Breeding strategies for identifying superior peach genotypes resistant to brown rot

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Running head: Screening peach germplasm for breeding purpose.

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Abstract

A sustainable approach to control the incidence of brown rot in pre- and post-harvest management is to select genotypes with high contents of antioxidant compounds and tolerance to *Monilinia laxa* (Aderh. and Ruhland) Honey. In this study, 68 progenies of the 'Babygold 9' × 'Crown Princess' population from the EEAD-CSIC breeding program were screened under controlled conditions for a period of 3 years (2013–2015). Susceptibility to brown rot was evaluated after inoculating 20 healthy fruits per genotype with *M. laxa*. Brown rot incidence, lesion diameter, and colonization extent, as well as the severities of these issues, were calculated after 5 days of incubation. Physicochemical traits, such as fruit firmness and soluble solids content, were also recorded before and after storage. Titratable acidity, pH, and antioxidant composition were measured at harvest. Significant differences were found for pathogenic traits, as well as for contents of vitamin C, total

phenolics, flavonoids, and anthocyanins, within genotypes in this population. Negative correlations were also found between the content of phytochemical compounds (such as anthocyanins and total phenolics), as well as disease incidence and severity. Differences in susceptibility to brown rot confirm the genetic variability available in these progeny. This allowed the selection of six genotypes highly resistant to brown rot of *M. laxa*, with high organoleptic properties and high phenol content, to be introduced in our peach breeding program.

Key words: Genetic tolerance, bioactive, susceptibility, screening, brown rot, plant breeding**.**

Abbreviations used

AsA: Ascorbic acid; **B9** × **CP**: 'Babygold 9' × 'Crown Princess'; **%BRI**: percentage brown rot incidence; **LD**: lesion diameter; **C3GE**: cyanidin-3-glucoside equivalents; **CE**: catechin equivalents; **CEx**: colonization extent; **LS**: lesion severity; **CS**: colonization severity; **%C**: percentage colonization; **GAE**: gallic acid equivalents; **HD**: harvest date; **FW**: fresh weight; **FtW**: fruit weight; **FF**: fruit firmness; **SSC**: soluble solids content; **TA**: titratable acidity; **Vit C**: Vitamin C; **TPC**: total phenolic content; **JDs**: Julian days; **Vs**: versus.

1. Introduction

The storage life and commercial shelf life of the peach [*Prunus persica* (L.) Batsch] are negatively influenced by pre- and post-harvest diseases that are principally associated with brown rot (Sisquella *et al*., 2014). Brown rot of stone fruits is a disease primarily caused by *Monilinia* species, such as: *M. laxa* (Aderh. and Ruhland) Honey; *M. fructigena* Honey; *M. fructicola* (G. Winter) Honey and *M. polystroma* (G. Leeuwen) L.M. Kohn (Jansch *et al*., 2012). In peach, the pathogen initiates and encourages flower blights, twig and branch death, spurs, and fruit rot in the field (Gell *et al*., 2007). The activity of the pathogen on peach is therefore highly destructive from the flowering stage, to fruit production, and storage (Thomidis and Exadaktylou, 2010; see Obi *et al*., 2018b for a review).

In Spain, *M. laxa* and *M. fructicola* have been the most recurrent pathogens since the dislodgment of *M. fructigena* from Spain in 2010 (Villarino *et al*., 2013). These species cause over 60% fruit loss after harvest (Villarino *et al*., 2012; Egüen *et al*., 2015), mostly under favourable environmental conditions for the commencement and growth of diseases in orchards.

Host tolerance to plant pathogens is important for the development of cost effective and environmentally safe strategies for disease management (Gradziel, 1994). Similarly, according to Gell *et al*. (2007) the use of resistant cultivars in crop improvement is critical for crop protection, since plants and plant products are usually protected from (prophylactic) (Mooney *et al*., 2012), rather than cured of, diseases (chemotherapeutic) (Obi *et al*., 2018b). The choice of cultivar significantly influences rot incidence and severity among other potential factors in stone fruits (Tarbath *et al*., 2014). and, therefore, are effective at disease control (Kreidl *et al*., 2015). The long-term prophylactic treatment of peach, using *M. laxa* resistant cultivars, will ensure prevention of pathogenic problems in orchards. Resistant genotypes will allow sustainable control with zero pesticide residues

on fruits, improving the safety of harvesting and decreasing disease problems during storage, thereby leading to enhanced economic benefits. The total absence of pesticide residues in prophylactic resistant peach cultivars would be environmentally beneficial (Usall *et al*., 2016). However, disease resistant cultivars are not readily available for many fruit crops (Spiers *et al*., 2005), including commercial peach cultivars.

Developing peach cultivars that are resistant to *M. laxa* pathogen requires, in the first instance, the identification of existing resistant and susceptible genotypes by screening individuals from a germplasm (Rubos *et al*., 2008). Although most commercial peach cultivars are susceptible to *Monilinia* spp., a few resistant cultivars have been identified (Gradziel and Wang, 1993; Martínez-García *et al*., 2013; Oliveira-Lino *et al*., 2016; Obi *et al*., 2017). The relative tolerance or susceptibility of fruit to disease has therefore often been used to select disease resistant genotypes for the purpose of breeding peach (Gradziel 1994). Selection within breeding descendant populations has been carried out for both peach and nectarine (Bassi *et al*., 1998; Pacheco *et al*., 2014; see Oliveira-Lino *et al*., 2016 and Obi *et al*., 2018b for details), and for other fruit germplasm such as apricot (Walter *et al*., 2004), plum (Pascal *et al*., 1994), and apple (Biggs and Miller, 2004). Previous studies have demonstrated that powerful antioxidants such as phenolic acids, flavonoids, and anthocyanins are present in the phytochemical compounds produced by peach cultivars (Giménez, 2013; Ágreda, 2016; Saidani *et al*., 2017). These bioactive compounds, especially chlorogenic and neochlorogenic acids, may confer important preservative functions during postharvest handling in the peach industry (Villarino *et al*., 2011; Pacheco *et al*., 2014; see Oliveira-Lino *et al*., 2016 and Obi *et al*., 2018b, in details). In addition, considering the recent drive for alternative technologies that can effectively control postharvest diseases of stone fruits (Mari *et al*., 2015; Usall *et al*., 2015, 2016), any

evidence regarding compounds inhibitory to brown rot development would influence breeding schemes, and would be useful for the postharvest peach industry.

There is limited information on peach pathogenic tolerance to *M. laxa* brown rot in their breeding descendants, and their relationships with quality and phytochemical traits in fruits during postharvest handling. This study aimed to identify superior Spanish peach cultivars that exhibit high tolerance to *M. laxa* brown rot, and possess high levels of antioxidants. The specific objectives of this work, therefore, were to evaluate tolerance to *Monilinia laxa* brown rot within the breeding descendant population of 'Babygold 9' × 'Crown Princess', and to examine whether fruit quality and phytochemical composition correlate with pathogen tolerance. Finally, the identification of biochemical compounds associated with brown rot tolerance would impact breeding strategies, beneficial to the postharvest industry, and facilitate environmental sustainability.

