccioni and Guizzardi, 1994; Caccioni et al., 1995; Tsao and Zhou, 2000, Neri et al., 2007).

Inoculation done 7 hours after wounding fruit results in slowed and reduced brown rot infection compared to inoculation done immediately after wounding (Dini et al., 2019), Suggesting that the fact healing may induce defense pathways.

The disease is mainly controlled with the use of fungicides (Thomidis et al., 2009) and resistance of fungus isolates to the main fungicidal molecules used for brown rot control has been reported (Luo et al., 2010; Hily et al., 2011; Zhu et al., 2012; Chen et al., 2017). Considereing the challenge for a more sustainable production, friendly to the environment and to the health of workers and consumers, the search for genetic resistance in peach has become increasingly important. Thus, the aim of this study was to analyse the effect of fruit reaction after wounding on the development of *Monilinia laxa* infection and to identify phenolic and volatile compounds specific to wounded fruit.

2. Materials and methods

2.1. Plant material

Two nectarine genotypes, the cultivar Zephyr and the advanced selection C216 of the 'Génétique et Amélioration des Fruits et Légumes' (GAFL) research unit of INRA Avignon germplasm collection were used. Several plants obtained by grafting of each genotype were located at the experimental orchard at Saint Paul station (INRA Avignon). Full bloom and harvest date, in 2018 season, was registered on February 10th and July 23th for 'Zephyr' and February 23th and July 4th for C216, respectively.

Three analyses were carried out to evaluate the effect of wounding fruit brown rot evaluation, HPLC analysis and GC-MS analysis, to quantify phenolic and volatile compounds respectively.

2.2. Brown rot evaluation

The evaluation of the incidence and the kinetics of lesion and sporulation growth caused by *M. laxa* were performed for both genotypes 130 days after full bloom (DAFB). For 'Zephyr' it was on June 20th (immature fruits) and for C216 July 4th (ripe fruits).

For that, fruits were collected and taken to the laboratory of the INRA Avignon - Saint Maurice (GAFL). The procedure adopted for inoculations and evaluations was the same as described by Dini et al. (2019). Briefly, the *M. laxa* isolate was obtained from INRA collection preserved in Petri dishes with PDA and for inoculation the concentration was adjusted to 1.0×10^5 conidia mL^{-1}. The fruits were wounded with a razor blade making a longitudinal cut, with a depth not exceeding 3 mm. Inoculation was carried out with 10 µL of inoculum on the wound of each fruit. Inoculations were made either immediately after wounding or seven hours after. The boxes containing ten fruits were placed in a growth chamber at 25±1°C, relative humidity 80% and a photoperiod of 12 hours. Measurements of diameters of lesion and sporulation of *M. laxa* in each fruit were taken perpendicular to the wound, in the equatorial region, every 24 hours after inoculation (HAI) during ten days.

2.3. HPLC analysis

This analysis was carried out in parallel to the brown rot evaluation, with only one day of difference for each of the two genotypes studied. For 'Zephyr' it was on June 19th and for C216 on July 3th. For each genotype, 60 fruits were collected, which were selected for absence of apparent lesions and infections. In the laboratory, they were separated into two groups of 30 fruits and placed on metal rings inside clear glass boxes, previously disinfested with 75% alcohol. In the fruits of one of the groups were made multiple longitudinal wounds (apex to the peduncle) with razor blade. The other group of 30 fruits remained non-wounded.

Samples were collected at two different times: 1 hour and 7 hours after fruits were wounded. One sample consisted in three fruits, and five replications were collected for each time (1 and 7 hours) and each treatment (wounded, unwounded). These samples consisted of all the fruit skin removed, with a depth that did not exceed 3 mm (the same depth of the wounds made in one of the groups). After collected, skin samples were immediately frozen in liquid nitrogen, and kept at -80°C. Then they were ground to a fine powder and lyophilized for three days.

The identification of the phenolic and triterpenoid compounds was performed by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) in INRA´s laboratories (INRA Avignon, Saint Maurice, GAFL).

The procedure adopted for phenolic compounds and triterpenoid extractions was the same described by Dini et al. (2019). Briefly, they were extracted from 50 mg of the lyophilized-dry powder. The material was homogenized in an Ultra-turrax homogenizer with 8 mL of extraction solution (ethanol 95%) and placed in rotary shaker, under controlled environment at 4°C for 4 hours, followed by centrifugation. The recovered supernatant was placed in pyrex tube (12 mL) which was placed in a Speed Vac Concentrator for the solvent evaporation and the resultant residue was dissolved in 1000 µL methanol 100%, filtered (membrane PTFE 0.45 µm), and collected into an autosampler vial 1.5 mL for HPLC analysis. As a control, 25 µl to 0.4 mg L^{-1} of pure compound 6-methoxyflavone was added. Extracts were analyzed using an HPLC system (Shimadzu - Prominence) equipped with a reversed phase C18 column (Merck Superspher RP18 end capped) coupled with a photodiode array detector, operated by Shimadzu software (LC Solutions). A 10 µL aliquot of the filtered extract was injected into a HPLC and the column was maintained at 30°C. The methanol extracts were analyzed simultaneously for free forms of triterpenoids (210nm), flavan-3-ols (280 nm), hydroxycinnamic acids and derivate forms of triterpenoids (315 nm), flavonols (350 nm), and anthocyanidin (520 nm). The phenolic compounds were characterized according to their UV lambda maximum, retention times and co-chromatography with known standards when available.

2.4. GC-MS analysis

This analysis was carried out in parallel to the previous one, with only two days of difference with the brown rot evaluation and one day with the HPLC analyses, for each of the two genotypes studied. For 'Zephyr' it was on June 18th and for C216 on July 2th.

The identification of the volatile compounds was performed by Gas Chromatograph with Mass Spectrometer (GC-MS) in immature fruits of 'Zephyr' nectarine and ripe fruits of C216 nectarine, subjected to wounded or non-wounded and samples collected after seven hours only. The fruits were wounded with a razor blade as for the HPLC analysis. Immediately after the wounds were made, they were individually placed inside glass jars (500 mL) with hermetic lid and in the same way the fruits that were not wounded. After 7 hours, and individually, the glass jars lids were quickly changed by one adapted with two holes. In one of the holes, a carbon filter was placed whereas in the other, stainless steel tubes for GC-MS (TENAX TA 60/80) and a pump (Supelco, Personal Air Sampler, PAS-500). Immediately, the pump was turned on and left on for 10 minutes (0.2 L min^{-1}). Fourteen replicates were used for wounded fruits, 14 for non-wounded fruits and two jars without fruit as control. This procedure was performed for 'Zephyr' and C216 on the respective dates, using two pumps in parallel and every 12 minutes.

The tubes were desorbed 10 min at 250°C with a Turbomatrix ATD 650 (PerkinElmer) thermal desorbed and then separated on a capillary column Elite 5-MS 0.25 mm × 30 m × 0.5 µm (PerkinElmer) using a Trace-ISQ (Thermo) GC-MS. Helium was used as carrier gas (0.8 mL min^{-1}) and the oven temperature program was 40°C for 4 minutes, after 3°C per minute up to 190°C, then 10°C per minute up to 280°C, the final temperature (280°C) was maintained 15 minutes.

2.5. Statistical analysis

Analyses of variance (ANOVA) were performed by F-test considering a general linear model for volatile compounds and a mixed-model by Satterthwaite F-test for the phenolic and triterpenoid compounds. Significant differences were determined at $p < 0.01$. Statistical analyses and graphical representations were performed using R software (R Core Team, 2018). A multivariate analysis was performed for all volatile compounds detected, using a methodology of principal component analysis (PCA) and Infostat software (InfoStat, 2019).

3. Results

The cultivar Zephyr showed differences in lesion and sporulation caused by *M. laxa* when inoculated immediately after wounding or after seven hours ($p < 0.01$). All the fruits inoculated immediately after wounding presented lesions and sporulation of *M. laxa*. The first symptoms and signs were visualized after 24 HAI and 72 HAI, respectively. In the case of inoculations seven hours after wounding, only 10% of the fruits presented lesion and sporulation. The first symptoms and signs appeared after 128 HAI and 168 HAI, respectively. In the selection C216, there were no significant differences between inoculation times after wounding ($p > 0.01$) (Fig. 1), reaching 100% of the fruits with presence of lesions and sporulation of *M. laxa*.

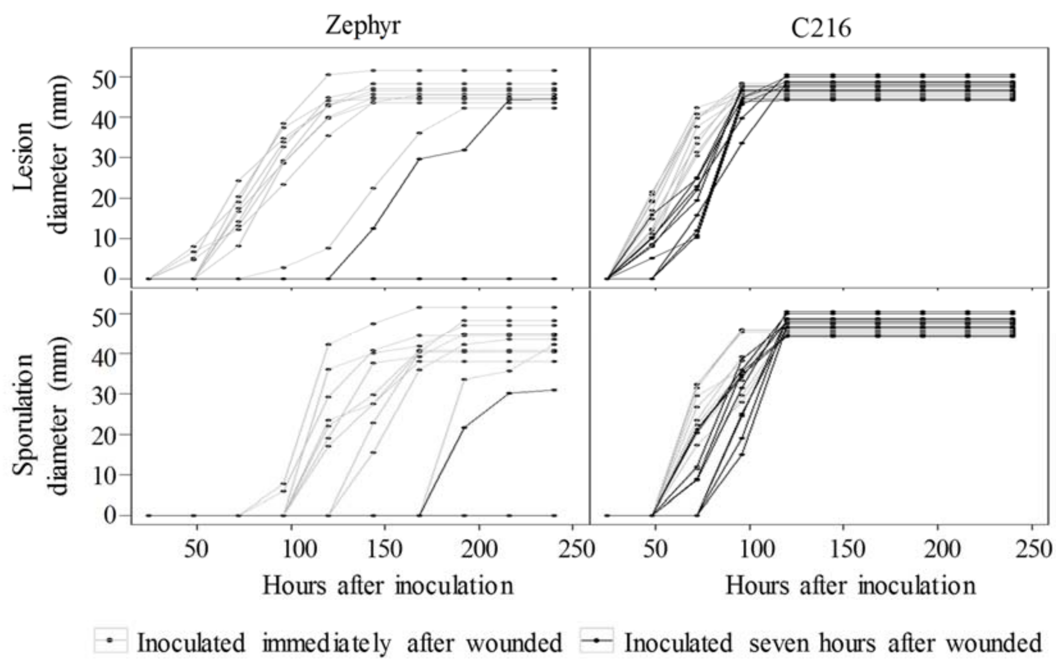


Fig. 1. Lesion and sporulation diameter in 'Zephyr' and C216 nectarine, inoculated with *M. laxa* 130 days after full bloom, immediately and seven hours after wounded. INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France.

A total of 30 phenolic and terpenoid compounds were identified in the performed HPLC analysis, being four triterpenoid (Tri), two flavan-3-ol (Fla), seven hydroxycinnamic derivatives (HCD), eleven triterpenoid derivatives (Trid), four flavonol (Flo) and two anthocyanin (Ant) (Table 1). Two compounds, both hydroxycinnamic derivatives, were present only in one of the genotypes. 5-p-Coumaroylquinic acid (pCQ) was only identified in 'Zephyr' fruits, and 3,4-Dicaffeoylquinic acid (3,4diCQ) only in C216 fruits (Fig. 2).

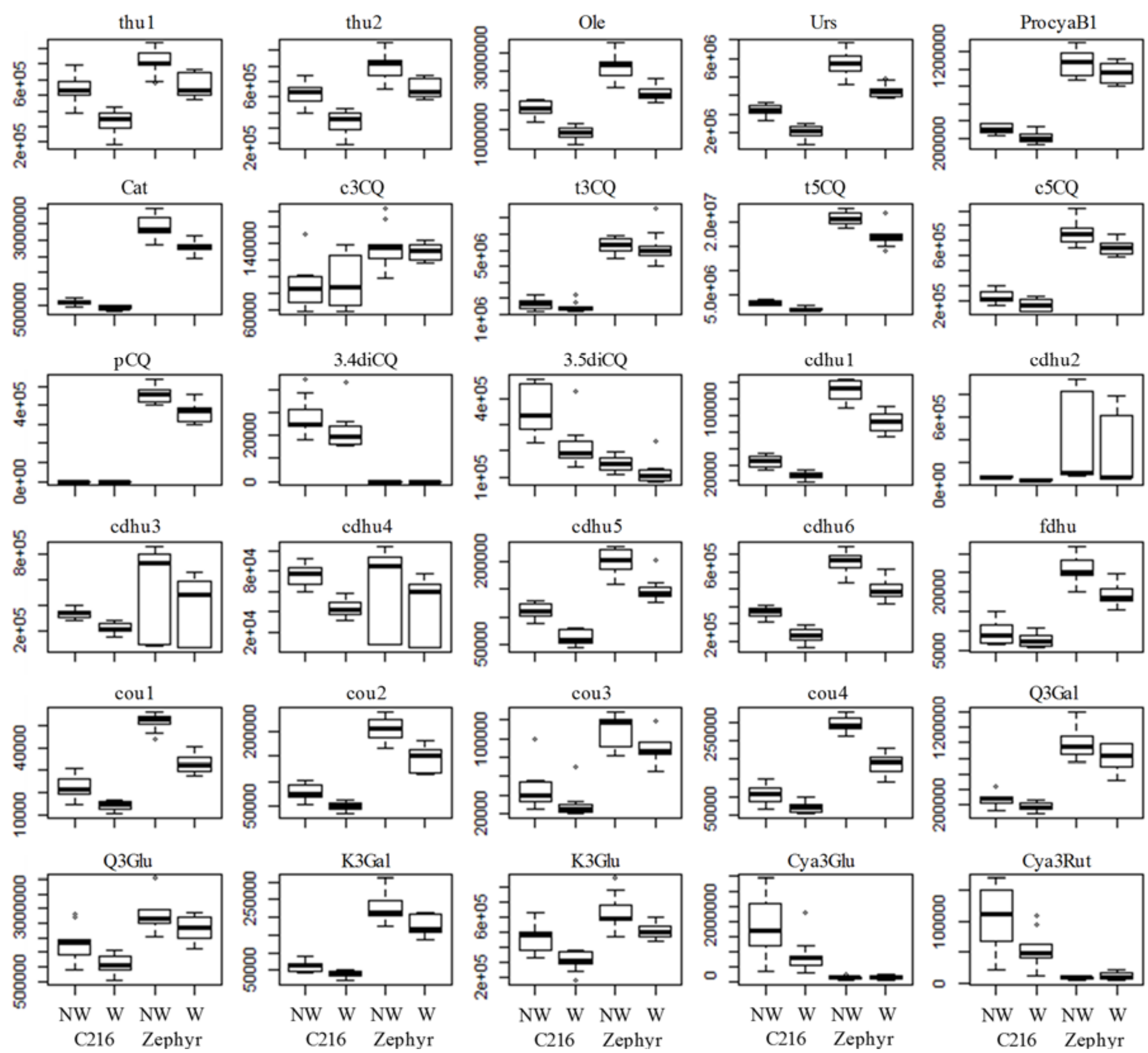


Fig. 2. Boxplot of the HPLC-DAD peaks area of compounds identified in fruits of 'Zephyr' (June 19, 2018) and C216 nectarine (July 03, 2018), subjected to wounded (W) or non-wounded (NW). Abbreviations of compounds in Table 1. INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France.

Table 1. Compounds identified by HPLC analysis, compounds family, wavelength (λ), lambda maximum (λ max), retention time (RT) and abbreviation used, 2018 season. INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France.

Compound	Family[1]	λ (nm)	RT (min)	λ max (nm)	Abbrev.
trihydroxy-urs-12-en-28-oic acid[2]	Tri	210	124.2	198	thu1
trihydroxy-urs-12-en-28-oic acid[2]	Tri	210	124.6	198	thu2
Oleanolic acid	Tri	210	131.8	198	Ole
Ursolic acid	Tri	210	132.5	198	Urs
Procyanidin B1	Fla	280	27.6	278	ProcyaB1
Catechin	Fla	280	34.0	278	Cat
6-methoxyflavone	Ctrl	280	116.7	268	6MF-Ctrl[3]
cis-Neochlorogenic acid	HCD	315	20.3	317	c3CQ
Neochlorogenic acid	HCD	315	23.3	324	t3CQ
Chlorogenic acid	HCD	315	43.2	326	t5CQ
cis-Chlorogenic acid	HCD	315	54.3	317	c5CQ
5-p-Coumaroylquinic acid	HCD	315	57.4	311	pCQ[4]
3,4-Dicaffeoylquinic acid	HCD	315	78.2	322	3.4diCQ[5]
3,5-Dicaffeoylquinic acid	HCD	315	79.0	323	3.5diCQ
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 1[2]	Trid	315	125.8	311	cdhu1
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 2[2]	Trid	315	126.2	313	cdhu2
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 3[2]	Trid	315	126.4	312	cdhu3
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 4[2]	Trid	315	126.8	288	cdhu4
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 5[2]	Trid	315	127.5	309	cdhu5
p-coumaroyl-2,3-dihydroxy-urs-12-en-28-oic acid 6[2]	Trid	315	128.4	308	cdhu6
feruloyl-2,3-dihydroxy-urs-12-en-28-oic acid[2]	Trid	315	128.9	321	fdhu
3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid 1[2]	Trid	315	135.3	289	cou1
3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid 2[2]	Trid	315	135.4	309	cou2
3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid 3[2]	Trid	315	135.8	314	cou3
3*β*-*p*-coumaroyloxy-urs-12-en-28-oic acid 4[2]	Trid	315	136.0	312	cou4
Quercetin-3-galactoside	Flo	350	81.6	354	Q3Gal
Quercetin-3-glucoside	Flo	350	83.4	354	Q3Glu
Kaempferol-3-galactoside	Flo	350	91.3	347	K3Gal
Kaempferol-3-glucoside	Flo	350	93.5	347	K3Glu
Cyanidin-3-glucoside	Ant	520	62.5	518	Cya3Glu
Cyanidin-3-rutinoside	Ant	520	64.6	529	Cya3Rut

(1) Tri = Triterpenoid; Fla = Flavan-3-ol; HC = Hydroxycinnamic derivatives; Trid = Triterpenoid derivatives; Flo = Flavonol; Ant = Anthocyanin. (2) Ursolic acid or Oleanolic acid as principal structure. (3) Control added, 25 µL to 0.4 mg L^{-1} of 6-methoxyflavone. (4) pCQ present only in 'Zephyr'. (5) 3.4diCQ present only in C216.

HPLC chromatography spectra, performed on the wounded and non-wounded samples, is available in Supplementary Fig. S1 for 'Zephyr' and Supplementary Fig. S2 for C216.

The triple interaction Genotype x Treatment x Hour (G$^{\times}$T$^{\times}$H) was only significant ($p < 0.01$) for two triterpenoid compounds (Oleanolic acid and Ursolic acid) and for three triperpenoid derivates (cdhu1, cdhu5 and cdhu6). Double interactions with the hour factor (G$^{\times}$H and T$^{\times}$H) were not significant for any compound ($p > 0.01$) and there was no simple effect of hour. This indicates that the hour factor is negligible (Supplementary Fig. S3 and Supplementary

Fig. S4). The genotype $^{\times}$ treatment interaction ($G^{\times}T$) was significant for eight compounds out of the 28 commun, indicating different effets of wounding depending on the genotype. Finally, the genotype factor proved to be significant for all but one of the identified compounds (cdhu4), and the treatment factor significant for all except six compounds (c3CQ, t3CQ, Cya3Glu, Cya3Rut, cdhu2 and cdhu3). It should be stressed that the control (Ctrl - 6MF) used did not present any significance for any of the interactions or main factors, which increases the reliability of the results (Table 2).

Table 2. ANOVA summary of the compounds identified by HPLC-DAD, using the peaks area in C216 and 'Zephyr' (Factor: Genotype) in immature fruits subjected to wounded or non-wounded (Factor: Treatment) and samples collected at 1 and 7 hours (Factor: Hour), 2018 season. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon. France.

	Triterpenoid				Flavan-3-ol		Ctrl	Hydroxycinnamic derivatives					Anthocyanin	
Compound	thu1	thu2	Ole	Urs	ProcyaB1	Cat	6MF	c3CQ	t3CQ	t5CQ	c5CQ	3.5diCQ	Cya3Glu	Cya3Rut
Genotype (G)	***	***	***	***	***	***	ns	***	***	***	***	***	***	***
Treatment (T)	***	***	***	***	**	***	ns	ns	ns	***	***	***	ns	ns
Hour (H)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$G^{\times}T$	ns	ns	ns	ns	ns	***	ns	ns	ns	***	ns	ns	ns	**
$G^{\times}H$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$T^{\times}H$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$G^{\times}T^{\times}H$	ns	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

	Triterpenoid derivatives											Flavonol			
Compound	cdhu1	cdhu2	cdhu3	cdhu4	cdhu5	cdhu6	fdhu	cou1	cou2	cou3	cou4	Q3Gal	Q3Glu	K3Gal	K3Glu
Genotype (G)	***	**	**	ns	***	***	***	***	***	***	***	***	***	***	***
Treatment (T)	***	ns	ns	**	***	***	***	***	***	**	***	***	***	***	***
Hour (H)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$G^{\times}T$	***	ns	ns	ns	ns	ns	**	***	**	ns	***	ns	ns	ns	ns
$G^{\times}H$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$T^{\times}H$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$G^{\times}T^{\times}H$	**	ns	ns	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns	ns

Significance (p-value): *** = $p < 0.001$, ** = $p < 0.01$, ^{ns} = non-significant ($p > 0.01$). Abbreviations of compounds in Table 1.

All 30 compounds identified according to the Treatment (wounded or non-wounded) and Genotype (C216 and 'Zephyr') are shown in Fig. 2 as box-plot. It is observed that, in general, within each genotype, the wounded fruits presented less proportion of the compounds than the ones without wound. Also, generally, within the wounded or non-wounded treatment, 'Zephyr' showed a higher proportion of compounds than C216.

In Supplementary Figure S5 the compounds detected according to their family (Tri, Fla, HCD, Trid, Flo and Ant) were summarized.

A total of 70 volatile compounds were detected by GC-MS analysis (Table 3), with slight differences between genotypes: C216 showed 67 of the detected volatile compounds (except 2,6-Dimethyl, trans-α-Bergamotene and cis-*β*-Farnesene) and 'Zephyr' showed 54 over the 70.

