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## Exploring sources of resistance to brown rot in an interspecific almond × peach population

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jsfa.9640](https://doi.org/10.1002/jsfa.9640)

**Abstract**

**BACKGROUND:** *Monilinia* spp. are responsible for brown rot, one of the most significant stone fruit diseases. Planting resistant cultivars seems a promising alternative, although most commercial cultivars are susceptible to brown rot. Therefore, the aim of this study was to explore the resistance to *M. fructicola* over two seasons in a backcross one interspecific population between almond ‘Texas’ and peach ‘Earlygold’ (named T1E).

**RESULTS:** ‘Texas’ almond was resistant to brown rot inoculation whereas peach was highly susceptible. Phenotypic data from the T1E population indicated wide differences in response to *M. fructicola*. Additionally, several non-wounded individuals exhibited resistance to brown rot. QTLs were identified in several linkage groups but only two proximally QTLs in G4 were detected over both seasons, and accounted for 11.3%-16.2% of the phenotypic variation.

**CONCLUSION:** The analysis of the progeny allowed the identification of some resistant genotypes that could serve as a source of resistance in peach breeding programs. Furthermore, the finding of loci associated with brown rot resistance would shed light on implementing a strategy based on marker-assisted selection (MAS) for introgression of this trait into elite peach materials. New peach cultivars resistant to brown rot may contribute to implement more sustainable crop protection strategies.

**Keywords:** *Monilinia fructicola*, disease resistance, *Prunus persica*, *Prunus dulcis*, phenotyping, QTL analysis.

## INTRODUCTION

Brown rot caused by *Monilinia* spp. is an economically important disease in stone fruit since it causes losses in the whole fruit production chain.<sup>1</sup> Under the difficulties to control this disease by chemical approaches and due to consumers concerns about health risk and environmental contamination, planting resistant cultivars would be a promising alternative against brown rot.<sup>2-4</sup> For this, it is necessary to find sources of resistance that could later be used in breeding programs to develop resistant or less susceptible varieties to *Monilinia* spp., and especially to *M. fructicola*. Until 2006, the species *M. fructigena* and *M. laxa*, were identified as the main causal agents of brown rot in pome and stone fruit, respectively.<sup>1</sup> However, over the last decade, *M. fructicola* has gained importance due to the higher aggressiveness and infection ability at higher temperatures (20-25 °C) than *M. laxa*,<sup>5,6</sup> becoming a threat in temperate regions. The application of standard methods to screen the resistance to *Monilinia* spp. on apricot,<sup>7</sup> cherry,<sup>8</sup> nectarine<sup>7,9-11</sup> and plum<sup>7</sup> highlighted a variable range of susceptibility in the materials studied, indicating that this trait has a genetic component. Phenotyping constitutes the basis for the identification of suitable parents for efficient breeding programs to improve this trait<sup>12</sup> and a requirement to find the genetic determinants underlying the observed variability. The susceptibility to fungal pathogens is also strongly affected by environmental conditions and fruit macroscopic characteristics (e.g., maturity date, developmental stage and cuticular cracks, among others)<sup>3,10</sup> which adds complexity to the phenotyping and consequent genetic analysis.

Peach (*Prunus persica* (L.) Batch) is one of the most important stone fruit crops due to its economic value and biological characteristics: small genome size, taxonomic proximity to other important species and relatively short juvenile period,<sup>13</sup> being a model for genomic studies of *Prunus* and other rosaceous fruit crops.<sup>14</sup> In peach, two varieties ‘Bolinha’<sup>15,16</sup> and ‘Contender’<sup>10,17</sup> have been used to improve tolerance for *M.*

*fructicola* and for both *M. laxa* and *M. fructigena*, respectively. Carrying out intraspecific and interspecific crosses, it has been proven that they confer certain tolerance to their offspring. To increase the genetic variability available, several studies have been carried out with germplasm from related species<sup>18</sup> and strategies to introgress this genetic variability in an efficient way have been proposed.<sup>19</sup> Among the varieties described with the highest level of tolerance is included a peach line with almond introgressions called 'F8, 1-42'.<sup>11</sup> Using segregating populations of crosses with 'Contender' and 'F8, 1-42', several QTLs of resistance to *Monilinia* spp. have been described and individuals with greater tolerance than that of the most resistant parent, both at the level of the epidermis and the flesh, have been identified.<sup>11,17</sup> These findings evidenced the interest of exotic sources as a potential suppliers of genes to improve fruit resistance against *Monilinia* spp. Furthermore, these results suggest that the identification of QTLs and the subsequent application of marker-assisted selection (MAS) could be an efficient strategy to develop resistant varieties to brown rot.<sup>20</sup> This type of approach is also used in peach to introduce resistance to powdery mildew<sup>19,21-23</sup> and in apple, where genes for scab resistance (*Venturia inaequalis*) have been introduced from a wild accession of *Malus floribunda*.<sup>24</sup>

The main obstacle for the development of brown rot highly resistant cultivars is the limited amount of tolerant sources that have been identified. Therefore, the search of resistant materials and further analyses to uncover regions associated with brown rot resistance are desirable. Recently, Donoso *et al.*<sup>21</sup> discovered genes and QTLs for fruit quality and powdery mildew resistance from almond that could enrich the peach gene pool analysing two interspecific populations (T×E and T1E). Similarly, the current study aimed to evaluate individuals of one of these populations (T1E) for resistance to *M. fructicola* under controlled conditions. This required the application of an artificial fruit inoculation methodology to evaluate skin and flesh resistance over two harvest seasons. Data obtained was analysed for the presence of QTLs associated with brown rot resistance.

