Short title: Protocols to evaluate brown rot tolerance in peach fruits

Title: Optimizing protocols to evaluate brown rot (*Monilinia laxa*) susceptibility in peach and nectarine fruits

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Abstract

This study assessed and optimized an *in vivo* method to evaluate the levels of susceptibility/resistance in fruit from the EEAD-CSIC peach germplasm to an isolate of *Monilinia laxa* (Aderhold & Ruhland) Honey from peach. A total of four commercial cultivars and six genotypes, descendants of three families, of peach [*Prunus persica* (L.) Batsch] were superficially inoculated in fruits as “uninjured inoculation fruit test”. Inoculum was obtained from artificially infected peach fruit after five days of incubation under a photoperiod of 12 h supplied by fluorescent lighting system. Spores were harvested from the fruit, incubated at 20-26 °C, of 40-60 % RH, being careful to avoid contamination. Production of inoculum (conidia) was rapid and adequate using these inoculation and incubation conditions (five days at 23 °C) indicating that the *M. laxa* used was highly pathogenic and inoculation protocol suitable to screen the peach material and commercial cultivars. Lesion diameter and colonization extent were measured on inoculated fruits to estimate disease severity (colonization severity and lesion severity) to establish levels of

susceptibility/resistance to brown rot. Results from the screening test showed that although all peach genotypes and commercial cultivars screened were susceptible to the *M. laxa* isolate, the lack of sporulation on lesions of ‘Calante’ cultivar and the significant differences in colonization and lesion severities among the genotypes indicated the existence of genetic tolerance to *M. laxa* infection. The reasons for the differences in tolerance to infection and implications for breeding peach fruit in the Ebro valley are discussed.

Additional key words: *Prunus persica*; *ex-situ*; *in-situ*; screening germplasm; fungus.

Introduction

Peach [*Prunus persica* (L.) Batsch] is the third most important global tree crop after apples and pears within the economically important Rosaceae family (FAOSTAT 2016). The largest producer is China, followed by Italy, Spain, and the United States (FAOSTAT 2016). Brown rot is a disease principally associated with the peach fruit. It is caused by *Monilinia* species which include: *Monilinia laxa* (Aderhold and Ruhland) Honey, *Monilinia fructigena* (Honey) and *Monilinia fructicola* (G. Winter) (Hu et al. 2011; Zhu et al. 2014). As polycyclic pathogens they produce numerous secondary cycles throughout the annual growth cycle of the host (Schumann and D`Arcy 2006) affecting harvest, storage and commercial shelf life of the product (Gell et al. 2007; Thomidis et al. 2008; Sisquella et al. 2014).

Until 2006, peach brown rot in Spain was only caused by either *M. laxa* (Aderh & Ruhl) Honey or *M. fructigena* Honey in Whetzel (Gell et al. 2008, 2009). Of the species, *M. laxa* was the most prevalent (85-90%), followed by *M. fructigena* (10-15%) (Larena et al. 2005). However, since 2006 both species, *M. laxa* and *M. fructigena*, may have co-existed

at the same relative frequency (Villarino et al. 2013; De Cal et al. 2014). The entrance of *M. fructicola* in peach orchards in the Ebro Valley, Lerida, Spain (Villarino et al. 2013) and cohabitation with *M. laxa*, since 2010 led to the displacement of *M. fructigena* (Villarino et al. 2016).

In Spain the disease causes damage of 59-100 % after harvest particularly within favourable environmental conditions (Villarino et al. 2012). Synthetic fungicides like benzimidazoles, dicarboximides and demethylation inhibitors (Egüen et al. 2015) are used to control the disease in orchards in Spain. Also antagonistic microorganisms such as *Penicillium frequentans* and *Epicoccum nigrum* fungi (Guijarro et al. 2008; Larena et al. 2010) are examples of pre-harvest control measures. Increasing concern about the effects of biochemical fungicides on the environment (Giobbe et al. 2007), human health (Thomidis and Exadaktylou 2010), and strain fungicide resistance (Ritchie 1982) views new alternatives, such as host resistance, as one of the most cost effective and environmentally safe strategies for disease control.

In fruit crops different methodologies and varying protocols have been assayed in breeding programs to evaluate genetic resistance to fungi (Gradziel 1994, 2003; Byrne 2005; Biggs and Miller 2005). Different parts have been used to study genetic resistance such as flowers in apricot (Christen et al. 2012), twigs and shoots in pears, quince and blueberry (Bell et al. 2004; Polashock and Kramer 2006), or fruits in peach and citrus (Gradziel and Wang 1993; Sanzani et al. 2014). Moreover, evaluations have consistently been performed *in-situ* with attached fruits in natural ambient situation in the field on peaches and nectarines (Rubos et al. 2008) or *ex-situ* in apples (Biggs and Miller 2005); involving detached apricot fruits under controlled systems (Walter et al. 2004).