Table 3. Volatile compounds, abbreviation and retention time, identified by GC-MS in fruits of 'Zephyr' (June 18, 2018) and C216 nectarine (July 02, 2018), subjected to wounded or non-wounded and samples collected at 7 hours. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Volatile compound	Abbreviation	Family	RT	Zephyr		C216	
				W	NW	W	NW
Acetic acid, methyl ester	Aac,methyl_ester	Ester	2.89			x	
2-Butanone	2-Butanone	Ketone	3.56	x		x	
Ethyl Acetate	Ethyl_Acetate	Ester	3.80			x	
Tropilidene and/or Toluene	Tropilidene_Toluene	Hydrocarbon	8.20			x	x
Hexanal	Hexanal	Aldehyde	9.64		x	x	x
Unknown1	Unknown1		11.98		x	x	x
Unknown2	Unknown2		12.30		x	x	x
Ethylbenzene	Ethylbenzene	Hydrocarbon	12.53	x	x	x	x
1-Methoxy-2-propyl acetate	1-Met_2prop_acet	Ether	12.80			x	
p-Xylene (A)	p-Xylene_A	Hydrocarbon	13.00	x	x	x	x
3-Methyl-3-buten-1-ol, acetate	3-Met_3but1ol,acet	Ester	13.68			x	x
p-Xylene (B)	p-Xylene_B	Hydrocarbon	14.17	x	x	x	x
n-Amyl acetate	n-Amyl_acetate	Ester	15.26			x	
α-Pinene (A)	α-Pinene_A	Terpene	16.36	x	x	x	x
Benzaldehyde	Benzaldehyde	Aldehyde	17.88	x	x	x	x
5-Hepten-2-one, 6-methyl-	5-Hep_2one,6met	Ketone	19.04	x	x	x	x
β-Myrcene or *β*-Pinene	*β*-Myrcene	Terpene	19.29	x		x	x
Furan, 2-pentyl-	Furan,2pentyl	Furan	19.31	x	x		x
Heptane, 2,2,6,6-tetramethyl-4-methylene-	Heptane	Hydrocarbon	19.62	x	x	x	x
Decane	Decane	Hydrocarbon	19.86		x	x	x
Octanal	Octanal	Aldehyde	20.01		x		x
3-Hexen-1-ol, acetate, (Z)-	3-Hexen_1ol,acet	Ester	20.09			x	
3-Heptene, 2,2,4,6,6-pentamethyl-	3-Heptene	Hydrocarbon	20.17	x	x	x	x
α-Phellandrene	α-Phellandrene	Hydrocarbon	20.20	x		x	x
α-Pinene (B)	α-Pinene_B	Terpene	20.35	x	x	x	x
Acetic acid, hexyl ester	Aac,hexyl_es	Ester	20.49			x	
2-Hexen-1-ol, acetate	2-Hexen_1ol,ac	Ester	20.60	x		x	
Terpinolene (A)	Terpinolene_A	Terpene	20.77			x	x
o-cymene or p-cymene (o m p?-cymene)	omp-cymene	Terpene	21.18	x		x	x
Limonene	Limonene	Terpene	21.42	x	x	x	x
o-cymene	o-cymene	Terpene	21.72	x		x	x
β-Ocimene	*β*-Ocimene	Terpene	22.29	x		x	
γ-Caprolactone	γ-Caprolactone	Lactone	22.58			x	x
γ-Terpinene	γ-Terpinene	Terpene	22.94			x	
Acetophenone	Acetophenone	Ketone	23.33	x	x	x	x
1-Pentene, 5-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene-	1-Pentene	Hydrocarbon	23.92	x		x	
Terpinolene (B)	Terpinolene_B	Terpene	24.37			x	
p-Cymenene	p-Cymenene	Terpene	24.63			x	
2-tert-Butyltoluene	2-tert_Butyltoluene	Hydrocarbon	24.66	x	x		x
Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-(A)	Cyclohexane_A	Hydrocarbon	24.70	x		x	
Undecane	Undecane	Hydrocarbon	25.05		x		x
β-Linalool	*β*-Linalool	Alcohol	25.11			x	
Nonanal	Nonanal	Aldehyde	25.29	x	x	x	x
Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-(B)	Cyclohexane_B	Hydrocarbon	25.75	x		x	

neo-allo-Ocimene	neo_allo_Ocimene	Terpene	26.48	x		x	
2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	2,6-Dimethyl	Sulfur	26.60	x			
4E,6Z-allo-Ocimene	allo-Ocimene	Terpene	27.10			x	
Unknown3	Unknown3		28.57			x	x
Diisopropyl xanthate	Diisopropyl_xanth	Ester	29.55	x	x	x	x
Dodecane	Dodecane	Hydrocarbon	30.02	x	x	x	x
Decanal	Decanal	Aldehyde	30.33	x	x	x	x
Benzothiazole	Benzothiazole	Sulfur	31.44	x	x	x	x
cis-3-Hexenyl isovalerate	cis_3Hexenyl_isoval	Ester	31.70			x	
n-Hexyl iso-valerate	n_Hexyl_iso_val	Ester	31.99	x		x	
trans-2-Hexenyl valerate	trans_2Hexenyl_val	Ester	32.09	x		x	
Unknown4	Unknown4		33.75				x
Tridecane	Tridecane	Hydrocarbon	34.73	x	x	x	x
Undecanal	Undecanal	Aldehyde	35.11		x	x	x
Unknown5	Unknown5		36.11	x	x	x	x
Unknown6	Unknown6		36.62		x	x	x
Unknown7	Unknown7		38.67		x	x	x
cis-Jasmone	cis-Jasmone	Lactone	39.03	x		x	
Tetradecane	Tetradecane	Hydrocarbon	39.19	x	x	x	x
trans-α-Bergamotene	trans-α-Bergamo	Terpene	40.73	x	x		
trans-Geranylacetone	trans-Geranylacet	Ketone	41.24	x	x	x	x
cis-*β*-Farnesene	cis-*β*-Farnesene	Terpene	41.44	x	x		
Pentadecane	Pentadecane	Hydrocarbon	43.40	x	x	x	x
Butylated Hydroxytoluene	Butylated_Hydroxy	Phenol	43.61	x	x	x	x
Heneicosane	Heneicosane	Hydrocarbon	47.40	x	x	x	x
Di-n-octyl ether	Di_n_octyl_ether	Ether	49.79		x	x	x

x = Volatile compound detected.

The genotype × treatment interaction was significant for 20 volatile compounds (Table 4). This interaction was significant, in the majority of cases, due to the presence of compounds detected only or in greater proportion in the C216 wounded fruits (Supplementary Figure S6). The genotype principal effect was significant for 49 volatile compounds, and in general the C216 selection presented a higher proportion than 'Zephyr', except for the compounds *β*-Ocimene, trans-α-Bergamo and cis-*β*-Farnesene which were significantly higher in fruits of 'Zephyr' (Supplementary Figure S6).The treatment (wounded or non-wounded fruits) effect was significant for 33 volatile compounds, and 13 compounds of them were present only in case of wounded samples, for both genotypes. These compounds were: 2-Butanone, α-Phellandrene, 2-Hexen_1ol,ac, omp-cymene, o-cymene, *β*-Ocimene, 1-Pentene, Cyclohexane_A, *β*-Linalool, Cyclohexane_B, neo-allo-Ocimene, n-Hexyl_iso_val, trans_2Hexenyl_val and cis-Jasmone (Supplementary Figure S4).

Table 4. ANOVA summary of the volatile compounds identified by GC-MS in immature fruits of C216 and 'Zephyr' (Factor: Genotype), subjected to wounded or non-wounded (Factor: Treatment), year 2018. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Volatile compound	Genotype	Treatment	Genotype × Treatment	Volatile compound	Genotype	Treatment	Genotype × Treatment
Aac,methyl_ester	4.06^{-3} **	4.06^{-3} **	4.06^{-3} **	1-Pentene	0.528^{ns}	8.17^{-8} ***	0.528^{ns}
2-Butanone	0.189^{ns}	1.35^{-6} ***	0.189^{ns}	Terpinolene_B	6.40^{-9} ***	6.40^{-9} ***	6.40^{-9} ***
Ethyl_Acetate	0.017^{ns}	0.017^{ns}	0.017^{ns}	p-Cymenene	0.015^{ns}	0.015^{ns}	0.015^{ns}
Tropilidene_Toluene	1.93^{-7} ***	0.617^{ns}	0.617^{ns}	2-tert_Butyltoluene	0.046^{ns}	0.457^{ns}	0.121^{ns}
Hexanal	0.010^{ns}	0.558^{ns}	0.263^{ns}	Cyclohexane_A	0.984^{ns}	4.35^{-7} ***	0.984^{ns}
Unknown1	0.039^{ns}	0.053^{ns}	0.390^{ns}	Undecane	3.30^{-5} ***	1.38^{-8} ***	3.30^{-5} ***
Unknown2	2.35^{-4} ***	3.57^{-3} **	0.085^{ns}	*β*-Linalool	2.45^{-3} **	1.95^{-3} **	2.45^{-3} **
Ethylbenzene	0.020^{ns}	0.899^{ns}	0.087^{ns}	Nonanal	1.53^{-7} ***	0.837^{ns}	0.997^{ns}
1-Met_2prop_acet	5.34^{-3} **	0.333^{ns}	0.070^{ns}	Cyclohexane_B	1.06^{-3} **	6.52^{-9} ***	1.21^{-3} **
p-Xylene_A	8.37^{-12} ***	0.538^{ns}	0.800^{ns}	neo-allo-Ocimene	1.12^{-4} ***	5.78^{-10} ***	1.12^{-4} ***
3-Met_3but1ol,acet	7.22^{-3} **	7.22^{-3} **	7.22^{-3} **	2,6-Dimethyl	0.012^{ns}	0.012^{ns}	0.012^{ns}
p-Xylene_B	7.27^{-8} ***	0.211^{ns}	0.623^{ns}	allo-Ocimene	2.11^{-4} ***	2.11^{-4} ***	2.11^{-4} ***
n-Amyl_acetate	0.049^{ns}	0.049^{ns}	0.049^{ns}	Unknown3	3.40^{-5} ***	0.017^{ns}	0.017^{ns}
α-Pinene_A	0.016^{ns}	3.65^{-3} **	0.024^{ns}	Diisopropyl_xanth	1.45^{-6} ***	4.83^{-3} **	0.070^{ns}
Benzaldehyde	3.89^{-4} ***	0.513^{ns}	0.807^{ns}	Dodecane	1.52^{-7} ***	0.307^{ns}	0.928^{ns}
5-Hep_2one,6met	4.86^{-6} ***	0.491^{ns}	0.425^{ns}	Decanal	1.78^{-5} ***	0.759^{ns}	0.314^{ns}
β-Myrcene	1.83^{-7} ***	4.11^{-8} ***	4.17^{-6} ***	Benzothiazole	1.67^{-5} ***	0.018^{ns}	0.482^{ns}
Furan,2pentyl	7.96^{-4} ***	0.021^{ns}	8.00^{-3} **	cis_3Hexenyl_isoval	6.98^{-12} ***	6.98^{-12} ***	6.98^{-12} ***
Heptane	0.070^{ns}	0.064^{ns}	0.247^{ns}	n-Hexyl_iso_val	1.88^{-8} ***	5.02^{-12} ***	1.88^{-8} ***
Decane	5.80^{-5} ***	7.83^{-8} ***	7.54^{-4} ***	trans_2Hexenyl_val	0.031^{ns}	2.60^{-6} ***	0.031^{ns}
Octanal	0.039^{ns}	8.31^{-4} ***	0.039^{ns}	Unknown4	0.018^{ns}	0.018^{ns}	0.018^{ns}
3-Hexen_1ol,acet	7.06^{-6} ***	9.58^{-6} ***	9.58^{-6} ***	Tridecane	1.39^{-7} ***	0.997^{ns}	0.333^{ns}
3-Heptene	0.175^{ns}	0.661^{ns}	0.712^{ns}	Undecanal	1.63^{-4} ***	0.674^{ns}	0.657^{ns}
α-Phellandrene	0.070^{ns}	1.57^{-4} ***	0.350^{ns}	Unknown5	2.86^{-3} **	0.067^{ns}	0.194^{ns}
α-Pinene_B	2.70^{-3} **	0.015^{ns}	0.021^{ns}	Unknown6	4.20^{-4} ***	0.543^{ns}	0.111^{ns}
Aac,hexyl_es	3.70^{-6} ***	3.63^{-6} ***	4.48^{-6} ***	Unknown7	5.74^{-3} **	0.012^{ns}	0.099^{ns}
2-Hexen_1ol,ac	0.404^{ns}	3.98^{-3} **	0.404^{ns}	cis-Jasmone	0.066^{ns}	1.18^{-8} ***	0.066^{ns}
Terpinolene_A	0.027^{ns}	0.018^{ns}	0.018^{ns}	Tetradecane	7.94^{-6} ***	0.812^{ns}	0.877^{ns}
omp-cymene	1.70^{-3} **	3.80^{-4} ***	0.013^{ns}	trans-α-Bergamo	6.34^{-8} ***	7.38^{-4} ***	7.38^{-4} ***
Limonene	1.36^{-3} **	4.27^{-4} ***	0.076^{ns}	trans-Geranylacet	6.46^{-4} ***	0.818^{ns}	0.585^{ns}
o-cymene	6.03^{-8} ***	6.90^{-11} ***	1.11^{-7} ***	cis-*β*-Farnesene	1.32^{-5} ***	6.50^{-3} **	6.50^{-3} **
β-Ocimene	1.94^{-4} ***	1.27^{-7} ***	2.25^{-4} ***	Pentadecane	2.14^{-11} ***	0.683^{ns}	0.794^{ns}
γ-Caprolactone	5.04^{-5} ***	0.238^{ns}	0.238^{ns}	Butylated_Hydroxy	1.52^{-6} ***	0.021^{ns}	0.755^{ns}
γ-Terpinene	3.38^{-4} ***	3.38^{-4} ***	3.38^{-4} ***	Heneicosane	1.48^{-9} ***	0.195^{ns}	0.999^{ns}
Acetophenone	2.63^{-4} ***	0.201^{ns}	0.591^{ns}	Di_n_octyl_ether	6.54^{-6} ***	0.808^{ns}	0.671^{ns}

Significance (*p*-value): *** = $p < 0.001$, ** = $p < 0.01$, ^{ns} = non-significant ($p > 0.01$). Abbreviations in Table 3.

A principal component analysis (PCA) using 56 samples (2 genotypes × 2 treatments × 14 replications) and 70 variables (total volatile compounds detected), was performed to graphically summarize the distribution of the volatile compounds (Table 5 and Fig. 3).

Table 5. Eigenvectors and correlations of the principal components (PC) with the original variables (volatile compounds detected by GC-MS analysis). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Variables	Eigenvectors		Correlation*		Variables	Eigenvectors		Correlation*	
	e1	e2	PC 1	PC2		e1	e2	PC 1	PC2
Aac,methyl_ester	0.13	-0.12	0.81	-0.57	1-Pentene	0.01	-0.18	0.03	-0.89
2-Butanone	0.07	-0.18	0.44	-0.88	Terpinolene_B	0.13	-0.12	0.81	-0.57
Ethyl_Acetate	0.13	-0.12	0.81	-0.57	p-Cymenene	0.13	-0.12	0.81	-0.57
Tropilidene_Toluene	0.14	0.07	0.90	0.36	2-tert_Butyltoluene	-0.15	0.05	-0.94	0.25
Hexanal	0.16	-0.02	0.99	-0.08	Cyclohexane_A	0.03	-0.19	0.16	-0.91
Unknown1	0.07	0.18	0.47	0.87	Undecane	0.03	0.19	0.20	0.94
Unknown2	0.08	0.17	0.51	0.83	β-Linalool	0.13	-0.12	0.80	-0.57
Ethylbenzene	0.09	0.09	0.58	0.44	Nonanal	0.15	0.05	0.95	0.23
1-Met_2prop_acet	0.09	0.13	0.57	0.63	Cyclohexane_B	-0.06	-0.15	-0.38	-0.71
p-Xylene_A	0.15	0.06	0.94	0.31	neo-allo-Ocimene	0.11	-0.15	0.66	-0.75
3-Met_3but1ol,acet	0.13	-0.12	0.81	-0.57	2,6-Dimethyl	-0.10	-0.10	-0.62	-0.48
p-Xylene_B	0.14	0.09	0.86	0.46	allo-Ocimene	0.13	-0.12	0.81	-0.57
n-Amyl_acetate	0.13	-0.12	0.81	-0.57	Unknown3	0.09	0.15	0.55	0.74
α-Pinene_A	-0.09	-0.12	-0.56	-0.58	Diisopropyl_xanth	0.15	-0.06	0.95	-0.29
Benzaldehyde	0.15	0.08	0.92	0.39	Dodecane	0.15	0.02	0.95	0.11
5-Hep_2one,6met	0.14	0.09	0.85	0.42	Decanal	0.13	0.08	0.84	0.38
β-Myrcene	0.13	-0.12	0.81	-0.59	Benzothiazole	0.15	-0.05	0.94	-0.23
Furan,2pentyl	-0.12	-0.09	-0.75	-0.42	cis_3Hexenyl_isoval	0.13	-0.12	0.81	-0.57
Heptane	-0.10	-0.13	-0.62	-0.63	n-Hexyl_iso_val	0.12	-0.14	0.75	-0.66
Decane	0.04	0.19	0.24	0.95	trans_2Hexenyl_val	-0.05	-0.16	-0.31	-0.75
Octanal	0.03	0.20	0.17	0.96	Unknown4	0.04	0.18	0.28	0.86
3-Hexen_1ol,acet	0.13	-0.12	0.81	-0.56	Tridecane	0.15	0.04	0.98	0.20
3-Heptene	-0.14	-0.09	-0.90	-0.42	Undecanal	0.15	0.06	0.95	0.31
α-Phellandrene	0.09	-0.16	0.58	-0.77	Unknown5	0.15	-0.07	0.94	-0.34
α-Pinene_B	-0.11	-0.10	-0.70	-0.50	Unknown6	0.16	-0.01	1.00	-0.03
Aac,hexyl_es	0.13	-0.12	0.81	-0.57	Unknown7	0.07	0.18	0.42	0.87
2-Hexen_1ol,ac	0.07	-0.18	0.47	-0.86	cis-Jasmone	-0.03	-0.17	-0.17	-0.83
Terpinolene_A	0.13	-0.11	0.83	-0.53	Tetradecane	0.15	0.06	0.92	0.31
omp-cymene	0.13	-0.13	0.79	-0.61	trans-α-Bergamo	-0.13	-0.09	-0.80	-0.46
Limonene	0.13	-0.12	0.80	-0.58	trans-Geranylacet	0.14	0.07	0.87	0.36
o-cymene	0.12	-0.13	0.76	-0.65	cis-β-Farnesene	-0.12	-0.09	-0.79	-0.46
β-Ocimene	-0.08	-0.13	-0.48	-0.63	Pentadecane	0.15	0.06	0.92	0.31
γ-Caprolactone	0.16	-0.01	0.99	-0.07	Butylated_Hydroxy	0.12	0.13	0.78	0.62
γ-Terpinene	0.13	-0.12	0.81	-0.57	Heneicosane	0.14	0.09	0.90	0.41
Acetophenone	0.15	-0.02	0.97	-0.08	Di_n_octyl_ether	0.15	0.04	0.97	0.18

* Correlations with the original variables, cophenetic correlation coefficient (CCC) = 0.995.

The cumulative proportion of the total variance between the first two principal components was 90.8% (Fig. 3). The first principal component (PC1) which explained 57.1% of the variance mainly discriminated the volatile compounds associated with the genotypes . The second principal component (PC 2) accounts for 33.7% of the variance and mainly discriminated the two treatments.

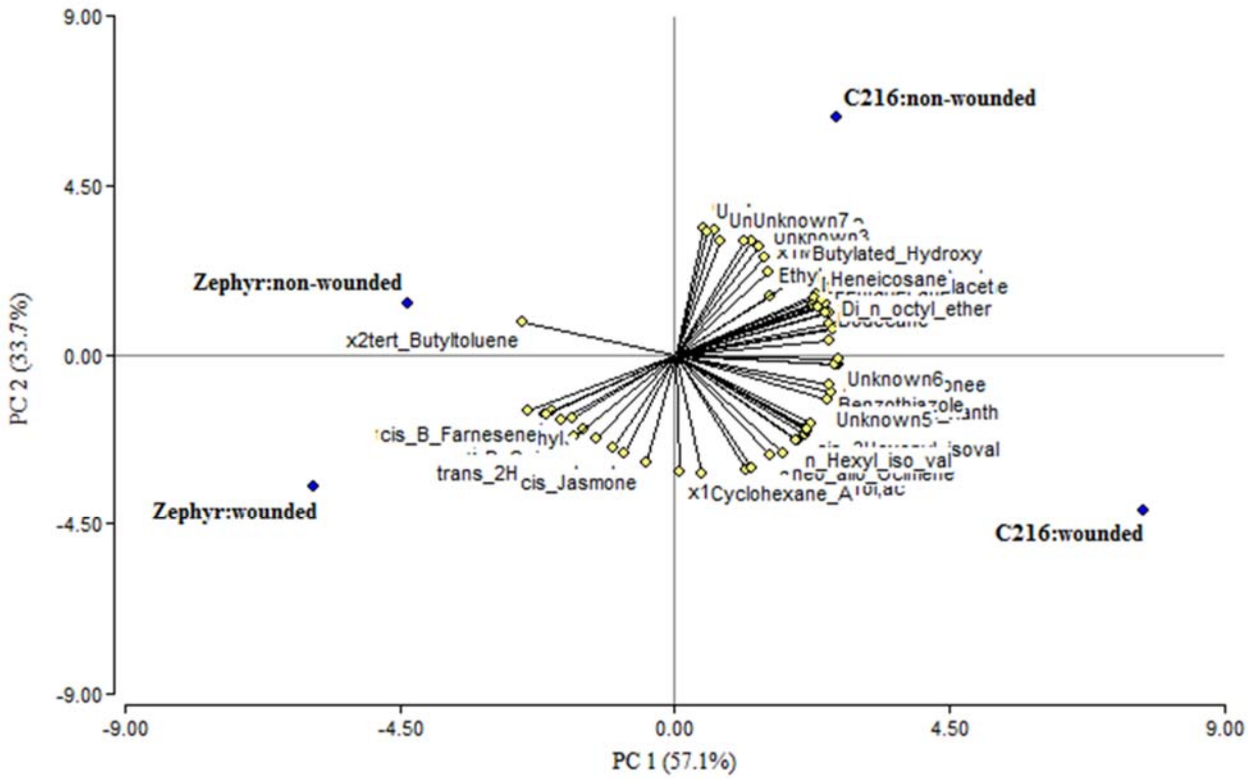


Fig. 3. Results from PCA analysis, projection of volatile compounds detected by GC-MS in fruits of 'Zephyr' (June 18, 2018) and C216 nectarine (July 02, 2018), subjected to wounded or non-wounded. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

4. Discussion

The differences found in terms of infection development between 'Zephyr' fruits inoculated immediately after the injury and seven hours later (Fig. 1), may be due to the start of process of healing of the wound, making more difficult the entrance of the *M. laxa* conidia. Wounds in plant organs, such as fruits, can heal in a period of minutes to a few hours, which will depend on the degree of the lesion, the fruit physiological stage and the environmental conditions (Dean and Kolattukudy, 1976; Spotts et al., 1998). It should be noted that although the inoculations were carried out at 130 DAFB both genotypes, have different cycle length. Thus, fruits of 'Zephyr' were 3 or 4 weeks ahead of the harvest, while C216 fruits were close to commercial ripening. Susceptibility to *Monilinia* spp. varies depending on the fruit development stage, being high in the early stages, lowering during the green fruit stages (around pit hardening) and again increasing as the fruit matures, being maximum in the pre and postharvest stages (Biggs and Northover, 1988; Gradziel, 1994; Emery et al., 2000; Mari et al., 2003; Adaskaveg et al., 2008). This may explain why there were no significant differences of infection development between the treatments for C216 fruits (Fig. 1).