## **MATERIAL AND METHODS**

## Plant material

A previously described interspecific backcross one (BC1) population of 185 individuals derived from a hybrid plant ('MB 1.37') from the cross between 'Texas' (almond) and 'Earlygold' (peach), backcrossed to 'Earlygold', for which a genetic map exists, was used in this research.<sup>25</sup> In this BC1 population (named T1E) all individuals are peach type.<sup>23</sup> Fruit that had not received any synthetic fungicide applications in the field nor postharvest and that were free of visible wounds and rot were obtained from the Experimental Station of Lleida in Gimenezs (Catalonia, Spain) at commercial maturity. The harvest date was defined in accordance to visual colour changes, manual evaluation of firmness and soluble solids content (SSC) measurements that were taken weekly from a sample of 3 fruit per tree. Besides, due to the difficulty in determining a uniform measure of maturity, during 2016 harvest season some individuals were evaluated for resistance at several time points (between two and three times).

In general, 60 fruit per genotype were harvested from the two tree replicates per each genotype, however, in some cases the number of available fruit was smaller. Upon arrival at the laboratory, fruit was homogenised based on the single index of absorbance difference ( $I_{AD}$ ) determined using a portable DA-Meter (TR Turoni, Forli, Italy). In general, from the set of 60 fruit, 10 were discarded, and the remaining 50—with homogenous medium apparent maturity based on a normal distribution—were randomly selected for further analysis and stored at 0 °C until the day of the assay.

## Pathogen and inoculum preparation

The strain of *M. fructicola* used in this study (CPMC3) belong to the collection of the Postharvest Pathology group of IRTA (Lleida, Catalonia, Spain) and was isolated from a latent infection of a peach fruit from a commercial orchard. This strain was identified by the Department of Plant Protection, INIA (Madrid, Spain) and maintained in aqueous solution amended with glycerol (200 g L<sup>-1</sup>) at -80 °C for long-term storage. This strain was subcultured periodically on Petri dishes containing potato dextrose agar (PDA; Biokar Diagnostics, 39 g L<sup>-1</sup>) supplemented with tomato pulp (2.5 g kg<sup>-1</sup>) at 25 °C in the dark for short-term storage.

Conidial suspensions of the fungal culture were prepared by adding 10 mL of sterile distilled water amended with Tween-80 (0.1 g L<sup>-1</sup>) as a wetting agent over the surface of 7-day-old cultures grown on PDA supplemented with tomato pulp, and scraping the surface of the agar with a sterile glass rod. The inoculum was filtered through two layers of sterile cheesecloth to minimize the presence of mycelial fragments. Then, conidia were counted in a haemocytometer and diluted to the desired concentration.

## **Phenotyping**

### **Screening for brown rot resistance**

To check the level of susceptibility to *M. fructicola*, 40 fruit per genotype were divided into two batches according to the method of inoculation applied —wounded (W) or non-wounded (UW)—, and infected following the methodology developed by Baró-Montel *et al.*<sup>26</sup>. Then, fruit were kept in a chamber for 7 days at 20 °C and 85% relative humidity (RH). After 5 days of storage, the number of brown rot infected fruit was recorded, and the lesion diameter was measured at each inoculation point. An additional measure was carried out for UW fruit after 7 days of incubation. Recorded data were expressed as rot diameter (RD) in cm and number of brown rot infected fruit (IN) in percentage. Lesions that did not originate from the inoculation points were considered natural infections and were not recorded. Experiments were carried out with four replicates of five fruit over two consecutive harvest seasons (2016 and 2017). The number of individuals harvested and used for the evaluation of resistance to brown rot, year and meteorological data is given in Table 1.

### **Fruit quality**

To qualitatively characterise the fruit at maturity stage and to explore correlations between pathological and quality data, 10 fruit per each genotype were assessed for value of I<sub>AD</sub>, fruit diameter (FD), flesh firmness (FF), SSC and titratable acidity (TA) following the methodology described elsewhere.<sup>26</sup>

### **Data analysis**

In all cases, the average value per replicate for each response variable was calculated and the data were collated and statistically analysed with the JMP<sup>®</sup> software version 8.0 (SAS Institute Inc., Cary, NC, USA). The non-

parametric Kruskal-Wallis rank sum test was used. For individuals evaluated for resistance at two or three time points, the least significance difference value test (LSD) or the Tukey's HSD test, respectively, at the level  $p < 0.05$  was performed for separation of means. Significance of correlations between traits was checked by Spearman's rank correlation coefficient.

### **QTL analysis**

For the QTL analysis, a highly saturated map of the T1E population described in Donoso *et al.*<sup>25</sup> was used. The map used was based on the subset of 2,032 markers heterozygous in the 'Texas' × 'Earlygold' hybrid parent and constructed using the MAPMAKER 3.0<sup>27</sup> and the Kosambi's mapping function. QTL analysis was performed using the MapQTL 6.0 software package<sup>28</sup> and the interval mapping (IM) method. Only phenotypic data from individuals bearing a minimal number of 10 fruits was used, since lower sample size was underpowered to be considered representative. A QTL was considered significant when presented a LOD  $e \geq 2.5$  in the IM.

## **RESULTS**

### **Meteorological data**

Seasonal maximum and minimum mean temperatures, RH and rainfall that occurred during the field experiments are reported in Table 1, jointly with other harvest information (harvest interval, number of harvest dates and total number of individuals evaluated). The data used came from a meteorological station located in Gimenez (41° 39' 9" N, 0° 23' 23" E, 259 m), a region with a semi-arid climate with Mediterranean precipitation pattern, foggy and cold winters and hot and dry summers. Overall, temperature and RH values were very similar between the two years. Regarding temperature, in 2016 monthly-average temperature was  $22 \text{ °C} \pm 2 \text{ °C}$ , and in 2017 around  $23.5 \text{ °C} \pm 1 \text{ °C}$  except in September, when the average value was lower ( $18 \text{ °C}$ ). Concerning RH, average values increased along the season from 53 to 65 in 2016, and from 60 to 68% in 2017. In contrast, rainfall showed significant differences between these two years due to drought conditions during 2016 (14 mm from mid-June to mid-



September) compared to 2017, that was consistently wetter (120.3 mm for the same harvest interval). However, the trend was irregular and a raise of precipitation in mid-June (64.7 mm) was monitored.

