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# Cultivar susceptibility and environmental parameters affecting symptom expression of red leaf blotch of almond in Spain

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## Abstract

Red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, is an important foliar disease of this nut tree in the Mediterranean basin and Middle East regions. In recent years, the incidence of this disease has increased in Spain corresponding to increases in the area of newly-planted orchards and the use of susceptible cultivars. In 2009, an experimental orchard including 21 almond cultivars was planted at Les Borges Blanques, Lleida, NE Spain. No fungicide treatments were applied during the 10-year experimental period (2009-2018) in order to allow natural disease development. Cultivar susceptibility to RLB was assessed each year, from 2011 to 2018, through visual observations of symptoms in naturally-infected trees. The experimental results led us to classify the cultivars into five susceptibility groups. The most susceptible were 'Tarraco', 'Guara', 'Tuono', 'Marinada', 'Desmayo Largueta', and 'Soleta', whereas 'Mardía' was the most tolerant. The annual incidence of disease was positively correlated with accumulated rainfall in spring, and especially in April, while it was negatively correlated with high spring and summer temperatures, especially in May. These findings could be used to improve disease management strategies by identifying the most susceptible cultivars and improving the timing of fungicide application.

## Introduction

Red leaf blotch (RLB) of almond, caused by the Ascomycota fungus *Polystigma amygdalinum* P.F. Cannon, is one of the most important leaf diseases affecting almond trees (*Prunus dulcis* (Mill.) Webb.) in the Mediterranean basin and Middle East regions (Cannon 1996). It has been widely reported in the main almond producing countries in these areas (Bayt Tork et al. 2014; Cannon 1996; Farr and Rossman 2019). The disease has not yet been detected in other important almond-growing regions such as the U.S.A. and Australia (Farr and Rossman 2019; Saad 2002). RLB is widespread in Spain, particularly in the southern part of the country, along the Mediterranean coast, and around the Ebro Valley in the Northeast. In recent years, the incidence of RLB in Spain has increased. This has mainly been due to the expansion of new almond orchards to inland areas where climatic conditions are more suitable for disease development (Almacellas 2014). The almond acreage in Spain, the country with the largest cropping area in the world, has increased in 100,000 ha in the last five years to a total of 657,771 ha (MAPA 2019a). The newly planted cultivars are more productive than the traditional ones and offer good agronomic characteristics, such as late-blooming and self-fertility; they are, however, susceptible to *P. amygdalinum* (Miarnau et al. 2010, 2013). Furthermore, the most intensive production systems introduced into Spain by the almond industry, such as super-high density plantations, have created particularly favorable conditions for the development of fungal diseases (Miarnau et al. 2013, 2016).

RLB only affects almond leaves (Banihashemi 1990), causing diffuse spots of different shapes and sizes. They are initially yellowish in color, later turning into reddish-brown blotches. These blotches are actually the stromata of the pathogen where pycnidia are formed in summer and perithecia develop in winter, once the leaves have fallen (Cannon 1996; Ghazanfari and Banihashemi 1976). In spring, under favorable temperature and humidity conditions, and especially after rain events, ascospores are released and primary infections occur on new almond leaves (Banihashemi 1990; Saad and Masannat 1997). The potential period for the production and release of *P.*

*amygdalinum* ascospores in Spain extends from February to July, but the highest inoculum potentials are in March-April (Zúñiga et al. 2020). In severe RLB infections, early defoliation may occur (Cannon 1996; Kranz 1962), with a subsequent decrease in tree photosynthetic activity and a possible reduction in yield (López-López et al. 2016; Saad and Masannat 1997). These effects are most intense in orchards that only receive natural rainfall, where water stress may contribute to greater defoliation (Almacellas 2014). As 86% of the Spanish almond growing area is under dry, non-irrigated conditions (MAPA 2019a), the RLB incidence is particularly severe in regions with these conditions.

According to Almacellas (2014), RLB management strategies are generally based on: i) crop management practices that reduce both primary inoculum and the risk of infection, ii) the use of fungicides, and iii) tolerant cultivars. The most commonly applied cultural practice is the elimination of the primary inoculum by removing the infected leaves that had fallen from trees in the previous year (Cannon 1996). This is commonly done through the application of crystalline urea which expedites the decomposition of the fallen leaves (Lin and Szteinberg 1992). In-season applications of fungicides, from petal fall until the end of summer, have also been shown to reduce infection (Banihashemi 1990; Bayt Tork et al. 2014; Sahragard et al. 2007). One major concern with this strategy is the limited number of approved fungicide products available to control this disease in Spain (MAPA 2019b) and some other European countries. In practice, it is difficult to implement an annual fungicide management program without applying the same modes of action repeatedly, which can eventually lead to resistance in pathogen populations.