The 30 compounds detected in the HPLC analyses (Table 1) were not affected by the time the samples were taken (1 or 7 hours) (Table 2). However, genotype and treatment (wounded or non-wounded fruits) factors affected most of the compounds. In general, triterpenoids, flavan-3-ols, hydroxycinnamic derivatives, triterpenoid derivatives and flavonols were higher in 'Zephyr' fruits, and within each genotype, they were higher in fruit without wound (Fig. 2). This suggests that green fruits have more phenolic and triterpenoid compounds than mature fruits. These results are supported by studies of Lee et al. (1990), Senter and Callahan (1990) and Villarino et al. (2011), that indicate a higher proportion of some of these compounds in immature peach fruits when compared to mature fruits. In addition, there are studies that report changes in phenolic and triperepoid compounds pattern and/or their degradation, after suffering some type of damage or stress, and these modifications may be associated with the activation of plant defenses against pathogens (Chistopher et al., 2004, Smith et al., 2004, Janisiewicz et al., 2016 Han et al., 2017).

The high levels of phenolic compounds in peach fruits such as the Brazilian cultivar Bolinha were associated with lower susceptibility to brown rot. Chlorogenic acid (t3CQ) and caffeic acid, two hydroxycinnamic derivatives, were reported as the main responsible for the high resistance of Bolinha fruits to *M. fructicola* (Gradziel et al., 1998). Working with *M. fructicola in vitro*, Bostock et al. (1999) showed that t3CQ suppressed cutinase production. The authors suggested that high levels of this compound in the peach epidermis could prevent fungus infection. In a later study, Lee and Bostock (2007) confirmed the earlier observation about cutinase and showed that t3CQ also suppressed polygalacturonase production and appressoria formation. Studying the relationship of compounds present in the skin and flesh of different peach genotypes with the susceptibility to *M. laxa* infection, Villarino et al. (2011) reported that high concentrations of t3CQ and neo-chlorogenic (t5CQ) in immature fruits inhibited fungal infection by interfering with melanin synthesis by the pathogen but not by interfering with fungal growth. These results suggest that t3CQ and t5CQ work by increasing resistance, interfering with the fungal processes required for the infection and not as an antimicrobial compound. In the present work, the majority of phenolic compounds were found to be higher in 'Zephyr', which showed more resistance to *M. laxa* infection when inoculated after seven hours of injury (Fig. 1). Among these compounds there were t3CQ and t5CQ, the first being in a higher proportion in the 'Zephyr' fruits and not affected by the wound, however the t5CQ was affected by the wound, being found to a lesser extent in the injured fruits, in both genotypes (Table 2 and Fig. 2).

Making weekly inoculations with *M. laxa* on non-wounded peach fruits Guidarelli et al. (2014), observed different susceptibilities to brown rot depending on the fruit development stage. In the same study, they performed a transcriptomic analysis to compare the gene expressions between susceptible (ripening stage) and resistant fruits (green fruits, on pit hardening stage), indicating that the genes related to flavonoids and phenylpropanoids are differentially expressed between the two stages, supporting the role of these metabolites in the response of the fruit to *M. laxa*.

It should be noted that in the present study, several compounds of two subgroups of flavonoids (flavan.3.ols and flavonols) and one subgroup of phenylpropanoids (hydroxycinnamic derivates) were detected in greater proportion in 'Zephyr' fruits when compared to C216 fruits (Table 2 and Supplementary Figure S3).

The results suggest that the phenolic and triterpenoid compounds, present in the fruits, may be increasing brown rot resistance. But they might act as a type of passive chemical resistance, since they do not increase after injury; They are even in lower proportion in the wounded fruit samples, perhaps because they are more exposed to oxidation and degradation (Table 2 and Fig. 2).

The anthocyanins were in a higher proportion in C216 fruits (Fig. 2), without significant differences between wounded or non-wounded fruits (Table 2). This higher proportion must be associated to the differences between the maturation stage of the fruits, when the experiment was performed (being more advanced in C216). A number of studies have indicated the highest proportion of anthocyanins in mature fruits and redder skin when compared to green fruits (Chaparro et al., 1995, Vizzotto et al., 2006, Reig et al., 2013, Rahim et al., 2014).

Most of the 70 volatile compounds detected in the fruits were identified (Table 3), belonging to the terpenes, esters, aldehydes, ketones, lactones, among others. Only seven compounds were not associated with any known volatile compounds (Unknown1, 2, 3, 4, 5, 6 and 7). Several of the compounds detected had already been reported in previous studies such as

Aubert and Milhet (2007) and Eduardo et al. (2010), in peach fruits. The majority of the volatile compounds detected were affected by genotype and/or treatment (wounded or non-wounded fruits) (Table 4). The compounds 2-Butanone, α-Phellandrene, 2-Hexen_1ol,ac, omp-cymene, *β*-Ocimene, 1-Pentene, Cyclohexane_A, Cyclohexane_B, neo-allo-Ocimene, n-Hexyl_iso_val, trans_2Hexenyl_val and cis-Jasmone were higher when fruits were wounded. The compounds Aac,methyl_ester, Ethyl_Acetate, 3-Met_3but1ol,acet, n-Amyl_acetate, *β*-Myrcene 3-Hexen_1ol,acet, Aac,hexyl_es, o-cymene, γ-Terpinene, Terpinolene_B, p-Cymenene, *β*-Linalool, allo-Ocimene and cis_3Hexenyl_isoval were only present in C216 wounded fruits and 2,6-Dimethyl in 'Zephyr' wounded fruits (Supplementary Figure S4).

The PCA confirmed the association of the compounds with the wounded fruits as detected by ANOVA (Table 5). Indeed, the compounds 2-Butanone, α-Phellandrene, 2-Hexen_1ol,ac, o-cymene, *β*-Ocimene, 1-Pentene, Cyclohexane_A, Cyclohexane_B, neo-allo-Ocimene, n-Hexyl_iso_val, trans_2Hexenyl_val and cis-Jasmone were negatively highly correlated (-0.65 to -0.91) with PC 2 (Fig. 3). On the contrary, high contents of Decane, Octanal, Undecane and five unknown compounds (Unknown1, 2, 3, 4, and 7) were associated with non-wounded fruit. They were highly correlated (0.74 to 0.96) with PC2 (Table 5 and Fig. 3).

Several of these compounds detected in a larger proportion in wounded fruits belong to the aldehydes, ketones, and terpenes groups (volatile organic compounds) (Table 5 and Supplementary Figure S4). Several of these were already associated to responses to wounds and/or antifungal activity and also with the increase in resistance to different pathogens, of different plant species, such as *Alternaria alternata* (Andersen et al., 1994), *M. laxa* (Caccioni et al., 1995; Neri et al., 2007), *Rhizopus stolonifer* (Caccioni et al., 1995), *Botrytis cinerea* (Kishimoto et al., 2005), *Alternaria dauci* (Koutouan et al., 2018) and *Xanthomonas oryzae* pv. *oryzae* (Taniguchi et al., 2014). Among the most studied compounds and recognized as an inducer of resistance in several species, mainly pests, is cis-Jasmone (Birkett et al., 2000; Bruce et al., 2003a, 2003b, 2008; Matthes et al., 2010; Hegde et al., 2012).

On the other hand, the compounds Furan,2pentyl, 3-Heptene, α-Pinene_B, 2-tert_Butyltoluene, trans-α-Bergamo and cis-*β*-Farnesene were highly negatively correlated (-0.70 to -0.94) with PC1, being mainly associated with Zephyr fruits. However, several volatiles were positively correlated with PC1, associated with C216 fruits, being the most correlated (> 0.90) were Hexanal, p-Xylene_A, Benzaldehyde, Nonanal, Diisopropyl_xanth, Dodecane, Benzothiazole, Tridecane, Undecanal, Unknown5, Unknown6 and Tetradecane (Table 5 and Fig. 3).

Testing volatile plant compounds for their activity against *M. laxa*, Neri et al. (2007) reported that trans-2-hexenal, carvacrol and citral showed a consistent fungicidal activity on conidial germination and *in vitro* mycelial growth of the fungus. However, in the present study these compounds were not detected in the peach (Table 4). In an another study, several volatile compounds characteristic of stone fruits aroma were tested on wounded peaches inoculated with *M. laxa* and *Rhizopus stolonifer* (Caccioni et al., 1995). The authors found best fungistatic effects with benzaldehyde and hexanal. In the present study, these two compounds were not associated with the wounded fruits (Table 4 and Supplementary Figure S4), but they were highly correlated with PC1 (0.92 and 0.99) associated with the C216 genotype (Table 5 and Fig. 3). Since this cultivar was more associated with high susceptibility to *M. laxa*, these results disagree with the work of Caccioni et al. (1995).

The experimentation performed does not allow to establish associations between the compounds, either detected in the HPLC-DAD and GC-MS analyses, and the susceptibility to *M. laxa*. However, it brings about new perspectives to explore. Indeed, this study revealed observations on the metabolic changes induced by healind of wounds in parallel to the peach response to fungal infection. A continuity of this work is necessary in order to better understand the mechanisms of defense, promoted by wound healing, that could play a role in fruit defense against brown rot. In this context, an analysis of genetic expression could help to decipher the pathways solicited.

Acknowledgements

To the Coordination of Improvement of Higher Education Personnel (CAPES-Brazil) for providing PhD scholarship founds, and GAFL - INRA Avignon staff, especially Marie-Noëlle Corre, Guy Costagliola, Laure Heurtevin and Véronique Signoret.

References

Adaskaveg, J.E., Schnabel, G., Forster, H., 2008. Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In: Layne D.R., Bassi, D. (Eds.), The Peach: Botany, Production and Uses. CAB International, Wallingford, UK, pp. 352-406.

Andersen, R.A., Hamilton-Kemp, T.R., Hildebrand, D. F, McCracken, C.T., Collins, R.W., Fleming, P.D., 1994. Structure-antifungal activity relationships among volatile C6 and C9 aliphatic aldehydes, ketones, and alcohols. J. Agric. Food Chem. 42, 7, 1563-1568. https://dx.doi.org/10.1021/jf00043a033.

Aubert, C., Milhet, C., 2007. Distribution of the volatile compounds in the different parts of a white-fleshed peach (*Prunus persica* L. Batsch). Food Chem. 102, 1, 375-384. https://dx.doi.org/10.1016/j.foodchem.2006.05.030.

Biggs, A.R., Northover, J., 1988. Early and late-season susceptibility of peach fruits to *Monilinia fructicola*. Plant Disease 72, 1070-1074. https://dx.doi.org/10.1094/pd-72-1070.

Birkett, M.A., Campbell, C.A.M., Chamberlain, K., Guerrieri, E., Hick, A.J., Martin, J.L., Matthes, M., Napier, J.A., Pettersson, J., Pickett, J.A., Poppy, G.M., Pow, E.M., Pye, B.J., Smart, L.E., Wadhams, G.H., Wadhams, L.J., Woodcock, C.M., 2000. New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc. Nat. Acad. Sci. 97, 9329-9334. https://dx.doi.org/10.1073/pnas.160241697.

Bostock, R.M., Wilcox, S.M., Wang, G., Adaskaveg, J.E., 1999. Suppression of *Monilinia fructicola* cutinase production by peach fruit surface phenolic acids. Physiol. Mol. Plant Pathol. 54, 37-50. http://dx.doi.org/10.1006/pmpp.1998.0189.

Bruce, T.J.A., Martin, J.L., Pickett, J.A., Pye, B.J., Smart, L.E., Wadhams, L.J., 2003a. *cis*-jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). Pest Man. Sci. 59, 1031-1036.

Bruce, T., Pickett, J., Smart, L., 2003b. *cis*-jasmone switches on plant defence against insects. Pestic. Outlook 14, 3, 96-98. https://dx.doi.org/10.1039/b305499n

Bruce, T.J.A., Matthes, M.C., Chamberlain, K., Woodcock, C.M., Mohib, A., Webster, B., Smart, L.E., Birkett, M.A., Pickett, J.A., Napier, J.A., 2008. *cis*-Jasmone induces Arabidopsis genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. Proc. Nat. Acad. Sci. 105, 4553–4558.

Caccioni, D.R.L., Guizzardi, M., 1994. Inhibition of germination and growth of fruit and vegetable postharvest pathogenic fungi by essential oil components. J. Essent. Oil Res. 6, 174-179. https://dx.doi.org/10.1080/10412905.1994.9698349.

Caccioni, D.R.L., Tonini, G., Guizzardi, M., 1995. Antifungal activity of stone fruit aroma compounds against *Monilinia laxa* (Aderh. Et Ruhl.) Honey and *Rhizopus stolonifer* (Ehrenb.): *in vivo* trials. J. Plant Dis. Prot. 102, 518-525.

Chaparro, J.X., Werner, D.J., Whetten, R.W. O`Malley, D.M., 1995. Inheritance, genetic interaction, and biochemical characterization of anthocyanin phenotypes in peach. J. Hered. 86, 1, 32-38. https://dx.doi.org/10.1093/oxfordjournals.jhered.a111522.

Chen, S., Yuan, N., Schnabel, G., Luo, C., 2017. Function of the genetic element 'Mona' associated with fungicide resistance in *Monilinia fructicola*. Mol. Plant. Pathol. 18, 1, 90-97. http://dx.doi.org/10.1111/mpp.12387.

Christopher, M.E., Miranda, M., Major, I.T., Constabel, C.P., 2004. Gene expression profiling of systemically wound-induced defenses in hybrid poplar. Planta 219, 936-947. http://dx.doi.org/10.1007/s00425-004-1297-3.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564-582. http://dx.doi.org/10.1128/cmr.12.4.564.

Dean, B.B., Kolattukudy, P.E., 1976. Synthesis of suberin during wound-healing in jade leaves, tomato fruit, and bean pods. Plant Physiol. 58, 3, 411-416. http://dx.doi.org/10.1104/pp.58.3.411.

Dini, M., Quilot-Turion, B., Raseira, M.C.B., 2019. Fruit reaction to brown rot at different development stages and inoculation time after wounded. Hort. Sci. (Amsterdam) (under review).

Eduardo, I., Chietera, G., Bassi, D., Rossini, L., Vecchitti, A., 2009. Identification of key odor volatile compounds in the essential oil of nine peach accessions. J. Sci. Food Agric. 90, 7, 1146-1154. https://dx.doi.org/10.1002/jsfa.3932.

Emery, K.M., Michailides, T.J., Scherm, H., 2000. Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. Plant Dis. 84, 8, 853-857. https://dx.doi.org/10.1094/pdis.2000.84.8.853.

Farmer, E.E., 2001. Surface-to-air signals. Nature 411, 854-856. https://dx.doi.org/10.1038/35081189.

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

Gell, I., De Cal, A., Torres, R., Usall, J., Melgarejo, P., 2008. Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. Eur. J. Plant Pathol. 121, 487-498. http://dx.doi.org/10.1007/s10658-008-9268-3.

Gradziel, T.M., 1994. Changes in susceptibility to brown rot with ripening in three clingstone peach genotypes. J. Am. Soc. Hortic. Sci. 119, 101-105. https://dx.doi.org/10.21273/jashs.119.1.101.

Gradziel, T.M., Bostock, R.M., Adaskaveg, J.E., 2003. Resistance to brown rot disease in peach is determined by multiple structural and biochemical components. Acta Hort. 622, 347-352. http://dx.doi.org/10.17660/ActaHortic.2003.622.34.

Gradziel, T.M., Thorpe, M.A., Bostock, R.M., Wilcox, S., 1998. Breeding for brown rot (*Monilinia fructicola*) resistance in clingstone peach with emphasis on the role of fruit phenolics. Acta Hortic. 465, 161-170. https://dx.doi.org/10.17660/actahortic.1998.465.18.

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., Baraldi, E., 2014. Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. Eur. J. Plant. Pathol. 140, 3, 503-513. http://dx.doi.org/10.1007/s10658-014-0484-8.

Han, C., Jin, P., Li, M., Wang, L., Zheng, Y., 2017. Physiological and transcriptomic analysis validates previous findings of changes in primary metabolism for the production of phenolic antioxidants in wounded carrots. J. Agric. Food Chem. 65, 7159-7167. http://dx.doi.org/10.1021/acs.jafc.7b01137.

Hegde, M., Oliveira, J.N., Costa, J.G., Loza-Reyes, E., Bleicher, E., Santana, A.G.N., Caulfield, J.C., Mayon, P., Dewhirst, S.Y., Bruce, T.J.A., Pickett, J.A., Birkett, M.A., 2012. Aphid antixenosis in cotton is activated by the natural plant defence elicitor *cis*-jasmone. Phytochemistry 78, 81-88. http://dx.doi.org/10.1016/j.phytochem.2012.03.004.

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 Manag. Sci. 67, 385-396. http://dx.doi.org/10.1002/ps.2074.

InfoStat, 2017. InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Córdoba, Argentina. (URL). http://www.infostat.com.ar/.

Isaman, M., 2000. Plant essential oils for pest and disease management. Crop Prot. 19, 603-608. https://dx.doi.org/10.1016/s0261-2194(00)00079-x.

Janisiewicz, W.J., Nichols, B., Bauchan, G., Chao, T.C., Jurick II, W.M., 2016. Wound responses of wild apples suggest multiple resistance mechanism against blue mold decay. Postharvest Biol. Technol. 117, 132-140. http://dx.doi.org/10.1016/j.postharvbio.2015.12.004.

Kishimoto, K., Matsui, K., Ozawa, R., Takabayashi, J., 2006. Components of C6-aldehyde-induced resistance in *Arabidopsis thaliana* against necrotrophic fungal pathogen, *Botrytis cinerea*. Plant Sci. 170, 4, 715-723. https://dx.doi.org/10.1016/j.plantsci.2005.11.002.

Koutouan, C., Le Clerc, V., Baltenweck, R., Claudel, P., Halter, D., Hugueney, P., Hamama, L., Suel, A., Huet, S., Merlet, M.H.B, Briard, M., 2018. Link between carrot leaf secondary metabolites and resistance to *Alternaria dauci*. Sci. Rep. 8, 13746. https://dx.doi.org/10.1038/s41598-018-31700-2.

Lee, M., Bostock, M., 2007. Fruit exocarp phenols in relation to quiescence and development of *Monilinia fructicola* infections in *Prunus* spp.: A role for cellular redox?. Phytopathology 97, 269-277. http://dx.doi.org/10.1094/phyto-97-3-0269.

Lee, C.Y., Kagan, V., Jaworski, A.W., Brown, S.K., 1990. Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. J. Agric. Food Chem. 38, 99-101. http://dx.doi.org/10.1021/jf00091a019.

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 Qol fungicides. Pest Manag. Sci. 66, 1308-1315. http://dx.doi.org/10.1002/ps.2016.

Mari, M., Casalini, L., Baraldi, E., Bertolini, P., Pratella, G.C., 2003. Susceptibility of apricot and peach fruit to *Monilinia laxa* during phenological stages. Postharvest Biol. Technol. 30, 1, 105-109. http://dx.doi.org/10.1016/S0925-5214(03)00138-8.

Matthes, M.C., Bruce, T.J.A., Ton, J., Verrier, P.J., Pickett, J.A., Napier, J.A., 2010. The transcriptome of *cis*-jasmone-induced resistance in *Arabidopsis thaliana* and its role in indirect defence. Planta 232, 1163-1180. http://dx.doi.org/10.1007/s00425-010-1244-4.

Neri, F., Mari, M., Brigati, S., Bertolini, P. Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. Plant Disease 91, 1, 30-35. https://dx.doi.org/10.1094/pd-91-0030.

Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., Uyemoto, J.K., 1995. Compendium of Stone Fruit Diseases, first ed. The American Phytopathological Society, Saint Paul, MN.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R Core Team, Vienna, Austria (URL). http://www.R-project.org/.

Rahim, M.A., Busatto, N., Trainotti, L., 2014. Regulation of anthocyanin biosynthesis in peach fruits. Planta 240, 5, 913-929. https://dx.doi.org/10.1007/s00425-014-2078-2.

Reig, G., Iglesias, I., Gatius, F., Alegre, S., 2013. Antioxidant capacity, quality, anthochyanins and nutrients content of several peach cultivars [*Prunus persica* (L.) Batsch] grown in Spain. J. Agric. Food Chem. 61, 26, 6344-6357. https://dx.doi.org/10.1021/jf401183d.

Roemmelt, S., Fischer, T.C., Halbwirth, H., Peterek, S., Schlangen, K., Speakman, J.B., Treutter, D., Forkmann, G., Stich, K., 2003. Effect of dioxygenase inhibitors on the resistance-related flavonoid metabolism of apple and pears: chemical, biochemical and molecular biological aspects. Europ. J. Hort. Sci. 68, 3, 129-136.

Senter, S.D., Callahan, A., 1990. Variability in the quantities of condensed tannins and other major phenols in peach fruit during maturation. J. Food Sci. 55, 1585-1587. http://dx.doi.org/10.1111/j.1365-2621.1990.tb03575.x.

Smith, C.M., Rodriguez-Buey, M., Karlsson, J., Campbell, M.M., 2004. The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. New Phytol. 164, 123-136. http://dx.doi.org/10.1111/j.1469-8137.2004.01151.x.

Spotts, R.A., Sanderson, P.G., Lennox, C.L., Sugar, D., Cervantes, L.A., 1998. Wounding, wound healing and staining of mature pear fruit. Postharvest Biol. Tec. 13, 27-36. http://dx.doi.org/10.1080/00221589.1981.11514980.

Taniguchi, S., Hosokawa-Shinonaga, Y., Tamaoki, D., Yamada, S., Akimitsu, K., Gomi, K., 2014. Jasmonate induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. Plant Cell Environ. 37, 2, 451-461. https://dx.doi.org/10.1111/pce.12169.

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. J. Phytopathol. 157, 194-200. http://dx.doi.org/10.1111/j.1439-0434.2008.01469.x.

Tomas-Barberán, F.A., Gil, M. I., Cremin, P., Waterhouse, A.L., Hess-Pierce, B., Kader, A.A., 2001. HPLC-DAD-ESIMS analysis of phenolic ocompounds in nectarines, peaches, and plums. J. Agric. Food Chem. 49, 4748-4760. http://dx.doi.org/10.1021/jf0104681.

Tripathi, P., Dubey, N.K., 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol. Technol. 32, 235-245. https://dx.doi.org/10.1016/j.postharvbio.2003.11.005.

Tsao, R., Zhou, T., 2000. Antifungal activity of monoterpenoids against postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. J. Essent. Oil Res. 12, 113-121. https://dx.doi.org/10.1080/10412905.2000.9712057.