2. Materials and Methods

2.1 Plant material

The plant materials are progenies from a controlled biparental cross of two commercial cultivars, 'Babygold 9' × 'Crown Princess' (B9 × CP). These genotypes were propagated during 2000 and 2001 in collaboration with Agromillora Catalana S.L. (Barcelona, Spain). Both the progenitors and the entire progeny are yellow fleshed, clingstone peach. The resulting seedlings were budded on GF677 rootstock, and established in 2002 at the Estación Experimental de Aula Dei-CSIC (Zaragoza, Spain). Trees were trained to the standard open vase system, hand thinned, and subsequently grown under standard conditions of irrigation, fertilization, and pest and disease control chemical spray programmes. For the 3 years of the study (2013–2015), any fungicide treatment was applied in the field prior to harvest with adequate consideration to the free entry period and

harvesting for evaluation. A total of 68 genotypes were harvested in the 2013 and 2014 seasons (Supplementary table 1). Seventeen genotypes with lesion severity (LS) < 40 mm were then pre-selected, either in 2013 or 2014, or when the mean value for both years was below 40 mm (Obi *et al*., 2017), and harvested in 2015 to validate results concerning *M. laxa* tolerance. The pathogenic traits [percentage of brown rot incidence (%BRI), lesion diameter (LD), and colonization extent (CEx)] were measured for each seedling tree separately over the 3-year period, and the means of the 17 selected genotypes were calculated. Fruits were subjectively selected and harvested based on optimum maturity [(Cantín *et al*., 2009) (expressed on visual colour change and manual evaluation of firmness, favouring apparently healthy fruit of uniform ripeness and size)]. Fruits were disinfected as described by Obi *et al*. (2017).

2.2 Pathogen culture, conidia production, and inoculation

The procedure adopted is as described by Obi *et al*. (2017). Briefly, the culture of *Monilinia laxa* (Alderh. & Ruhland) Honey, isolate number: CPML02, used in this study was supplied by the Collection of Postharvest Pathology Group of IRTA (Lleida, Spain). Conidia from wounded fruits were sampled into a solution of sterile distilled water and Tween® 80 (0.0005%) surfactant. Quantification of conidia in suspension was as in Obi *et al*. (2017), and adjusted to 25×10^3 mL^{-1} spore for fruit inoculation. To evaluate tolerance to brown rot, 20 disinfected fruits were inoculated with 25 μL of spore load of the virulent pathogen. Five fruits used as control were inoculated with 25 μL of sterile water. Both treatment and control were then incubated for five days in darkness at 23 °C.

2.3 Brown rot disease evaluation

144 Pathogenic traits were evaluated according to Obi *et al*. (2017). In brief, inoculated fruits
145 were observed daily during the five days of incubation. The %BRI was assessed using the
146 percentage fraction infected over the total number of inoculated fruits. Percentage of
147 colonization (%C) was assessed using the percentage colonised over the total number of
148 fruits. LD and CEx were also measured. These parameters were used in the determination
149 of brown rot disease severity for genotype tolerance rating, as has been reported previously
150 (Martínez-García *et al*., 2013; Obi *et al*., 2017). LS was calculated by the %BRI × LD/100
151 and colonization severity (CS) by the %C × CEx/100.
152
153 ***2.4 Fruit quality trait evaluation***
154 During the 2014 and 2015 seasons, twenty fruits were harvested to evaluate fruit quality
155 individually for each tree seedling. Harvesting date (Julian days, JDs) ranged from late-
156 May to mid-September, depending on the genotype of the population. Fruit weight (FtW)
157 and physicochemical traits were determined for each genotype. Titratable acidity (TA) and
158 pH were determined at harvest, as detailed in previous studies (Abidi *et al*., 2015; Zeballos
159 *et al*., 2016).
160 Fruits were evaluated for firmness (FF) and soluble solids content (SSC) at three different
161 levels: at harvest and after 5 days of storage (inoculated and uninoculated) at 23 °C. At
162 harvest, firmness was determined for 5 fruits and genotypes on opposite sides of the
163 equator of each fruit, after a section of the peel (approximately 2 cm^2) was removed using a
164 penetrometer fitted with an 8-mm diameter probe (Effegi, Milan, Italy). Both measures
165 were averaged for each fruit, and data are given in Newton (N). Firmness of uninoculated
166 and inoculated fruits were determined on 5 and 20 fruits and genotypes, respectively, in the
167 undamaged part of the fruit after 5 days of incubation. The SSC of the juice was also

168 measured at harvest and after incubation using a temperature compensated refractometer
169 (model ATC-1, Atago Co., Tokyo, Japan); and data are presented as °Brix.
170
171 ***2.5 Antioxidant compounds analysis***
172 For biochemical analysis on fruit pulp and peel, out of the 20 fruits used for the study, 10
173 were randomly selected, peeled using a mechanical peeler, and later cut into smaller pieces
174 for relative homogeneity. Then, 3 g of peel and 5 g of fresh fruit were weighed into 50 mL
175 transparent polypropylene jars, frozen in liquid nitrogen, and conserved at -20 °C for later
176 use in total phenolics (TPC), flavonoids, anthocyanins assays. For vitamin C (Vit C)
177 determination, samples were stored with metaphosphoric acid (HPO_3) and subsequently
178 conserved at -20°C prior to analysis. Biochemical extractions were performed as described
179 in Cantín *et al*. (2009).
180 Vit C, TPC, flavonoid, and anthocyanin contents were determined using colorimetric
181 methods (Cantín *et al*., 2009) and measured using a spectrophotometer ([BIOCHROM
182 ASYS UVM 340 microplate reader (see details in Ágreda, 2016)]. Standard calibration
183 curves were prepared daily for all determinations. For Vit C, absorbance was measured at
184 525 nm, and the amount of Vit C was expressed as milligrams (mg) of ascorbic acid (AsA)
185 per 100 g fresh weight (FW). For TPC, the colorimetric method based on the chemical
186 reduction of the Folin-Ciocalteu reagent was used. Absorbance was measured at 725 nm,
187 and the phenolic content was expressed in mg of gallic acid (3,4,5-trihydroxybenzoic acid)
188 equivalents (GAE) per 100 g of FW. Total flavonoid content was determined by measuring
189 the absorbance at 510 nm, and the results were expressed as milligrams of catechin
190 equivalents (CE) per 100 g of FW. The total anthocyanin content was evaluated using a
191 hydroalcoholic extract, and the absorbance was measured at 520 and 700 nm. Anthocyanin
192 concentration was calculated using the molar extinction absorptivity coefficient ε =