Breeding for cultivar tolerance to RLB should therefore be part of any long-term control strategy. The use of tolerant cultivars can help to reduce primary inoculum and the need for subsequent fungicide treatments. However, to date, no cultivars with resistance to *P. amygdalinum* have been identified. Differences in the degree of cultivar susceptibility to RLB have been reported in Spain (Egea et al. 1984; Malagón et al. 2017; Miarnau et al. 2010; Miarnau and Vargas 2013; Ollero-Lara et al. 2019). Previous studies have produced inconsistent results, possibly as a result of differences in experimental conditions that could have influenced the natural incidence of disease. Moreover, information is rather limited on the susceptibility of cultivars released after 2007 (Gradziel et al. 2017), although Ollero et al. (2019) recently reported on the susceptibility of 40 cultivars to RLB in Spain. It is therefore important to test a large number of cultivars in order to discover as much as possible about the range of susceptibility among cultivars.

One objective of this research was to assess RLB susceptibility among the main cultivars released in recent decades by some Spanish and French almond breeding programs. Some traditional cultivars from Italy and Spain, which have mainly been planted in Spain and other Mediterranean countries, were also included in a 10-year trial to monitor the incidence and severity of RLB symptoms among cultivars. In addition, intra-seasonal variations in RLB symptom expression were also analyzed for four selected cultivars. A second objective of our study was to evaluate the effect of several regional climatic factors on the incidence of RLB symptoms among cultivars in order to expand the available information on the epidemiology of RLB.

## Materials and Methods

**Almond cultivars.** Twenty-one almond cultivars were assessed. Fifteen cultivars were obtained from three different Spanish breeding programs: seven from IRTA ('Constantí', 'Francolí', 'Glorieta', 'Marinada', 'Masbovera', 'Tarraco', and 'Vairo') (Vargas and Romero 1994; Vargas et al. 2008); four from 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 CEBAS-CSIC ('Antoñeta', 'Marta', 'Penta', and 'Tardona') (Dicenta et al. 2008; Dicenta et al. 2018; Egea et al. 2000). Three cultivars were obtained from INRA, France ('Ferraduel', 'Ferragnès', and 'Lauranne') (Grasselly 1991; Grasselly and Duval 1997). Two traditional cultivars widely planted in Spain, 'Desmayo Largueta' and 'Marcona' (Felipe 2000), and one Italian cultivar commonly planted in some Mediterranean countries, 'Tuono' (Dicenta et al. 2015; Felipe 2000), were also included in the study. All cultivars were grafted onto INRA 'GF-677' rootstock.

**Experimental plot design and management.** The seedlings were planted in December 2009 as bare root trees (1 m in height) at the IRTA facilities at the Les Borges Blanques Experimental Station, Lleida, northeastern Spain (UTM coordinates: WGS84 Datum, 31 T $x = 320870$, $y = 4597530$). The trees were planted at 4 m × 2 m (distances between and within rows, respectively) in a randomized block design with four replicate blocks containing four trees for each cultivar. They were pruned as a central axis. The orchard was drip-irrigated, and pruning, soil management, and fertilization were based on the Spanish Integrated Production Management practices (BOE 2002). No fungicide treatments were applied during the experimental period.

**Disease assessment.** From 2011 to 2018, annual assessments of RLB symptoms were conducted during July-August, well before almond harvest (mid-September). A sample of 100 leaves of each cultivar per replicate block (25 leaves per tree) and year was evaluated. Fully expanded leaves were randomly collected from new shoots, at different heights and orientations, from the outer canopy of each tree. Disease incidence was recorded as the percentage of leaves showing at least one identifiable RLB lesion, regardless of its size. To estimate the severity of the disease, collected leaves were visually classified in one of five categories based on the percentage of RLB-affected leaf surface (Fig. 1): class 0 (0% affected leaf surface, apparently healthy), class 1 (1-10%), class 2 (11-20%), class 3 (21-50%), and class 4 (>50%). A mean leaf severity index for each sample was obtained from the weighted average of the midpoint percentages for each class (i.e., 0%, 5%, 15%, 35% and 75%) prior to statistical analysis (Chiang et al. 2017). Four cultivars with different levels of susceptibility to RLB (from low to high: 'Vairo', 'Lauranne', 'Guara', and 'Tarraco'), based on previous unpublished evaluations, were selected to describe the intra-seasonal progress of the disease. A random sample of 50 leaves per replicated block for each cultivar was evaluated, as described above, at 15-day intervals from April to September, of 2015 to 2017.