### **Phenotyping**

The phenotyping methodology was set up in order to generate data to be used later in QTL analysis. As shown in Table 1, a total of 89 and 120 individuals for the growing seasons of 2016 and 2017, respectively, were evaluated for brown rot resistance and assessed according to standard quality parameters. Between both years of study, 81 individuals were coincident.

In this study, a total of 9 traits were analyzed in the T1E population and in the parentals ('Texas', 'Earlygold' and 'MB 1.37') in two different seasons. Traits were related to: i) resistance to brown rot: severity and incidence for wounded and non-wounded fruit (W\_RD, W\_IN, UW\_RD and UW\_IN) and ii) to fruit quality: maturity date (MD), FD, FF, SSC and TA.

### **Screening for brown rot resistance**

The susceptibility of the fruit to *M. fructicola* displayed a high variability of lesion diameter (Fig. 1), and as shown in Fig. 2 significant differences were found among the parents. It is worth noticing that the almond parental 'Texas' was resistant using both wounded and non-wounded inoculation methodologies (Fig. 2A and 2B), whereas 'Earlygold' was highly susceptible as more than 80% of the fruit developed the disease (Fig. 2C and 2D). After 5 days of incubation, the lesion diameter of wounded 'Earlygold' fruit was approximately 7.3 cm in 2016 (Fig. 1A) and 7.4 cm in 2017 (Fig. 1B). With regard to 'MB 1.37', did not develop brown rot disease without the presence of a wound in any of the harvest seasons (Fig. 2F). Contrarily, wounded 'MB 1.37' fruits showed lesion diameters of approximately 7.3 cm in 2016 (Fig. 1A) and 8.6 cm in 2017 (Fig. 1B), after 5 days of incubation. Regarding brown rot incidence, 100% of wounded fruit developed the disease.

Concerning the T1E population, histograms for brown rot resistance traits indicated a non-normal distribution, especially for incidence that registered values mainly extreme (Supplemental Figure S1). For instance, most wounded individuals were in the range of 90-100%. For non-wounded fruit, it should be

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noted that more than half of non-wounded individuals exhibited no or low infection whereas the rest fell in other classes, especially 10-20% and 20-30%. For rot diameter, wounded fruit presented a wide range of values distributed in diverse frequencies whereas non-wounded fruit showed similar distribution to incidence. In 2016, the overall mean lesion diameter of UW and W fruit was 0.7 cm and 6.6 cm, respectively (Fig. S1A and S1B), whereas incidence values were 15% and 97%, respectively (Fig. S1E and S1F). Thirty out of the 89 non-wound-inoculated individuals were classified as resistant (34% of the T1E population) and the rest ranged from 0.1 cm and 5% of infection (T1E 101) to 4.9 cm and 86% of infection (T1E 243). Regarding wound-inoculated, there were no completely resistant individuals found and values ranged from 2 cm and 80% of infection (T1E 418) and to 9 cm and 55% of infection (T1E 219). In 2017, the mean lesion diameter of UW and W fruit was 1.38 cm and 7.39 cm, respectively (Fig. S1C and S1D) whereas incidence values were 18% and 99%, respectively (Fig. S1G and S1H). Thirty-nine out of the 120 non-wound-inoculated individuals were not infected by *M. fructicola* (33% of the T1E population) and the remaining showed values from 0.1 cm (T1E 22) to 7 cm (T1E 101). Regarding wound-inoculated individuals, minimum and maximum values were 2 cm (T1E 287) and 9.6 cm (T1E 344), respectively.

The application of the phenotyping methodology in non-wounded fruit allowed the identification of 7 coincident genotypes between both years of study (T1E 24, T1E 43, T1E 49, T1E 155, T1E 197, T1E 239 and T1E 340) with resistance to *M. fructicola*. Apart from these resistant genotypes, it is worthwhile pointing out that the following individuals: T1E 5, T1E 20, T1E 22, T1E 34, T1E 45, T1E 50, T1E 62, T1E 64, T1E 219, T1E 220, T1E 304, T1E 389 and T1E 491 were not infected in one year and low infected (RD <0.5 cm and d3 rotted fruit) in the other year.

Finally, correlations of all the traits are reported in Table 2. Focusing on resistance traits, comparison between years only resulted in significant correlations for W\_RD ( $R^2$  value of 0.43 ( $p < 0.0006$ )). Concerning correlations between traits for each year, W\_RD significantly correlated with W\_IN ( $R^2 = 0.31$ ;  $p < 0.0097$ ) only in 2016 whereas UW\_RD and UW\_IN were highly correlated in both years ( $R^2 = 0.96$ ;  $p < 0.0001$  in 2016 and  $R^2 = 0.97$ ;  $p < 0.0001$  in 2017).