In fruits, inoculum could be applied superficially on a specific spot (Northover and Biggs 1990; Walter et al. 2004) or placing mycelium agar plugs on the fruit (Biggs and Miller 2005); or dipping the fruit into an inoculum suspension (Denoyes-Rothan and Guerin 1996; Gordon et al. 2005), even though injecting spores beyond the epidermis (Pascal et al. 1994). Finally, different inoculum concentration, treatment load, incubation period and temperature have been assayed for screening in stone fruits: 10 μ L load of 2×10^4 spores mL^{-1} (Gradziel and Wang 1993) and 30 μ L of different spore density (10^6 to 10^3 mL^{-1}) (Northover and Biggs 1990; Bonaterra et al. 2003; Walter et al. 2004).

Nowadays, there are numerous methodologies describing partially similar protocols concerning spores concentration and inoculum load for screening brown rot (*Monilinia* spp) tolerance in peach and nectarine (Casals et al. 2012; Kreidl et al. 2015; Cindi et al. 2016). However, little information is available on the genetic screening of peach progenies for tolerance to brown rot of *Monilinia* spp. in the Ebro valley region. Casals et al. (2012), used 50 μ L of *M. fructicola* at different concentrations of 10^4 , 10^5 and 10^6 of spores mL^{-1} ; while other authors used 10^4 of spores mL^{-1} ; of 10 and 50 μ L inoculum load to study *Monilinia* species and *M. laxa*, respectively (Kreidl et al. 2015; Cindi et al. 2016). However, other factors, such as inoculum production, incubation time, etc., are not comparable. Kreidl et al. (2015) used fresh cultures of *Monilinia* grown on PDA media at 25 °C for 7 days (12 h light, 12 h dark) for enhancement of sporulation and conidia production. Casals et al. (2012) and Cindi et al. (2016) used 12 day old of *Monilinia* grown on PDA culture for inoculation.

Using the available methods, longer periods are required for obtaining the source of inoculum and the amount and purity of inoculum are not assured. Moreover, an easy

inoculation system would help to screen large populations and controlled environment would be preferable to avoid cross contamination. We have modified the existing protocols by shortening the inoculum production and also ensuring the accurate supply of inoculum. The objective of this study, therefore, was to have an evaluation method, by optimizing the available protocols, to screen tolerance to brown rot by *Monilinia* spp. in peach germplasm. We have evaluated brown rot tolerance on *Monilinia laxa ex situ* inoculated peach fruit by measuring brown rot incidence (%), lesion diameter (mm) and colonization extent (mm). In the Spanish peach collection at the EEAD-CSIC, screening for resistance to brown rot has shown that local germplasm accessions are susceptible although slight differences can be found.

Materials and Methods

Plant material

A total of two commercial peach ('Calante' and 'Catherina') cultivars, two nectarines ('Venus' and 'BigTop') and six progenies of peach [*Prunus persica* (L.) Batsch] were evaluated (Table 1). All cultivars, except 'Venus' and 'Calante', were grown in the experimental station of Aula Dei-CSIC. Orchards are all in the Ebro valley (Northern Spain, Zaragoza) under a Mediterranean climate. All progenies are descendant from the crossing of 'Babygold 9' × 'VAC-9510'; 'Babygold 9' × 'Crown Princess' and 'Andross' × 'Calante'. They were grown under standard cultural practices (pruning, collection and adequate disposal of mummies, irrigation and weeding) and chemical spray programs for pest and disease control. In 2012, any fungicide treatment was applied in the field before harvest. The preventive fungicide Teldor® 500 SC was only sprayed after regular irrigation or rainfall on July 16 and August 7. Fruits were harvested based on optimum maturity

(Cantín et al. 2009) (expressed on visual colour change and manual evaluation of firmness, selecting healthy fruit with uniform ripeness and size). Peaches were disinfected by immersion in aqueous solution of 1.6 % sodium hypochlorite (commercial), 0.005 % Tween® 80 (polysorbate surfactant) and 1.6 % ethyl alcohol for 4 minutes, rinsed in sterile distilled water, and spread out on sterile holding stone-fruit cardboard cells for 20 minutes ambient air drying in blossom-stem (upside down) position to avoid any possible percolation at the stem position.

