Susceptibility of almond (*Prunus dulcis*) cultivars to twig canker and shoot blight caused by *Diaporthe amygdali*

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Abstract. Twenty-five almond cultivars were assessed for susceptibility to *Diaporthe amygdali,* causal agent of twig canker and shoot blight disease. In laboratory experiments, growing twigs were inoculated with four *D. amygdali* isolates. Moreover, growing shoots of almond cultivars grafted onto INRA 'GF-677' rootstock were used in four-year field inoculations with one *D. amygdali* isolate. In both type of experiments, inoculum consisted of agar plugs with mycelium, which were inserted underneath the bark and the lesion lengths caused by the fungus were measured. Necrotic lesions were observed in the inoculated almond cultivars both in laboratory and field tests, confirming the susceptibility of all the evaluated cultivars to all the inoculated isolates of *D. amygdali*. Cultivars were grouped as susceptible or very susceptible according to a cluster analysis. The relationship between some agronomic traits and cultivar susceptibility was also investigated. Blooming and ripening times were found relevant variables to explain cultivars performance related to *D. amygdali* susceptibility. Late and very late blooming, and early and medium ripening cultivars were highly susceptible to *D. amygdali*. Our results may provide valuable information that could assist in ongoing breeding programs of this crop and additionally in the selection of cultivars for new almond plantations.

Keywords. Almond breeding, blooming time, fungi, nut crops, pathogenicity, ripening time.

INTRODUCTION

During the last 15 years, almond (*Prunus dulcis* (Mill.) D.A. Webb) crop has been experiencing a very favorable period worldwide (Gradziel et al. 2017). Consumption of almonds has several positive connotations with respect to health, as they are rich in nutrients like vitamin E, proteins, mono-unsaturated fatty acids, poly-unsaturated fatty acids, magnesium, potassium, and dietary fibers, which have been linked to lower cardiometabolic disease risk (Kalita et al. 2018). This fact, together with the opening of new markets in Asia, has resulted in an increase in both almond demand and prices (INC 2020). Moreover, almond growing in the Mediterranean area is currently evolving from a marginal rainfed crop to a very productive and profitable one, with new cultivars and production systems, thus increasing its planted area (Maldonado et al. 2019).

Spain stands out with the largest almond area in the world, with 718,540 ha (MAPA 2020), but yields per ha are below those obtained by other countries with less planted area such as the USA and Australia (FAOSTAT 2021). This represents a new challenge for Spanish almond growers, who aim at improving their orchard yields by opting for cultivars with favorable agronomic characteristics for intensive production (i.e., increased planting density, mechanized harvesting, and the use of drip irrigation). In addition, in recent years the crop is experiencing an active process of varietal renewal (Batlle et al. 2017). The new almond cultivars obtained in Spanish breeding programs aim to improve fruit quality (size, shape, weight, protein, oil content and stability and fatty acids), while selecting for late flowering, self-fertility bearing precocity, and tolerance to pathogens (Batlle et al. 2017). Nevertheless, potential yield of almond in Spain can be reduced by the reemergence of pests and diseases that were not usual in traditional almond growing or just showed a low

66 impact on production, and by the low number of fungicides currently authorized for the
67 control of almond pests and diseases (Torguet et al. 2019).

68 Almond crop can be affected by several fungal diseases, such as red leaf blotch
69 (*Polystigma amygdalinum* P.F. Cannon), shot hole (*Wilsonomyces carpophilus* (Lév.)
70 Adask., J.M. Ogawa & E.E. Butler), brown rot and blossom blight (*Monilinia* spp.), and
71 leaf curl (*Taphrina deformans* (Berk.) Tul.) (Miarnau et al. 2021; Ollero-Lara et al. 2019;
72 Teviotdale et al. 2002), as well as by the reemergence of old ones such as anthracnose
73 (*Colletotrichum acutatum* J.H. Simmonds) (López-Moral et al. 2019), and the new branch
74 canker and dieback diseases caused by trunk pathogens (Gramaje et al. 2012; Holland et al.
75 2021; Olmo et al. 2016). Among them, twig canker and shoot blight caused by *Diaporthe*
76 *amygdali* (Delacr.) Udayanga, Crous & K.D. Hyde is widespread in the Mediterranean
77 countries, and seriously compromise crop productivity (Adaskaveg 2002; Diogo et al.
78 2010; León et al. 2020). A recent study conducted in Spain, in which 225 *Diaporthe*
79 isolates from almond orchards were characterized by a multilocus DNA sequence analysis,
80 confirmed *D. amygdali* as a key pathogen of almond in Spain (Hilário et al. 2021; León et
81 al. 2020).

82 Symptoms of twig canker and shoot blight disease caused by *Diaporthe* spp. are
83 characterized by the quick desiccation of buds, flowers and leaves after infections produced
84 in late winter or early spring. The new shoots developing from infected buds usually wilt
85 and die (Adaskaveg 2002; Varjas et al. 2017b). Brown lesions (1 to 5 cm diameter),
86 initially formed around buds on green shoots, further develop into annual sunken cankers,
87 sometimes with a gummy exudate, as well as withering of twigs (Adaskaveg 2002). As a

result, leaves wilt and, when the disease is severe, defoliation may occur. In summer, pycnidia develop just under the dry canker bark (Adaskaveg 2002).