## Fruit quality

At maturity, the individuals of the BC1 progeny were assessed for  $I_{AD}$ , FD, FF, SSC and TA (data not shown). Most fruit quality traits, except those related to SSC and TA in 2016 and FD in 2017, presented a non-normal distribution (data not shown). Similar results were observed comparing the same quality parameters between years, except for TA. In 2016, the overall mean, minimum and maximum values (in parenthesis) for MD, FD, FF and SSC were 203 (163-248) Julian days, 49.4 (27.7-61.4) mm, 30 (1.6-80.5) N and 10.6 (6.2-16.2) °Brix. In 2017, the overall mean for MD, FD, FF and SSC were 195 (156-247) Julian days, 49.6 (27.3-67) mm, 29.6 (3.2-127.4) N and 10.6 (4.7-17.5) °Brix. For TA, significant differences were found between values in 2016 (15.1 g of malic acid L<sup>-1</sup>) and 2017 (11.6 g of malic acid L<sup>-1</sup>).

Overall, significant correlations were found between quality data obtained in both years (Table 2). Comparisons of the same traits between years resulted in significant correlations for MD, FD, SSC and TA. MD exhibited the highest correlation between years ( $R^2 = 0.93$ ;  $p < 0.0001$ ), indicating that Julian days were similar. FD, SSC and TA showed  $R^2$  values of 0.57 ( $p < 0.0001$ ), 0.46 ( $p < 0.0004$ ) and 0.45 ( $p < 0.0004$ ), respectively, whereas FF gave insignificant correlations between years.

Correlations between pathological and quality traits evidenced the effect of maturity date on the disease development in non-wounded fruit. Values for this trait were negatively correlated with rot diameter and incidence in 2016 ( $R^2 = 0.39$ ;  $p < 0.001$ . and  $R^2 = 0.47$ ;  $p < 0.001$ , respectively), and positively correlated in 2017 ( $R^2 = 0.20$ ;  $p < 0.05$  and  $R^2 = 0.25$ ;  $p < 0.01$ , respectively). Following on from these comparisons, for wounded fruit, rot diameter also correlated with fruit diameter in 2016 ( $R^2 = 0.33$ ;  $p < 0.0085$ ) and in 2017 ( $R^2 = 0.21$ ;  $p < 0.004$ ). For non-wounded fruit, it should be noted that in 2017 significant correlations were also found between fruit diameter and rot diameter ( $R^2 = 0.30$ ;  $p < 0.0029$ ) and fruit diameter and incidence ( $R^2 = 0.27$ ;  $p < 0.0069$ ). Similarly, rot diameter and incidence negatively correlated with flesh firmness and titratable acidity in 2016.

## QTL analysis

As shown in Table 1, a total of 68 and 100 individuals for the growing seasons of 2016 and 2017, respectively, were used for QTL analysis. Between both years of study, 58 individuals were coincident. The integration between phenotypic and genotypic dataset using IM analysis on the T1E integrated map allowed the identification of a total of 12 QTL regions involved in brown rot resistance that are summarized in Table 3. LOD scores were between 2.55 and 4.86 and variance explained between 11% and 23.5%. The location of these putative QTLs conferring resistance to brown rot were placed on all linkage group (G), except G1 and G3.

When QTLs for a specific trait are detected in the same chromosomal regions in both years, they can be considered stable. On this basis, no consistent QTLs could be found over the two harvest seasons, although two QTLs mapped in G4 were near stability. These QTLs for UW\_IN trait, explaining 16.2% (LOD 2.69) and 11.3% (LOD 2.66) of phenotypic variance in 2016 and 2017, respectively, were found between markers SNP\_IGA\_407115 and SNP\_IGA\_440110.

In addition, several QTLs for MD in G4 and in the same region of QTLs for UW\_IN were found, explaining around 19.6% and 57% and between 11.8% and 72.2% of the trait variability in 2016 and 2017, respectively (data not shown).

## DISCUSSION

One of the most challenging issues facing crop improvement is breeding for disease resistance.<sup>29</sup> For this reason, breeders and pathologists have focused on obtaining new cultivars resistant to pathogens. This work, which incorporates phenotyping on an almond × peach population under controlled laboratory conditions, represents an important step forward to achieve this goal. The two-year results revealed statistically significant differences in lesion diameter but not in incidence. Since outbreaks of brown rot are dependent on prevailing environmental conditions,<sup>30</sup> analysis of the two-year meteorological conditions is advisable. In this study, the clearest differences between seasons were the slightly higher temperatures, together with the presence of wet periods (from 2<sup>nd</sup> to 4<sup>th</sup> June, 27<sup>th</sup> June and 8<sup>th</sup> July)

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during the 2017 that could have accounted for significantly higher disease severity. Mild winters, rainfall and warmer summers favour the occurrence of *Monilinia* spp.<sup>30-32</sup> Future scenarios indicate that these trend will continue, so these changes demand for disease assessment in future environmental conditions, which are favourable for the infection, development and spread of brown rot.

In the present study, the effect of wounding the fruit on *M. fructicola* development could be clearly confirmed after 5 days of incubation. For lesion diameter, the distributions were methodology-dependent evidencing that the need of a wound is one of the crucial factors for pathogen colonisation.<sup>33</sup> A wounded area is an open way to infections because automatically implies disruption of membranes, loss of integrity and thus, is easily accessible to penetration of pathogens.<sup>34</sup> In this study, as similarly observed by other authors, response to pathogen attack after wounding led to a wide range of values without any resistant individual. Conversely, half of non-wounded individuals exhibited no or low infection confirming the critical role of skin on fruit defence against this pathogen, as pointed out by several authors.<sup>7,16,35-37</sup> The correlations of resistance traits between both harvest seasons were weak. This lack of correlation may indicate a strong seasonal influence in the development of the disease at the skin and flesh levels,<sup>17</sup> supporting the fact that genetic control of this trait is low.