Pathogen culture

The culture of the *Monilinia laxa* (Aderh. & Ruhl.) Honey used in this study was supplied by the Post-harvest Pathology Unit, IRTA Lerida, and it was isolated from peach from the Cataluña region of Spain (isolate number: CPML02). The strain was maintained at 4 °C in the dark on 39 gL⁻¹ solid potato dextrose agar (PDA) medium (Panreac, Spain). The fungal culture was replicated biweekly and maintained at 23 °C under continuous darkness during the periods in which the experiments were conducted. There was also periodic inoculation on peach fruit for preservation of pathogenicity according to Koch-Pasteur's postulates (De Cal et al. 1990).

Conidia production and inoculation

To ensure sufficient conidia production (Kreidl et al. 2015) for the susceptibility studies, the isolate was inoculated onto surface sterilized peach fruits ('Catherina' cv.), to avoid surface contaminants, by wounding the fruit (1 x 2 mm) with a sterile surgical blade and transferring mycelium plug from the PDA culture to the wound site using a sterile inoculating needle. We have used wounded non-inoculated fruits as control to determine the existence of latent infections. The commercial cardboard box hosting the fruits was

covered with sterile transparent polythene sheet, with the ends slightly sealed with sellotape. Fruits were incubated at 20-26 °C, of 40-60 % RH and photoperiod of 12 h supplied by a mixture of two fluorescent lighting systems (Sylvania Gro-Lux, F36W / GRO and Ogram Daylight, F36W / 840DL of the Fridger Growth Chamber, Spain) for 4 to 6 days.

Conidia were efficiently harvested into a solution of sterile distilled water and Tween® 80 (0.0005%) surfactant by mildly rubbing over the infected regions of the fruit using a sterile dental brush. This involves superficial submerge of sample in the harvest solution and relative slight spore wash with the brush (restrained harvest as against profound harvest method). The spore solution was stirred at 1500 r.p.m. for 3 min, and then filtered through a sterile fourfold cheese cloth to remove mycelia and inert particles. Quantification of conidial suspension was determined on a hemocytometer (Neubauer new improved chamber) and the density adjusted to $25 \times 10^3 \text{ mL}^{-1}$ for inoculation purposes.

To evaluate tolerance to brown rot, 20 disinfected fruits from each genotype were inoculated with the isolate of the virulent pathogen. On the equatorial position of each unwounded fruit, 25 μL spore load (one drop) of the $25 \times 10^3 \text{ mL}^{-1}$ conidial suspension of *M. laxa* was deposited. Five fruits used as control were loaded with 25 μL sterile water accordingly.

Incubation and brown rot evaluation.

Inoculated fruits were incubated for 5 days in darkness at 23 °C and 40-60% RH (Climatronic 2132-model growth incubator, Germany). Brown rot incidence was assessed using the fraction infected over total number of inoculated fruits. Lesion diameter and colonization extent were measured across perpendicular sectors (longitudinal and

latitudinal fruit sectors) with a digimatic venier caliper (Mitutoyo CD-15 DCX, Tokyo Japan). These parameters were used in the determination of brown rot disease severity for genotype tolerance rating in the study according to (Martínez-García et al. 2013). Lesion severity (LS) was calculated by the percentage of brown rot incidence (% BRI) x lesion diameter (LD) /100 and colonization severity (CS) by the percentage of colonization (% C) x colonization extent (CE)/100. Mean, standard errors (SE) and Pearson correlations were carried out with SPSS-19 statistical software (Statistical Product and Service Solutions Inc., Chicago USA). Statistical significance was judged at the level $P < 0.05$, and the Duncan test was used for mean comparison (ANOVA test).

Results

As indicated in Table 1, there was 100 % brown rot incidence (BRI) in all genotypes except for the 3_(A × C) genotype with 80 %, and the ‘Venus’ cultivar with 60 %. There were significant differences within the mean colonization extent and lesion diameters (Table 1), as well as in the colonization and lesion severities (Fig. 1).

The 3_(A × C) genotype, and ‘Big Top’, ‘Venus’ and ‘Calante’ cultivars exhibited brown rot severity (BRS) lower than 40 mm (Fig. 1) but only significant in ‘Calante’. Lesion severity (LS) was between 62.52 and 20.97 mm, colonization severity (CS) was between 50.68 mm and zero. The ‘Venus’ and ‘Big Top’ cultivars and 2_(B9 × CP) and 3_(A × C) genotypes showed colonization severity below 40 mm (16.22 mm, 32.96 mm, 28.78 mm and 21.65 mm, respectively). The ‘Calante’ was the only cultivar without colonization owing to no or restricted sporulation and therefore, zero CS.