193 26,900/cm and was expressed in milligrams of cyanidin-3-glucoside equivalents (C3GE)
194 per 100 g of FW (Liu *et al*., 2015; Saidani *et al*., 2017).
195
196 ***2.6 Statistical analysis***
197 Means, standard errors (SE), and Pearson's correlation were calculated using SPSS 25
198 (IBM Inc, Armonk, NY, USA) statistical software. The incidence and severity of brown
199 rot, including the influence of quality parameters, were also analysed using an analysis of
200 variance (ANOVA) with SPSS 25 statistical software. Statistical significance was set at the
201 $p < 0.05$ level, and the Duncan's test was used for the comparison of means.
202
203 **3. Results**
204 We studied a total of 68 descendants from the 'Babygold 9' × 'Crown Princess' population
205 over a period of 3 years (2013, 2014, and 2015) for tolerance to *Monilinia laxa* brown rot
206 (Supplementary table 1). The disease parameters used included: %BRI, LD, LS, %C, CEx,
207 and CS. As previously mentioned, we selected 17 genotypes that exhibited a *M. laxa* LS of
208 < 40 mm, either in 2013 or 2014, or with the mean value for both years (Supplementary
209 table 2), to evaluate and validate the *M. laxa* tolerance of these genotypes in 2015.
210 For the 17 genotypes studied, the harvest date (HD) was recorded and the physicochemical
211 traits [FtW, FF, SSC, pH, and TA] were evaluated over a period of 3 years [2013–2015
212 (Table 1)], and a parametric test of Pearson correlation was conducted within pairs of fruit
213 quality traits (Table 2, Supplementary table 3). We also determined phytochemical trait
214 compounds as Vit C and total phenolic, flavonoid, and anthocyanin contents in flesh
215 (2014–2015, Table 3) and in peel (2015 only, Table 4, Supplementary table 4).
216
217 ***3.1 Effect of phytopathogen activities***

The evaluation of the 68 genotypes of 'Babygold 9' × 'Crown Princess' for brown rot tolerance in 2013 and 2014 is presented in Supplementary table 1. The %BRI in both years was between 50 to 100%. Differences exist between the years, although a similar average %BRI was found for 2013 (91.9%) and 2014 (91.6%). The average %C in 2013 was 84.8%, but was lower in 2014 (80.2%). The average LD in 2013 was 56.5 mm, while the average LD in 2014 was 48.9 mm. The mean LS was 52.5 mm in 2013, and 45.3 mm in 2014. A corresponding pattern was repeated in both years in the range of CS, with an average CS of 44.0 mm in 2013 and 36.6 mm in 2014. Almost all the associated pathological parameters indicate that the progeny showed fewer symptoms of *M. laxa* infection in 2014 than in 2013. However, only colonization extent and CS were positive correlated in 2013 *vs* 2014 ($r = 0.388$, and $r = 0.338$ at $P \leq 0.05$, respectively). Briefly, in 2015, the 17 genotypes, the average %BRI (92.9%) and %C (89.4%) was higher than in the previous years. In contrast, the %LD and LS were lower, with averages of 48.1 and 44.7 mm, respectively.

From the mean of these 17 genotypes evaluated in 2013, 2014, and 2015, only six genotypes (BC1, BC48, BC58, BC63, BC67, and BC68) showed a lesion severity of <40 mm and a colonization severity below 32 mm (Supplementary table 2). An analysis of the brown rot tolerance between the 3 years of the study shows that the 17 genotypes exhibited high variability in most of the pathogenic parameters studied (Supplementary table 2). The lowest %BRI (73.3%) and %C (51.7%) occurred in the BC67 genotype, while the lowest LD (41.98 mm), LS (31.75 mm), CEx (39.05 mm), and CS (21.75 mm) were observed in the BC58 genotype. The highest values for %BRI (100%) occurred in four different genotypes (BCs: 11, 24, 61, and 66), while for LD (52.34 mm), LS (50.27 mm), and CS (42.51 mm) the highest values were recorded in the BC11 genotype. For %C (91.7%) and

CEx (49.47 mm), the highest values were observed in the BC61 and BC60 genotypes, respectively.

The Pearson correlation between pairs of traits for pathological traits showed significant positive correlation coefficients ranging from 0.406 to 0.959 at $P \leq 0.01$. Among the strongest are %BRI with %C (r = 0.814, $P \leq 0.01$); LD with CEx (r = 0.859, $P \leq 0.01$), and LS with CS (r = 0.959, $P \leq 0.01$) (Figure 1).

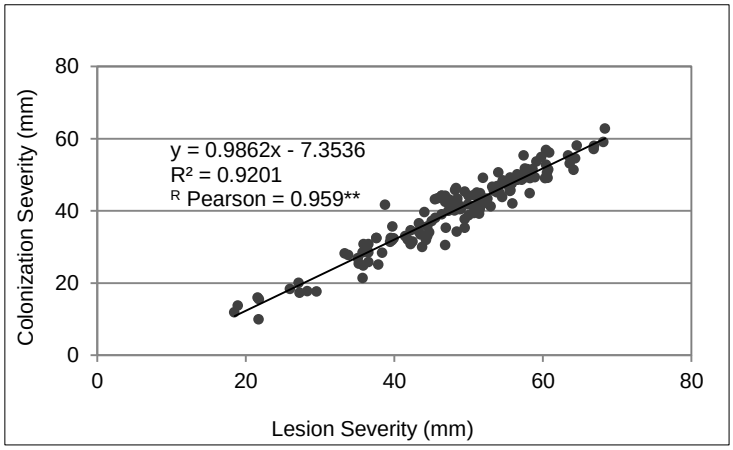

Figure 1 Correlation between lesion and colonization severities in all the 'B9' × 'CP' genotypes evaluated over 3 years (2013–2015). N = 138.

Within the phytopathogenic activities in fruits, we have found that genotypes with smaller fungus injury diameters correlated with smaller colonization diameters. In addition, these genotypes are also associated with a lower incidence of disease, that is, a lower percentage of damaged fruits (susceptibility).

3.2 Effect of storage, inoculation and physicochemical traits on fruits.

Table 1 shows the effect of storage and inoculation on FF, SSC, and physicochemical traits in 17 selected genotypes evaluated over a period of 3 years (2013–2015). Genotypes from these progeny were harvested between 175 and 227 JDs, which is late June and mid-August, respectively. The six resistant genotypes (shown in bold in Table 1) matured between 175 and 224 JDs. The FtW ranged between 143 g and 241 g. Marked variability

was encountered in the FF at harvest and after storage. In the 17 genotypes selected, the mean FF at harvest was 32.47 N. Specifically, the lowest FF at harvest (17.51 N) was recorded for BC68 genotype, while the highest FF (51.16 N) was recorded for the BC11 genotype. The mean FF at harvest (32.47 N) was lower than the mean FF at storage (33.06 N) for all 17 genotypes. The mean SSC at harvest was 9.3 °Brix (from 7.7 °Brix in BC58 to 11.1 °Brix in BC48). Within the stored peach, the mean SSC of uninoculated fruit was 8.7 °Brix, and 8.3 °Brix for inoculated fruit. After storage, significant differences were found in SSC among the 17 selected genotypes. There was also marked variability in pH (3.62–4.17), TA (0.40–0.60%), and ripening index (RI, 12.68–21.20). As shown in Table 2, there were significant positive correlations between most of the physicochemical traits. HD showed a significant positive correlation with FtW, FF, SSC, pH, and RI. The FtW showed a significant positive correlation with FF and SSC (at harvest, inoculated, and at storage), and pH and RI. The FF and SSC at harvest was highly correlated with both parameters at storage.