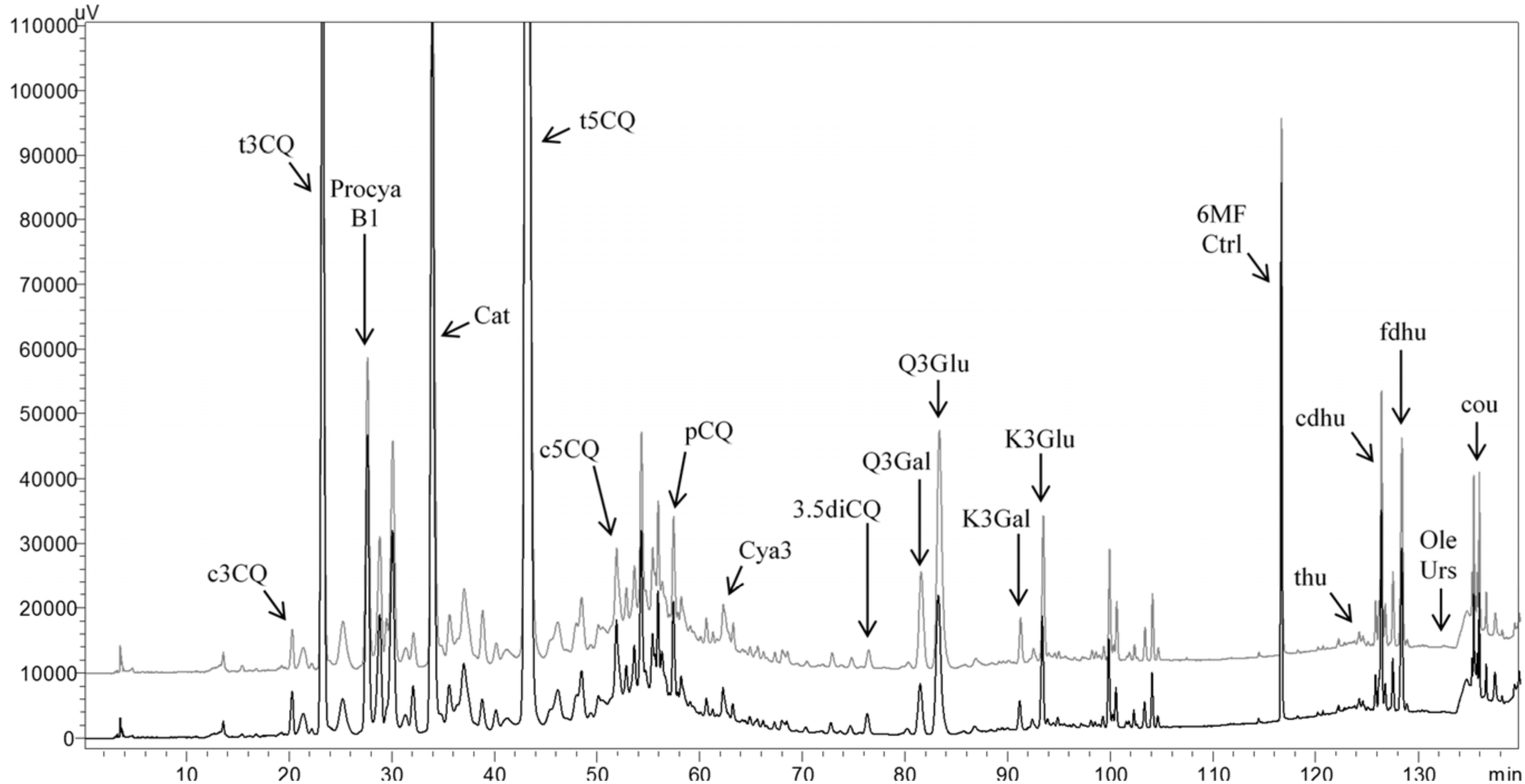
Tsuda, T., Yamaguchi, M., Honda, C., Moriguchi, T., 2004. Expression of anthocyanin biosynthsis genes in the skin of peach and nectarine fruit. J. Amer. Soc. Hort. Sci. 129, 6, 857-862. https://dx.doi.org/10.21273/jashs.129.6.0857.

Villarino, M., Sandín-España, P., Melgarejo, P., De Cal, A., 2011. High chlorogenic and neochlorogenic acid levels in immature peaches reduce *Monilinia laxa* infection by interfering with fungal melanin biosynthesis. J. Agric. Food Chem. 59, 3205-3213. http://dx.doi.org/10.1021/jf104251z.

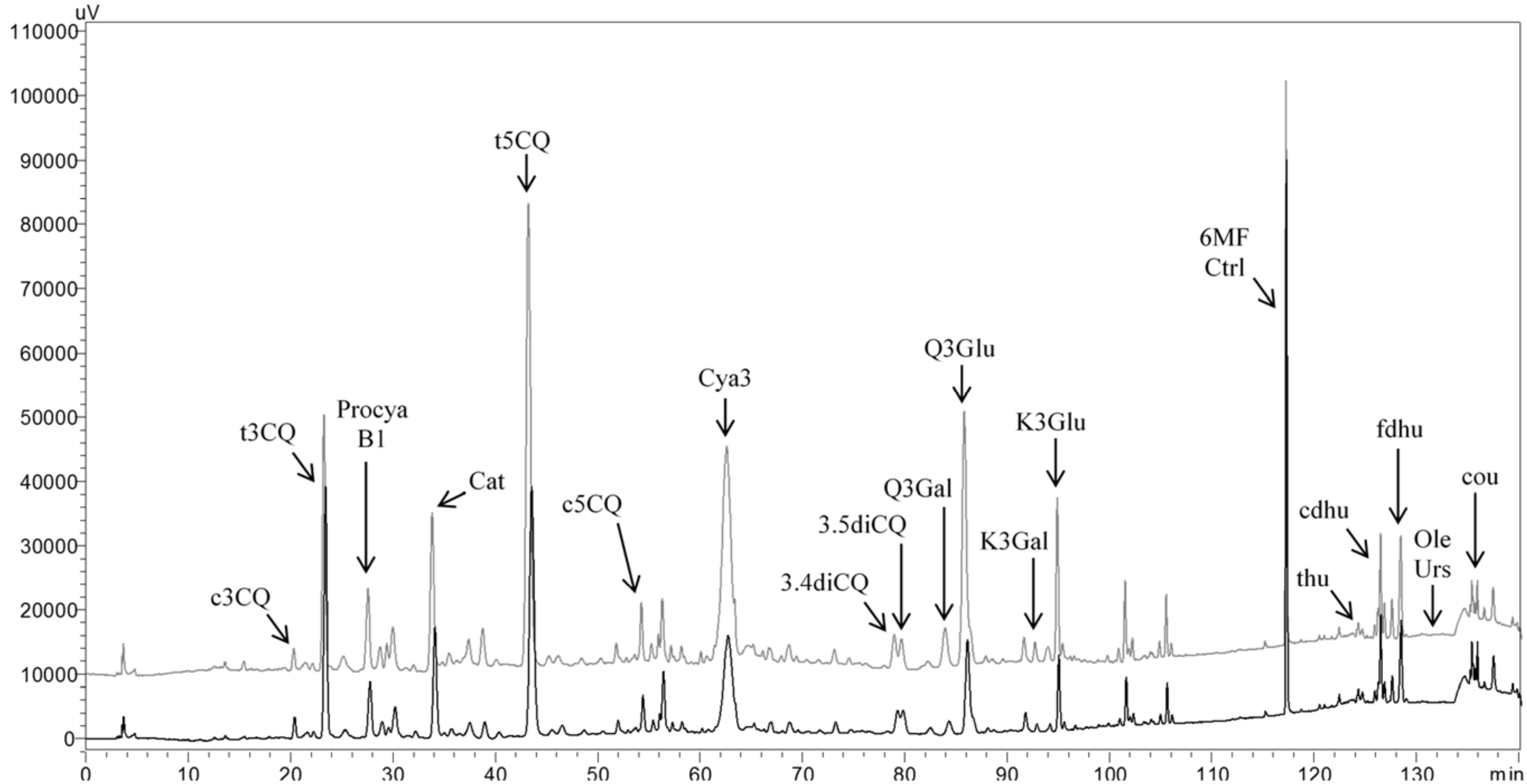
Vizzotto, M., Cisneros-Zevallos, L., Byrne, D.H., Ramming, D.W., Okie, W.R., 2006. Total phenolic, carotenoid, and anthocyanin content and antioxidant activity of peach and plum genotypes. Acta Hortic. 713, 453-356. https://dx.doi.org/10.17660/actahortic.2006.713.67.

Wilson, C.L., Franklin, J.D., Otto, B.E., 1987. Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. Plant Dis. 71, 4, 316-319. https://dx.doi.org/10.1094/pd-71-0316.

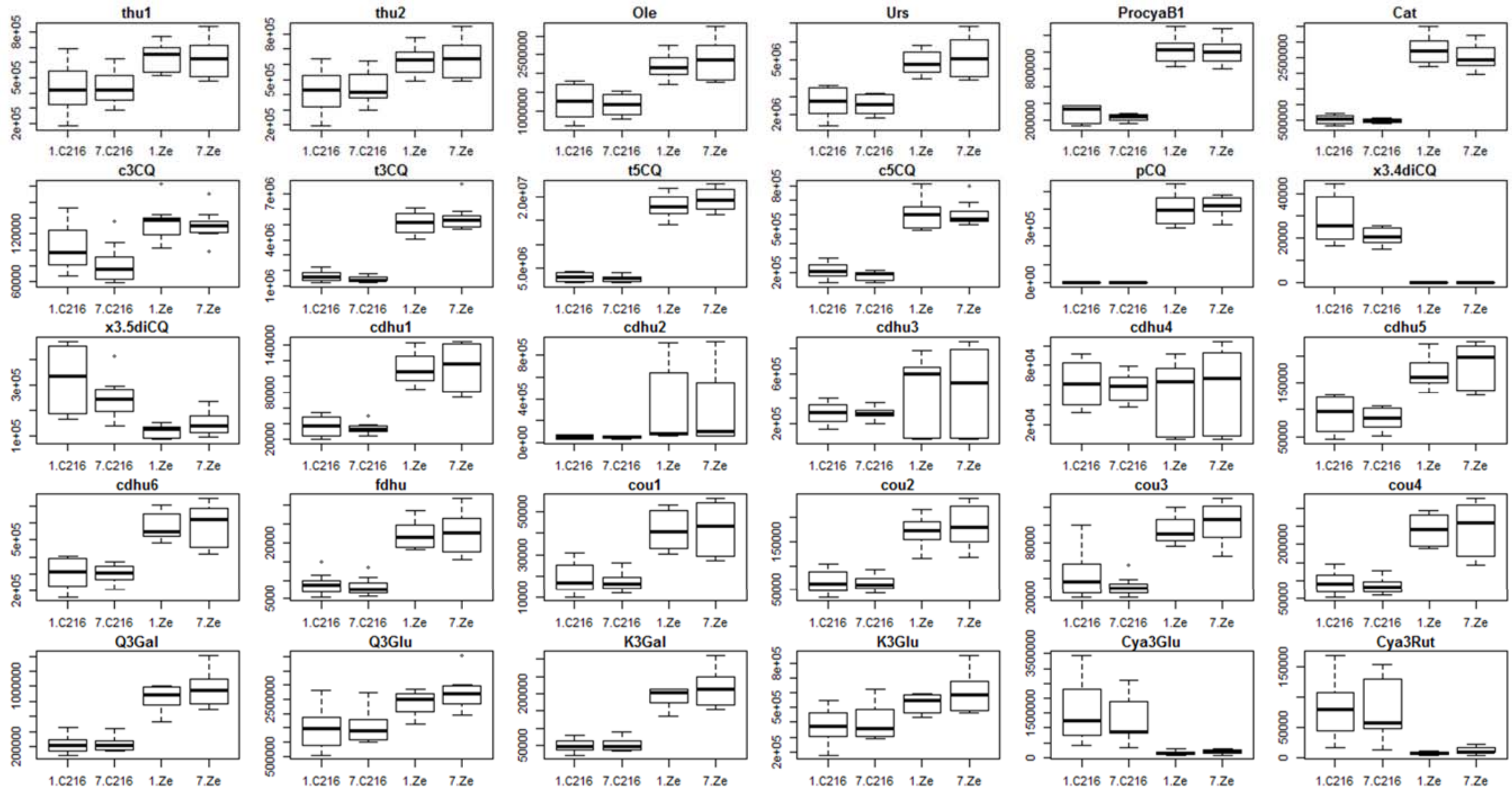
Zhu, F., Bryson, P.K., Schnabel, G., 2012. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. Pest Manag. Sci. 68, 7, 1003-1009. http://dx.doi.org/10.1002/ps.3255.



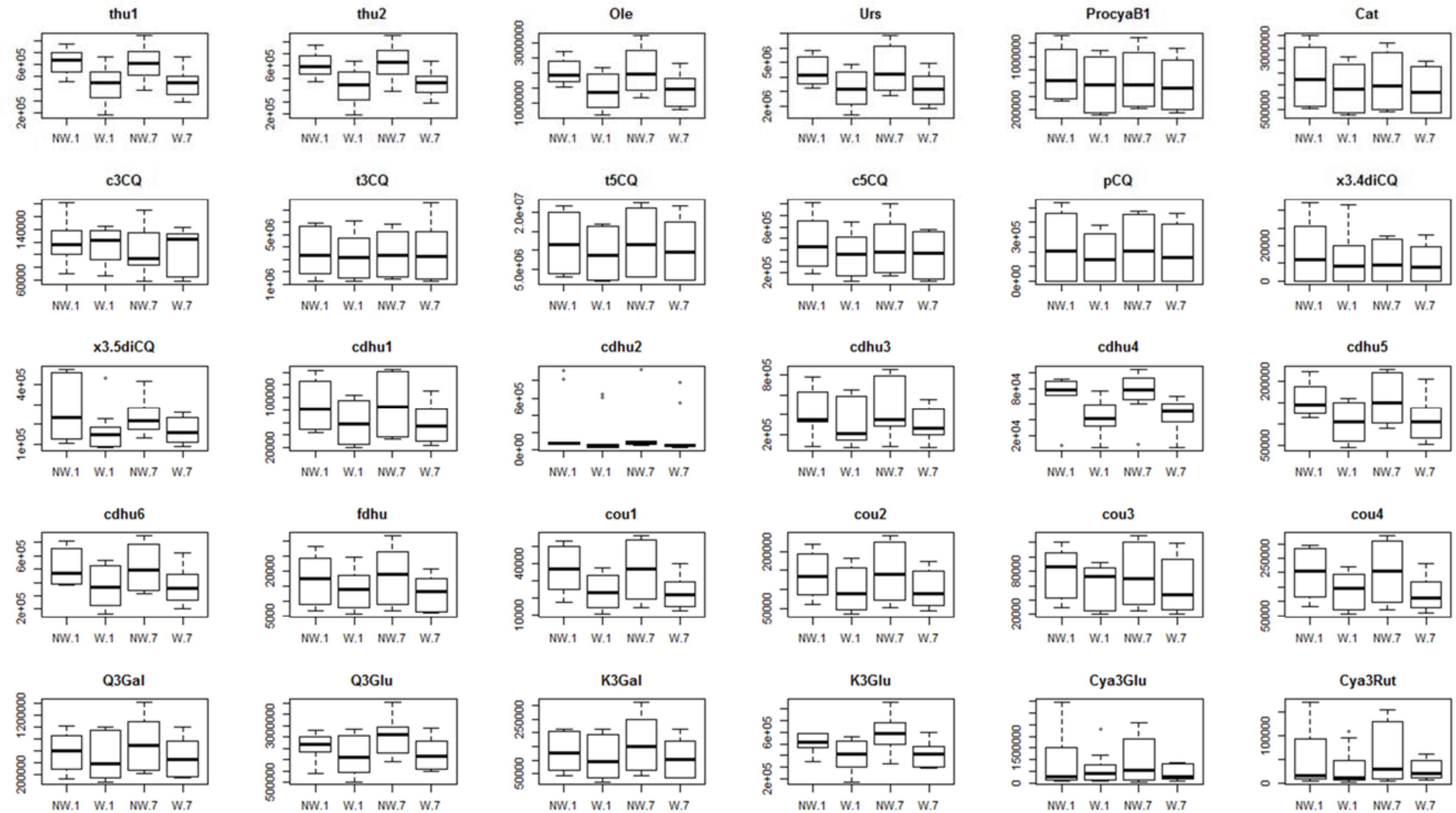
Supplementary Figure S1. HPLC-DAD chromatography (280nm) of Zephyr, samples taken in wounded immature fruits (black line) and non-wounded immature fruits (gray line), June 19, 2018. Abbreviations of compounds in Table 2. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.



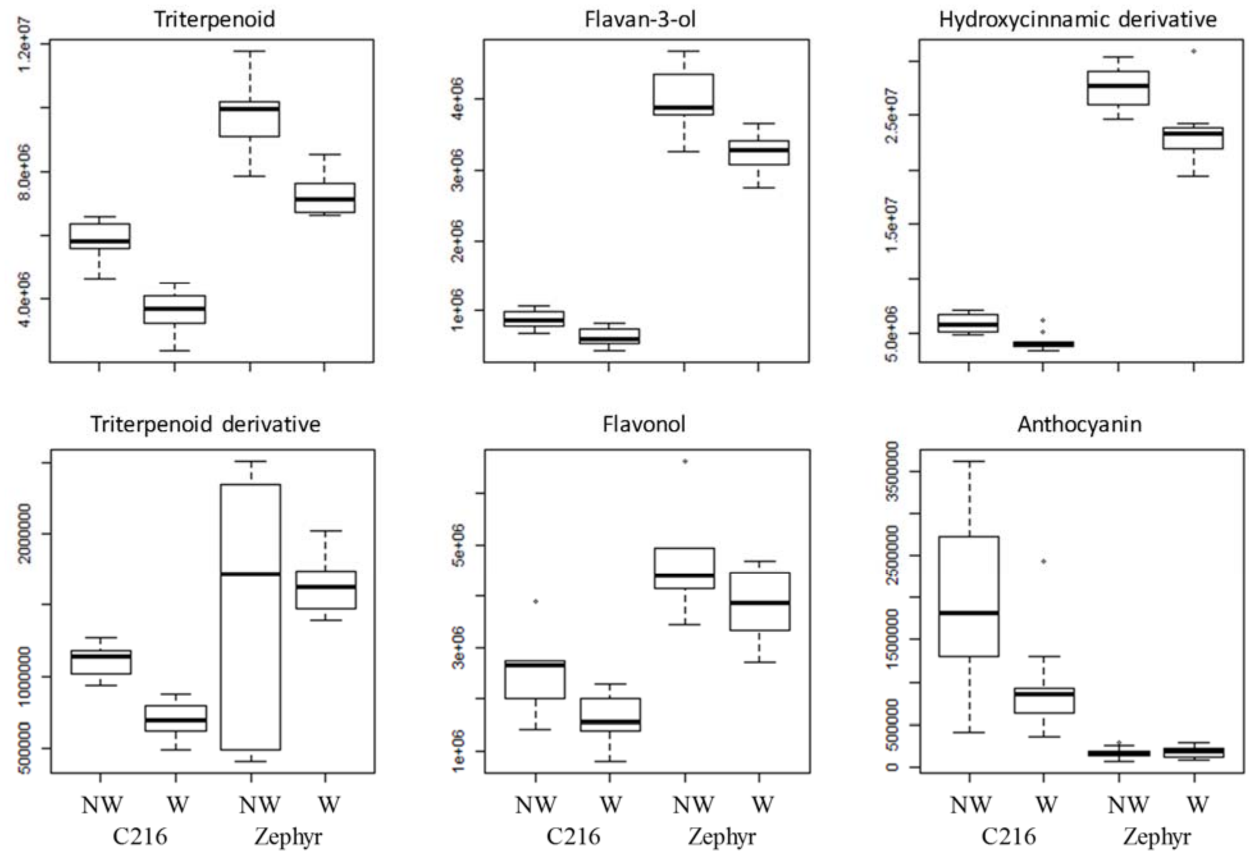
Supplementary Figure S2. HPLC-DAD chromatography (280nm) of C216, samples taken in wounded ripe fruits (black line) and non-wounded ripe fruits (gray line), July 03, 2018. Abbreviations of compounds in Table 2. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.



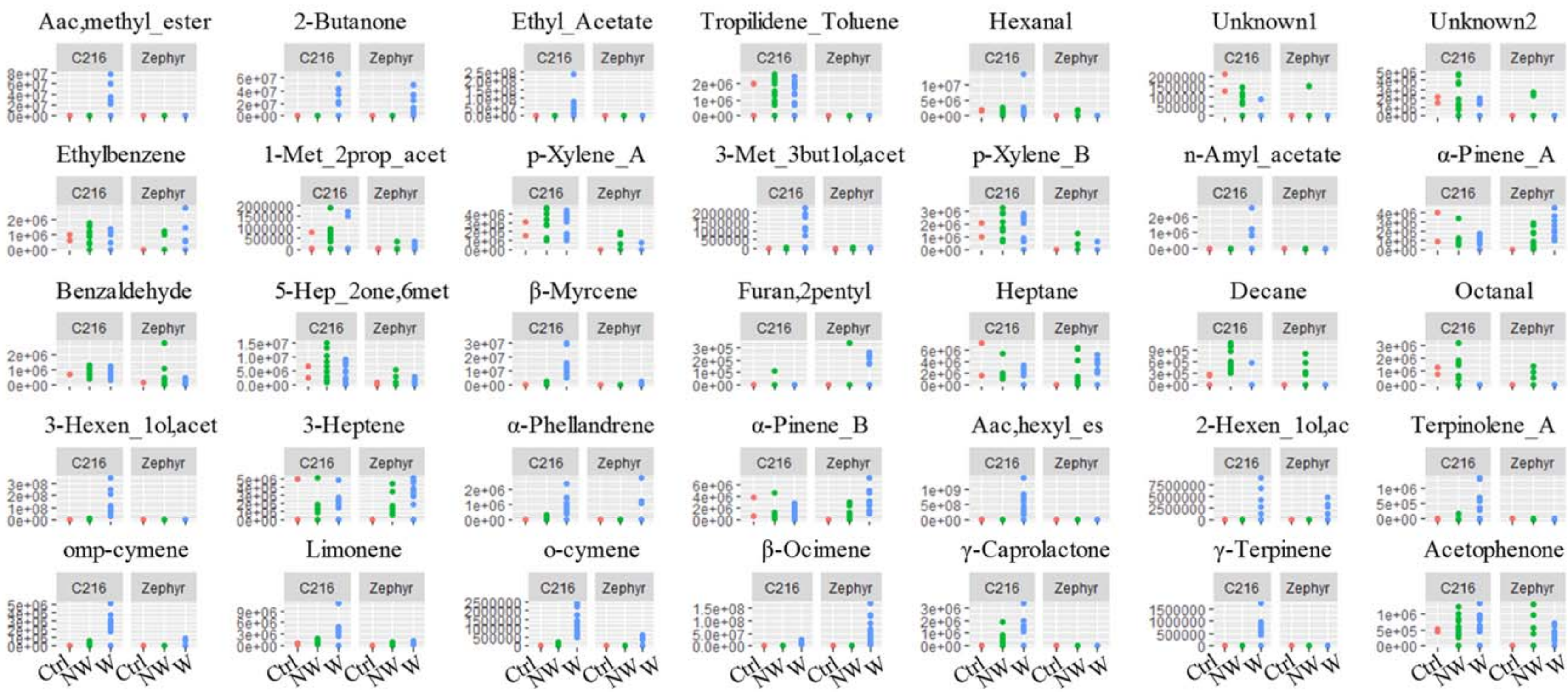
Supplementary Figure S3. Boxplot of the HPLC-DAD peaks area of compounds identified in fruits of 'Zephyr' (Ze) (June 19, 2018) and C216 nectarine (July 03, 2018), samples collected at 1 and 7 hours (1 and 7, respectively). Abbreviations of compounds in Table 1. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.