Fruit quality traits were significantly correlated between both harvest seasons, evidencing the role of genetic factors rather than environmental. Among them, maturity date was the most strongly correlated. When it comes to fungal-host interactions, the influence of some quality traits such as acidity and maturity, is documented. Effect of maturity on infection capacity in other pathosystems such as *Penicillium digitatum*-oranges and *P. expansum*-apples has already been described.<sup>38,39</sup> In the current study, while exploring possible correlations between pathological and quality traits, maturity date was found to be significantly correlated with rot diameter and incidence for non-wounded fruits in both years. Notwithstanding, correlation coefficients were low, and negatively correlated in 2016 whereas in 2017 were positively correlated. This last result is in consonance with Pacheco *et al.*<sup>15</sup> who also obtained significant correlations for maturity date and rot diameter for both, wounded and non-wounded fruits. A positive correlation with maturity date could suggest that earlier fruits are less susceptible to brown rot

than late-ripening ones as pointed by several authors, but this pattern was not clearly demonstrated in this study. However, to further investigate the effect of MD in the QTL analysis, the closest marker to a major QTL for MD on G4 identified both in this study and using several populations from different *Prunus* species,<sup>23,40</sup> was used as a cofactor in MQM mapping. Notably, neither other QTL nor variations in fungal decay at several time points were detected.

To develop infection in the absence of a wound, the fungi need to pass physical and biochemical barriers. For instance, flesh firmness is related to the first type of barrier, whereas acidity to the second one. Therefore, it would make sense the negative correlation found between these quality traits and disease traits for non-wounded fruit because generally, both parameters decrease during peach maturation.<sup>41</sup> Interestingly, the results presented herein showed that TA was highly correlated between both years, although the coefficients were low. Remarkably, these levels were higher in 2016 than in the next year, when disease severity was lower, and consequently, let us to hypothesise that the less favourable environmental conditions for *M. fructicola* growth and colonisation —compared to 2017—, jointly with the higher levels of malic acid monitored during this year may explain such difference in severity. In relation to malic, is the predominant organic acid in mature peach fruit followed by citric acid,<sup>42</sup> and studies recently conducted by Baró-Montel *et al.* (unpublished data) with ‘Merryl O’Henry’ peaches, pointed out the importance of organic acids in determining peach susceptibility to brown rot. Regarding flesh firmness, it is noteworthy to mention that the negative correlation with pathological traits may be at least partly attributable to the width and fleshiness of the pulp due to the sporadic nature of fruit bearing within the T1E population. Taken together, these results indicate that the disease was easily developed and spread in fruit with softer pulp and/or lower malic acid levels, both characteristics of mature fruit, the stage most susceptible to infection as reported by various works that have focused on understanding how susceptibility to brown rot changes with ripening.<sup>10,36,43–46</sup>

In the present work, connection between genotype and phenotype allowed the identification of several QTLs. Between one and three QTLs were detected for each trait and year. Some of these QTLs were found in positions where brown rot QTLs were already detected using other populations, for instance that

on G4<sup>11,17</sup> while others were located in regions where no previous brown rot QTLs were reported (G6). From the brown rot management point of view, skin resistance is more interesting, since once fruit has been wounded there is no way to avoid the development of the disease. According to this, non-wounded inoculations were the most informative and allowed the discovery of two proximal resistance QTLs for incidence in G4 that showed significant effects in the two years of study, although in 2016 ‘Texas’ alleles were increasing resistance while in 2017 were decreasing it. As no major QTLs were detected in 2016 and to verify if some recessive alleles could be involved in almond resistance, in 2017 we evaluated fruit from a F2 population (named T×E) derived from the same cross. Despite the low number of individuals producing fruit in the F2 was very low (n = 34), we found a significant QTL in G6 (data not shown), but on the other side of the chromosome where it was detected the QTL in the T1E population. By contrast, the QTLs found for wounded inoculations were of greater magnitude, but inconsistent due to the changing position between the two traits —W\_RD and W\_IN— and the two years of experimentation, suggesting that infection is largely controlled by environmental factors. Moreover, as brown rot resistance is of polygenic nature, multiple genes with minor effects may be involved,<sup>11,16,17,37</sup> that could not be detected due to the relatively low population size and the limitation of the phenotypic tools used.

Little information exists referring to genomic regions involved in brown rot resistance and in general, for resistance to necrotrophic pathogens in fruit. Martínez-García *et al.*<sup>9</sup>, using an almond × peach progeny identified QTLs in G1. This region included two potential candidate genes, coding for pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) proteins. In this same field, Pacheco *et al.*<sup>15</sup>, using an F1 progeny from the cross ‘Contender’ (moderately resistant) × ‘Elegant Lady’ (susceptible), uncovered three genomic regions associated with brown rot resistance: G2 and G4 for skin resistance, and G3 for flesh resistance. For G2, the presence of several putative resistance gene analogues (RGAs) have been reported.<sup>47</sup> More recently, Fu *et al.*<sup>44</sup>, evaluated allelic variability in these brown rot associated genomic regions and phenotyped a progeny from crosses with ‘Bolinha’, that in combination with genotyping data could provide an important basis for developing predictive DNA information tools for brown rot resistance.

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Finally, using The Genome Database for Rosaceae (GDR)<sup>49</sup> it was possible to search *in silico* genes of interest. Within the position of markers for the QTL for non-wounded incidence trait located on G4 (9,947,470-16,076,720 bp), was retrieved the NB-ARC domain-containing disease resistance protein involved in regulation of programmed cell death.<sup>50,51</sup> In the same chromosome, there are candidate genes encoding endopolygalacturonases (endo-PGs). Plant cell wall has three main components: cellulose, hemicellulose and pectin that necrotrophic pathogens break down and utilise as nutrients.<sup>52</sup> Pectin is a complex polymer constituted by units of homogalacturonan, rhamnogalacturonan and xylogalacturonan that determines the cohesion and porosity of cell wall.<sup>53</sup> Endo-PG are cell wall hydrolases that attack internal linkages in cell wall pectin and as a consequence, accelerate the rate of softening and diminish resistance to fungal infections and cracking during postharvest handling.<sup>54</sup> Hence, studying the activity and changes in endo-PGs levels of expression may help in further understanding the role of skin in contributing to pathogenicity, and could somehow be related to levels of resistance or susceptibility of certain individuals.