Table 1 Effect of storage and inoculation on FF, SSC, and physicochemical traits in the 17 descendants of the 'B9' × 'CP' population. Data are mean ± SE of the 3 years (2013–2015). Resistant genotypes are shown in bold.

Genotype	HD (JDs)	FtW (g)	FF at harvest (N) [1]	FF uninoculated (N) [1]	FF inoculated (N)[1]	SSC at harvest[a] (°Brix)	SSC after storage uninoculated (°Brix)[1]	SSC after storage inoculated (°Brix)[1]	pH[a]	TA (%)[a]	RI[a]
BC1	**175 ± 5**	**175 ± 21.1**	**26.50 ± 1.9 ab**	**33.40 ± 1.9 bcde**	**28.10 ± 0.8 b**	**8.2 ± 0.4**	**8.0 ± 0.5ab**	**8.0 ± 0.3 b**	**3.62 ± 0.0**	**0.6 ± 0.0**	**13.29 ± 0.8**
BC11	227 ± 6	209 ± 27.1	51.16 ± 3.8 d	48.13 ± 4.6 f	41.55 ± 3.0 d	9.8 ± 0.7	10.0 ± 0.4 cd	9.2 ± 0.2 c	3.96 ± 0.1	0.5 ± 0.1	17.70 ± 1.3
BC19	175 ± 5	186 ± 21.0	18.58 ± 2.3 a	24.05 ± 1.2 a	22.31 ± 0.8 ab	9.7 ± 0.1	8.0 ± 0.6 ab	7.5 ± 0.3 ab	3.82 ± 0.1	0.4 ± 0.0	20.05 ± 0.5
BC24	226 ± 7	208 ± 32.6	39.31 ± 3.3 c	34.18 ± 2.1 cde	34.35 ± 1.9 c	10.4 ± 0.4	9.2 ± 0.3 bc	9.2 ± 0.3 c	3.96 ± 0.1	0.6 ± 0.1	17.31 ± 1.6
BC44	175 ± 5	171 ± 21.5	20.35 ± 2.1 ab	19.07 ± 1.5 a	17.65 ± 0.6 a	8.2 ± 0.2	7.9 ± 0.3 ab	7.5 ± 0.2 ab	3.84 ± 0.3	0.5 ± 0.1	15.88 ± 3.1
BC48	**224 ± 4**	**208 ± 15.7**	**47.51 ± 2.9 cd**	**36.23 ± 1.4 e**	**35.11 ± 0.9 c**	**11.1 ± 0.7**	**11.1 ± 0.4 d**	**9.3 ± 0.2 c**	**3.88 ± 0.1**	**0.6 ± 0.1**	**16.56 ± 0.9**
BC51	175 ± 5	181 ± 21.0	24.17 ± 4.4 ab	25.15 ± 2.8 ab	25.50 ± 1.2 b	8.9 ± 0.9	8.0 ± 0.5 ab	7.9 ± 0.2 b	3.71 ± 0.1	0.5 ± 0.1	16.17 ± 2.9
BC53	178 ± 3	143 ± 16.2	19.22 ± 0.9 a	24.50 ± 0.8 a	23.73 ± 0.7 b	8.5 ± 0.3	7.1 ± 0.4 a	7.6 ± 0.3 ab	3.68 ± 0.0	0.5 ± 0.1	14.63 ± 1.2
BC57	227 ± 6	241 ± 18.0	48.19 ± 4.9 cd	46.76 ± 3.9 f	49.82 ± 3.1 e	9.3 ± 0.5	9.6 ± 0.5 c	7.9 ± 0.3 b	3.95 ± NA	0.5 ± NA	17.44 ± NA
BC58	**180 ± 6**	**187 ± 15.5**	**23.79 ± 2.1 ab**	**27.20 ± 1.1 abcd**	**24.27 ± 0.7 b**	**7.7 ± 0.9**	**7.6 ± 0.5 a**	**7.1 ± 0.3 a**	**3.62 ± 0.0**	**0.6 ± 0.1**	**12.98 ± 1.3**
BC59	176 ± 8	163 ± 24.3	29.08 ± 1.5 b	34.06 ± 1.7 cde	28.07 ± 0.8 b	9.7 ± 1.8	8.0 ± 0.3 ab	8.0 ± 0.3 b	3.76 ± 0.1	0.5 ± 0.0	17.16 ± 3.0
BC60	224 ± 7	187 ± 13.2	51.09 ± 3.1 d	52.29 ± 3.6 f	42.00 ± 1.6 d	11.0 ± 0.6	10.3 ± 0.3 cd	9.0 ± 0.2 c	3.92 ± 0.2	0.7 ± 0.2	14.40 ± 2.7
BC61	227 ± 6	220 ± 22.4	41.27 ± 2.6 c	36.26 ± 3.2 e	33.56 ± 1.5 c	9.2 ± 0.7	10.0 ± 0.3 cd	9.6 ± 0.2 cd	4.17 ± 0.0	0.4 ± 0.1	21.20 ± 2.1
BC63	**222 ± 2**	**235 ± 18.0**	**43.28 ± 1.9 cd**	**35.91 ± 1.6 de**	**36.37 ± 1.0 c**	**9.4 ± 1.0**	**9.5 ± 0.4 c**	**9.1 ± 0.2 c**	**3.89 ± 0.1**	**0.6 ± 0.1**	**14.28 ± 0.6**
BC66	216 ± NA	151 ± NA	29.29 ± 2.1 b	33.87 ± 1.6 bcde	27.22 ± 1.3 b	8.8 ± NA	9.5 ± 0.5 c	10.1 ± 0.3 d	3.87 ± NA	0.6 ± NA	12.68 ± NA
BC67	**180 ± 6**	**169 ± 10.6**	**21.70 ± 1.1 ab**	**25.60 ± 1.2 abc**	**24.73 ± 0.5 b**	**8.5 ± 1.2**	**7.6 ± 0.5 a**	**6.9 ± 0.2 a**	**3.67 ± 0.0**	**0.5 ± 0.1**	**17.01 ± 0.3**
BC68	**180 ± 6**	**186 ± 4.2**	**17.51 ± 1.0 a**	**25.32 ± 0.7 abc**	**24.05 ± 0.5 b**	**9.4 ± NA**	**7.2 ± 0.5 a**	**7.1 ± 0.2 a**	**3.76 ± 0.0**	**0.4 ± 0.1**	**18.01 ± NA**

a: No replication (data from pooled fruits of 5). Abbreviations: HD, harvest date; JDs, Julian days; FtW, fruit weight; FF, fruit firmness; SSC, soluble solids content; TA, titratable acidity; RI, ripening index (SSC/TA); SE, standard error; NA, not available, because replications were less than 3 or harvested once a year. [1] Different letters show differences among genotypes at $P \leq 0.05$.