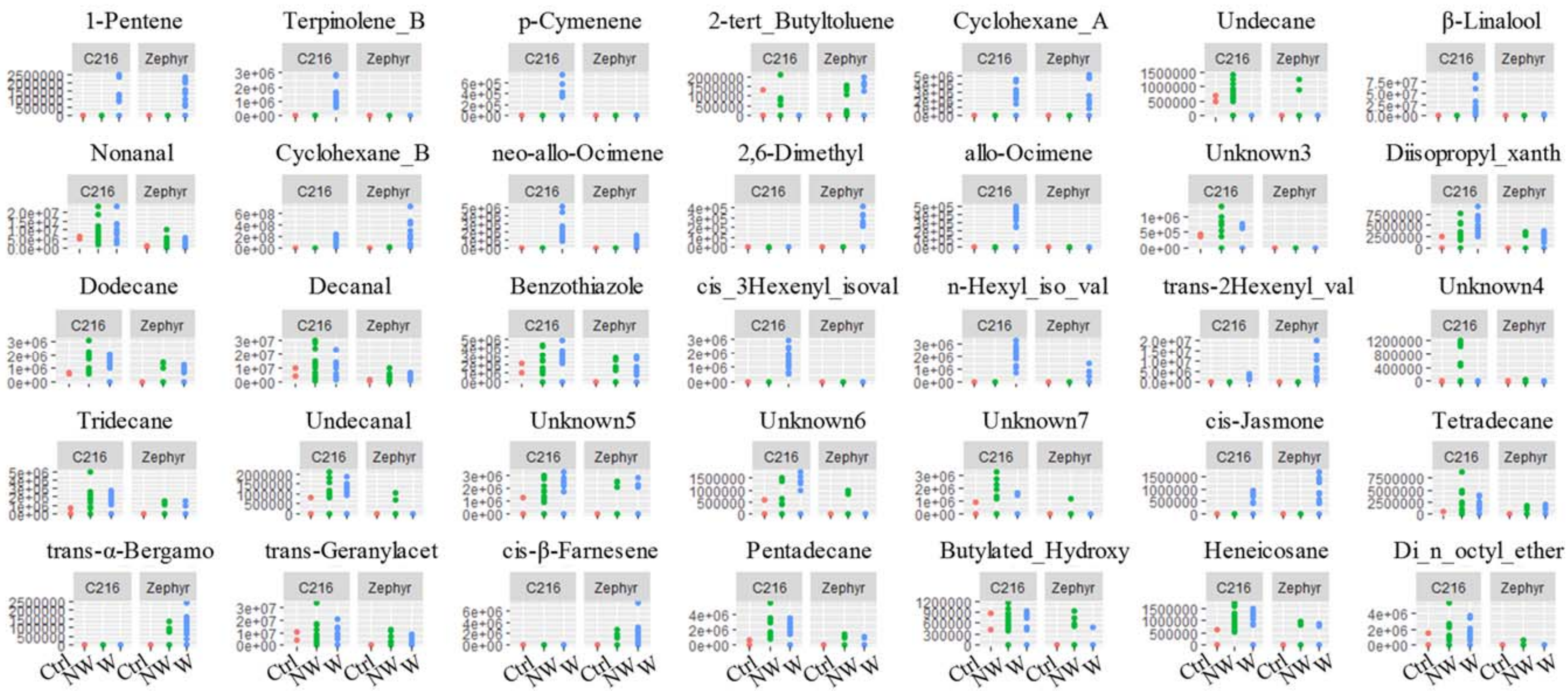
Supplementary Figure S4. Boxplot of compounds family identified by HPLC-DAD in 'Zephyr' (June 20, 2018) and C216 nectarine (July 03, 2018), subjected to wounded (W) or non-wounded (NW) and samples collected at 1 and 7 hours (1 and 7, respectively). INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.



Supplementary Figure S5. Boxplot of compounds family identified by HPLC-DAD in immature fruits of 'Zephyr' nectarine (June 20, 2018) and ripe fruits of C216 nectarine (July 03, 2018), subjected to wounded (W) or non-wounded (NW). INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France.



Supplementary Figure S6. Volatile compounds detected by Gas Chromatograph with Mass Spectrometer (GC-MS) in immature fruits of 'Zephyr' nectarine (June 18, 2018) and ripe fruits of C216 nectarine (July 02, 2018), subjected to wounded (W) or non-wounded (NW) and samples collected after 7 hours. Ctrl = Control, without fruits. INRA Avignon – ‘Génétique et Amélioration des Fruits et Légumes’, Avignon, France.



Supplementary Figure S6. Continuation.

4.9 Artigo 9. Influence of wounded nectarine fruit on *Monilinia laxa* infection

Artigo a ser submetido na Revista *“Journal of Agricultural Science”*

Influence of wounded nectarine fruit on *Monilinia laxa* infection

Maximiliano Dini[1], Bénedicte Quilot-Turion[2], Maria do Carmo Bassols Raseira[3]

[1] Postgraduate Program in Agronomy (PPGA), Faculty of Agronomy 'Eliseu Maciel', Federal University of Pelotas (FAEM-UFPel), Pelotas/RS, Brazil.

[2] GAFL, INRA, 84143, Montfavet, France.

[3] Fruit Breeding Laboratory, Embrapa Temperate Agriculture, Pelotas/RS, Brazil.

Correspondence: Maximiliano Dini, Federal University of Pelotas, Faculty of Agronomy Eliseu Maciel, Departament of Horticulture and Crop Science, Campus Capão do Leão, s/n, 96160-000, Rio Grande do Sul, Brazil. Tel: +55-53-999886095 E-mail: maxidini@hotmail.com; mdini@inia.org.uy.

Abstract

Three experiments were carried out in order to evaluate the influence of wounds on immature nectarine fruits of 'Zephyr' on *Monilinia laxa* infection *in vitro* and *in vivo*. In the first experiment, fruits were subjected to three different preconditions (non-wounded, wounded on one side of the fruit in the laboratory, and wounded on one side of the fruit in the tree). Seven hours later, all fruits were wounded with a razor blade and inoculated with *M. laxa* in this same spot. Wounding fruit in advance did not affect the level of brown rot susceptibility in the nectarine. The second experiment, was conducted to study the influence of fruit presence on the growth of *M. laxa* colonies *in vitro*. For this purpose, Petri dishes with PDA media were inoculated with the fungus, and placed in three boxes. In one of them, only Petri dishes were placed; in the second, non-wounded fruits were added in the same box; and in the third, wounded fruits were added next to Petri dishes. In this experiment, it was identified that *M. laxa* perceived the fruit and its growth was accelerated in the boxes that contained fruit. The third experiment was carried out to test the reaction of fruit inoculated with *M. laxa* after being in the presence of wounded fruit in the same environment. For that, one of treatment consisted of boxes with five fruits along with ten wounded fruits, and in the other only the fruits without wounds. After 7 hours, the previously wounded fruits were eliminated, and the intact fruits of the two treatments were wounded and inoculated with *M. laxa*. In this experiment, the presence of wounded fruits increased the brown rot susceptibility. Revealing host-pathogen interactions related to different responses to mechanical stress such as wounding is critical for the understanding of resistance mechanisms. Further studies with more genotypes and different fruit development stages are necessary to confirm the results.

Keywords: *Prunus persica* (L.) Batsch, brown rot, host-pathogen interaction.

1. Introduction

Numerous diseases attack stone fruits, being brown rot the most economically important fungal disease. In Europe, it is mainly caused by *Monilinia laxa* (Aderh. & Ruhl.) Honey. This disease can infect peaches and nectarines at any stage of their growth cycle, from bloom to harvest (Ogawa et al., 1995; Adaskaveg et al., 2008). However, the fruit development stage determines its susceptibility to brown rot infection, and three phases were established: a first phase of high susceptibility; a second phase when fruit are low susceptible (wich coincides with the pit hardening); and in the third, the susceptibility increases again until be maximum for mature fruits (Mari et al., 2003; Gell et al., 2008; Guidarelli et al., 2014).

Plants react to stress by activating several different mechanisms depending on their development stage, intensity and duration of stress and the tissue type (Tosetti et al., 2013). Among the abiotic stresses are mechanical damage, such as physical injuries, which can occur in the orchard and after harvesting. Physiological, biochemical and molecular responses to plant wounds result in metabolic alterations that target different purposes such as: sealing and healing of the wounded tissue, developing mechanical barriers to pathogenic or opportunistic invading organisms, and activating defense mechanisms against invading organisms (Zhou and Thornburg, 1999; Bruxelles and Roberts, 2001; Cheong et al., 2002; Shanker and Venkateswarlu, 2011). These changes involve the synthesis of various hormones such as ethylene, abscisic acid, and jasmonic acid (Birkenmeier and Ryan, 1998; León et al., 2001; Delessert et al., 2004; Broekaert et al. 2006; Koo and Howea, 2009) and also the selective modulation of gene expression, several wound-related genes have been identified and their expression studied (Christopher et al., 2004; Mitsuda et al., 2007; Koo and Howea, 2009; Trinidade et al., 2011; Leide et al., 2012; Tsaballa et al., 2015). Several identified genes encode signaling molecules. Cheong et al. (2002) working with Arabidopsis, suggested that a cascade of gene regulation is activated after injury, where early genes encode regulatory proteins (transcription factors), modulating the expression of other response genes. Wound-induced transcription factors were identified in several cultures, such as kaki (*Diospyros kaki* Thunb.) (Akagi et al., 2010), tabacco (*Nicotiana tabacum* L.) (Hara et al., 2000), poplar (*Populus* spp.) (Christopher et al., 2004; Smith et al., 2004) and rubber tree (*Hevea brasiliensis* Muell. Arg.) (Chen et al., 2012). Response genes, modulated by transcription factors, encode primarily for effector proteins, including those that enhance resistance or recovery of stress or damage cells, such as heat shock proteins, cell wall modifying enzymes, secondary metabolites, and related proteins to the pathogenesis (Cheong et al., 2002).

In comparison to other plant organs/tissues, little information is available on the molecular mechanisms of wounded fruit (Tosetti et al., 2013). In ripe fruit, wounds cause an increase in respiration rate and ethylene production, and generally lead to flesh softening, membrane rupture, browning, accelerated senescence, weight and water loss, and microbial diseases development (Toivonen and Brummell, 2008). A positive regulation of the expression of a phenylalanine ammonia lyase gene was reported in mature banana flesh after accumulation of RNAs from different heat shock proteins (Chen et al., 2009). However, responses to mechanical stress may change depending on the development stage. Working with 'Gala' apples harvested at different development stages, Su et al. (2011) demonstrated that wounds can activate the initial accumulation of H_2O_2 and the wound healing capacity to defend against the penetration of *Botrytis cinerea*. Tosetti et al. (2013) studied the molecular and biochemical responses of peach mesocarp to wounds in minimally processed products. In this study, up to 2218 genes were reported there were differentially expressed between the minimally processed fruits and the control fruits. However, there are no references to brown rot infection response. Therefore, the objective of this study was to evaluate the influence of wounds on immature nectarine fruits on *M. laxa* infection *in vitro* and *in vivo* conditions.

2. Method

2.1 Plant Material

Zephyr nectarine cultivar from the germplasm collection of 'Génétique et Amélioration des Fruits et Légumes' (GAFL) research unit of INRA Avignon was used. The plants were located at the experimental orchard at Saint Paul station (INRA Avignon). The cv. Zephyr had full bloom on February 10th and the harvest was initiated on July 4th, in the 2018 season. Fruit were collected and taken to the laboratory of Saint Maurice station (INRA Avignon), where they were selected for absence of apparent lesions or infections. Fruit disinfection was done with hot water at 60°C for 30 seconds. All experiments were installed on June 14th, 124 days after full bloom (DAFB) and 20 days before harvest date (DBHD).

2.2 Pathogen Culture and Incubation Conditions

The inoculum used was prepared under aseptic conditions on the day of inoculation, using the same strain of *M. laxa* (Ml3) preserved in Petri dishes with PDA media. *M. laxa* suspension was adjusted to 1.0×10^5 conidia mL^{-1} using a Mallassez chamber and an optical microscope. Distilled water with a drop of Tween-80® was used to break the surface tension and improve the homogeneity of the suspension. The fruits were placed on metal rings inside clear plastic boxes (40 × 28 × 18 cm), previously disinfected with 75% alcohol. The boxes were maintained in a growth chamber at 25±1°C, 80% relative humidity and a photoperiod of 12 hours.

2.3 Experiments

Three experiments were carried out to evaluate the effect of wounded fruit on infection development. They will be described separately.

2.3.1 Effect of prior wounding on M. laxa infection development (1st experiment)

To test the existence of a systemic reaction on brown rot infection triggered by wounding fruit in advance, 20 immature fruits were subjected to each of three different preconditions.

For the first condition, fruits were collected and taken to the laboratory of the INRA Avignon - Saint Maurice (GAFL), placed inside the boxes without wounds.

The second condition consisted on the same procedure as the previous one, but multiple longitudinal wounds (apex to the peduncle) were made with razor blade on one side of the fruit.

For the third condition, the fruits were marked and wounded in the orchard and left on the trees. The wounds were made as in the previous condition. After 7 hours, the fruits were harvested and taken to the laboratory. They were conditioned like the fruits of the other two conditions.

After 7 hours, all the fruits (three conditions), were wounded with a razor blade with a longitudinal cut from the apex to the peduncle, a depth not exceeding 3 mm. In the case of the second and third conditions, the fruits were wounded on the other side, unwounded previously. Then, the inoculation was made immediately after the wound, with a micropipette, depositing 10 µL of inoculum on the wound of each fruit. The boxes were closed and placed in the growth chamber.

The incidence and growth kinetics of the lesion and the sporulation caused by *M. laxa* were evaluated. Evaluations were performed on each fruit every 24 hours, for a period of ten days post-inoculation. The lesion and sporulation of *M. laxa* measurement was taken perpendicular to the wound, in the equatorial region, using a digital caliper.

2.3.2 Effect of presence of fruit next to M. laxa cultures on fungal growth (2nd experiment)
This experiment was conducted to study the growth of *M. laxa* colonies *in vitro* when fruits were present. For that, 30 Petri dishes of 5 cm diameter containing PDA media were inoculated. The inoculum used was prepared in the same conditions as in the first experiment, using 10 µL per Petri dish. Petri dishes without a lid, separated into three groups of ten, were placed in three boxes.
One of the boxes was hermetically closed and placed in the growth chamber. Ten immature fruits were placed in the second box before closing it and placing under the same condition as the first one. In the third box, ten fruits with multiple wounds (made with razor blade) were placed in the box, and then it was closed and conditioned like the previous ones.
Visual daily follow-up was done but the boxes were opened only 168 hours after inoculation to perform the final evaluation. This final evaluation consisted of two perpendicular measurements of the *M. laxa* colony, for each Petri dish. For the statistical analysis, the mean values of each Petri dish were used, considering each one as a replication.

2.3.3 Effect of the presence of wounded fruit next to fruit inoculated with M. laxa on the infection development (3rd experiment)
This experiment was carried out to test the reaction of fruits inoculated with *M. laxa* after being in the presence of wounded fruits. For that, two treatments were carried out. One of the treatments consisted in placing 20 fruits inside boxes. The other treatment was the same as the first, but for each box of five fruits, another ten fruits with multiple wounds were added. In other words, the first treatment consisted of boxes of five unwounded fruits, and the second had five unwounded fruits along with ten wounded fruits.
After 7 hours, the previously wounded fruits were eliminated. The fruits of the two treatments were wounded with a razor blade with a longitudinal cut from the apex to the peduncle, with a depth not exceeding 3 mm. Immediately after that, the inoculation was made with a micropipette, depositing 10 µL of inoculum on the wound of each fruit. The boxes were hermetically sealed and placed in the growth chamber.
The evaluations were performed in the same way as in the first experiment.

2.4 Statistical analyses
For all experiments, a completely randomized design was considered using each fruit (experiment 1 and 3) or Petri dish (experiment 2) as replication. For the first and third experiments, in order to estimate the disease progress, growth lesion scored data were plotted in graphs. Delay was calculated by the time at which the infection was first observed (in hours), and the progression rate was calculated by maximum progression of infection between two observation dates. For the sporulation data, the same graphs were plotted and parameters were calculated.
Analyses of variance (ANOVA) were performed by F-test ($p \leq 0.01$) and means were compared by Tukey's multiple range test.
Statistical analyses and graphical representations (ggplot2 package) were performed using R software (R Core Team, 2018).

3. Results and Discussion
3.1 Effect of prior wounding on M. laxa infection development (1st experiment) (1st experiment)
In the treatment where the fruits were not previously wounded, 65% of them were infected (Figure 1A), whereas, in the treatment where the fruits were previously wounded on one side, 70% of fruits were infected when wounds were made in laboratory (Figure 1B) and 50% when they were wounded in the tree (Figure 1C).

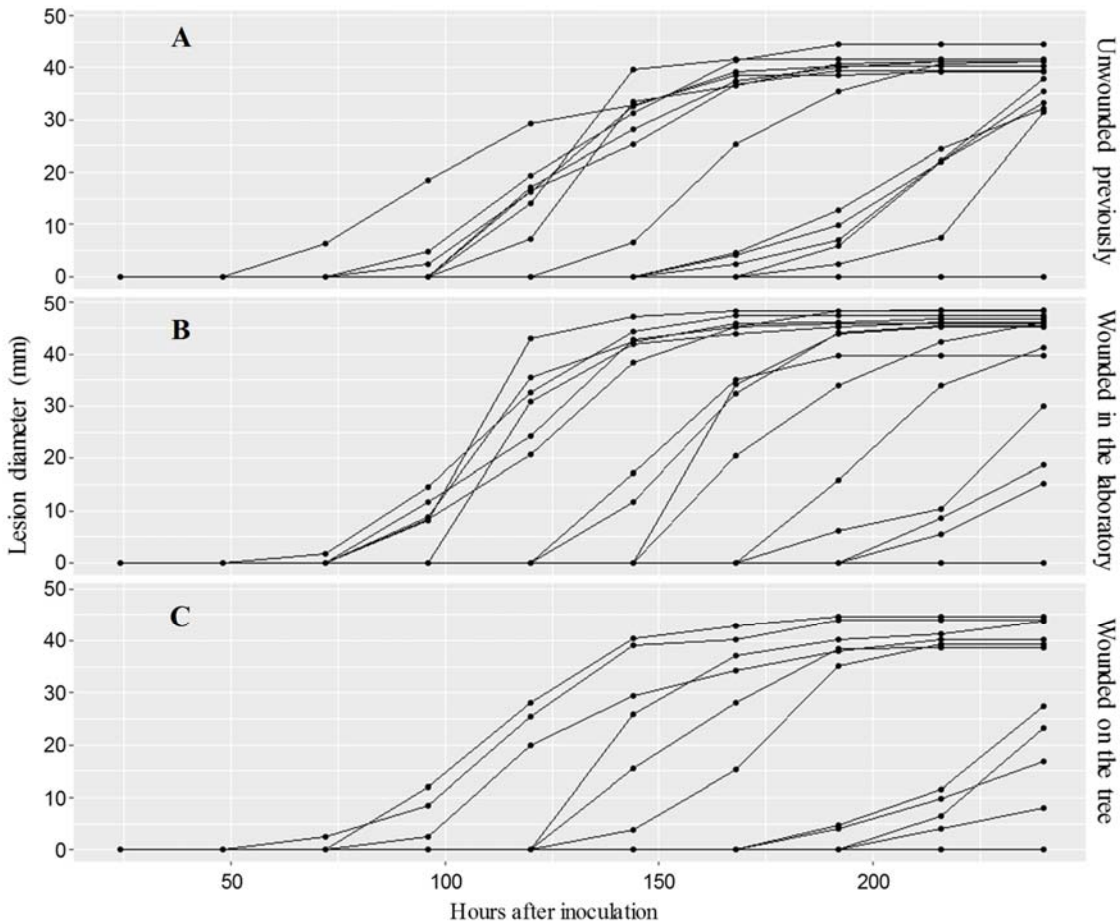


Figure 1. Brown rot lesion growth in 'Zephyr' immature fruits wounded and immediately inoculated with *Monilinia laxa,* which were submitted to three previous conditions: unwounded; wounded on one side in the laboratory; wounded on one side when still on the tree. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

In the case of sporulation (Figure 2), 55% of the fruits of the treatment without previous wounds (Figure 2A), 60% in the treatment where the fruits were previously wounded in the laboratory (Figure 2B), and 30% in the treatment when they were wounded in the tree (Figure 2C), presented the presence of sporulation of *M. laxa*.