As a concluding remark, it is worthwhile to mention that different studies carried out worldwide with different hosts (berries, citrus, pome and stone fruit) in order to study the inheritance of resistance traits, among others, reported the difficulty, complexity and long-duration of this type of research. For instance, Norelli *et al.*<sup>55</sup>, took a similar approach to identify blue mould resistance in a mapping population of a cross between ‘Royal Gala’ × *Malus sieversii* (PI613981) and indicated the difficulty in determining a uniform measure of maturity when phenotyping for resistance as each individual is a distinct genotype with different characteristics. However, a much stronger QTL was identified in the former study although it was also highlighted that some LOD did not account for the observed differences in resistance among the progeny. Besides, consumer expectations are lacking in current peach germplasm as characteristics associated with fruit resistance may conflict with commercial aptitude as some traits associated with host resistance are present in cultivars of poor commercial and productive quality.<sup>10</sup> Hence, information in this area is still scarce and more is needed to satisfy the increased awareness of clean-label products among producers and consumers.



## CONCLUSIONS

The current work presents a phenotypic analysis performed in an almond × peach population over two consecutive harvest seasons by an artificial inoculation procedure that measured skin and flesh resistance to *M. fructicola*. The characterization of the different individuals under different conditions (wounded and non-wounded) lead to significant differences among the studied plant material. Besides, the identification of genotypes with low levels of infection or no infection at all, indicates that genetic resources for the development of peach cultivars resistant to brown rot are available in the almond species. The study has already enabled the detection of QTLs, most with small effect and low reproducibility and thus, indicative of the great complexity of this trait. In this context, peach breeding programs would benefit from the identification of genomic regions involved in *Monilinia* spp. resistance which could be used later for MAS and thus, provide an alternative approach to chemical sprays and may contribute to improve sustainable crop protection strategies.

## ACKNOWLEDGEMENTS

This work was supported by national projects AGL2014-55287-C02-02-R and RTA2015-00050-00-00 from Ministry of Economy, Industry and Competitiveness (MINECO), by a PhD grant 2017FI\_B1\_00153 (Baró-Montel, N.) from the Catalan Government (Generalitat de Catalunya) and by the funding received from CERCA Programme / Generalitat de Catalunya and the Severo Ochoa Program for Centres of Excellence in R&D 2016-2019 (SEV-2015-0533).