Table 2 Pearson correlations (parametric test) within pairs of fruit quality traits in the 'B9' × 'CP' population studied over a period of 3 years (2013–2015).

	FtW	FF at harvest	FF un-inoculated	FF inoculated	SSC at harvest	SSC un-inoculated	SSC inoculated	pH	TA	RI
HD (JDs)	0.554******	0.602**	0.385*	0.552**	0.677**	0.630**	0.687**	0.759**	0.092	0.497**
FtW		0.220*****	0.200*	0.319**	0.334**	0.463**	0.445**	0.464**	0.167	0.421**
FF at harvest			0.833**	0.800**	0.418**	0.514**	0.363**	0.316**	0.261*	0.115
FF uninoculated				0.837**	0.367**	0.547**	0.386**	0.369**	0.245*	0.175
FF inoculated					0.391**	0.562**	0.407**	0.415**	0.260*	0.173
SSC at harvest						0.786**	0.829**	0.667**	0.174	0.586**
SSC uninoculated							0.810**	0.667**	0.133	0.518**
SSC inoculated								0.696**	0.199	0.514**

Abbreviations: HD, harvest date; JDs, Julian days; FtW, fruit weight; FF, fruit firmness; SSC, soluble solids content; TA, titratable acidity; RI, ripening index (SSC/TA).

***, **:** Correlations significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; N = 138

3.3 Effect of antioxidant compound contents

Table 3 shows the levels of all antioxidant compounds (ascorbic acid, TPC, flavonoids, and anthocyanins) in the flesh of the 17 genotypes evaluated in 2014 and 2015. In addition, we included as preliminary results the content of these compounds in the peel measured in 2015, to determine whether any compounds were associated with tolerance to *M. laxa* (Table 4). Significant differences were found between genotypes for all antioxidant contents in both flesh and peel tissues.

Among the 17 selected genotypes, the AsA content in flesh ranged from 2.55 to 9.20 mg AsA/100 g FW, TPC ranged from 27.90 to 63.73 mg GAE/100 g FW, and flavonoid contents ranged from 9.48 to 35.45 mg CE/100 g FW. The variation in anthocyanins, particularly in fruit flesh, was from 0.09 to 0.40 mg C3GE/100 g FW. A wide range of antioxidant contents were found in the peel of the 17 genotypes studied. In general, Vit C, total phenolics, and flavonoid contents were higher in the peel than in the flesh. The TPCs of the BC67 genotype, and AsA and anthocyanin contents of the BC1 and BC67 genotypes, were significantly higher than for the other genotypes. Flavonoid content was not significantly different in the resistant compared to non-resistant genotypes. As shown in Table 4, the AsA content in the peel of the 17 genotypes studied ranged from 5.89 to 16.29 mg AsA/100 g FW.

306 **Table 3** Antioxidant compound contents in the flesh of the 17 genotypes of the 'B9' ×
307 'CP' population evaluated over a period of 2 years (2014–2015). Data are mean ± SE
308 (N=4-6 from 10 pooled fruits). Resistant genotypes are shown in bold.

Genotype	Ascorbic acid (mg AsA/100 g FW)	Total phenolics (mg GAE/100 g of FW)	Flavonoids (mg CE/100 g FW)	Anthocyanins (mg C3GE/100 of FW)
BC1	**9.20 ± 3.3 d**	**51.08 ± 1.9 efg**	**17.99 ± 1.8 abc**	**0.13 ± 0.0 a**
BC11	4.41 ± 0.3 abc	63.73 ± 2.6 i	33.49 ± 7.6 d	0.16 ± 0.0 ab
BC19	7.89 ± 0.5 cd	49.32 ± 0.7 def	24.46 ± 1.1 abcd	0.14 ± 0.0 a
BC24	7.74 ± 1.3 cd	58.15 ± 3.0 ghi	35.10 ± 3.5 d	0.17 ± 0.0 ab
BC44	6.12 ± 0.7 abcd	34,81 ± 1.1 ab	12.08 ± 1.2 ab	0.09 ± 0.0 a
BC48	**5.22 ± 0.5 abc**	**48.84 ± 1.4 def**	**17.69 ± 1.5 abc**	**0.09 ± 0.0 a**
BC51	3.64 ± 0.9 ab	61.26 ± 1.6 hi	35.45 ± 6.9 d	0.15 ± 0.0 ab
BC53	6.47 ± 0.9 bcd	27.90 ± 0.9 a	10.02 ± 1.9 a	0.17 ± 0.0 ab
BC57	2.76 ± 0.3 a	37.54 ±0.5 bc	10.96 ± 0.6 ab	0.16 ± 0.0 ab
BC58	**3.17 ± 0.2 ab**	**42.98 ± 0.5 cde**	**17.48 ± 2.9 abc**	**0.22 ± 0.0 ab**
BC59	5.69 ± 1.3 abc	50.54 ± 5.1 efg	25.86 ± 9.9 bcd	0.10 ± 0.0 a
BC60	5.26 ± 1.0 abc	29.23 ± 2.3 a	09.95 ± 3.0 a	0.30 ± 0.1 bc
BC61	6.36 ± 0.4 bcd	45.33 ± 3.9 cde	13.44 ± 2.1 ab	0.20 ± 0.0 ab
BC63	**2.55 ± 0.5 a**	**53.85 ± 4.9 fgh**	**29.04 ± 8.7 cd**	**0.16 ± 0.0 ab**
BC66	3.86 ± 0.6 ab	39.10 ± 2.9 bc	19.04 ± 1.4 abc	0.12 ± 0.0 a
BC67	**4.88 ± 1.9 abc**	**50.39 ± 1.4 efg**	**19.09 ± 2.2 abc**	**0.17 ± 0.0 ab**
BC68	**4.66 ± 0.3 abc**	**41.87 ± 2.3 bcd**	**09.58 ± 0.7 a**	**0.40 ± 0.1 c**

309

310 Abbreviations: AsA, ascorbic acid; GAE, gallic acid equivalents; CE, catechin equivalents;
311 C3GE, cyaniding-3-glucoside equivalents. For each column, different letters show
312 significant differences among genotypes ($P \leq 0.05$, Duncan's test).
313

Table 4 Antioxidant compound contents in the peel of the 17 genotypes of 'B9' × 'CP' evaluated in 2015. Data are mean ± SE (N=3 from 10 pooled fruits per genotype). Resistant genotypes are shown in bold.