Studies on the susceptibility of almond cultivars to fungal diseases are increasing in literature, mainly within the last decade. In Spain, Egea et al. (1984) carried out an evaluation of the susceptibility to red leaf blotch with 81 almond cultivars. In California, Gradziel and Wang (1994) evaluated the fruit susceptibility of different almond cultivars to *Aspergillus flavus* Link, and Diéguez-Uribeondo et al. (2011) determined the susceptibility of four almond cultivars to *C. acutatum*. In Australia, Horsfield and Wicks (2014) studied the susceptibility of 34 almond cultivars to the rust pathogen *Tranzschelia discolor* (Fuckel) Tranzschel & M.A. Litv. in field conditions following natural and artificial infections. In Spain, López-Moral et al. (2019) evaluated the susceptibility of 19 almond cultivars to *C. acutatum* and *C. godetiae* Neerg., and additional studies have evaluated the susceptibility of early and late flowering almond cultivars to foliar diseases caused by *Monilinia laxa* (Aderh. & Ruhland) Honey*, P. amygdalinum, T. deformans* and *W. carpophilus* (Miarnau et al. 2021; Ollero-Lara et al. 2019).

Regarding *D. amygdali*, its pathogenicity to almond trees has been widely documented (Adaskaveg et al. 1999; Diogo et al. 2010; León et al. 2020; Teviotdale et al. 2002; Varjas et al. 2017b), and the susceptibility of almond cultivars to this pathogen has also been investigated. In Chile, *D. amygdali* was inoculated in three almond cultivars (‘Carmel’, ‘Nonpareil’ and ‘Price’), being ‘Nonpareil’ and ‘Price’ more susceptible than ‘Carmel’ (Besoain et al. 2000). In Portugal, a local almond cultivar (‘Barrinho Grado’) showed a higher tolerance to *D. amygdali* than ‘Ferragnès’ (Cabrita et al. 2004). In Spain, Vargas and Miarnau (2011) evaluated more than 70 almond cultivars and 36 selections in field

conditions with natural infections, and showed a broad gradient of susceptibility to Diaporthe dieback among cultivars. In Hungary, pathogenicity tests were carried out in 162 almond genotypes with *D. amygdali* (Varjas et al. 2017a). Thirty-one of them were found to be highly tolerant according to 4-year observations. Specifically, 'Budatétényi-70' and 'Tétényi keményhéjú' cultivars showed a significantly higher tolerance to this pathogen compared with other Hungarian cultivars, and the results also showed a wide range of variability among the genotypes and cultivars studied.

The main objective of this research was to obtain new information about the susceptibility of a collection of 25 almond cultivars to *D. amygdali*, with experiments conducted both *in vitro* and *in vivo* conditions. We focused our attention on evaluating the susceptibility to *D. amygdali* of the most recently-obtained Spanish cultivars in the last two decades, in order to provide breeders and farmers with tools to obtain and grow more tolerant cultivars in the future. Additionally, some of the most planted cultivars in Europe, including France and Italy, and the USA were included in our trials for comparison purposes.

MATERIALS AND METHODS

Almond cultivars. In this study, twenty-five almond cultivars were assessed for susceptibility to *D. amygdali*. Fifteen cultivars were obtained from three different Spanish breeding programs: seven from Institut de Recerca i Tecnologia Agroalimentàries (IRTA) ('Constantí', 'Francolí', 'Glorieta', 'Marinada', 'Masbovera', 'Tarraco', and 'Vairo') (Vargas and Romero 1994; Vargas et al. 2008); four from Centro de Investigación y

Tecnología Agroalimentaria de Aragón (CITA) ('Belona', 'Guara', 'Mardía', and 'Soleta') (Dicenta et al. 2015; Felipe and Socias i Company 1987; Socias i Company and Felipe 2006; Socias i Company et al. 2008) and four from Centro de Edafología y Biología Aplicada–Consejo Superior de Investigaciones Científicas (CEBAS-CSIC) ('Antoñeta', 'Marta', 'Penta', and 'Tardona') (Dicenta et al. 2008; Dicenta et al. 2018; Egea et al. 2000). Three cultivars were obtained from Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), France ('Ferraduel', 'Ferragnès', and 'Lauranne') (Grasselly 1991; Grasselly and Duval 1997). Two traditional cultivars widely planted in Spain, 'Desmayo Largueta' and 'Marcona' (Felipe 2000), one Italian cultivar commonly planted in some Mediterranean countries, 'Tuono' (Dicenta et al. 2015; Felipe 2000), and four American cultivars ('Fritz', 'Independence', 'Monterey' and 'Nonpareil') (Batlle et al. 2017) were also included in this study. A single clone per cultivar was used in both laboratory and field evaluations.

Fungal isolates. Four fungal isolates of *D. amygdali* (DAL-4, DAL-34, DAL-138 and DAL-174) were used in the laboratory evaluation, and one isolate of *D. amygdali* (DAL-138) was used in the field inoculations. All isolates were obtained from diseased almond shoots showing twig cankers and shoot blight in different almond growing areas of Spain, and characterized as described in previous studies (Hilário et al. 2021; León et al. 2020). The isolates were stored in 15% glycerol solution at -80 ºC in 1.5 mL cryovials in the fungal collection of the Instituto Agroforestal Mediterráneo–Universitat Politècnica de València (IAM-UPV) (Spain). The fungal inocula used in the laboratory and field inoculations were obtained by previously growing the isolates on potato dextrose agar (PDA; Biokar-Diagnostics, Zac de Ther, France) for 10 d at 26 ºC in the dark.