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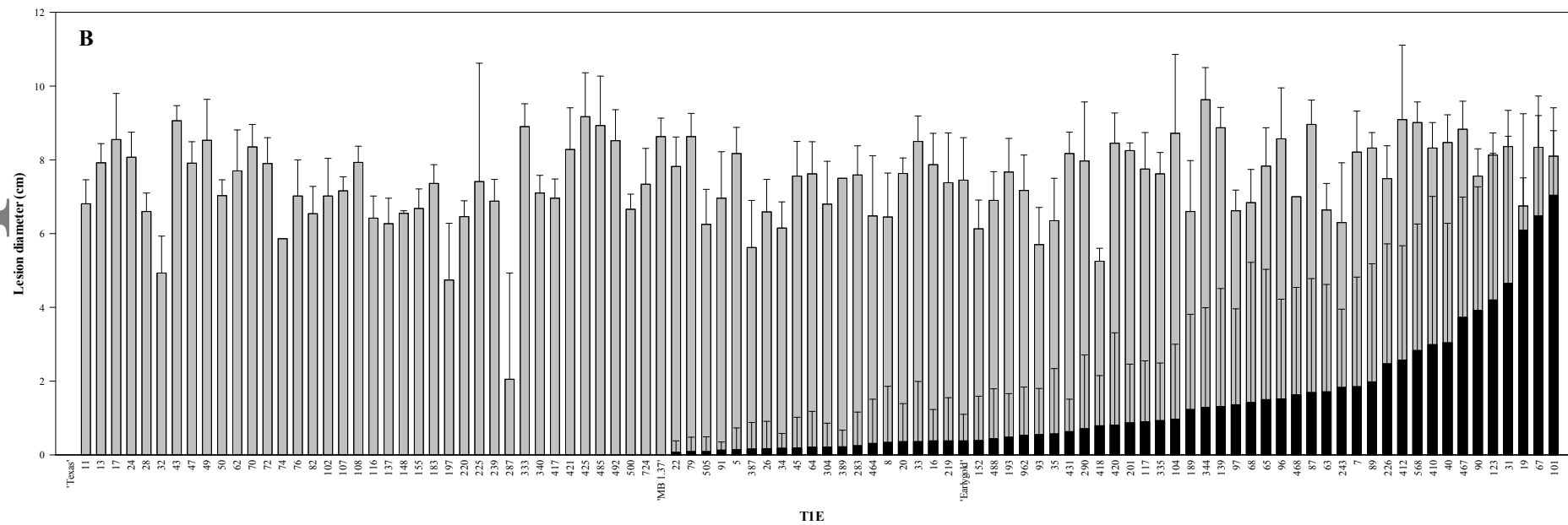
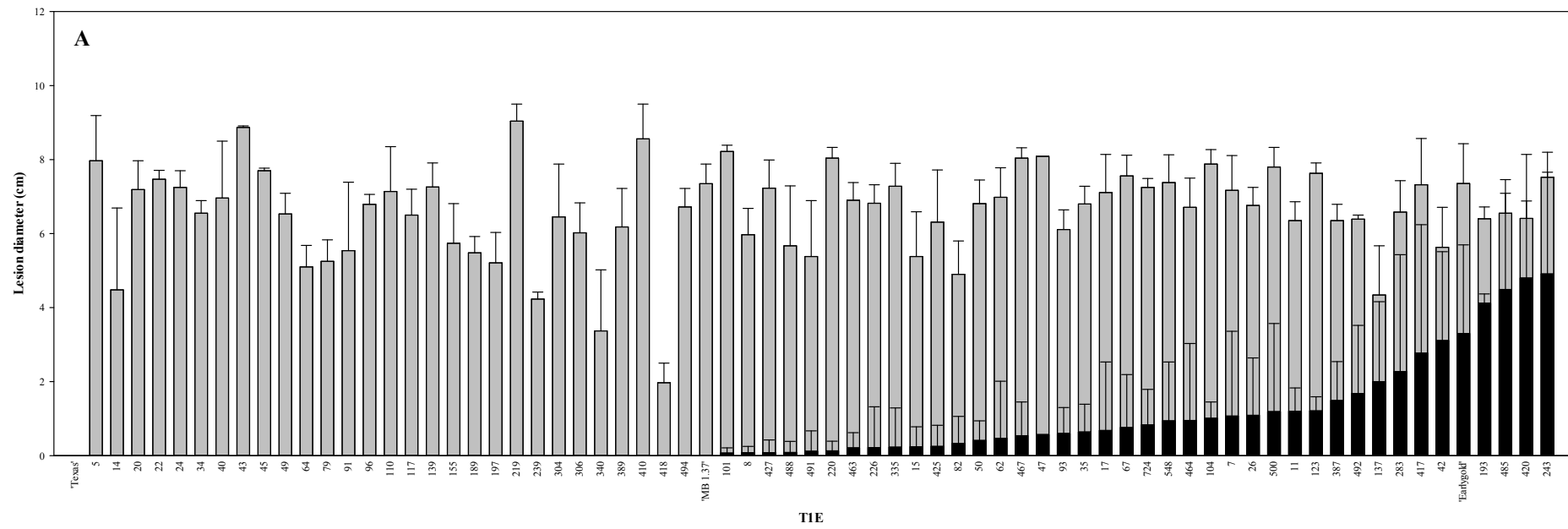
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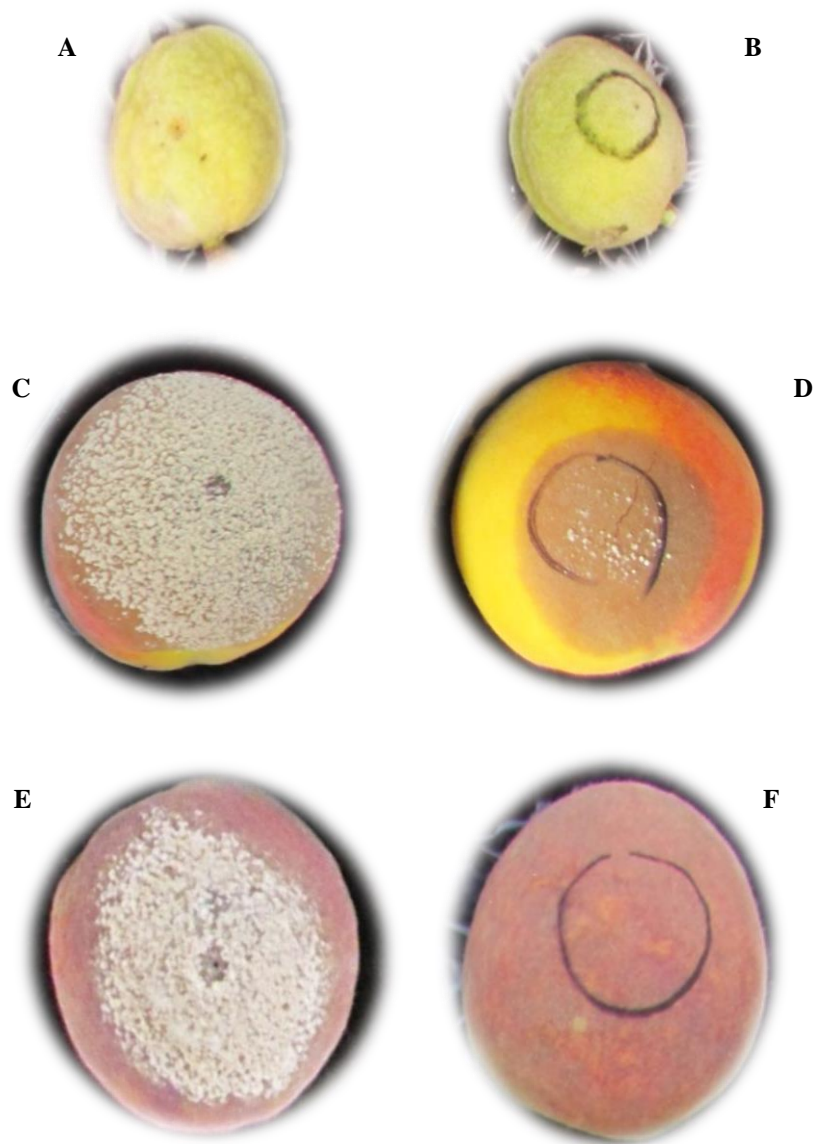
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**Figure 1.** Lesion diameter of wounded (■) and non-wounded (■) individuals of the T1E population including the parents (almond ‘Texas’, peach ‘Earlygold’ and the hybrid ‘MB 1.37’), for the 2016 (A) and 2017 (B) harvest seasons in order of increasing values for non-wounded fruit. Wounded fruit were inoculated with 10  $\mu\text{L}$  of strain CPMC3 of *Monilinia fructicola* at  $10^4$  (100 conidia per fruit) and non-wounded fruit were inoculated with 10  $\mu\text{L}$  at  $10^5$  conidia  $\text{mL}^{-1}$  (1000 conidia per fruit) and incubated for 5 days at 20 °C and 100% relative humidity. Data represent the mean of 20 fruit for each inoculation methodology and genotype assessed. Bars indicate standard deviation of the mean.