Genotype	Ascorbic acid (mg AsA/100 g FW)	Total phenolics (mg GAE/100 g FW)	Flavonoids (mg CE/100 g FW)	Anthocyanins (mg C3GE/100 g FW)
BC1	**15.48 ± 0.7 e**	**153.54 ± 1.1 hi**	**96.42 ± 2.7 fg**	**9.66 ± 0.1 i**
BC11	9.01 ± 0.7 abcd	158.92 ± 5.0 ij	106.18 ± 3.4 g	4.17 ± 0.0 e
BC19	9.24 ± 0.9 abcd	112.17 ± 1.2 bcd	75.70 ± 4.5 de	6.00 ± 0.2 f
BC24	8.45 ± 0.5 abcd	168.24 ± 2.8 j	128.13 ± 2.4 h	0.62 ± 0.0 a
BC44	10.58 ± 0.4 bcd	116.81 ± 1.1 cde	67.74 ± 0.4 cd	2.42 ± 0.0 c
BC48	**9.87 ± 0.6 bcd**	**141.01 ± 7.5 gh**	**74.25 ± 6.7 de**	**5.99 ± 0.1 f**
BC51	8.12 ± 0.4 abcd	150.15 ± 6.7 hi	142.04 ± 5.4 hi	2.81 ± 0.0 d
BC53	11.10 ± 0.9 cd	89.98 ± 3.2 a	50.00 ± 0.8 b	4.36 ± 0.1 e
BC57	7.37 ± 0.7 abc	123.00 ± 2.4 de	61.69 ± 7.6 bcd	1.69 ± 0.0 b
BC58	**10.89** ± 0.1 **cd**	**128.13 ± 5.2** ef	**86.47 ± 6.6 ef**	**8.26 ± 0.2 h**
BC59	9.48 ± 0.1 abcd	144.07 ± 3.0 gh	132.70 ± 1.3 h	2.17 ± 0.0 c
BC60	11.31 ± 0.2 d	106.19 ± 4.5 ab	56.30 ± 7.5 bc	6.94 ± 0.2 g
BC61	8.45 ± 0.3 abcd	135.70 ± 3.1 fg	105.45 ± 5.5 g	2.94 ± 0.0 d
BC63	**5.89** ± 0.3 **a**	**115.61 ± 3.8 bcde**	**88.72 ± 1.4 ef**	**4.28 ± 0.1 e**
BC66	08.42 ± 0.3 abcd	103.73 ± 2.7 b	74.16 ± 1.0 de	0.68 ± 0.0 a
BC67	**16.29** ± 1.1 **e**	**189.43 ± 5.7 k**	**148.61 ± 3.4 i**	**12.94 ± 0.2 j**
BC68	**6.77** ± 0.1 **ab**	**148.59 ± 2.5 hi**	**36.54 ± 6.9 a**	**2.18 ± 0.0 c**

Abbreviations: AsA, ascorbic acid; GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyaniding-3-glucoside equivalents. For each column different letters show significant differences among genotypes ($P \leq 0.05$, Duncan's test).

Notably. pathologic variables, %BRI, %C, LS, and CS correlated negatively with peel anthocyanin contents (r = -0.551, r = -0.552, r = -0.481, r = -0.491, $P \leq 0.05$ respectively (Figure 2). However, only %BRI correlated negatively with fruit flesh anthocyanin contents (r = -0.219, $P \leq 0.05$).

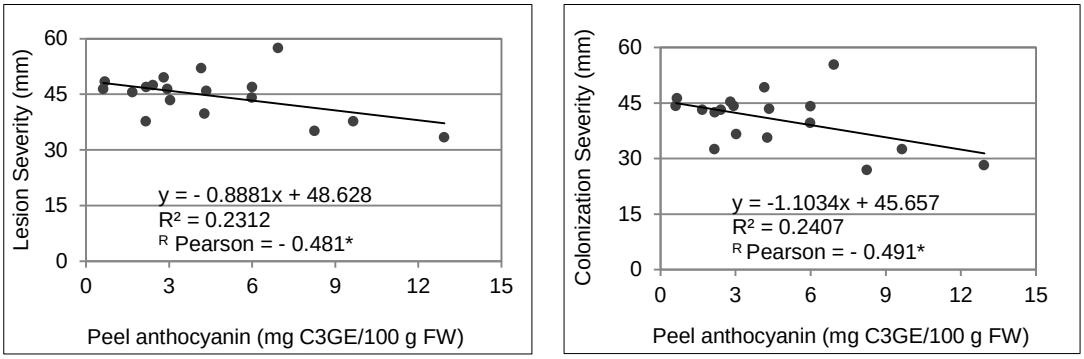

Figure 2 Correlation between lesion severity and peel anthocyanin contents (left), and colonization severity and peel anthocyanin contents (right) in the 17 'B9 × CP' genotypes evaluated for 2015. N = 17.

4 Discussion

The annual disparity found in the responses of the genotypes to brown rot after inoculation may be due to different levels of cuticular cracking or fractures, as has been reported for stone fruits by other authors (Gradziel *et al*., 2003; Kappel and Sholberg 2008). Cuticular cracks are considered to be the preferential portal of entry for fungi pathogens in the *Monilinia* genus (Gibert *et al*., 2007), and the incidence of fruit infection increases with increasing fruit cuticular crack surface area (Borve *et al*., 2000; Gibert *et al*., 2009). In the present study, fruits were not wounded prior to inoculation; therefore, the brown rot pathogen would require naturally occurring wounds or micro-cracks in the cuticle to gain entry into the fruit (Oliveira-Lino *et al*., 2016). The yearly variation is likely due to natural differences in surface cuticular cracks, since a uniform quantity of artificial inoculum density was used in this study; Ágreda (2016) reported similar results for a different peach population evaluated under the same conditions.

The significant positive correlation observed between pairs of pathological traits in our study is typical (Obi *et al*., 2017). This undoubtedly indicates that the level of infection significantly influenced the LD and CE, including the severity of the disease situation (Michailides *et al*., 2000). Therefore, LD and CEx are two brown rot parameters that are usually associated, and are useful in evaluating the brown rot tolerance of peach. Information for these two traits is important in the evaluation of disease tolerance from genetic or pathogenic points of view, respectively (Xu *et al*., 2008; Burnett *et al*., 2010).

Considering the physicochemical variables, the observations of HD in this study are in agreement with that of Giménez (2013), in which the studied population was harvested during 2009, 2010, and 2011, between 169 and 248 JDs. All the genotype-pathogen interactions indicated variable degrees of susceptibility, and occurred in genotypes harvested both in the early- or late-season. Nevertheless, the susceptibility of peach to brown rot depends on the interaction between the host (cultivar) and the pathogen (Obi *et al*., 2018a, 2018b), not on the season or ripening time. However, when fruits are harvested later in the season, they are sweeter and larger, and have higher total phenolics, flavonoids (Font i Forcada *et al*., 2013, Abdelghafat *et al*., 2018), and total sugar contents (Font i Forcada *et al*., 2013). Both very early-maturing and very late-maturing peach genotypes are of significant interest for the peach industry, particularly in the Mediterranean area.