**Monitoring of environmental factors.** Environmental factors, namely daily temperature (maximum, minimum and mean), relative humidity and accumulated rainfall during the experimental period, were obtained from an automatic weather station located at Castelldans, Lleida (UTM coordinates: $x$ = 312540, $y$ = 4599934) (Catalan Weather Service, https://www.meteo.cat/). This station was located about 8.5 km from the experimental orchard. Temperature and humidity data were averaged for the following time intervals: i) monthly for the period of October of the previous year through July (10 time periods), and ii) *n*-month periods (two to five consecutive months), from March to July within the same year: 9 periods in total. Accumulated rainfall was also calculated for the same periods. These combinations produced a total of 95 weather-related variables (5 weather variables × 19 time intervals), which were used in the correlation analyses between disease-related and environmental data.

**Data analysis.** Experimental data were analyzed using JMP Pro (Version 14.0.0, SAS Institute. Inc.). A linear mixed model, which included cultivar (20 df), year (7 df) and cultivar × year (140 df) as the fixed factors, and block (3 df) as a random factor, was separately fitted to disease incidence and severity data in order to evaluate the effects of the independent variables cultivar and year. Percentage data were arcsine-transformed to normalize the data distribution, and residuals were plotted to determine their distribution and influence. Data were analyzed using a repeated-measures design model, which included a compound symmetry of unequal variances structure in the variance-covariance matrix. The latter was selected according to the lowest Corrected Akaike's Information Criteria ($AIC_c$). Mean comparisons were made by the Tukey-Kramer's test at $\alpha = 0.05$.

A regression model was fitted to describe the relationship between incidence and severity data for all the cultivars during 2011 to 2018. In addition, a hierarchical cluster analysis was performed with Ward's criterion (Kuiper and Fisher, 1975; Ward, 1963) to characterize the susceptibility of the almond cultivars to RLB; this was based on a combined analysis of all annual incidence and severity values.

The potential influence of environmental conditions on RLB incidence was evaluated using Pearson’s correlation analyses. Linear regressions were also performed when highly statistically significant correlations were detected. The data included in the analyses corresponded to the 2010-2016 period, as the environmental data from the winter of 2017 onwards were incomplete.

## Results

**Cultivar susceptibility.** All 21 almond cultivars evaluated in this study developed various degrees of RLB symptoms during each season of the experimental period. The relationship between the annual indices of incidence and severity data for the different cultivars was best fitted to an exponential regression model ($P < 0.001$; $R^2 = 0.697$) (Fig. 2). Due to this good relationship, disease incidence was chosen to describe the RLB symptom expression and its relationship with environmental parameters. Although incidence values over 80% were not uncommon, their associated severity indices only had maximum values in the range 20% to 40% (Fig. 2).

Analyses of the whole dataset of incidence scores showed that statistical significance was detected for the following factors: cultivar ($P < 0.001$), monitoring year ($P < 0.001$), and their interaction ($P < 0.001$). Given this interaction, the mean comparisons for the different cultivars were made separately in each year. The mean values of incidence for all cultivars and years during the experimental period ranged from 0.0% ('Mardía') to 96.3% ('Desmayo Largueta') (Fig. 3). 'Mardía' showed the lowest yearly incidence values in the eight-year monitoring period, with annual values ranging from 0.0% to 54.2% (overall mean: 15.3%). This cultivar did not show any RLB symptoms in 2012, 2015, and 2016. 'Mardía' was the only cultivar that consistently showed significant differences to the other cultivars in most years (Fig. 3). 'Vairo' had the second lowest overall RLB incidence (47.1%), with annual mean values ranging from 22.6% to 69.6%. In contrast, the most susceptible cultivar 'Tarraco' had an average incidence over 75% and annual values ranging from 56.0% to 89.8%. 'Desmayo Largueta', 'Guara', 'Marinada' and 'Tuono' showed average incidences for the whole experimental period of between 70% and 75% and annual values of between 37.3% and 96.3%. The remaining 14 cultivars showed mean annual incidences of between 23.5% and 90.2% without any significant differences between them in most years (Fig. 3). Regarding the variation in annual incidence values per cultivar, 'Penta' (23.8% to 90.2%) and 'Tardona' (23.5% to 83.7%) showed the widest ranges, whereas 'Tarraco' was the cultivar with the least variability from year to year (56.0% to 89.8%). The cultivar groupings based on the comparison of mean annual incidence values were not consistent over the whole experimental period (Fig. 3). Mean values of severity indices for all cultivars and years are given in Supplementary Fig. S1.