**Figure 2.** Disease assessment of wounded (A, C and E) and non-wounded (B, D and F) 'Texas', 'Earlygold' and 'MB 1.37' hybrid fruits, respectively, inoculated with *Monilinia fruticola* and incubated for 5 days at 20 °C and 100% relative humidity.

**Table 1.** Summary of individuals harvested from the backcross one (BC1) population derived from the cross between the hybrid ‘MB 1.37’ (almond ‘Texas’ × peach ‘Earlygold’) and the peach ‘Earlygold’ (T1E) used for the evaluation of resistance to brown rot (BR) and for the QTL analysis and meteorological data of the two consecutive harvest seasons.

Year	Total number of individuals evaluated		Seasonal temperature (° C)	Seasonal relative humidity (%)	Total seasonal precipitation (mm)	Harvest interval	Total number of harvest dates
	For BR resistance	For QTL analysis					
2016	89	68	15.8 – 31.9	54.5	14	13 Jun. – 12 Sep.	14
2017	120	100	16.4 – 31.4	61.2	120.3	5 Jun. – 12 Sep.	15

Weather data was obtained from the Department of Agriculture of the Catalan Government (RuralCat) and minimum, maximum or mean season values were calculated.

**Table 2.** Spearman's rank correlations between traits in the T1E population for the two consecutive

	W_RD	W_IN	UW_RD	UW_IN	MD	FD	FF	SSC	TA
W_RD	<b>0.43</b>	<b>0.31</b>	0.15	0.11	0.16	<b>0.33</b>	0.22	-0.17	0.22
W_IN	0.10	<b>-0.06</b>	0.12	0.19	-0.08	0.21	-0.16	0.10	-0.14
UW_RD	0.20	<b>0.21</b>	<b>-0.03</b>	<b>0.96</b>	<b>-0.39</b>	-0.09	<b>-0.32</b>	-0.15	<b>-0.29</b>
UW_IN	0.12	<b>0.21</b>	<b>0.97</b>	<b>-0.08</b>	<b>-0.47</b>	-0.15	<b>-0.37</b>	-0.11	<b>-0.38</b>
MD	0.15	0.09	<b>0.20</b>	<b>0.25</b>	<b>0.93</b>	0.14	<b>0.62</b>	0.05	<b>0.37</b>
FD	<b>0.21</b>	-0.03	<b>0.30</b>	<b>0.27</b>	<b>0.30</b>	<b>0.57</b>	0.01	0.12	<b>0.26</b>
FF	<b>-0.24</b>	-0.14	-0.11	-0.10	-0.17	<b>-0.41</b>	<b>0.03</b>	0.02	<b>0.37</b>
SSC	0.04	-0.01	-0.06	-0.04	<b>0.38</b>	<b>0.28</b>	<b>-0.33</b>	<b>0.46</b>	0.09
TA	0.05	<b>-0.22</b>	-0.04	-0.05	<b>0.39</b>	0.06	<b>0.39</b>	0.02	<b>0.45</b>

harvest seasons.

In the diagonal correlations between the 2 years of testing. Values above and below the diagonal reported the correlation coefficients between traits in 2016 and 2017, respectively. Significant correlations ( $p < 0.05$ ) are given in bold. Wounded rot diameter (W\_RD); wounded incidence (W\_IN); non-wounded rot diameter (UW\_RD); non-wounded incidence (UW\_IN); maturity date (MD); fruit diameter (FD); flesh firmness (FF); soluble solids content (SSC) and titratable acidity (TA).

**Table 3.** Linkage group locations, nearest marker position, LOD score and proportion of phenotypic variation explained (%) of each putative QTL controlling traits analysed for the two consecutive harvest seasons in the T1E population using the T1E map.

Trait	Year	Linkage group	Nearest marker	Position (cM)	LOD	Variance explained (%)
<b>W_RD</b>	2016	6	SNP_IGA_679852	38.2	3.76	22.5
	2017	7	SNP_IGA_781455	38.8	4.86	20.6
<b>W_IN</b>	2016	8	SNP_IGA_884329	51.7	3.96	23.5
		5	SNP_IGA_560796	4.7	2.71	16.8
<b>UW_RD</b>	2016	2	SNP_IGA_144913	6.1	2.74	17
		5	SNP_IGA_552254	1.9	2.55	15.8
	2017	6	SNP_IGA_694830	52.1	2.60	11.3
<b>UW_IN</b>	2016	5	BPPCT037	18.6	2.82	16.9
		<b>4</b>	<b>SNP_IGA_407115</b>	<b>38.2</b>	<b>2.69</b>	<b>16.2</b>
		2	SNP_IGA_144913	6.1	2.60	15.7
	2017	<b>4</b>	<b>SNP_IGA_440110</b>	<b>47.0</b>	<b>2.66</b>	<b>11.3</b>
		6	SNP_IGA_683611	42.8	2.58	11

QTLs detected in both seasons in proximal regions are given in bold. Wounded rot diameter (W\_RD); wounded incidence (W\_IN); non-wounded rot diameter (UW\_RD) and non-wounded incidence (UW\_IN).