Contrary to our expectation, mean FF at harvest (32.47 N) was lower than mean FF at storage (33.06 N) for all 17 genotypes studied. However, there were no significant differences, indicating that the incubation conditions did not particularly affect fruit firmness. A correlation analysis indicated a significant decrease ($P \leq 0.05$) in FF during storage for uninoculated (33.06 N) vs inoculated (30.49 N). This suggests that the decrease in FF in inoculated fruit could be due to the activity of *M. laxa*, and that this may have affected the surrounding tissues. Our results may explain the observation of Yaghmour *et*

365 *al.* (2011), who that found that rates of infection increased as the FF decreased. Our
366 analysis revealed a broad range of FF, from 17.51 to 47.51 N, within the genotypes with a
367 LS below 40 mm, indicating that brown rot may be dependent on fruit firmness.

368 The present study also revealed that the SSC decreased from the levels observed at harvest
369 during storage, for both inoculated and uninoculated fruits. In the 17 peach genotypes
370 studied, the mean SSC at harvest was 9.3 °Brix, which ranged from 7.7 °Brix in the BC58
371 genotype to 11.1 °Brix in the BC48 genotype. In stored peach, the mean SSC in
372 uninoculated fruits was 8.7 °Brix, and 8.3 °Brix in inoculated fruit. After storage,
373 significant differences were found in SSC among the 17 selected genotypes. This trend of
374 the decrease in SSC during storage (for uninoculated fruit) is, however, contrary to our
375 hypothesis and contradict the results of previous studies (Amodios *et a*l., 2007 and Liu *et*
376 *al*., 2012), although in distinct crop populations. Conversely, the decrease in SSC observed
377 in peach during storage (for inoculated fruit) could be attributed to the pathogenic activities
378 of the fungus on the inoculated host; inferring that as the pathogen preys on the host, the
379 interaction leads to the depletion of SSC as sugars can be used for mycelia biosynthesis,
380 growth, and development.

381 The SSC of inoculated peaches showed a negative significant correlation with CEx, LD,
382 and LS ($r = -0.273$, $P \leq 0.01$; $r = -0.236$, $P \leq 0.01$; and $r = -0.178$, $P \leq 0.05$; respectively).
383 These findings are in agreement with those of Biggs and Miller, (2004), that showed
384 negative correlations between disease severity and sugar content; they are also in
385 agreement with Gradziel, (1994), who found that lesion development progressed as SSC
386 content increased, becoming highest at the fully ripe stage, depending on the peach
387 cultivar.

388 The relationship between disease parameters and FF within the 17 genotypes is also of
389 interest. The BC58 genotype recorded one of the lowest FF at harvest (23.79 N), which

was associated with the lowest disease parameters for LD (41.98 mm), LS (31.75 mm), CEx (39.05 mm), and CS (21.75 mm), while the BC11 genotype recorded the highest FF at harvest (51.16 N), and the highest disease parameters for LS (50.27 mm) and CS (42.51 mm). However, the BC44 genotype, which demonstrated an FF of 20.35 N, did not correspond to either a resistant or susceptible genotype (LS = 43.64 mm and CS = 34.96 mm). Hence the state of FF, especially at harvest, does not seem to significantly influence brown rot development. Consequently, the level of susceptibility to brown rot depends largely on peach genotype (Gradziel, 1994).

In the same manner the BC58 genotype, which was recorded as having the lowest SSC at harvest (7.7 °Brix), was associated with the lowest disease parameters, as was the BC67 genotype (6.9 °Brix). However, the BC48 genotype, which was recorded as having the highest SSC (11.1 °Brix), also exhibited only low levels of damage from the pathogen. Genotypes that had intermediate SSC contents at harvest, such as BC44 (9.8 °Brix), showed the highest brown rot severities.

The significant positive correlation of HD with FtW, FF, SSC, and pH observed in this study is in agreement with what has been reported by other authors (Giménez, 2013; Font *et al*., 2013; Ágreda, 2016). The correlation observed between FF and SSC at harvest with same parameters after storage ($r = 0.418$, $P \leq 0.01$) is similar to that reported by Giménez (2013) ($r = 0.226$, $P \leq 0.01$), who studied 100 progenies of the same population. This positive correlation between FF and SSC in resistant genotypes is important, because the genotypes with high SSC are selected aiming firstly for higher firmness, and secondly for lower pathogen susceptibility, to prevent mechanical damage during handling and transport (Crisosto *et al*., 2001).

The variation of pH from pH 3.62 to pH 3.89 in our six resistant genotypes are typical values for fruit acidity, since a pH lower than 4.0 at maturity is considered acidic (Abidi *et*

415 *al.*, 2015). The negative and significant correlations found between pH vs TA (r = -0.327,
416 $P \leq 0.01$) and TA vs ripening index (r = -0.665, $P \leq 0.01$), are similar to that reported by
417 other authors (Giménez, 2013; Abidi *et al.*, 2015). In previous experiments, we have
418 observed that the pH of the fruit increased as fruit maturity increased, while the TA
419 decreased (Obi *et al.*, 2018a). These parameters can be important, since it has been
420 reported that acidity preserve fruits from pathogen damage (Hajilou and Fakhimrezaei
421 2011; Cropotova *et al.*, 2013; Tarabih and El-Metwally, 2014).
422 Regarding the bioactive compounds, the AsA content in the flesh ranged from 2.55 to 9.20
423 mg AsA/100 g FW, as reported by Giménez, (2013) for the same population. However, the
424 TPC (27.90 to 63.73 mg GAE/100 g FW) among the selected 17 genotypes was in excess
425 of the range found by Giménez, (2013) (11.22 to 37.42 mg GAE/100 g FW) for the same
426 progeny studied over a period of 3 years (2009–2011). The differences found here may be
427 due to the screening of genotypes for an LS that is lower than 40 mm. Flavonoid contents
428 varied from 9.58 to 35.45 mg CE/100 g FW, and were also higher than those obtained in
429 previous studies of different peach progenies by Giménez, (2013) (1.6 to 13.7 mg CE/100
430 g FW); Abidi *et al.*, 2015 (2.3 to 18.0 mg CE/100 g FW) and Ágreda (2016) (3.79 to 27.63
431 mg CE/100 g FW). The average total phenolic and flavonoid accumulation (46.23 mg
432 CE/100 g FW and 20.04 mg CE/100 g FW, respectively) were higher than the range
433 reported by Abdelghafar *et al*. (2018) in early-, mid-, and late-season peach germplasm
434 evaluated in 2013, but below the values found in 2014, for TPC in peach harvested in any
435 season (over 51.4 mg CE/100 g FW), and for flavonoids in late-season peach genotypes
436 (28.7 mg of CE/100 g of FW). Abdelghafar *et al*. (2018) also found that the annual
437 variation in the antioxidant composition was independent of season and peach germplasm.
438 Environmental variables such as temperature, solar radiation, photoperiod, precipitation,
439 and soil profile affect the growing environment and result in wide variations in peach fruit

harvest quality (Lopresti et al., 2014). The effects of environment and orchard practices on peach fruit quality attributes are extensively reviewed by Minas *et al.* (2018). The anthocyanins, particularly in fruit flesh, varied from 0.09 to 0.40 mg C3GE/100 g FW. These values were below those reported by other authors [(0.7 to 12 mg C3GE/100 g FW) from a broad germplasm collection (Font i Forcada *et al*., 2013); (0.23–11.83 mg C3GE/100 g FW), for the same progeny (Giménez, 2013)]. These differences may be due to the flesh, with the 17 'B9' × 'CP' genotypes selected having yellow flesh, and/or due to the different methods used for quantification.