The Pearson’s correlation coefficients (r) between pairs of traits are shown in Table 2. Brown rot incidence (% BRI) significantly correlated with all pathologically associated

factors [LD ($r = 0.293$, $P \leq 0.01$); LS ($r = 0.471$, $P \leq 0.01$); CE ($r = 0.349$, $P \leq 0.01$) and CS ($r = 0.473$, $P \leq 0.01$)]. This indicates that the level or frequency of infection will always and significantly influence the lesion diameter and colonization extent including severities in a disease situation. All pathological traits showed significant correlation higher than 0.883. Also harvest date showed a positive and significant correlation with % BRI ($r = 0.314$, $P \leq 0.01$); LS ($r = 0.405$, $P \leq 0.01$) and CS ($r = 0.313$, $P \leq 0.01$).

Discussion

In this study, we have validated the inoculation method finding differences in the susceptibility to the pathogenic activities of *M. laxa* in two peaches ('Catherina' and 'Calante'); two nectarines ('Venus' and 'Big Top') and six peach hybrids. In order to screen a large germplasm sample we have modified the existing methods. Incubation factors as temperature (20-26 °C), relative humidity (40-60%) and 12 h photoperiod enhanced colonization and sporulation for substantial conidia production. We have found that the peculiar lighting source (mixture of two fluorescent lighting systems, Sylvania Gro-Lux, F36W / GRO and Ogram Daylight, F36W / 840DL) was positive to obtain enough inoculum in a short period of time. We have also found that artificial injury ensured adequate and prompt infection of host (Casals et al. 2010; De Cal et al. 2013). Fruit injury encouraged short period of incubation making available inoculum source within 3 days post inoculation and avoiding cross-contamination of other fungi such as *Botrytis* and *Rhizopus* species. Moreover, the purity of inoculum load was ensured with this method because potential contaminants such as nematodes were avoided during conidia harvest by mildly rubbing over the infected regions of the fruit using a sterile dental brush. Initially enough *M. laxa* inoculum was produced from PDA culture incubated (Memmert CO₂ incubator

INCO, Germany) at least 35 days at 23 °C in the dark. Nevertheless, we tried to reduce the time to obtain inoculum adapting the methods used by Gell et al. (2007) and Jansch et al. (2012). They incubated prepared PDA cultures of *Monilinia* spp in the dark at 20-25 °C for 7 to 10 days for adequate sporulation. But our preliminary trials (data not shown) indicated poor inoculum production when incubated in the dark even with more than 6 days of incubation at 23 °C. Finally we have assured effective inoculum in 4 to 6 days after incubation of inoculated peaches under at temperature (20-26 °C), relative humidity (40-60%) and 12 h photoperiod.

Furthermore, the composition of inoculum for susceptibility screening could involve one pathogenic strain (Gradziel and Wang 1993) or two or more pathogenic isolates pooled (Biggs and Miller 2005) to enhance pathogen virulence. A mono-conidial culture was used in our study because our inoculum source was tested for stable virulence and was stable enough to initiate pathogenesis without need for strain synergism. We established effective inoculum density of $25 \times 10^3 \text{ mL}^{-1}$ with inoculating load of 625 spores per fruit. Pascal et al. (1994) though employed a mono-conidial isolate but higher loading of 20,000 spores/fruit that may be less sustainable.

We have evaluated 20 fruits per genotype as enough sample size to assess representative evaluation considering the large population involved in plant breeding. The number of samples used to evaluate disease tolerance against brown rot in stone fruit varies among researchers from 10 peaches per genotype (Gradziel and Wang 1993) to 30 fruits in apricot, plum and peach (Pascal et al. 1994). Moreover, the preference for the *ex-situ* evaluation is that the methodology enables for effective and efficient handling of large population and sample material. The *ex-situ* system also enables for thorough selection of

healthy fruits and disinfection from insects and potential contaminants. Also essential is that direct influence such as variation in temperature, precipitation and relative humidity are avoidable environmental factors when screening, for brown rot tolerance, under a controlled environment. The germination and other pathogenic activities of conidia are markedly influenced by the interaction of the above mentioned factors (Xu et al. 2001; Casals et al. 2010).