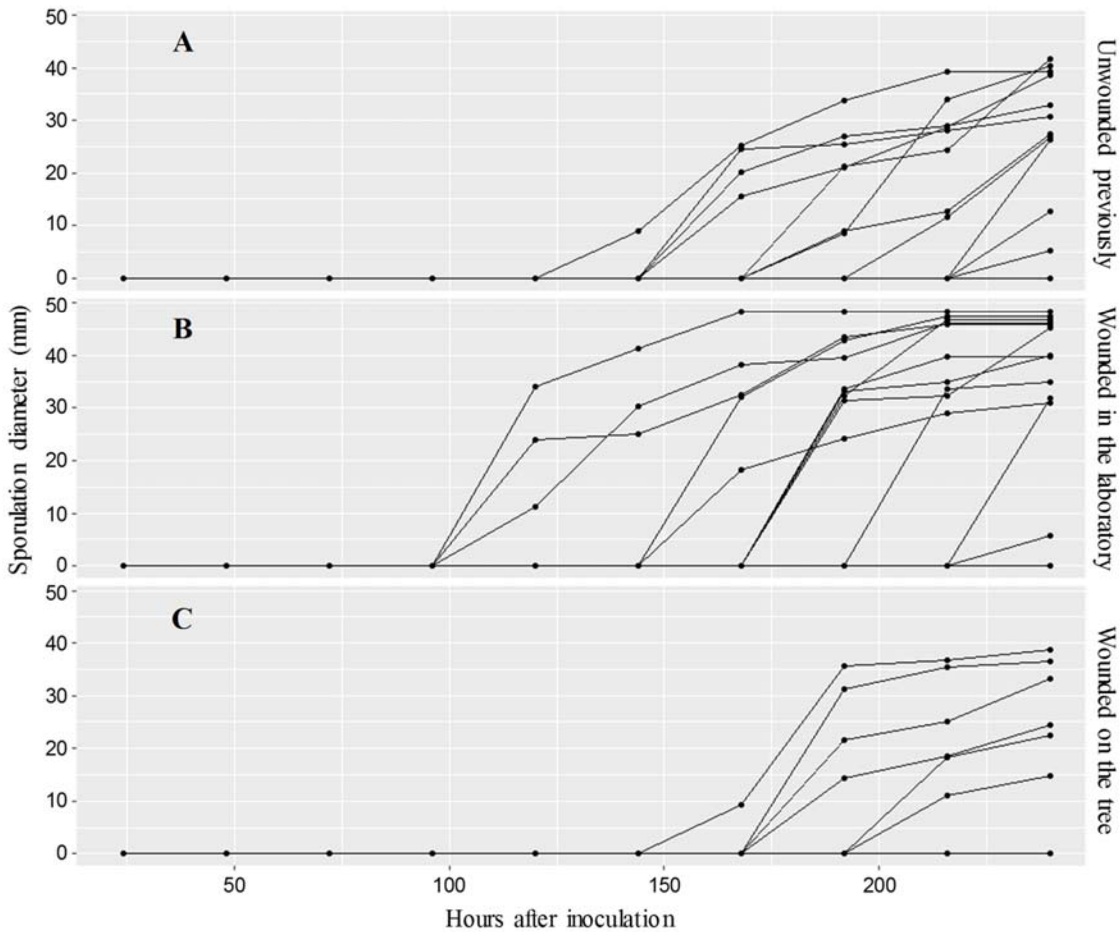


Figure 2. *Monilinia laxa* sporulation growth in 'Zephyr' immature fruits wounded and immediately inoculated, which were submitted to three previous conditions: unwounded; wounded on one side in the laboratory; wounded on one side when still on the tree. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

Although these percentages of infected fruit and sporulation *M. laxa* are different, there were large variations in the kinetics of diameter development within each conditions. Indeed, the parameters calculated to compare disease progression between treatments, Delay, Progression rate and final Diameter (240 hai), did not present significant differences ($p > 0.01$) (Figure 3).

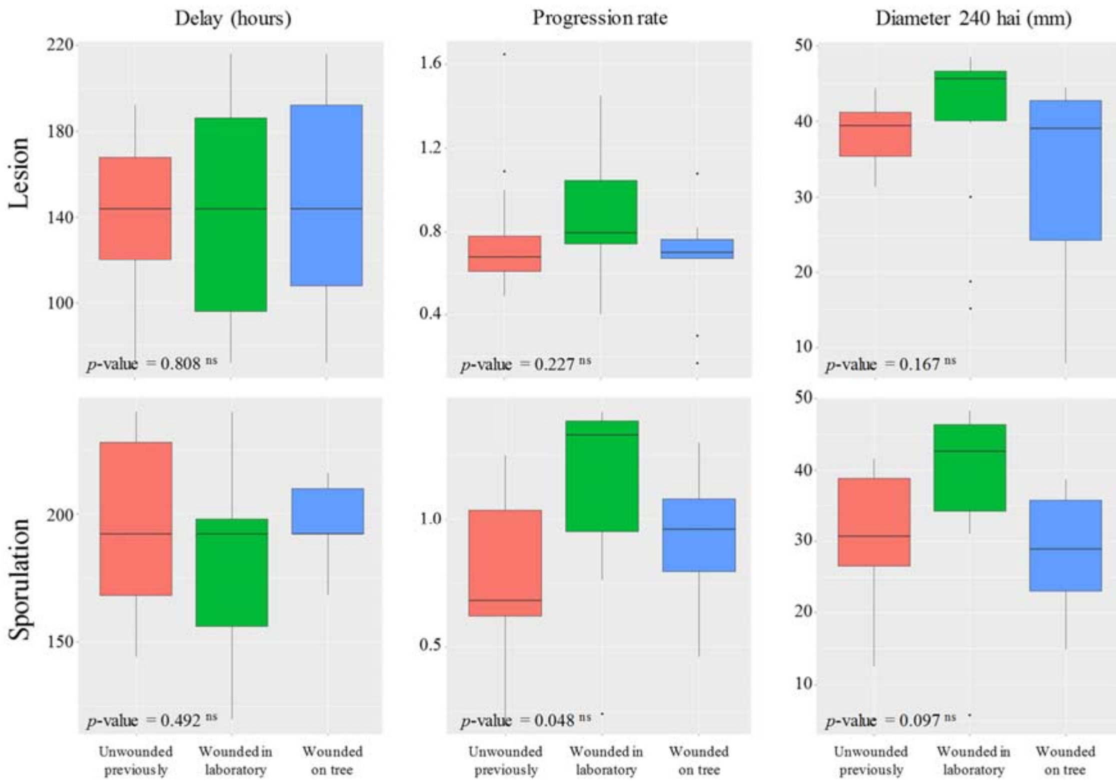


Figure 3. Boxplots of the delay, progression rate and diameter at 240 hours after inoculation (hai) of *Monilinia laxa* lesion and sporulation, inoculated immediately after wounded in 'Zephyr' immature fruits, in three conditions: unwounded previously (red); previously wounded in the laboratory in one side of the fruit (green); previously wounded on the tree in one side of the fruit (blue). ^{ns} not significantly significantly by F-test at $p \leq 0.01$. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

In this same experiment, red reactions associated with double stress (wound plus *M. laxa* inoculation) were detected (Figure 4). Such a reaction was not detected in the multiple wounds performed both in the laboratory and in the tree, without inoculation, as can be observed on the right side of the fruits of Figures 4A and 4B.

These red reactions have already been reported in an earlier study (Dini et al., 2019a). In this study, one of the five nectarine genotypes used was 'Zephyr', and these reactions in this cultivar were associated with inoculation with *M. laxa* immediately after wounding the fruits, at the development stage near to pit hardening. In the same study, these red areas were isolated and submitted to HPLC analysis, finding several new compounds, mainly from the flavonones family, being Eriodictyol-7-glucoside and Naringenine-7-glucoside (syn .: Prunin) the compounds that were found in greater proportion. It was suggested that these compounds may be involved in active mechanisms of resistance to the pathogen. However, in the present study, this hypothesis was not confirmed, because the reaction was not associated with resistance, since all fruits presented the red reaction and the disease usually developed anyway (Figure 4B). On the other hand, it may only be a question of stage of development: fruit can be too 'mature' here to react enough and stop the infection, or it can be that this reaction is a defense reaction of the fruit in case of double attack. But not enough after stage II or the defense mechanisms can no longer be activated

Several studies reported that wounds might increase levels of resistance to pathogens (Zhou and Thornburg, 1999; Léon et al., 2001; Bruxelles and Roberts, 2001; Cheong et al., 2002; Su et al., 2011). However, according to the present results the wounds do not appear to have a systemic effect on whole fruit. They may increase the resistance to pathogens though a local reaction linked to sealing that prevents the entry of pathogens and/or activate other defense mechanisms (Cheong et al., 2002). As mentioned in the previous study by Dini et al. (2019a) seven hours delay between wounding and inoculation resulted in less susceptibility.

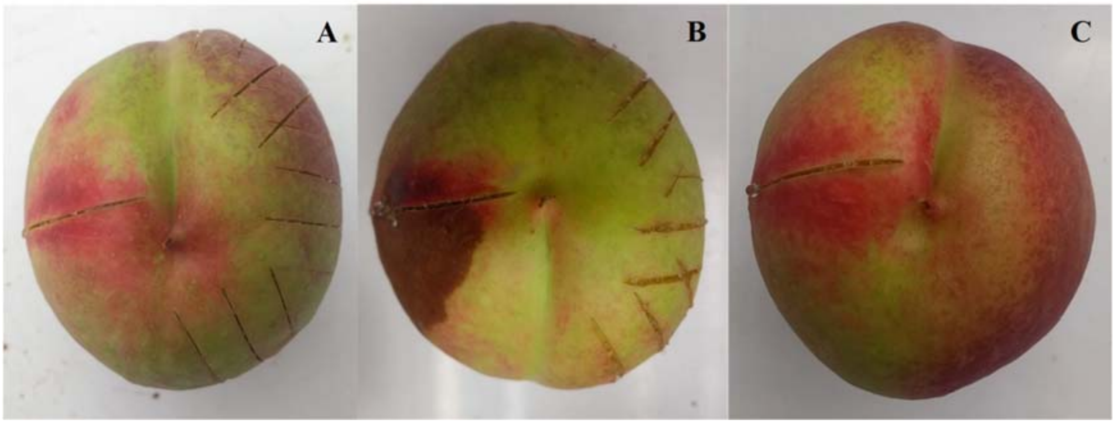


Figure 4. Reaction red detected in immature fruits wounded and immediately inoculated with *Monilinia laxa*. A: Red reaction is highlighted in the inoculated wound on the left side of the fruit, on the right side of the fruit multiple wounds without inoculation reactionless. B: Infection above red reaction in the inoculated wound on the left side of the fruit, on the right side of the fruit multiple wounds without inoculation reactionless. C: Red reaction in the inoculated wound on the left side of the fruit, on the right side of the fruit unwounded and reactionless. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

It should be noted that in parallel to these experiments four types of controls were installed: non-wounded inoculated fruits; non-wounded fruits and "inoculated" only with water (without Tween-80®); wounded fruits; and wounded fruits "inoculated" with water plus Tween-80®. In all of these cases, they did not present *M. laxa* infection and the red reactions did not occur. Only clearer spots than the normal colour were detected in the fruit skin, in the two controls, "inoculated" with water plus Tween-80®, being associated with the use of Tween-80® surfactant.

3.2 Effect of presence of fruit next to M. laxa cultures on fungal growth (2nd experiment)

When *M. laxa* was tested under *in vitro* conditions, there were significant differences (p-value = 8.66^{-10}) among the treatments tested. The diameter of the colony was larger in the treatments where the Petri dishes were incubated together with fruits (43.77 and 40.83 mm), without presenting significant differences between the wounded and non-wounded fruits (Figure 5).

In a previous study, Dini et al. (2019b) identified 70 volatile compounds in fruits of two genotypes of nectarines. In that study, we observed a change in the volatile compounds patterns between non-wounded and wounded fruits, identifying several compounds associated with wounded fruits only. It was hypothesized that these volatile compounds may be influencing plant-pathogen relationships. In the present study, the higher growth of *M. laxa* colonies in the presence of fruits may be associated with some mechanism of detection of the pathogen to its host, probably through some volatile compounds released by the fruits (Figure 5). This theory is supported by some reports of how host volatile compounds can be perceived by their pathogens (Knogge, 1996; Cowan, 1999; Kishimoto et al., 2005; Chisholm et al., 2006).

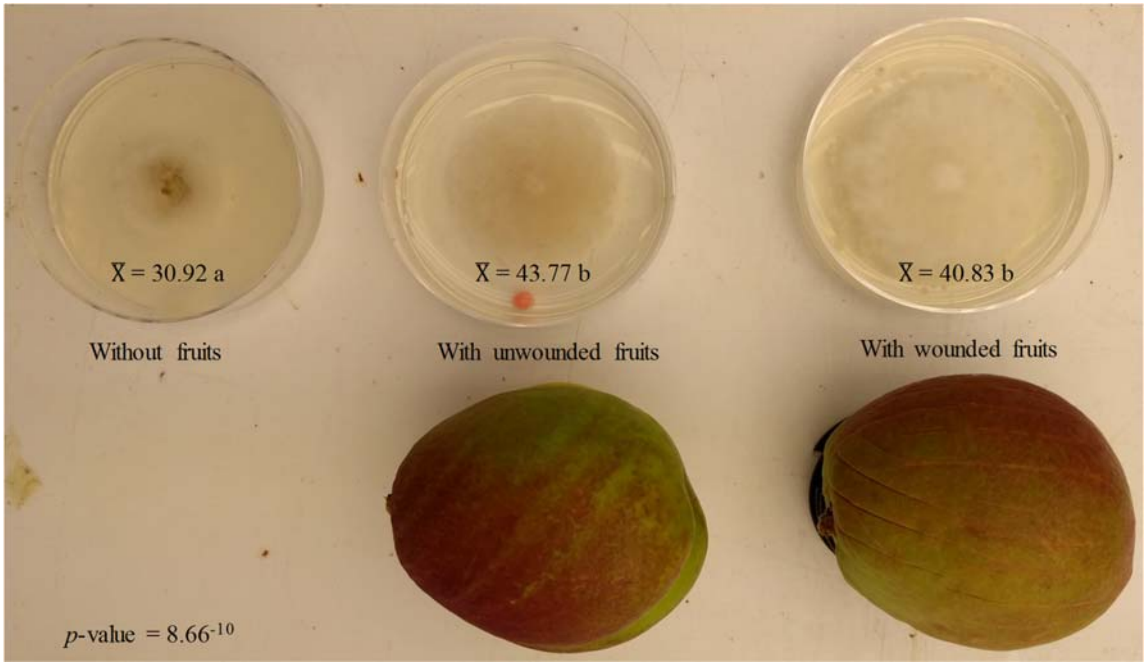


Figure 5. *Monilinia laxa* inoculated in open Petri dishes with PDA, placed inside a sealed plastic box in three conditions: without fruits, with unwounded fruits and with wounded fruits. Means of the diameter of *M. laxa* colony at 168 hours after inoculation followed by different letters are significantly different by the Tukey's test at $p \leq 0.01$. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes'. Avignon. France.

In the study of Wilson et al. (1987), they evaluated 16 natural volatile fruit compounds for the control of *M. fructicola* and *Botrytis cinerea* in peach at post-harvest period. Some of them had an inhibitory effect on the germination and growth of the pathogens such as Benzaldehyde, Methyl salicyate, and Ethyl benzoate. On the other hand, other compounds such as *d*-Limonene favored the fungus, increasing the percentage of spore germination. In that case, the volatile compounds were applied as isolated compounds and at known concentrations, however the fruits have a great complexity of volatile compounds (Aubert and Milhet, 2007; Eduardo et al., 2009; Dini et al., 2019b), which certainly affects their influence on pathogens.

3.3 Effect of the presence of wounded fruit next to fruit inoculated with M. laxa on the infection development (3rd experiment)

When fruits were inoculated after being seven hours in a closed box with other wounded fruit, 80% of them showed brown rot infection (Figure 6A), whereas 65% of fruit inoculated without being in presence of other wounded fruits presented infection (Figure 6B). For sporulation, 70% and 55% of fruits presented *M. laxa* sporulation, respectively (Figure 6C and 6D).

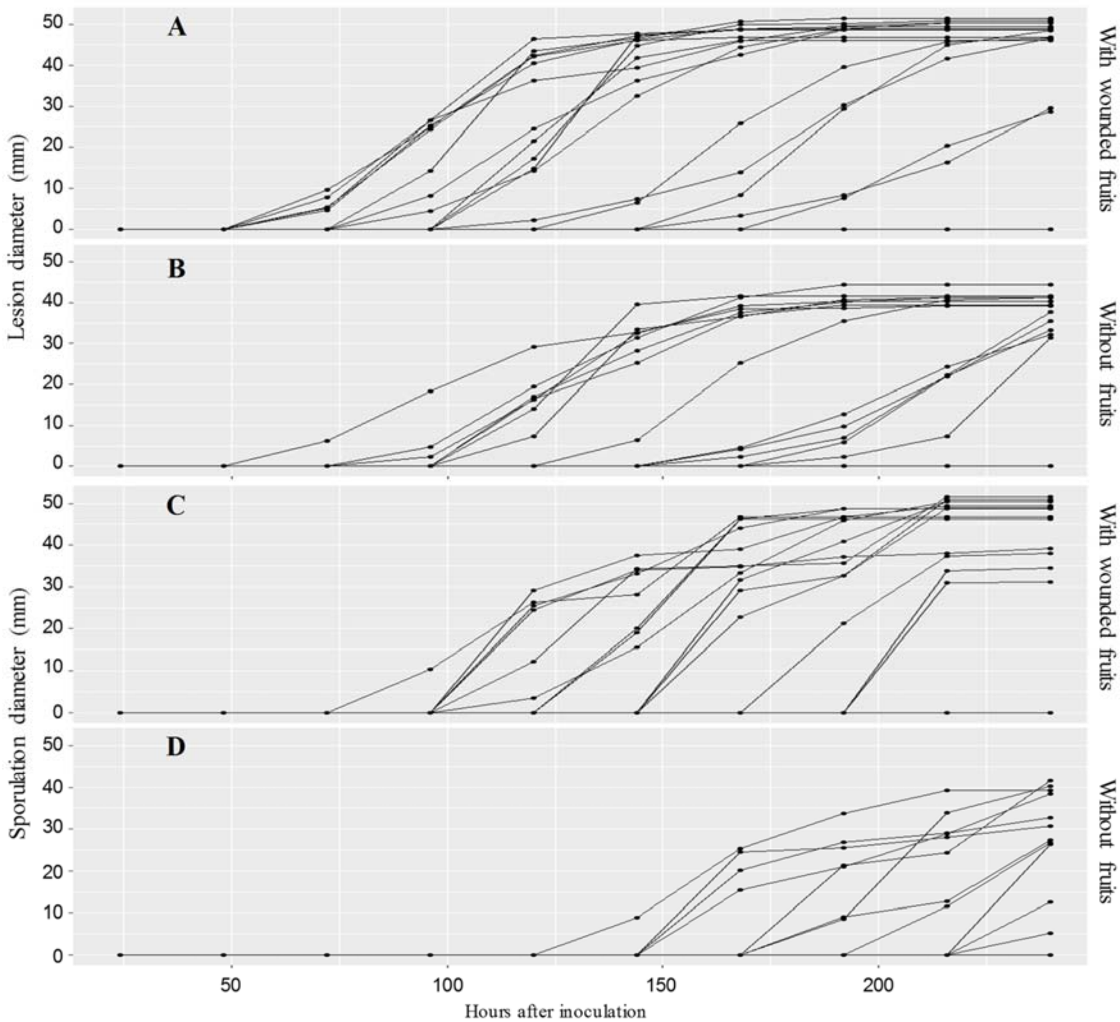


Figure 6. *Monilinia laxa* lesion and sporulation growth in 'Zephyr' immature fruits wounded and immediately inoculated with *M. laxa*, in two conditions: previously with wounded fruits in a closed box for 7 hours, and without the presence of wounded fruits. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

The delay and progression rate of the lesion did not present significant differences between the two treatments ($p > 0.01$). However, the final lesion diameter (240 hai) was significantly higher (p-value $= 1.03^{-3}$) in the treatment that was previously maintained with wounded fruits for seven hours (Figure 7).

In reference to sporulation, the three calculated parameters had significant differences between the two treatments ($p \leq 0.01$). Delay was lower in the treatment where the fruits were maintained for seven hours along with wounded fruits before the inoculation, demonstrating that the *M. laxa* sporulate faster on these fruits. The same way, the Progression rate and the final diameter (240 hai) of sporulation were higher, which indicated that *M. laxa* sporulation, occurred faster, reaching a larger final diameter, under this condition (Figure 7).

M. laxa seemed to be boosted by the presence of (wounded) fruits (Figures 6 and 7). This emphasizes the hypothesis that *M. laxa* presents mechanisms that perceive some signs (volatile compounds or hormones) of the nectarines (host). There are reports in other species of this perception involved in plant-pathogen interactions either with host or non-host plants (Cowan, 1999; Kishimoto et al., 2005, Broekaert et al., 2006, Lee et al., 2017).

It should be noted that, similarly to the first experiment, red reactions on, or close to, the wounds were observed (Figure 4C) as reported by Dini et al. (2019a).

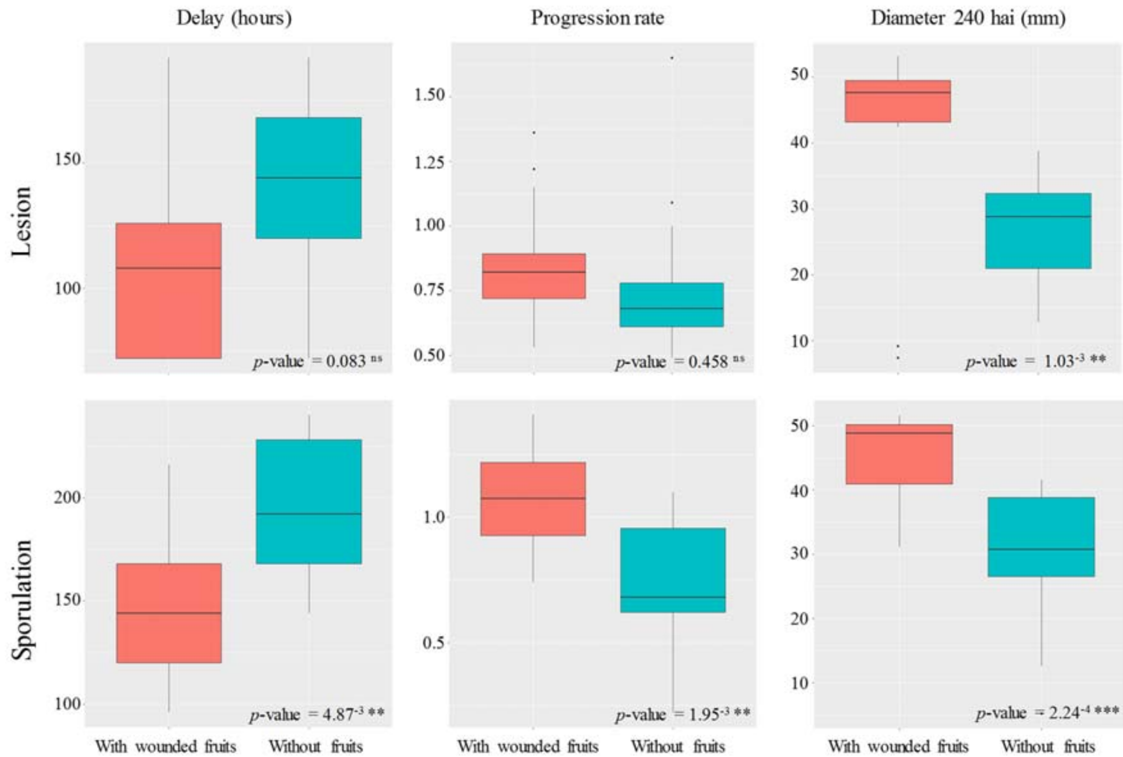


Figure 7. Boxplots of the delay, rate and diameter at 240 hours after inoculation (hai) of *Monilinia laxa* lesion and sporulation, inoculated immediately after wounded in 'Zephyr' immature fruits, in two conditions: with wounded fruits in a closed box for 7 hours (red), and without the presence of wounded fruits (blue). ^{ns}, ** and ***, not significantly ($p > 0.01$), significantly at $p \leq 0.01$ and $p \leq 0.001$ by F-test. INRA Avignon – 'Génétique et Amélioration des Fruits et Légumes', Avignon, France.

4. Conclusions

Wounds in another part of the fruit do not affect the degree of nectarine susceptibility to brown rot.