A wide range of antioxidant contents was found in the peel of the 17 studied genotypes. In general, Vit C, total phenolics, and flavonoid contents were higher in peel than in the flesh in, which is in agreement with previous reports (Ágreda, 2016; Saidani *et al*., 2017). We found that around 65% of Vit C, 75% of TPC, 81% of flavonoids and 96% of anthocyanin contents are concentrated in the peel of our progeny. The TPC in the BC67 genotype, and AsA and anthocyanins in the BC1 and BC67 genotypes, were significantly higher than that of other genotypes. However, flavonoid contents were not significantly different for the resistant compared to the non-resistant genotypes. As shown in Table 4, the AsA content in the peel of the 17 genotypes ranged from 5.89 to 16.29 mg AsA/100 g FW, similar to what other investigators have recently reported (Ágreda, 2016; Saidani *et al.*, 2017). The content of anthocyanins varied from 0.62 to 12.94 mg C3GE/100 g FW in the peel tissue of the 17 selected genotypes, and this reveals that most resistant genotypes had 27–81 times higher contents of anthocyanins in their peel than in their flesh. These values agree with previous reports (Prior *et al*., 1998; Gil *et al*., 2002; Saidani *et al*., 2017), supporting the inference that anthocyanins are more concentrated in the fruit peel than in the flesh.

An unequal distribution of Vit C and TPC in the flesh (≈ 25–30%) and peel (≈ 65–70%) of peach has also been documented (Ágreda, 2016; Saidani *et al*., 2017). It is of great

465 significance, therefore, that the high levels of these bioactive compounds in the peel
466 provide protection from abiotic stresses (Cantín *et al*., 2009), which often predispose peach
467 fruits to pathogen invasion. Fruit peel has frequently been suggested to be important in
468 broad range resistance against opportunistic pathogens such as *Monilinia* spp. (Pacheco *et*
469 *al*., 2014).
470 Pathologic variables (%BRI and %C) and severities (LS and CS) correlated negatively
471 with peel anthocyanin contents (Figure 2). However, only %BRI was negatively correlated
472 with flesh anthocyanin contents ($r = -0.219$, $P \leq 0.05$). Anthocyanins are the most common
473 pigment in nature (Khoddami *et al*., 2013), a class of phytochemicals that give plants their
474 colour and protect tissues from oxidative abiotic stress, which invariably extends the life
475 span of the plant organ. They are therefore more concentrated in the skin portion of fruit,
476 particularly as maturity approaches (Prior *et al*., 1998), to provide a protective barrier
477 against potential phytopathogenic invaders. This could be advantageous in providing
478 tolerance to our genotypes.
479 Nevertheless, only TPC from flesh showed a significant negative correlation in this
480 progeny with LD, LS, and CEx ($r = -0.282$, $r = -0.279$, and $r = -0.225$, all at $P \leq 0.05$,
481 respectively), as has been shown by Ágreda (2016). Other authors also have reported
482 significant negative correlations between phenolic acids and %BRI in immature peach and
483 nectarine cultivars (Villarino *et al*., 2011). High contents of antioxidants influence brown
484 rot negatively by reducing pathogenic activities (see Supplementary table 3); however, in
485 the present study, the genotypes with LS < 40 mm were not those with the highest TPC,
486 and vice-versa. Major phenolic acids such as chlorogenic and neochlorogenic acids
487 (Villarino *et al*., 2011), which have highly potent antioxidant properties (Dai and Mumper,
488 2010; Khoddami *et al*., 2013), may protect the plant and plant materials against fungi and
489 other phytopathogenic organisms (Prasad *et al*., 2014; Spadoni *et al*., 2014). However, fruit

phenolic contents decrease at harvest, and their effectiveness in controlling brown rot infection can vary with peach cultivar (Cindi *et al*., 2016; Obi *et al*., 2018b).

Pearson's correlation coefficients for bioactive compounds were between 0.790 and 0.506. TPC in the flesh showed significant positive correlations with flesh and peel flavonoids (r = 0.790, r = 0.718, respectively), all at $P \leq 0.01$. Moreover, TPC and flavonoid levels in the peel were also strongly correlated (r = 0.722, $P \leq 0.01$). The results found for this progeny were in agreement with previous studies in this and other progenies or peach germplasm (Giménez 2013; Font *et al*., 2014; Abidi *et al*., 2015). The strong association found in this study between the biochemical compounds implies that they are important antioxidant phytochemicals that act in coordination to induce tolerance to brown rot in peach. However, further studies are required to determine this.

Infection or incidence, sporulation, and dissemination are the three major stages of the fungal pathogen life cycle in a disease situation (Agrios, 2005). From a genetic point of view, lesion severity is a good parameter to consider during selection for breeding; although there is damage from the fungi, the dispersion of pathogens is limited by the lack of sporulation. However, from a pathogenic point of view, colonization severity is a better factor for consideration because there is the possibility of sporulation due to colonization, which leads to spore dispersal within the environment that can cause further damage.

5. Conclusions

The selection of genotypes for peach breeding that are rich in bioactive compounds, and which are possess brown rot tolerance, may avoid negative outcomes in the industry, and provide safe alternative to the use of pesticides. Based on our 3-year screening protocol, we found phenotypic differences in the susceptibility to brown rot caused by *Monilinia laxa* in the 'Babygold 9' × 'Crown Princess' population. It was also found that FF

515 decreased due to 5 days of storage and to the activity of *M. laxa* in the surrounding tissues.
516 It was possible to identify and select six genotypes (BC1, BC48, BC58, BC63, BC67, and
517 BC68) for low brown rot susceptibility and high fruit quality from the germplasm of the
518 Estación Experimental de Aula Dei-CSIC. Although genotypes that possess bioactive
519 compounds such as AsA, phenolics, flavonoids, and anthocyanins were associated with
520 potential brown rot tolerance, not all genotypes with a lesion of less than 40 mm contained
521 the highest levels of bioactive compounds. The BC1 and BC67 genotypes had significantly
522 higher levels of AsA, phenolics, and anthocyanins. However, flavonoid levels were not
523 significantly different in the resistant compared to the non-resistant genotypes. The
524 negative correlations observed between anthocyanin and brown rot severity highlight their
525 potential influence on susceptibility to *M. laxa*. This interaction is of paramount
526 importance, and consideration should be taken in breeding programs to select cultivars
527 with high levels of bioactive compounds, health-enhancing properties, and good
528 postharvest performance.
529

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536

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