The annual RLB incidence of the whole set of cultivars was highly variable over the experimental period. In the first two years (2011, 2012) RLB incidence was lower (≈50%) than in the following two years (i.e., over 70% on average). A minimum peak in average incidence (≈35%) was detected in 2015; this was followed by an increase in 2016, with the incidence then remaining at around 60% until the end of the experiment.

The cluster analysis for the combination of RLB incidence and severity data showed that almond cultivars clustered in five well-distinguished groups (Fig. 4). These were classified as: highly susceptible (three cultivars), susceptible (three cultivars), moderately susceptible (six cultivars), tolerant (eight cultivars) and highly tolerant (one cultivar).

Intra-seasonal symptom expression in four cultivars with different levels of RLB susceptibility ('Guara', 'Lauranne', 'Tarraco' and 'Vairo') followed a similar pattern among cultivars (Fig. 5). Depending on the year, the first RLB symptoms developed at the beginning of May (2017) or in the first half of June (2015-2016). The percentages of infected leaves for all cultivars increased at the end of May and during June. Moreover, the increase in RLB incidence was developed in shorter time periods in 'Tarraco' than in 'Vairo' after the first RLB symptom appearance. During July and August, RLB incidence remained relatively stable. A decrease in the incidence of RLB was recorded at the end of the monitored period, in September (Fig. 5). In 2015, RLB incidence fluctuated from 30% to 60% in summer. The same occurred in 2016: from 45% to 90%, and in 2017: from 60% to 80%. The observed RLB incidences among cultivars were consistent with their respective overall susceptibility rankings. 'Vairo' therefore exhibited the lowest incidence values for the three years that were monitored, followed by 'Lauranne', 'Guara' and 'Tarraco', with the latter two performing similarly in 2015 and 2017. However, in 2016, 'Tarraco' showed higher incidence values than 'Guara' at all evaluation times (Fig. 5).

**Influence of environmental conditions on RLB symptom expression.** The summarized monthly environmental conditions recorded over the period 2010-2016 (Supplementary Table S2) showed that the accumulated monthly rainfall ranged between 0.2 mm (July 2016) and 103.4 mm (October 2012); and the average relative humidity ranged from 50.5% (May 2015) to 94.4% (December 2016). The average mean temperature ranged from 3.2ºC (December 2013) to 26.4ºC (July 2015); the average maximum temperature ranged from 6.9ºC (December 2016) to 35.4ºC (July 2015); the average minimum temperature ranged from -2.2ºC (February 2012) to 18.3ºC (July 2015).

In some cases, the accumulated rainfall and some temperature-related variables were significantly correlated with the annual incidence of RLB (Supplementary Table S3). In contrast, no correlation was ever found between RLB incidence and humidity values. In general, spring and summer environmental data correlated better with RLB incidence than those of the previous autumn and winter months. The accumulated rainfall recorded in April and during the periods April to May and April to June showed higher Pearson's correlation coefficients (all $r > 0.90$, $P < 0.001$) than for the March to May and April to July periods (all $r > 0.80$, $P < 0.05$). The accumulated rainfall and temperature data in the previous autumn and winter periods did not correlate with RLB incidence, except for maximum temperature data in November ($r = -0.78$, $P < 0.05$). In contrast, some significant correlations were found between spring temperature and disease incidence. All temperature-related variables in May were negatively correlated with RLB incidence (all $r < -0.84$, $P < 0.05$). In addition, some significant negative correlations were found between multi-monthly mean temperature data (March to July, April to July, and May to July) and the incidence of disease ($-0.88 < r < -0.83$, all $P < 0.05$). The relationships between environmental data and RLB incidence were further studied through linear regression modeling (Fig. 6). The goodness of fit of the best linear models were $R^2 \geq 0.768$ in the variables studied. Higher levels of disease incidence were detected in summer when the accumulated rainfall in April was over 60 mm or when it was more than 100 mm over longer periods (Fig. 6a,b). Mild temperatures in spring and summer (below 18ºC in May and below 21ºC in May to July) corresponded to higher incidence of disease, whereas higher temperatures recorded in May and in the May to July period resulted in a reduced RLB incidence (Fig. 6c,d).