M. laxa in vitro can perceive the fruits and their growth is more accelerated.

The presence of wounded fruits seems to increase the brown rot susceptibility.

Revealing host-pathogen interactions related to these different responses to mechanical stress such as wounds is critical for the understanding of resistance mechanisms. This type of study, accompanied by analyzes of genetic expression, must be continued in order to understand these important plant-pathogen interactions.

Acknowledgments

To the Coordination of Improvement of Higher Education Personnel (CAPES-Brazil) for providing PhD scholarship founds, and GAFL - INRA Avignon staff, especially Marie-Noëlle Corre, Laure Heurtevin and Véronique Signoret.

References

Adaskaveg, J. E., Schnabel, G., Forster, H. (2008). Diseases of peach caused by fungi and fungal-like organisms: biology, epidemiology and management. In D.R., Layne, D. Bassi, (Eds.), *The peach: botany, production and uses* (pp. 352-406). Wallingford, UK: CAB International.

Akagi, T., Ikegami, A., Yonemori, K. (2010). DkMyb2 wound-induced transcription factor of persimmon (*Diospyros kaki* Thunb.), contributes to proanthocyanidin regulation. *Planta, 232*(5), 1045–1059. https://doi.org/10.1007/s00425-010-1241-7

Aubert, C., Milhet, C. (2007). Distribution of the volatile compounds in the different parts of a white-fleshed peach (*Prunus persica* L. Batsch). *Food Chemistry, 102*(1), 375-384. https://doi.org/10.1016/j.foodchem.2006.05.030.

Birkenmeier, G. F., Ryan, C. A. (1998). Wound signalling in tomato plants – evidencethat ABA is not a primary signal for defense gene activation. *Plant Physiology*, *117*(2), 687–693. https://doi.org/10.1104/pp.117.2.687

Broekaert, W. F., Zelauré, S. L., De Bolle, M. F. C., Cammue, B. P. A. (2006). The role of ethylene in host-pathogen interactions. *Annual Review of Phytopathology, 44*(1), 393-416. https://doi.org/10.1146/annurev.phyto.44.070505.143440

Bruxelles, G. L., Roberts, M. R. (2001). Signals regulating multiple responses to wounding and herbivores. *Critical Reviews in Plant Sciences, 20*(5), 487-521. https://doi.org/10.1080/07352689.2001.10131828

Chen, J., He, L., Jiang, Y., Kuang, J., Lu, C., Joyce, D., Macnish, A., He, Y., Lu, W. (2009). Expression of PAL and HSPs in fresh-cut banana fruit. *Environmental and Experimental Botany, 66*(1), 31–37. https://doi.org/10.1016/j.envexpbot.2008.12.019

Chen, Y. Y., Wang, L. F., Dai, L. J., Yang, S. G., Tian, W. M. (2012). Characterization of HbEREBP1, a wound-responsive transcription factor gene in laticifers of *Hevea brasiliensis* Muell. Arg. *Molecular Biology Reports 39*(4), 3713–3719. https://doi.org/10.1007/s11033-011-1146-y

Cheong, Y. H., Chang, H. S., Gupta, R., Wang, X., Zhou, T., Luan, S. (2002). Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. *Plant Physiology, 129*(2), 661–677. https://doi.org/10.1104/pp.002857

Chisholm, S. T., Coaker, G., Day, B., Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. *Cell, 124*(4), 803-814. https://doi.org/10.1016/j.cell.2006.02.008

Christopher, M. E., Miranda, M., Major, I. T., Constabel, C. P. (2004). Gene expression profiling of systemically wound-induced defenses in hybrid poplar. *Planta 219*(6), 936-947. http://doi.org/10.1007/s00425-004-1297-3

Delessert, C., Wilson, I. W., Van Der Straeten, D., Dennis, E. S., Dolferus, R. (2004). Spatialand temporal analysis of the local response to wounding in Arabidopsis leaves. *Plant Molecular Biology, 55*(2), 165–181. https://doi.org/10.1007/s11103-004-0112-7

Dini, M., Quilot-Turion, B., Raseira, M. C. B. (2019a). Fruit reaction to brown rot at different development stages and inoculation time after wounded. *Scientia Horticulturae* (under review).

Dini, M., Quilot-Turion, B., Raseira, M. C. B. (2019b). Reaction of wounded nectarine fruits: brown rot infection, phenolic and volatile compounds. *Scientia Horticulturae* (under review).

Eduardo, I., Chietera, G., Bassi, D., Rossini, L., Vecchitti, A. (2009). Identification of key odor volatile compounds in the essential oil of nine peach accessions. *Journal of the Science of Food and Agriculture, 90*(7), 1146-1154. https://doi.org/10.1002/jsfa.3932

Gell, I., De Cal, A., Torres, R., Usall, J., Melgarejo, P. (2008). Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. *European Journal of Plant Pathology, 121*(4), 487-498. http://dx.doi.org/10.1007/s10658-008-9268-3

Guidarelli, M., Zubini, P., Nanni, V., Bonghi, C., Rasori, A., Bertolini, P., Baraldi, E. (2014). Gene expression analysis of peach fruit at different growth stages and with different susceptibility to *Monilinia laxa*. *European Journal of Plant Pathology, 140*(3), 503-513. http://doi.org/10.1007/s10658-014-0484-8

Hara, K., Yagi, M., Kusano, T., Sano, S. (2000). Rapid systemic accumulation of transcripts encoding a tobacco WRKY transcription factor upon wounding. *Molecular and General Genetics 263*(1), 30–37. https://doi.org/10.1007/PL00008673

Kishimoto, K., Matsui, K., Ozawa, R., Takabayashi, J. (2005). Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant Cell Physiology, 46*(7), 1093-1102. https://doi.org/10.1093/pcp/pci122

Knogge, W. (1996). Molecular basis of specificity in host/fungus interactions. *European Journal of Plant Pathology, 102*(9), 807-816. https://doi.org/10.1007/bf01877049

Koo, A. J. K., Howea, G. A. (2009). The wound hormone jasmonate. *Phytochemistry, 70*, 1571–1580. https://doi.org/10.1016/j.phytochem.2009.07.018

Lee, H. A., Lee, H. Y., Seo, E., Lee, J., Kim, S.B., Oh, S., Choi, E., Choi, E., Lee, S. E., Choi, D. (2017). Current understandings of plant nonhost resistance. *Molecular Plant-Microbe Interaction, 30*(1), 5-15. https://doi.org/10.1094/mpmi-10-16-0213-cr

Leide, J., Hildebrandt, U., Hartung, W., Riederer, M., Vogg, G. (2012). Abscisic acid mediates the formation of a suberized stem scar tissue in tomato fruits. *New Phytologist, 194*(2), 402–415. https://doi.org/10.1111/j.1469-8137.2011.04047.x

León, J., Rojo, E., Sánchez-Serrano, J. J. (2001). Wound signalling in plants. *Journal of Experimental Botany, 52*(354), 1-9. https://doi.org/10.1093/jxb/52.354.1

Mari, M., Casalini, L., Baraldi, E., Bertolini, P., Pratella, G. C. (2003). Susceptibility of apricot and peach fruit to *Monilinia laxa* during phenological stages. *Postharvest Biology and Technology, 30*(1), 105-109. http://doi.org/10.1016/S0925-5214(03)00138-8.

Mitsuda, N., Iwase, A., Yamamoto, H., Yoshida, M., Seki, M., Shinozaki, K., Ohme-Takagia, M. (2007). NAC transcription factors, NST1 and NST3, are key regulatorsof the formation of secondary walls in woody tissues of Arabidopsis. *Plant Cell, 19*, 270–280. https://doi.org/10.1105/tpc.106.047043

Ogawa, J. M., Zehr, E. I., Bird, G. W., Ritchie, D. F., Uriu, K., Uyemoto, J. K. (1995). *Compendium of stone fruit diseases* (1st ed.). Saint Paul, MN: The American Phytopathological Society.

Shanker, A. K., Venkateswarlu, B. (2011). Abiotic stress response in plants – physiological, biochemical and *genetic perspectives* (1st ed.). Rijeka, Croatia: Intech.

Smith, C. M., Rodriguez-Buey, M., Karlsson, J., Campbell, M. M. (2004). The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. *New Phytologist, 164*(1), 123-136. http://doi.org/10.1111/j.1469-8137.2004.01151.x

Su, J., Tu, K., Cheng, L., Tu, S., Wang, M., Xu, H., Zhan, G. (2011). Wound-induced H_2O_2 and resistance to *Botrytis cinerea* decline with the ripening of apple fruit. *Postharvest Biology and Technology, 62*(1), 64–70. https://doi.org/10.1016/j.postharvbio.2011.05.001

Toivonen, P. M. A., Brummell, D. A. (2008). Biochemical bases of appearance and tex-ture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology, 48*, 1–14. https://doi.org/10.1016/j.postharvbio.2007.09.004

Tosetti, R., Tardelli, F., Tadiello, A., Zaffalon, V., Giorgi, F. M., Guidi, L., Trainotti, L., Bonghi, C., Tonutti, P. (2013). Molecular and biochemical responses to wounding in mesocarp of ripe peach (*Prunus persica* L. Batsch) fruit. *Postharvest Biology and Technology, 90*, 40-51. https://doi.org/10.1016/j.postharvbio.2013.12.001

Trinidade, I., Santos, D., Dalmay, T., Fevereiro, P. (2011). Facing the environment: small RNAs and the regulation of gene expression under abiotic stress in plants. In A.K. Shanker, B. Venkateswarlu (Eds.), *Abiotic Stress Response in Plants – Physiological, Biochemical and Genetic Perspectives* (pp. 113–136). Rijeka, Croatia: Intech.

Tsaballa, A., Nikolaidis, A., Trikka, F., Ignea, C., Kampranis, S. C., Makris, A. M., Argiriou, A. (2015). Use of the de novo transcriptome analysis of silver-leaf nightshade (*Solanum elaeagnifolium*) to identify gene expression changes associated with wounding and terpene biosynthesis. *BMC Genomics, 16*(1), 504. https://doi.org/10.1186/s12864-015-1738-3

Wilson, C. L., Franklin, J. D., Otto, B. E. (1987). Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. *Plant Disease, 71*(4), 316-319. https://doi.org/10.1094/pd-71-0316

Zhou, L., Thornburg, R. (1999). Wound-inducible genes in plants. In P. H. S. Reynolds (Ed.), *Inducible Gene Expression* (pp. 127–158). Wallingford, UK: CAB International.

5 Considerações finais

O objetivo central deste trabalho foi buscar fontes de resistência à podridão-parda, assim como contribuir com o entendimento dos mecanismos envolvidos. Com esta finalidade, foram desenvolvidos vários experimentos, primeiramente no Brasil, e no ano 2018, na França.

Em relação aos caráteres fenológicos, foi verificada uma alta herdabilidade. Foi detectada, na segregação dos mesmos, uma tendência na direção do genitor feminino, o que poderia estar sugerindo um efeito materno na transmissão destes caráteres, embora possa estar associado apenas às populações estudadas. Outra particularidade, foi que a data de colheita (DC) apresentou correlação negativa com a incidência da podridão-parda (IPP), indicando que quanto mais tardia era a DC, menor a IPP. Isto pode ser explicado devido ao fato de que as progênies de maturação mais tardias foram originadas por genitores que apresentam certo grau de resistência à infecção de *M. fructicola*, podendo, portanto, estar associado apenas às populações estudadas.

Quanto à reação das flores à *M. fructicola*, todos os genótipos estudados se apresentaram suscetíveis ou muito suscetíveis. Isto pode estar associado tanto à baixa resistência genética como às condições de inoculação e incubação muito favoráveis ao desenvolvimento da doença. Por isso, o ajuste da técnica de fenotipagem foi o objetivo de um novo experimento. O uso de flores abertas ou em estádio de balão, com uma suspensão de 20-200 conídios de *M. fructicola* por flor, realizando as avaliações 96 horas após inoculação, foi a melhor combinação que possibilitou diferenciar genótipos quanto a diferentes níveis de suscetibilidade. Entretanto, se um dos objetivos prioritários for a resistência à podridão de flores, novos genótipos deverão ser introduzidos, já que nenhum deles mostrou-se resistente. Trabalhos com diferentes dosagens de conídios, com menor intervalo das concentrações (entre 20-200 de *M. fructicola*), deveriam ser conduzidos para ajustar uma concentração exata, ressaltando que isso poderia mudar dependendo do inóculo latente no ambiente estudado.

O conteúdo de compostos fenólicos, antocianinas e atividade antioxidante das pétalas foi correlacionado negativamente com a suscetibilidade à podridão das flores. Sugere-se que novos trabalhos sejam realizados com maior número de genótipos com flores de diferentes tonalidades. Esse estudo deverá ser conduzido de modo a confirmar ou refutar a hipótese de que flores mais escuras apresentam menor suscetibilidade à podridão. Caso a hipótese seja confirmada, possibilitaria o uso da seleção indireta de fácil fenotipagem.

Em relação à podridão-parda em frutos, as populações estudadas apresentaram variabilidade quanto à suscetibilidade/resistência, sendo a herdabilidade de média a baixa. Utilizando uma pressão de seleção dos genótipos iguais ou melhores que a cv. Bolinha, foram estimados avanços genéticos aceitáveis, considerando que a suscetibilidade/resistência é um caráter poligênico. Além disso, vários dos genótipos com suscetibilidade igual ou menor que 'Bolinha' produzem frutos de melhor qualidade, demostrando o avanço genético do programa de melhoramento da Embrapa, não apenas na resistência à podridão-parda, senão também na qualidade dos frutos.

Assim, com base nos resultados obtidos na Tese, sugere-se utilizar as seleções Conserva 1600 e Conserva 947, e alternativamente, Conserva 672 e Conserva 1662, além da cv. Bolinha como genitores em hibridações que busquem a resistência à *M. fructicola* em frutos. Indica-se a inclusão da cv. Aldrighi em novos trabalhos, já que todos os genótipos menos suscetíveis têm como ancestral em comum esta cultivar.

A fenotipagem nos frutos deve ser realizada em frutos próximo à maturação e com pequeno ferimento para introdução do patógeno. O ferimento é uma forma de padronizar os testes, uma vez que os microferimentos são comuns sob condições de campo. A inoculação sem ferimento ainda é necessária, porém os erros associados a esta técnica são maiores, levando a falsos positivos de resistência.

A observação da incidência da podridão-parda e a presença de esporulação – mesmo a campo – são bons parâmetros para fazer uma pré-seleção. Após este passo, os melhores genótipos deverão ser inoculados, reduzindo tempo e trabalho.

Na França, os experimentos desenvolvidos concentraram-se em testar o efeito dos ferimentos nos frutos e a sua relação com a infecção da podridão-parda (*M. laxa*). Quando inoculados com *M. laxa* e com ferimento, em um estádio coincidente com o endurecimento do caroço, os frutos mostraram-se resistentes e foi identificada uma reação vermelha associada a alguns genótipos e ao duplo estresse causado por ferimento seguido de inoculação imediata. Essa reação foi isolada e analisada por HPLC, identificando compostos novos nos frutos, principalmente da família das flavononas, sugerindo que os mesmos podem estar envolvidos nas interações planta-patógeno, sendo necessários mais estudos para confirmar sua influência.

A reação de frutos ao ferimento foi estudada de forma paralela, tanto quanto à infecção com *M. laxa*, quanto ao conteúdo de compostos fenólicos e triterpenoides (por

HPLC), e compostos voláteis (por cromatografia gasosa). Neste estudo foi demostrado como os ferimentos modificam os padrões dos compostos presentes nos frutos, sugerindo que vários destes poderiam estar determinando a suscetibilidade/resistência à podridão-parda. Esta associação é muito difícil de ser estabelecida sem ter o acompanhamento de uma análise de expressão gênica, a qual foi feita, mas os resultados ainda não se encontram disponíveis.

No mesmo sentido, foram realizados outros experimentos menores com *M. laxa*, in vitro e in vivo. Em um primeiro experimento foram testados ferimentos prévios nos frutos, realizados de um lado do fruto, no laboratório ou na planta, e após sete horas os frutos foram feridos e inoculados no lado oposto ao ferimento. Os resultados indicaram que não existe um efeito sistêmico da ferida que aumente ou reduza a suscetibilidade. No segundo experimento, *M. laxa* foi inoculada in vitro e incubada junto com frutos feridos, frutos sem ferir e na ausência de frutos. O crescimento mais rápido deu-se nos dois tratamentos que contavam com frutos, sugerindo que a *M. laxa* tenha algum mecanismo de captar a presença de seu hospedeiro, mesmo sem ter contato físico com o mesmo. Um terceiro e último experimento foi feito deixando frutos feridos junto com outros frutos, e logo ferindo e inoculando os que estavam ilesos. Esses frutos foram comparados com outros que não tinham sido expostos a frutos feridos previamente. Os resultados indicaram que a suscetibilidade à *M. laxa* foi maior nos frutos que foram expostos previamente aos frutos feridos. Isto pode estar sugerindo algum tipo de percepção, pela *M. laxa*, de compostos liberados pelos frutos feridos, mesmo eles sendo retirados antes da inoculação, hipótese que deveria ser confirmada em futuros estudos. Nestes experimentos também foram detectadas as reações vermelhas relatadas em um experimento prévio (associadas ao ferimento + inoculação com *M. laxa*), sendo descartado a influência do surfactante como o responsável pelas mesmas.

A presente pesquisa apresentou uma importante contribuição ao conhecimento da reação de um elevado número de genótipos quanto à *M. fructicola*, tanto em flores quanto em frutos, segregação, herdabilidade e ganho genético. Resultou também em uma contribuição para o entendimento da influência dos ferimentos nos frutos na suscetibilidade à infecção com *M. laxa*, relacionando os mesmos com o conteúdo de compostos fenólicos e triterpenoides, além dos compostos voláteis.

Os resultados serão úteis aos programas de melhoramento genético, na medida em que: indicaram genótipos com menor ou similar suscetibilidade à *M. fructicola* do que a cultivar Bolinha, hoje utilizada como padrão de resistência nos frutos; foi estabelecida uma metodologia mais adequada à fenotipagem das flores quanto à podridão; foram indicados genótipos mais interessantes a serem utilizados como genitores em programas que visem à resistência à podridão-parda em frutos; evidenciou-se a necessidade de tratamentos fitossanitários durante a floração, uma vez que, nas condições de Pelotas e região, há grande quantidade de inóculo latente no pomar. Além disso, trabalhos realizados na França mostraram que há diferenças bioquímicas entre genótipos mais suscetíveis ou menos suscetíveis e que o nível dos compostos é alterado de acordo com a existência de ferimentos. Indicaram, também, que a presença de frutos, principalmente se danificados, é reconhecida pelo fungo.

Estudos nesta área deverão ter uma continuidade para responder muitas questões que surgiram neste trabalho e que poderiam ser objetivo ou ponto de partida para outras futuras pesquisas, como:

- Existe efeito materno na transmissão dos caráteres fenológicos, como plena floração, data de colheita e período de desenvolvimento do fruto?
- Existe uma maior resistência dos genótipos mais tardios? Por quê?
- Os genótipos de menor suscetibilidade à podridão de flores apresentam maior conteúdo fenólico, antocianinas e capacidade antioxidante nas suas pétalas? Essa maior resistência se mantém a nível de campo? É possível selecionar genótipos com pétalas mais escuras como uma forma de seleção indireta para aumentar a resistência à *Monilinia* spp.?
- Até onde pode chegar o avanço genético com respeito à resistência dos frutos à podridão-parda aumentando a resistência do tipo horizontal?
- O maior conteúdo de compostos da família das flavononas nos frutos aumenta ou diminui a suscetibilidade às infeções de podridão-parda?
- Algum mecanismo de resistência a patógenos é ativado através de ferimentos mecânicos?
- A *Monilinia* spp. tem mecanismos de percepção e reconhecimento de seu hospedeiro? Quais? Se existirem, os mesmos poderiam ser silenciados?

Enfim, mais uma vez ficou demostrado que, para cada avanço científico, novas questões são levantadas, o que leva a um dinamismo da pesquisa.

6 Referências (Introdução geral)

ADASKAVEG, J.E.; SCHNABEL, G.; FORSTER, H. Diseases of peach caused by fungi and fungal-like organisms: biology, epidimiology and management. In: LAYNE D.R.; BASSI, D. **The peach**: botany, production and uses. Wallingford: CAB International. p. 352-406, 2008.

BARÓ-MONTEL, N.; EDUARDO, I.; USALL, J.; CASALS, C.; ARÚS, P.; TEIXIDÓ, N.; TORRES, R. Exploring sources of resistance to brown rot in an interspecific almond × peach population. **Journal of the Science of Food and Agriculture**, v. 99, n. 8, p. 4105-4113, 2019.

BIRKENMEIER, G.F.; RYAN, C.A. Wound signalling in tomato plants – evidencethat ABA is not a primary signal for defense gene activation. **Plant Physiology**, v. 117, n. 2, p. 687–693, 1998.

BROEKAERT, W.F., ZELAURÉ, S.L., DE BOLLE, M.F.C., CAMMUE, B.P.A. The role of ethylene in host-pathogen interactions. **Annual Review of Phytopathology**, v. 44, n. 1, p. 393-416, 2006.

BRUXELLES, G.L.; ROBERTS, M.R. Signals regulating multiple responses towounding and herbivores. **Critical Reviews in Plant Sciences**, v. 20, n. 5, p. 487-521, 2001.

CHEN, S.; YUAN, N.; SCHNABEL, G.; LUO, C. Function of the genetic element ‘Mona’ associated with fungicide resistance in *Monilinia fructicola*. **Molecular Plant Pathology**, v. 18, n. 1, p. 90-97, 2017.

CHEONG, Y.H.; CHANG, H. S.; GUPTA, R.; WANG, X.; ZHOU, T.; LUAN, S. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. **Plant Physiology**, v. 129, n. 2, p. 661–677, 2002.

CITADIN, I.; SCARIOTTO, S.; SACHET, M.R.; ROSA, F.J.; RASEIRA, M.C.B.; WAGNER JUNIOR, A. Adaptability and stability of fruit set and production of peach trees in a subtropical climate. **Scientia Agricola**, Piracicaba, v. 71, n. 2, p. 133-138, 2014.

ELSHAFIE, H.S.; MANCINI. E.; CAMELE, I.; DE MARTINO, L.; DE FEO, V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. **Industrial Crops and Products**, v. 66, p.11-15, 2015.

FABIANE, K.C. **Reação de pessegueiros a *Monilinia fructicola* (Wint.) Honey e sua relação com componentes bioquímicos**. 2011. 130f. Dissertação (Mestrado em Agronomia) Universidade Tecnológica Federal do Paraná, Pato Branco, 2011.

FACHINELLO, J.C.; PASA, M.S.; SCHMTIZ, J.D.; BETEMPS, D.L. Situation and perspectives of temperate fruit crops in Brazil. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 33, n. spe1, p. 109-120, 2011.

FAO. **FAOSTAT**. Food and Agriculture Organization of the United Nations; Statical Division. Roma. Disponível em: <http://fenix.fao.org/faostat/internal/en/#data/QC> Acesso em: jun. 2019.