156 ***Laboratory evaluation.*** In 2020, growing twigs (30 cm long) of the 25 almond cultivars
157 used in this study were obtained from IRTA facilities located in Les Borges Blanques,
158 Lleida, northeastern Spain (UTM coordinates: WGS84 Datum, 31 T x=320870,
159 y=4597530), and they were inoculated with isolates DAL-4, DAL-34, DAL-138 and DAL-
160 174. The twigs were surface sterilized by immersion in 70% ethanol for 30 s, 1.5% sodium
161 hypochlorite solution for 1 min, and again in ethanol 70% for 30 s. Then, they were air-
162 dried in a laminar flow cabinet. Wounds were made in the center of each twig with a 5-mm
163 cork borer. Mycelium agar plugs (5-mm-diameter), which were obtained from active 15-
164 day-old colonies of the *D. amygdali* isolates growing on PDA, were inserted under the bark
165 and the wounds were sealed with Parafilm. Inoculated twigs were kept in an upright
166 position with their lower ends immersed in 1 L jars with 500 mL of sterile water in a
167 growth chamber at 23 ºC with 12 h light per day. The twigs were covered with a plastic bag
168 during the first 7 days to keep a moist environment. Five twigs per isolate were used and a
169 control was prepared using uncolonized PDA plugs. Jars were arranged in a completely
170 randomized design and the water was changed every 3 days. Lesion lengths were measured
171 15 days after inoculation. The experiment was repeated once.

172 Immediately after lesion measurements, two representative shoots per inoculated isolate
173 and repetition were surface sterilized as described above. Small internal fragments were cut
174 from the margin of the healthy and necrotic tissue and placed onto PDA supplemented with
175 0.5 g/L of streptomycin sulphate (PDAS). Plates were incubated at 25 °C in the dark for 7
176 to 10 days, and all fungal growth resembling *D. amygdali* were transferred to PDA for
177 morphological identification to satisfy Koch's postulates.

178 ***Field evaluation.*** The 21 European cultivars used in this study were grafted onto INRA
179 'GF-677' rootstock and planted in December 2009 as bare root trees (1 m in height) at the
180 IRTA facilities previously indicated. The experimental plot consisted of 16 trees per each
181 cultivar. The trees were planted at 4 m × 2 m (distances between and within rows,
182 respectively) and pruned as a central axis. The orchard was drip-irrigated, and pruning, soil
183 management, and fertilization were based on the Spanish Integrated Production
184 Management practices (BOE 2002). No fungicide treatments were applied during the
185 experimental period.

186 Every year in July 2012-2015, six growing shoots were randomly chosen per cultivar.
187 All shoots were located outside the tree in a north-east orientation and were about 30-35 cm
188 long. An incision (1.5 to 2 cm long) was made in the basal part of each shoot with a scalpel
189 and the bark partially removed. A colonized agar plug (~5-mm-diameter), obtained from
190 the margin of a 15-day-old colony of DAL-138, was placed on the wound with the
191 mycelium facing the inner wood tissues, and the wound was sealed with Parafilm. Non-
192 inoculated controls were prepared using uncolonized PDA plugs. About 3-4 weeks after
193 inoculation, the lesion length caused by the fungus, upwards and downwards from the
194 inoculation point, was measured. The pathogen was reisolated from three of the inoculated
195 shoots per cultivar, as it has been described above for the laboratory trial. The experiment
196 was repeated four times within the years 2012 to 2015.

197 ***Data analyses.*** Lesion length means were calculated for each isolate and cultivar. These
198 values were additionally grouped and analyzed according to four common agronomic traits:
199 blooming time, ripening time, tree vigor and branching density (Table 1). Blooming and
200 ripening times were classified into four levels (early, medium, late, and very late), whereas

branching density and vigor were similarly classified into four levels (low, medium, high, and very high).

Analysis of variance (ANOVA) assumptions were checked prior to the analysis and data were transformed (squared) to meet analysis requirements. One-way ANOVA was performed to detect any statistically significant effect ($P < 0.05$) of the cultivar variable on the lesion length caused by the fungus. The Least Significant Difference (LSD) test was further used to compare the mean lesion length of each cultivar. All calculations were performed using Statgraphics Centurion XVI (Statgraphics Technologies, Inc., The Plains, VA, USA).

In addition, a cluster analysis was conducted in R (R Core Team 2021) to characterize the response of the almond cultivars to the inoculation with *D. amygdali* isolates; this was based on a combined analysis of all mean lesion lengths obtained in the field and laboratory experiments. The optimal number of clusters was estimated using the function *NbClust* of the *NbClust* package (Charrad et al. 2014). The cluster analysis was performed using the function *pam* in the *cluster* package, which specifically uses the Partitioning Among Medoids (PAM) algorithm (Kaufman and Rousseeuw 2009). The results were visualized using the *fviz_cluster* function of the *factoextra* package (Kassambara and Mundt 2020), which combines the clustering results with a Principal Component Analysis of the original data matrix. The cluster means obtained in this analysis were compared with the Student's *t*-test.