The 3_(A × C) genotype, and ‘Big Top’, ‘Venus’ and ‘Calante’ cultivars exhibited brown rot severity (BRS) lower than 40 mm (Fig. 1). Paunovic and Paunovic (1996) have reported similar susceptibility values in peach germplasm in Yugoslavia. However, their investigation was done *in-situ* involving five pathogens (*M. laxa*, *M. fructicola*, *Sphaerotheca pannosa*, *Tapharina deformans* and Sharka virus).

As shown in Table 2, brown rot incidence (% BRI) showed positive and significant correlations with all pathologically associated factors (LS, CE and CS). This indicates that the level or frequency of infection will always and significantly influence the lesion diameter and extent of colonization including severities in a disease situation. Also harvest date showed a positive and significant correlation with % BRI, LS and CS. Inferring that genotypes that mature later generally tended to have bigger lesions and would be less tolerant to fungal pathogen. This is the first time to find that the pathological parameters correlate, positively, with Julian days. However, an ANOVA showed that there were scarcely any significant differences in pathological traits between early and late harvested genotypes (Table 1).

There was a particular interest on the ‘Calante’ cultivar which, though produced lesion, was devoid of colonization (sporulation) (Table 1), an important factor in disease

spread. This feat is biologically and epidemiologically an advantage for plant breeding. Its high level of tolerance may be related to a late harvest or to the agronomical practices performed in Alcañiz. In these orchards, due to the singularity of this late cultivar harvested in October, fruits are protected to pathogen injuries using paper bags. Other authors have found annual variation for brown rot infection in ‘Calante’ (Obi et al. 2015; Ágreda et al. 2016). It was described that colonization in nectarines and peaches fruit infected by *Monilinia* spp. could be associated with the local acidification of the host tissue (De Cal et al. 2013) and that acidic environment can prevent brown rot colonization on peach (Bonaterra et al. 2003), however, it is not the explanation for our results since the fruit has at maturity the same pH or acidity year by year. Other uncontrolled factors such as environmental instead of genetic may be responsible for non-colonization in ‘Calante’ harvested in the 2012. Moreover, colonization on host (extensive differentiation of hyphae at the surface of host) is not a genetic trait (Giobbe et al. 2007).

According to this research we have not found differences indicating the impact of hairiness on susceptibility although other authors have proposed that fruit hairiness could encourage susceptibility to disease infection in some stone fruits (Wade and Cruickshank, 1992; Xu et al. 2007; Garcia-Benitez et al. 2016). In our plant material using this protocol we have demonstrated a relatively similar degree of tolerance/susceptibility between peach and nectarine fruits. An example are the low levels of *Monilinia laxa* incidence registered with the ‘Venus’ nectarine and 3_(A × C) peach genotype or the lower LS found in ‘Venus’ in comparison with the rest of cultivars.

Conclusions

In this study an efficient *ex-situ* procedure through artificial skin inoculation to assess the susceptibility to brown rot (*Monilinia laxa*) of commercially ripe fruits was performed in ten peach and nectarine genotypes. We have modified the existing protocols by shortening the inoculum production and also ensuring the accurate supply of inoculum. The methodology has been tested and shall be applied in our breeding program.

The artificial injury ensured adequate and prompt availability of inoculum source. Incubation factors as temperature (20-26 °C), relative humidity (40-60%) and 12 h. photoperiod with two fluorescent lighting systems enhanced colonization and sporulation for substantial conidia production. In addition, this method ensured between 3 to 6 days the optimum conidia purity considering the ‘restrained’ harvest system we adopted to obtain inoculum.

Finally, it was possible to discriminate between highly and less susceptible peach germplasm at the EEAD. However, we have observed on a few of the genotypes the interaction of firmness and soluble solid compounds with pathologic factors. For further studies we suggest to consider adequately in the screening for tolerance, firmness, soluble solid compounds (at harvest and incubation) and other agronomic and quality traits. On the development of brown rot the effect of the agronomical practices shall be surveyed to ascertain influence on evaluation of the tolerance.

Acknowledgments

Dr. Josep Usall of the Phytopathology Unit, IRTA Lleida for the original inoculum of the *M. laxa* (isolate number: CPML02). Jose Luis Espada of the Department of Agriculture, Government of Aragon, provided us with the ‘Calante’ cultivar. The Research Center and Food Technology of Aragón (CITA) supplied the ‘Venus’ cultivar and also allowed us the

use of its plant protection facilities. Dr. Antonieta De Cal of the National Institute of Technology and Agricultural Research (INIA) Madrid for her useful professional input. We thank all of them. This work was financed by the MINECO with projects AGL2011-24576 and AGL2014-52063R; and the Government of Aragón with project A44, co-financed with FEDER and ESF, respectively

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447 **Table 1.** List of accessions, harvest date, brown rot incidence and mean $\pm$ SE of the pathological parameters evaluated in 2012.