FRANZON, R.C.; RASEIRA, M.C.B. Origem e história do pessegueiro. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília, DF: Embrapa, p. 19-23, 2014.

HU, M.J.; COX, K.D.; SCHNABEL, G.; LUO, C.X. *Monilinia* species causing brown rot of peach in China. **PLoS ONE**, v. 6, n. 9, e24990, 2011.

HILY, J.M.; SINGER, S.D.; VILLANI, S.M.; COX, K.D. 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**, v. 67, p. 385-396, 2011.

IBGE. Produção Agrícola Municipal. **Sistema IBGE de Recuperação Automática (SIDRA)**. Banco de Dados Agregados. Disponível em: <https://sidra.ibge.gov.br>. Acesso em: jun. 2019.

KOO, A.J.K., HOWEA, G.A. The wound hormone jasmonate. **Phytochemistry**, v. 70, p. 1571–1580, 2009.

LEÓN, J.; ROJO, E.; SÁNCHEZ-SERRANO, J.J. Wound signalling in plants. **Journal of Experimental Botany**, v. 52, n. 354, p. 1-9, 2001.

LUO, C.X.; HU, M.J.; JIN, X.; YIN, L.F.; BRYSON, P.K.; SCHNABEL, G. An intron in the cytochrome *b* gene of *Monilinia fructicola* mitigates the risk of resistance development to QoI fungicides. **Pest Management Science**, v. 66, p. 1308-1315, 2010.

MAY-DE-MIO, L.L.; MOREIRA, L.M.; MONTEIRO, L.B.; JUSTINIANO JÚNIOR, P.R. Infection of *Monilinia fructicola* in budding stages and incidence of brown rot on fruits in two peach production systems. **Tropical Plant Pathology**, v. 33, n. 3, p. 227-234, 2008.

MAY-DE-MIO, L.L.; GARRIDO, L.R.; UENO, B.; FAJARDO, T.V.M. Doenças da cultura do pessegueiro e métodos de controle. In: Raseira, M.C.B.; Pereira, J.F.M.; Carvalho, F.L.C. **Pessegueiro**. Brasília, DF: Embrapa, p. 355-432, 2014.

MITSUDA, N.; IWASE, A.; YAMAMOTO, H.; YOSHIDA, M.; SEKI, M.; SHINOZAKI, K.; OHME-TAKAGIA, M. NAC transcription factors, NST1 and NST3, are key regulatorsof the formation of secondary walls in woody tissues of Arabidopsis. **Plant Cell**, 19, 270–280, 2007.

MONDINO, P.; ALANIZ, S.; LEONI, C. Manejo integrado de las enfermedades del duraznero en Uruguay. In: SORIA, J. **Manual del duraznero; Manejo integrado de plagas y enfermedades**. Montevideo: INIA. p. 45-76, 2010.

OGAWA, J.M.; ZEHR, E.I.; BIRD, G.W.; RITCHIE, D.F.; URIU, K.; UYEMOTO, J.K. **Compendium of stone fruit diseases**. Saint Paul: The American Phytopathological Society, 1995. 98 p.

RASEIRA, M.C.B.; BARBOSA, W.; NAKASU, B.H.; PEREIRA, J.F.M. Pêssego. In: ALBUQUERQUE, A.C.S., SILVA, A.G., **Agricultura Tropical; Quatro décadas de inovações tecnológicas, instituicionais e políticas**. v.1 Produção e produtividade agrícola. Brasília, DF: Embrapa, p. 519-529, 2008.

RASEIRA, M.C.B.; CENTELLAS-QUEZADA, A. **Pêssego**: Produção. Brasília: Embrapa-SPI, Pelotas: Embrapa Clima Temperado, 2003. 162 p.

RASEIRA, M.C.B.; FRANZON, R.C. Melhoramento genético. In: RASEIRA, M.C.B.; PEREIRA, J.F.M.; CARVALHO, F.L.C. **Pessegueiro**. Brasília: Embrapa, p. 57-72, 2014.

SANTOS, J.; RASEIRA, M.C.B.; ZANANDREA, I. Resistência à podridão-parda em pessegueiro. **Bragantia**, Campinas/SP, v. 71, n. 2, p. 219-225, 2012.

SANTOS, J.; UENO, B. Controle da podridão-parda no Brasil. In: MITIDIERI, M.; CASTILLO, J.A. **Manejo de la podredumbre morena (*Monilinia fructicola* y *M. laxa*) en huertos frutales de Uruguay, Chile, Bolivia, Brasil y Argentina**. Santiago de Chile, p. 73-77, 2014.

SHANKER, A.K.; VENKATESWARLU, B. **Abiotic stress response in plants – physiological, biochemical and genetic perspectives**. Rijeka, Croatia: Intech, 2011. 346 p.

TOIVONEN, P.M.A.; BRUMMELL, D.A. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. **Postharvest Biology and Technology**, v. 48, p. 1–14, 2008.

TOSETTI, R.; TARDELLI, F.; TADIELLO, A.; ZAFFALON, V.; GIORGI, F.M.; GUIDI, L.; TRAINOTTI, L.; BONGHI, C.; TONUTTI, P. Molecular and biochemical responses to wounding in mesocarp of ripe peach (*Prunus persica* L. Batsch) fruit. **Postharvest Biology and Technology**, v. 90, p. 40-51, 2013.

TRINIDADE, I.; SANTOS, D.; DALMAY, T.; FEVEREIRO, P. Facing the environment: small RNAs and the regulation of gene expression under abiotic stress in plants. In: Shanker, A.K.; Venkateswarlu, B. **Abiotic Stress Response in Plants** – **Physiological, Biochemical and Genetic Perspectives**, p. 113–136. Rijeka, Croatia: Intech, 2011.

TSABALLA, A.; NIKOLAIDIS, A.; TRIKKA, F.; IGNEA, C.; KAMPRANIS, S.C.; MAKRIS, A.M.; ARGIRIOU, A. Use of the de novo transcriptome analysis of silver-leaf nightshade (*Solanum elaeagnifolium*) to identify gene expression changes associated with wounding and terpene biosynthesis. **BMC Genomics**, v. 16, n. 504, 2015.

WAGNER JÚNIOR, A. **Avaliação de germoplasma de pessegueiro, quanto à reação à *Monilinia fructicola* (Wint.) Honey**. 2003. 62f. Dissertação (Mestrado em Fruticultura de Clima Temperado), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2003.

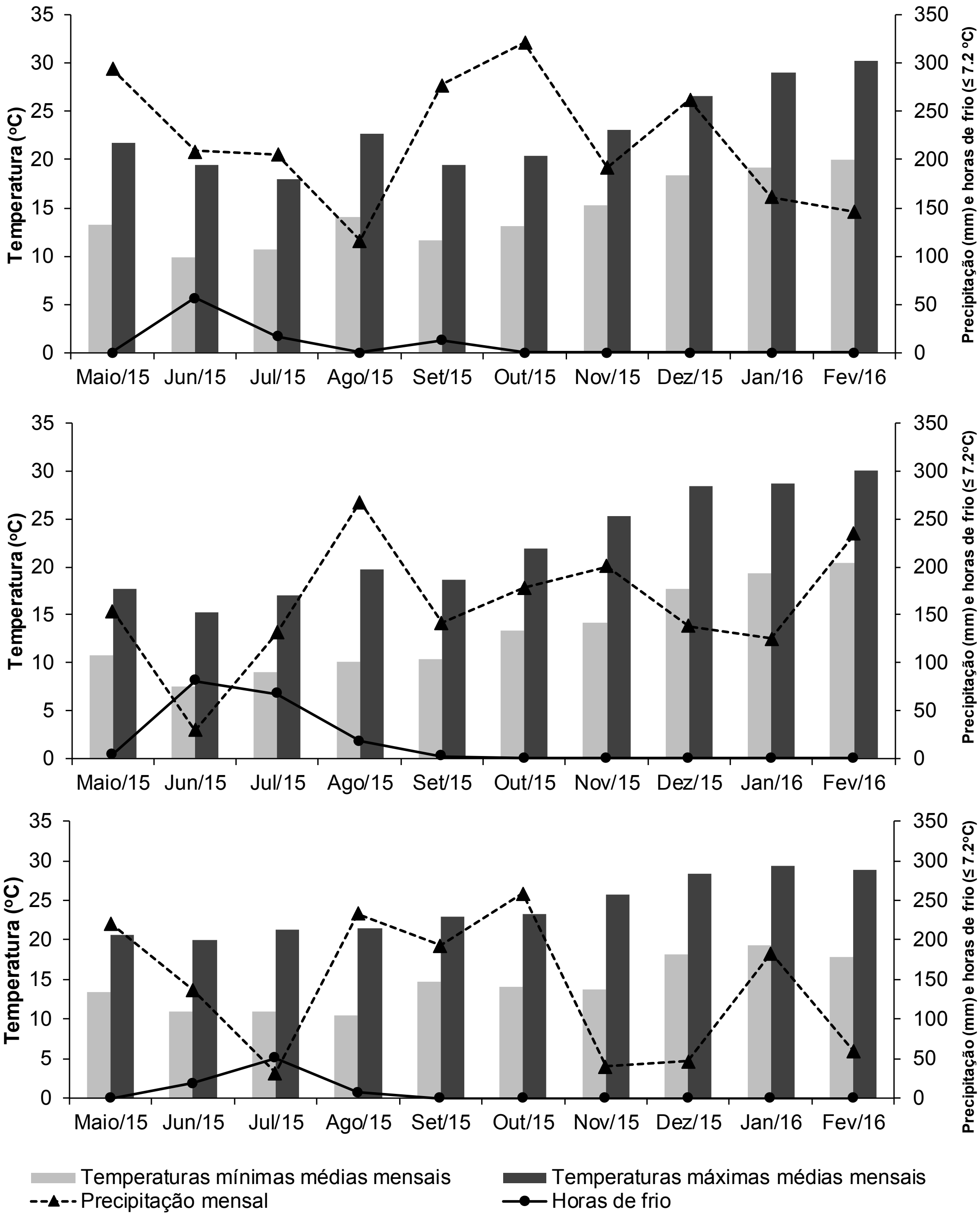
WAGNER JÚNIOR, A.; RASEIRA, M.C.B.; FORTES, J.F.; PIEROBOM, C.R.; SILVA, J.B. Non-correlation of flower and fruit resistance to brown rot (*Monilinia fructicola* (Wint.) Honey) among 27 peach cultivars and selections. **Journal of the American Pomological Society**, v. 59, n. 3, p. 148-152, 2005.

ZHOU, L.; THORNBURG, R. Wound-inducible genes in plants. In: Reynolds, P.H.S. **Inducible Gene Expression**. Wallingford, UK: CAB International, p. 127–158, 1999.

ZHU, F.; BRYSON, P.K.; SCHNABEL, G. Influence of storage approaches on instability of propiconazole resistance in *Monilinia fructicola*. **Pest Management Science**, v. 68, n. 7, p. 1003-1009, 2012.

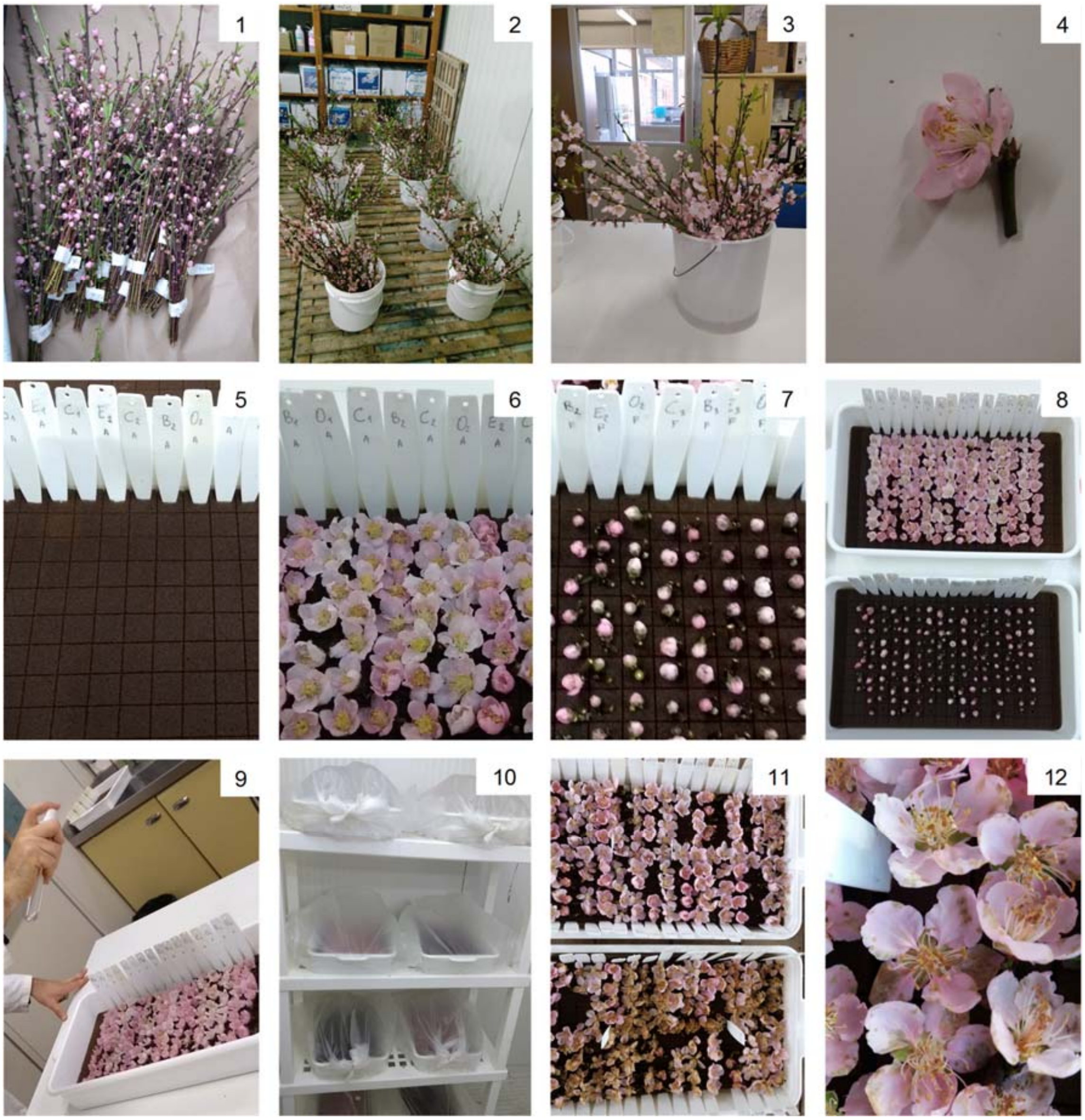
Apêndices

Apêndice A – Temperaturas mínimas e máximas médias mensais, precipitação mensal e horas de frio acumuladas abaixo de 7,2°C, nas safras 2015-2016 (A), 2016-2017 (B) e 2017-2018 (C). Embrapa Agricultura Temperada, Pelotas, Rio Grande do Sul, Brasil.



Fonte: AGROMET/CPACT/EMBRAPA (Pelotas, Rio Grande do Sul, Brasil).

Apêndice B – Fenotipagem da suscetibilidade/resistência à podridão das flores causada pela *Monilinia fructicola*, utilizando a técnica de flores destacadas, Embrapa Clima Temperado, Pelotas, Brasil.

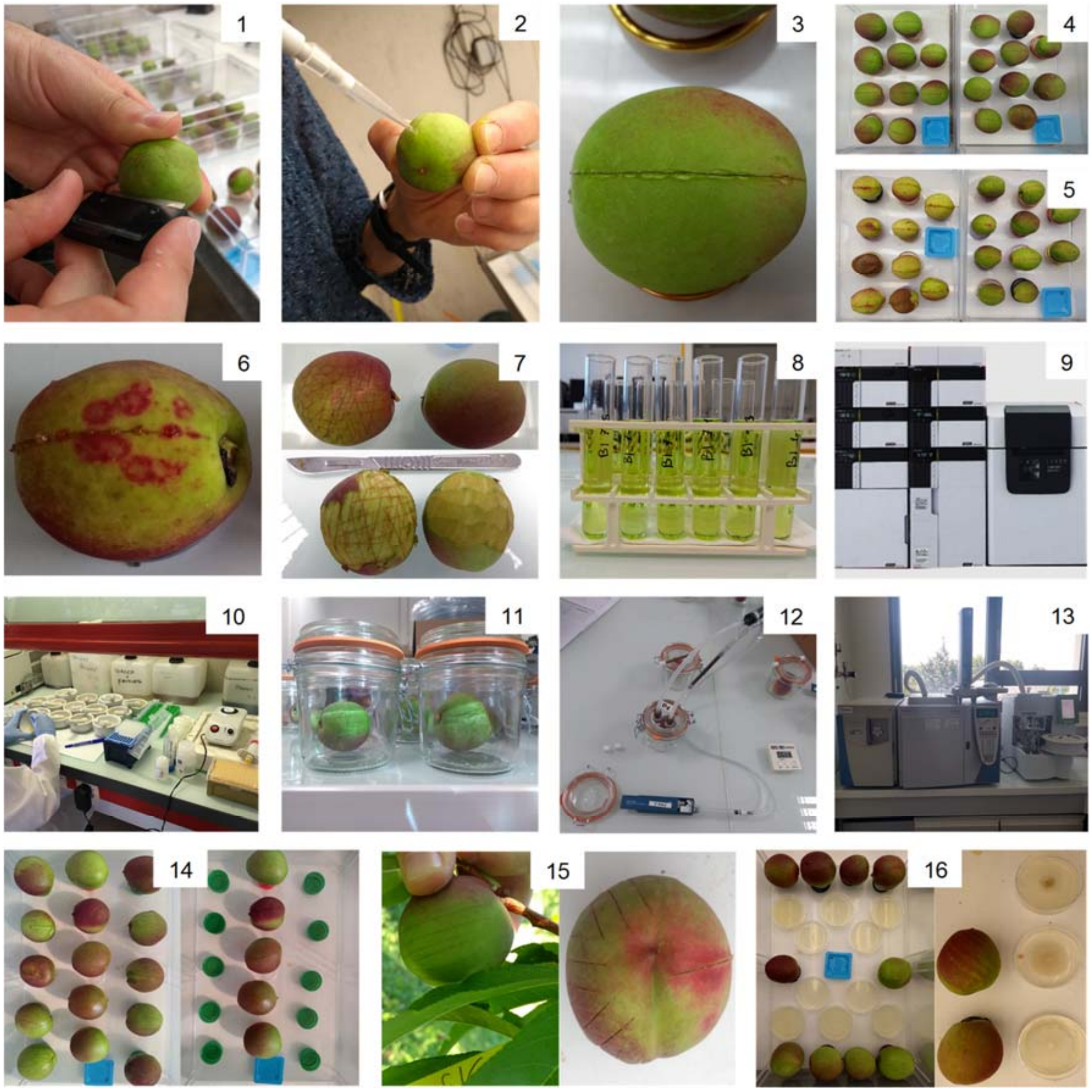


Ramos produtivos coletados e identificados segundo o genótipo (**1**), flores abertas, danificadas ou com infecção aparente foram descartadas; Ramos mantidos em água em câmara fria 4±1°C por 48 horas (**2**); Ramos depois de sair da câmara fria mantidos por mais 24 horas a temperatura ambiente, no laboratório (**3**); Flores destacadas com uma porção do ramo (**4**); Bandejas plásticas com espuma fenólica Green-up® (**5**); Flores abertas (**6**) e em estado de balão (**7**) dispostas na espuma fenólica para realizar as inoculações; Bandejas contendo as flores prontas para a inoculação (**8**); Inoculação feita com borrifador de gota fina com uma suspensão de *Monilinia fructicola* (**9**); Bandejas cobertas por plástico em câmara de crescimento a 23±1°C, 75% umidade relativa e 12 horas de fotoperíodo (**10**); Flores depois de 72 horas de incubação, sem inoculação (arriba) e com inoculação (abaixo) (**11**); Detalhe de flores com sintomas de podridão das flores 72 horas após a inoculação.

Apêndice C – Fenotipagem da suscetibilidade/resistência à podridão-parda causada pela *Monilinia fructicola*, utilizando a técnica de deposição em gota com e sem ferimento, Embrapa Clima Temperado, Pelotas, Brasil.

Frutos colhidos e identificados no laboratório (**1**); Processo de desinfestação dos frutos, um minuto a álcool 70%, três minutos cloro 0,5% (**2**), e tríplice lavagem com água destilada (**3**); Frutos desinfestados sobre anéis nas caixas plásticas (**4**); Microseringa de 100 µL acoplada em um dispensador de repetição 50x (Hamilton®), utilizada para fazer o ferimento e inocular os frutos (**5**); Frutos inoculados com (**6**) e sem (**7**) ferimento com uma suspensão de *Monilinia fructicola* de 2,5 x 10^4 conídios mL^{-1}; Incubação dos frutos dentro das caixas plásticas, em câmara de crescimento a 23±1°C, 75% umidade relativa e 12 horas de fotoperíodo (**8**); Frutos inoculados com (**9**) e sem (**10**) ferimento após 72 horas de incubação; Avaliação do diâmetro da lesão (**11**) e da esporulação (**12**) de *M. fructicola*; Material utilizado para a retirada de amostras da zona de esporulação de *M. fructicola*, utilizando um cortador circular de 5 mm, conservados em vidros com 1 mL de ácido láctico (**13**).

Apêndice D – Resumo das técnicas e equipamentos utilizados nos experimentos desenvolvidos no estágio no GAFL, INRA Avignon, França.



Corte longitudinal (3 mm de profundidade) para lesionar os frutos (**1**); Inoculação dos frutos com 10 µL de suspensão de *Monilinia laxa* a 1,0 x 10^5 conídios mL^{-1} (**2**); Fruto inoculado (**3**); Frutos logo depois (**4**) e 216 horas depois da inoculação (**5**); Detalhe da reação vermelha detectada em frutos imaturos inoculados com *M. laxa* imediatamente depois de realizada a ferida (**6**); Frutos feridos (esquerda) e não feridos (direita) antes e depois de retiradas as amostras para as análises de HPLC e transcriptômica (**7**); Extratos metanólicos para realizar as análises de HPLC (**8**); Equipamento Shimadzu (Prominence) utilizado para as análises HPLC (**9**); Extração de RNA para transcriptômica (**10**); Vidros vedados (**11**) e equipamentos (**12**) utilizados para a extração de compostos voláteis; Equipamento Trace-ISQ (Thermo) utilizado para as análises de cromatografia gasosa (**13**); Frutos feridos dentro de uma caixa vedada com frutos ilesos (esquerda), retirada dos frutos feridos e inoculação dos outros frutos (direita) (**14**); Frutos feridos em uma face na árvore (esquerda) e frutos inoculados na outra face (direita) (**15**); Frutos feridos dentro de uma caixa vedada com placas de Petri contendo BDA e *M. laxa* (esquerda), placas de Petri depois de 168 horas de incubação (**16**).