RESULTS

223 ***Laboratory evaluation.*** Inoculation of twigs of 25 almond cultivars with four *D.*
224 *amygdali* isolates resulted in necrotic lesions and canker development in all inoculated
225 twigs of all cultivar and isolate combinations. Lesions were variable in length depending on
226 the cultivar studied and the isolate used (Fig. 1). The uninoculated controls did not show
227 any measurable lesion and the fungus was not reisolated in any case. Therefore, lesion
228 length data for non-inoculated controls are not included in Fig. 1.

229 The significance of the interaction between cultivar and isolate factors (P<0.001) was
230 confirmed through a two-way ANOVA on the whole dataset (results not shown). Therefore,
231 one-way ANOVA analyses were conducted separately for each isolate. ANOVA results
232 indicated that significant differences (P<0.05) in mean lesion lengths among cultivars were
233 detected for each isolate. Mean lesion lengths ranged from 7 cm in 'Ferragnès' inoculated
234 with isolate DAL-138 to 24 cm in 'Penta' inoculated with isolate DAL-34. Some cultivars,
235 such as 'Soleta' and 'Penta', usually showed longer mean lesions with the four isolates of
236 *D. amygdali*. In contrast, 'Desmayo Largueta' usually showed shorter lesions. Regarding
237 the mean lesion length caused by each isolate, the minimum mean lesion value recorded for
238 DAL-4 was 11.6 cm in 'Ferragnès' and the maximum 23.6 cm in 'Constantí'. In the case of
239 DAL-34, minimum and maximum mean lesion values were 9.7 cm and 24.0 cm, obtained
240 in 'Marta' and 'Penta', respectively. In the case of DAL-138, minimum and maximum
241 mean lesion values were 7.0 cm and 18.4 cm, obtained in 'Ferragnès' and 'Glorieta',
242 respectively. Regarding DAL-174, minimum and maximum mean lesion values were 11.2
243 cm and 23.6 cm, obtained in 'Fritz' and 'Tardona', respectively.

244 Mean lesion lengths caused by four *D. amygdali* isolates in 25 almond cultivars grouped
245 according to the four agronomic traits are shown in Fig. 2. Regarding the effect of
246 blooming time, in all *D. amygdali* isolates the lowest lesion lengths were obtained in the

247 early-blooming cultivars whereas late-blooming cultivars showed longer lesions, although
248 with no consistent differences between means across cultivars. Regarding the ripening time,
249 the longest lesions were observed in early-ripening cultivars with a trend to decrease in
250 late-ripening cultivars, with or without statistically significant differences depending on the
251 isolate. In the case of vigor, the longest mean lesions were observed in the low vigor
252 cultivars for isolates DAL-4, DAL-34 and DAL-174, with a general trend to decrease
253 within the cultivars with higher vigor classes. In contrast, cultivars inoculated with isolate
254 DAL-138 behaved the opposite to the other *D. amygdali* isolates, as low-vigor cultivars
255 inoculated with DAL-138 showed shorter mean lesion values than the other groups.
256 Finally, when the cultivars were grouped by branching density, no statistically significant
257 differences among groups were found, except for isolate DAL-174, in which the cultivars
258 with high branching density showed the shorter mean lesion value, statistically significant
259 when compared to the rest of the groups.

260 ***Field evaluation.*** Mean lesion lengths caused by *D. amygdali* DAL-138 on 21 almond
261 cultivars in field trials are shown in Fig. 3. In general, a range of variation was found, being
262 'Tardona' the cultivar with the longest mean lesion length (5.41 cm), and 'Tarraco' the one
263 with the smallest lesion length (4.03 cm). The remaining cultivars showed intermediate
264 mean lesion lengths in a progressive trend (Fig. 3).

265 According to the agronomic traits of blooming and ripening times, early-blooming
266 cultivars showed the shortest lesions whereas early-ripening cultivars showed the longest
267 lesions (Fig. 4), as similarly observed in the laboratory trial. Regarding the vigor, the
268 shortest mean lesion lengths were obtained in high vigor cultivars, but differences with the
269 means of low and very high vigor cultivars were not statistically significant. Finally, no

significant differences were detected among groups when cultivars were grouped according to the branching density.

Susceptibility groupings. Cluster analysis (Fig. 5) separated the 21 evaluated cultivars into two well-defined different groups, which were statistically different according to Student's *t*-test comparisons between the mean lesion lengths of each group. These two groups were classified as very susceptible (longer lesions), which included 'Belona', 'Constantí', 'Ferraduel', 'Glorieta', 'Guara', 'Lauranne', 'Marinada', 'Masbovera', 'Penta', 'Soleta', 'Tardona', 'Tuono', 'Vairo', 'Francolí', and 'Tarraco'; and susceptible (shorter lesions), including 'Antoñeta', 'Desmayo Largueta', 'Ferragnès', 'Marcona', 'Mardía', and 'Marta'.