Accession name	Fruit type	Harvest date (Julian days)	Brown rot incidence (BRI) (%)	Colonization extent (CE) (mm)	Colonization severity (CS) (mm)	Lesion diameter (LD) (mm)	Lesion severity (LS) (mm)	Source
1_(B9×VAC)	Peach	208	100	51.50 $\pm$ 3.6 c	51.50 $\pm$ 3.6 d	57.10 $\pm$ 1.4 b	57.10 $\pm$ 1.4 c	EEAD
2_(B9×CP)	Peach	219	100	28.78 $\pm$ 5.7 b	28.78 $\pm$ 5.7 bc	49.85 $\pm$ 3.2 ab	49.85 $\pm$ 3.2 bc	EEAD
3_(A×C)	Peach	219	80	27.07 $\pm$ 7.0 b	21.65 $\pm$ 5.6 bc	47.06 $\pm$ 2.7 ab	37.65 $\pm$ 2.3 b	EEAD
4_(A×C)	Peach	240	100	49.76 $\pm$ 2.4 c	49.76 $\pm$ 2.4 d	55.40 $\pm$ 3.5 b	55.40 $\pm$ 3.5 c	EEAD
5_(A×C)	Peach	240	100	49.68 $\pm$ 2.6 c	49.68 $\pm$ 2.6 d	61.95 $\pm$ 2.1 b	61.95 $\pm$ 2.1 c	EEAD
6_(A×C)	Peach	240	100	50.68 $\pm$ 3.9 c	50.68 $\pm$ 3.9 d	62.52 $\pm$ 2.8 b	62.52 $\pm$ 2.8 c	EEAD
Big Top	Nectarine	218	100	32.96 $\pm$ 8.8 b	32.96 $\pm$ 8.8 c	38.26 $\pm$ 8.6 a	38.26 $\pm$ 8.6 b	EEAD
Calante	Peach	291	100	00.00 $\pm$ 0.0 a	00.00 $\pm$ 0.0 a	38.00 $\pm$ 5.8 a	38.00 $\pm$ 5.8 b	Alcañiz
Catherina	Peach	208	100	48.56 $\pm$ 1.4 c	48.56 $\pm$ 1.4 d	54.33 $\pm$ 0.9 b	54.33 $\pm$ 0.9 c	EEAD
Venus	Nectarine	218	60	27.04 $\pm$ 6.9 b	16.22 $\pm$ 4.2 b	34.95 $\pm$ 6.0 a	20.97 $\pm$ 3.6 a	CITA

448 SE: Standard error (n = 5-20); source: location of sample orchard. B9: ‘Babygold 9’, VAC: ‘VAC 9510’, A: ‘Andross’; CP: ‘Crown
449 Princess’; ‘Calante’ was grown in Alcañiz (Teruel) and ‘Venus’ in orchards of CITA-Aragón. Means with different letters show
450 significant differences at $P \leq 0.05$ (Duncan’s test).

Table 2 Pearson`s correlation coefficients among pathological traits and harvest date

Traits	LD	LS	CE	CS	Harvest date
	(mm)	(mm)	(mm)	(mm)	(Julian days)
BRI (%)	0.293 ^{**}	0.471 ^{**}	0.349 ^{**}	0.473 ^{**}	0.314 ^{**}
LD (mm)		0.980 ^{**}	0.901 ^{**}	0.908 ^{**}	0.374 ^{**}
LS (mm)			0.883 ^{**}	0.920 ^{**}	0.405 ^{**}
CE (mm)				0.989 ^{**}	0.285 ^{**}
CS (mm)					0.313 ^{**}

Abbreviations: BRI = Brown rot incidence; LD = Lesion diameter; LS = Lesion severity;
 CE = Colonization extent; CS = Colonization severity; ** = Correlations significant at $P \leq$
 0.01.

Fig. 1 Brown rot severity (BRS, in mm) on the studied genotypes. Lesion severity = %BRI x Lesion Diameter. Colonization severity = % Colonization x Colonization extent. Bars represent the standard errors (n = 5-20). The dotted line indicates brown rot severity less than 40 mm. Means with different letters show significant differences at $P \leq 0.05$ (Duncan's test).