DISCUSSION

Necrotic lesions and cankers observed in the inoculated almond cultivars both in laboratory and field tests coincided with those described as characteristic for twig canker and shoot blight disease caused by *D. amygdali* (Adaskaveg 2002; Diogo et al. 2010; León et al. 2020). Our results evidenced the susceptibility of all the cultivars evaluated to all the inoculated isolates of *D. amygdali*. Lesion length measurements showed a wide range of variation among cultivars in all experiments. Moreover, in the laboratory evaluation there were differences in pathogenicity among *D. amygdali* isolates as previously reported by Diogo et al. (2010) and León et al. (2020), when these authors inoculated this pathogen on the cultivars 'Ferragnès' and 'Vairo', respectively.

292 Almond cultivars were grouped as susceptible or very susceptible according to a cluster
293 analysis. It is interesting to remark that cultivars classified as very susceptible showed
294 approximately a 30% increase in mean lesions length compared to those susceptible. We
295 intentionally avoided the use of the concepts like tolerant or very tolerant when classifying
296 cultivars for their susceptibility to *D. amygdali*, because we think that colonization of
297 almond twig tissues by *D. amygdali* was biologically relevant among all cultivars.
298 Nevertheless, the cultivar susceptibility/tolerance concept can be easily managed by
299 farmers and agronomists if cultivars are placed into distinct ordinal classes (Pataky et al.
300 2011), and this was the goal of the cluster analysis used in this study.

301 Previous works had already studied the susceptibility of almond cultivars to *D. amygdali*
302 (Besoain et al. 2000; Cabrita et al. 2004; Diogo et al. 2010; Vargas and Miarnau 2011;
303 Varjas et al. 2017a), with some of them also included in our study. Besoain et al. (2000)
304 evaluated the cultivar 'Nonpareil', which showed significant lesions when inoculated with
305 *D. amygdali* on both non-lignified and semi-lignified almond tissues, thus being considered
306 as susceptible. These results agree with those obtained in our study, which confirm an
307 intermediate susceptibility of 'Nonpareil' for all *D. amygdali* isolates. Later, Cabrita et al.
308 (2004), evaluated the susceptibility of the Portuguese 'Barrinho Grado' and the French
309 'Ferragnès' cultivars to *D. amygdali*, showing that 'Ferragnès' was more susceptible than
310 the Portuguese cultivar because it showed longer lesions in artificially inoculated twigs, in
311 inoculations with either mycelium plugs or conidial suspensions. Diogo et al. (2010)
312 confirmed the susceptibility of 'Ferragnès' to *D. amygdali*, when they compared the lesions
313 caused by this fungus with those caused by *D. foeniculina* (syn. *D. neotheicola* A.J.L.
314 Phillips & J.M. Santos), being the mean length of lesions of the first species significantly

longer. Similar results were obtained in our studies, in which the cultivar 'Ferragnès' showed considerable lesions in both laboratory and field tests. In Spain, Vargas and Miarnau (2011) established five categories of susceptibility among 70 almond cultivars after conducting a study on naturally-infected trees. The cultivars ranged from very susceptible for the Spanish cultivars 'Desmayo Largueta' and 'Marcona', and the French ones 'Ferragnès' and 'Lauranne', to very tolerant for the cultivars 'Masbovera' and 'Tarraco'. This is in contrast with our results, in which these last two cultivars were considered very susceptible. The other cultivars included in the evaluation of Vargas and Miarnau (2011) had intermediate susceptibility ranges; for instance 'Antoñeta' and 'Marta' resulted susceptible, in agreement with our results. Data regarding the high susceptibility of 'Lauranne' to *D. amygdali* reported by Vargas and Miarnau (2011) are also consistent with our results. It is also important to note that cultivars 'Ferraduel', 'Glorieta', 'Marinada', 'Masbovera', 'Nonpareil', 'Tarraco', and 'Vairo' evaluated in this study did not exactly match the susceptibility range assigned by Vargas and Miarnau (2011) (i.e., medium to very tolerant).

Some disagreements in cultivar susceptibility among different evaluation studies can be due to the type of inoculation (artificial vs. natural). In artificial inoculations some natural barriers from the cultivar are eliminated, with the wounds facilitating the introduction of the pathogen. In contrast, each cultivar can behave differently in response to the pathogen penetration under natural conditions. For instance, Mathew et al. (2018) compared different inoculation methods to study the aggressiveness of *D. helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov isolates causing Phomopsis stem canker of sunflower. These authors found a significant interaction between inoculation methods and isolates, confirming that the

inoculation method influenced the disease caused by *D. helianthi*, and pointed out that although inoculation by mycelial plugs has many advantages, such as the efficiency to detect significant differences in the severity of the disease, and the efficient use of space and the time required to inoculate the plants, it does not replicate the natural infection process by *Diaporthe* spp. Ghimire et al. (2019), stated that inoculation methods have a significant impact on the development of symptoms caused by some *Diaporthe* species on soybean, indicating that wound-based inoculation methods resulted in the greatest disease severity ratings.

Regarding the relationship between agronomic traits and cultivar susceptibility, blooming and ripening times were found relevant variables to explain cultivars performance related to *Diaporthe* dieback susceptibility. Late and very late blooming, and early and medium ripening cultivars, such as 'Constantí', 'Lauranne', 'Penta', and 'Tardona' were highly susceptible to *D. amygdali*. These later cultivars are releases from different breeding programs which share late blooming and early ripening time as two major desired goals (Batlle et al. 2017), but these selected characters seem to be related to a higher susceptibility to *D. amygdali*. Moreover, these four cultivars have been obtained from crosses of 'Tuono' (Pérez de los Cobos et al. 2021), an Italian cultivar classified as susceptible in our study and also in previous ones (Martins et al. 2005; Vargas and Miarnau, 2011).

It is generally agreed that vigor of an organism and its susceptibility to disease are antithetic variables, meaning that one increases as the other diminishes, and also that cultural practices aiming at improving the vigor of the plant often help increase its tolerance to pathogens (Agrios 2005; Raines 1922). This is in agreement with our results because, in

general, we observed longest lesions in low vigor cultivars although, in the particular case of the laboratory experiment, this was depending on the inoculated isolate. To the best of our knowledge, very few studies have addressed the influence of agronomic traits on the disease tolerance of fruit tree cultivars to dieback diseases. Willingham et al. (2004) reported a contradictory observation: avocado (*Persea americana* Mill.) fruits from non-vigorous tress affected by root rot pathogens were less susceptible to anthracnose caused by *C. gloeosporioides* (Penz.) Penz. & Sacc. than the fruits from healthy vigorous trees. This was related to a 40% increase in the concentration of calcium (Ca) in the flesh of fruits from non-vigorous trees, but their size make them unmarketable. In our case, the relationship of blooming and ripening times, and vigor with an eventual increased susceptibility of almond cultivars to *D. amygdali* remains to be further investigated.

Information about the susceptibility of almond cultivars to different fungal pathogens could assist in ongoing breeding programs of this crop, in order to achieve simultaneous tolerance to several economically important fungal pathogens. But certainly, it is in short term when the information generated in this study can be very valuable by selecting less susceptible almond cultivars to *Diaporthe* spp. for the new almond orchard plantations and, specifically, in the Iberian Peninsula.

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Table 1. Blooming time, ripening time, tree vigor and branching density of all tested cultivars. Agronomic traits adapted from Felipe (2000), Arquero et al. (2013), and Miarnau et al. (2016).

Cultivar	Blooming time[1]	Ripening time[1]	Vigor[2]	Branching density[2]
'Antoñeta'	Medium	Early	Very high	High
'Belona'	Medium	Medium	Very high	Low
'Constantí'	Late	Medium	High	Medium
'Desmayo Largueta'	Early	Very late	Medium	Very high
'Ferraduel'	Medium	Medium	Medium	High
'Ferragnès'	Medium	Medium	High	Medium
'Francolí'	Medium	Medium	High	Medium
'Fritz'	Early	Late	High	Medium
'Glorieta'	Medium	Medium	High	Medium
'Guara'	Medium	Early	Medium	Low
'Independence'	Early	Early	High	Medium
'Lauranne'	Late	Early	Medium	Medium
'Marcona'	Early	Medium	High	High
'Mardía'	Very late	Medium	High	Low
'Marinada'	Late	Late	Low	Low
'Marta'	Medium	Medium	Very high	Medium
'Masbovera'	Medium	Medium	High	Medium
'Monterey'	Early	Late	High	Medium

'Nonpareil'	Early	Medium	High	Medium
'Penta'	Very late	Early	Medium	High
'Soleta'	Medium	Very late	Medium	Very high
'Tardona'	Very late	Early	Medium	Very high
'Tarraco'	Late	Very late	High	Low
'Tuono'	Medium	Early	Medium	Low
'Vairo'	Medium	Medium	Medium	Medium

Note: [1]Blooming and ripening time: early, medium, late, and very late. [2]Vigor and branching density: low, medium, high, and very high.

Figure 1. Mean lesion length caused by four isolates of *D. amygdali* (DAL-4, DAL-34, DAL-138 and DAL-174) on 25 almond cultivars 15 days after inoculation in laboratory conditions. The vertical bars represent the standard error of the mean. The letters in the horizontal bars indicate significant differences (LSD; $P < 0.05$) among the cultivar means.

Figure 2. Mean lesion length caused by four isolates of *D. amygdali* (DAL-4, DAL-34, DAL-138 and DAL-174) on 25 almond cultivars 15 days after inoculation in laboratory conditions. Cultivars were grouped by blooming time, ripening time, vigor and branching density. The vertical bars represent the standard error of the mean. The letters indicate significant differences (LSD; $P < 0.05$) between the level means of each grouping factor.

Figure 3. Mean lesion length caused by *D. amygdali* DAL-138 on 21 almond cultivars 3-4 weeks after inoculation in field conditions. The vertical bars represent the standard error of the mean. The horizontal bars with different letters indicate significant differences (LSD; $P<0,05$) among the cultivar means.

Figure 4. Mean lesion length caused by *D. amygdali* DAL-138 in 21 almond cultivars 3-4 weeks after inoculation in field conditions. Cultivars were grouped by blooming time, ripening time, vigor and branching density. The vertical bars represent the standard error of the mean. The letters indicate significant differences (LSD; $P < 0.05$) between the level means of each grouping factor.

Figure 5. Cluster analysis of the mean lesion length caused by four *Diaporthe amygdali* isolates (DAL-4, DAL-34, DAL-138 and DAL-174) and one isolate (DAL-138) in laboratory and field experiments, respectively, on 21 almond cultivars: (1) ‘Antoñeta’, (2) ‘Belona’, (3) ‘Constantí’, (4) ‘Desmayo Largueta’, (5) ‘Ferraduel’, (6) ‘Ferragnès’, (7) ‘Francolí’, (8) ‘Glorieta’, (9) ‘Guara’, (10) ‘Lauranne’, (11) ‘Marcona’, (12) ‘Mardía’, (13) ‘Marinada’, (14) ‘Marta’, (15) ‘Masbovera’, (16) ‘Penta’, (17) ‘Soleta’, (18) ‘Tardona’, (19) ‘Tarraco’, (20) ‘Tuono’, and (21) ‘Vairo’. Two categories of susceptibility were defined as follows: susceptible (light gray) and very susceptible (gray). Ellipses include the 95% confidence interval for the centroids (black solid dots).

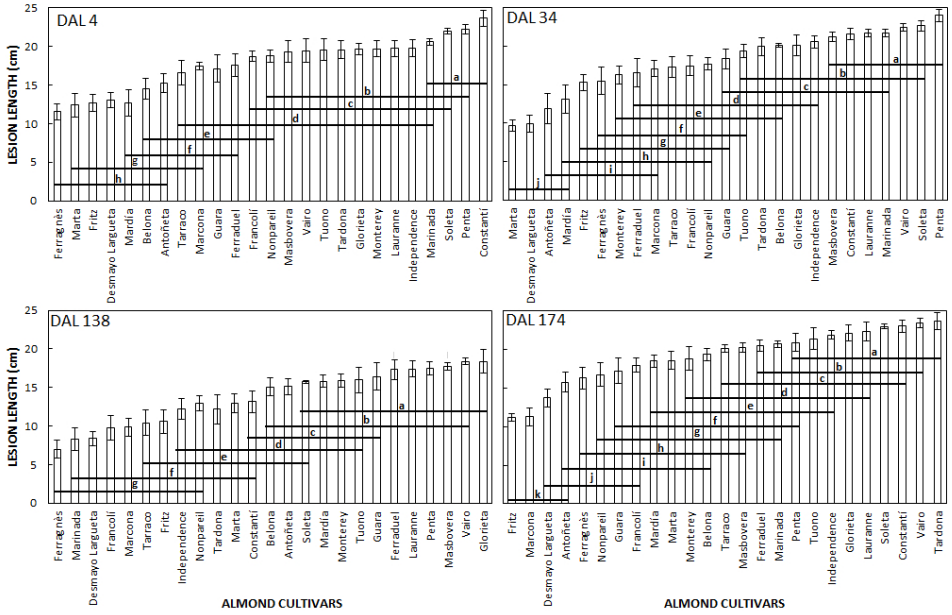

Figure 1

708x445mm (38 x 38 DPI)

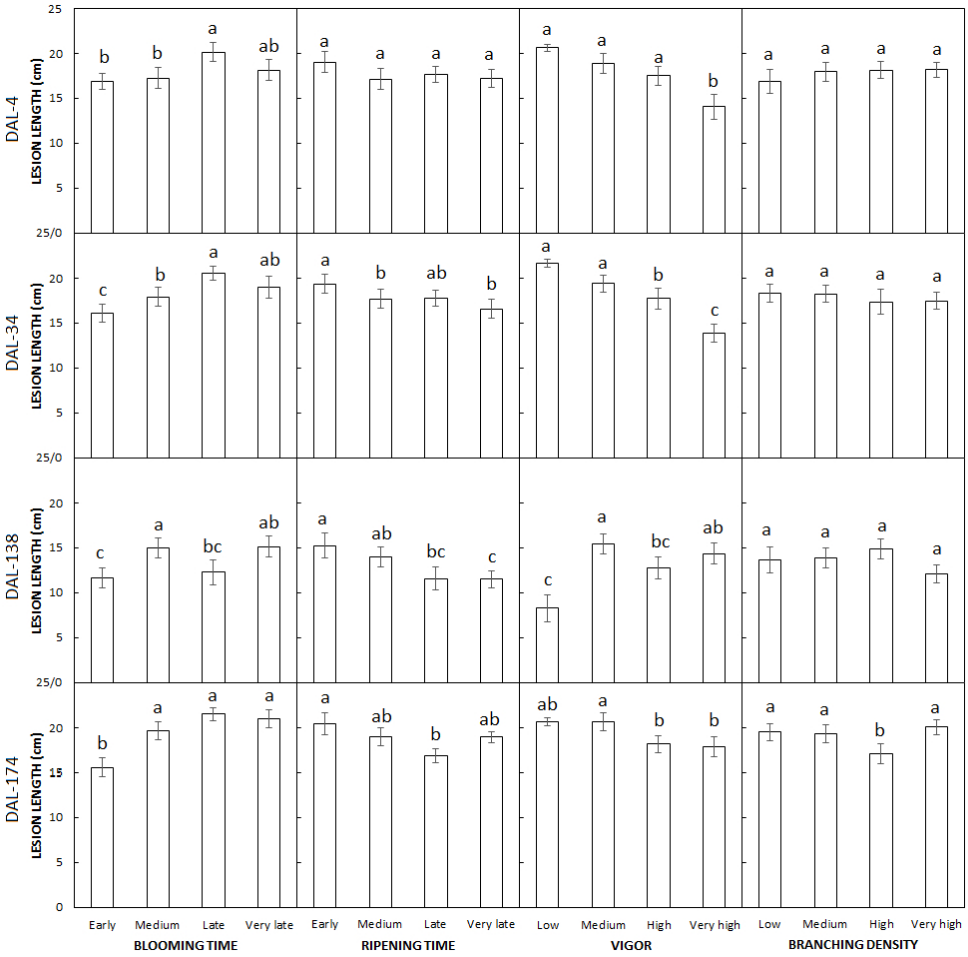

Figure 2

541x533mm (47 x 47 DPI)

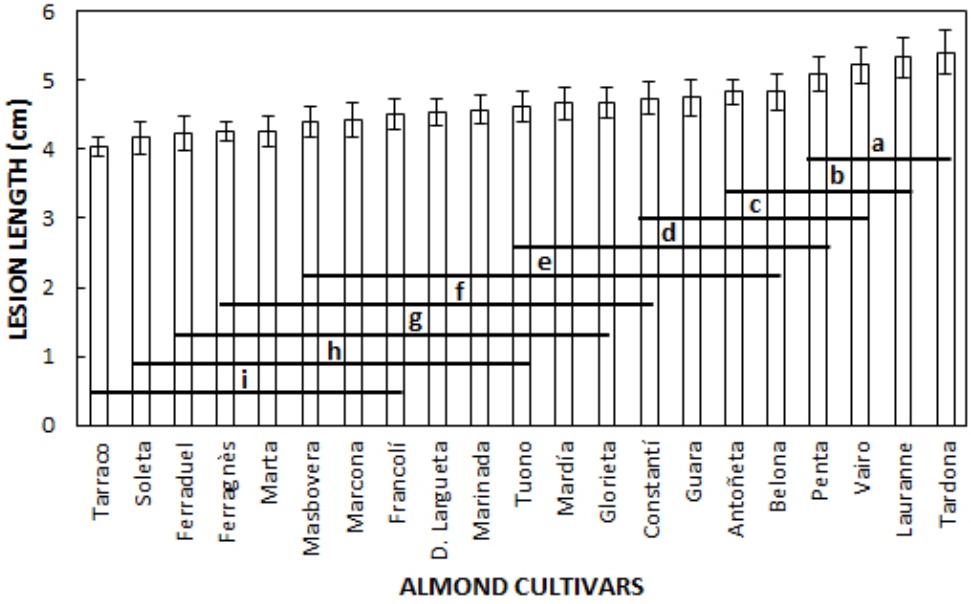

Figure 3

349x220mm (38 x 38 DPI)

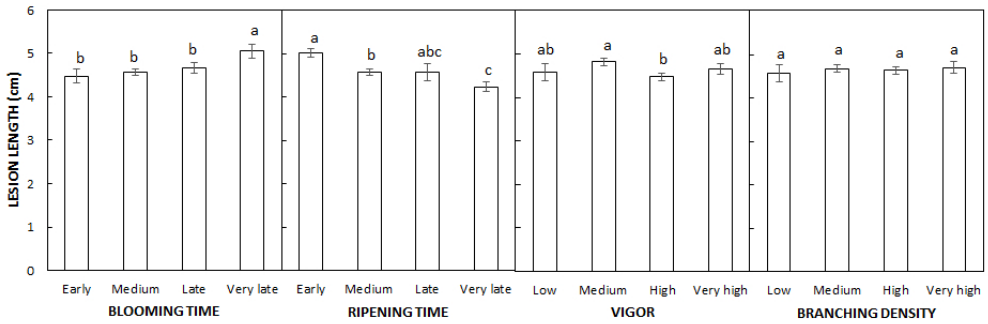

Figure 4

623x206mm (38 x 38 DPI)

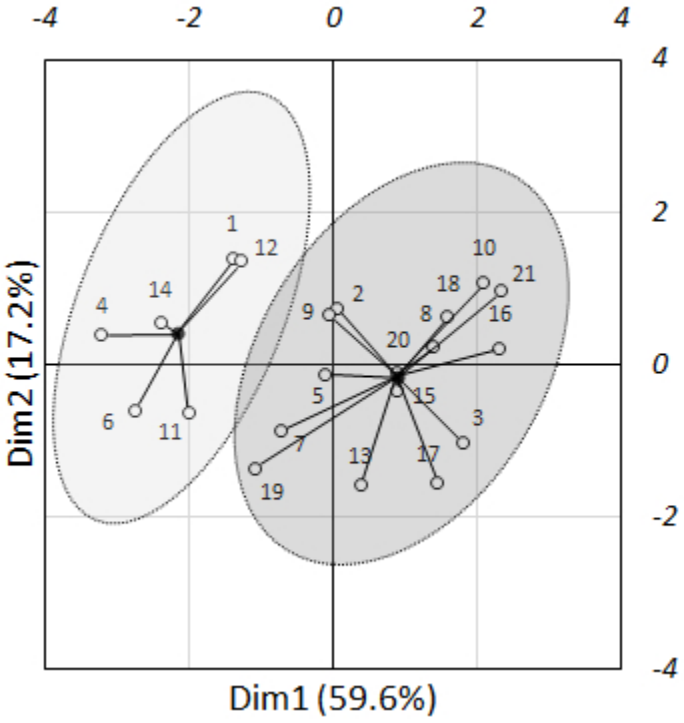

Figure 5

245x254mm (38 x 38 DPI)