## Discussion

*P. amygdalinum* is a hemibiotrophic pathogen (Zúñiga et al. 2019) that cannot be isolated and grown on synthetic culture media (Banihashemi 1990; Saad and Masannat 1977) or artificially inoculated. Consequently, evaluation of cultivar susceptibility must be performed under natural field conditions that are suitable for both host and pathogen development. Our trial took place in an area that allowed good disease development and cultivar characterization. According to the Köppen-Geiger climate classification (Kottek et al. 2006), the experimental area has a cold semi-arid climate (BSk). Disease development is favored because the region has: 1) cold and humid winters (about 40 to 50 days a year with fog and temperatures below 0°C) that favor ascocarp maturation (Ghazanfari and Banihashemi 1976); 2) spring rains (annual average 340 mm) that promote ascospore release, germination, and infection (Banihashemi 1990; Saad and Masannat 1997; Zúñiga et al. 2017); and 3) warm, dry summers that favor fungal growth (Zúñiga et al. 2017). To the best of our knowledge, this is the first time that an almond trial was established to specifically evaluate the susceptibility of cultivars to RLB over a ten-year period.

The cultivars evaluated were affected by *P. amygdalinum* at different levels of incidence and severity; this suggested that RLB development involves a cultivar component. RLB symptom expression also varied from year to year, although a good exponential correlation was found between the incidence and severity of RLB. The almond cultivars were classified into five susceptibility groups based on the hierarchical cluster analysis performed on incidence and severity data. We decided to include both incidence and severity data in this analysis due to the non-linear relationship between these variables; this also increased the amount of information to evaluate cultivar susceptibility. The cluster analysis indicated that 'Tuono', 'Guara' and 'Tarraco' (in order of increasing susceptibility) were the almond cultivars most susceptible to *P. amygdalinum*, whereas 'Mardía' was the most tolerant cultivar. Most of the cultivars assessed were grouped in three intermediate clusters which were susceptible to tolerant. However, none of the cultivars evaluated was identified as resistant to RLB.

Several attempts to assess cultivar susceptibility to RLB have been reported from different countries. In Iran, Heydarian and Moradi (2005) found that 'Ferragnès' showed a tolerance level similar to some Iranian selections ('Shahrud-6', 'Shahrud-12' and 'Shekofeh'). Previously, in Spain, Egea et al. (1984) evaluated 81 cultivars from a germplasm bank and found that 'Marcona' and 'Tuono' were very susceptible to RLB, whereas 'Desmayo Largueta' was tolerant, although this last finding differed from that obtained in our study. Miarnau et al. (2010) reported similar results to ours, with 'Guara' and 'Tarraco' as the most susceptible cultivars, whereas 'Vairo' was rated as the most tolerant. More recently, in a study in Southern Spain involving organically- and conventionally-managed orchards, Ollero-Lara et al. (2019) did not find any cultivar more susceptible than 'Guara'. They also reported 'Tarraco' and 'Tardona' to be tolerant, which was not confirmed by our results. The short evaluation period for 'Tarraco' in the study conducted by Ollero-Lara et al. (2019) and the high annual variability in RLB symptom expression for 'Tardona' found in ours could perhaps have been responsible for these contrasting results. In another previous study, Malagón et al. (2017) also reported different degrees of almond susceptibility to RLB as found in our study, with the authors considering 'Lauranne' to be as tolerant as 'Mardía', and 'Vairo' to be as susceptible as 'Soleta'. The latter study used only a single replication of four trees which was yearly evaluated for three years, but we showed in our study that a higher number of observations are needed because of the annual fluctuation in RLB development. In general, patterns of almond susceptibility to RLB are consistent across studies, but some cultivars show differential susceptibility responses. Cultivar phenology, local inoculum potential and proximity to inoculum sources, tree canopy architecture, orchard watering methods (e.g. natural rainfall vs. irrigation), and different local environmental conditions can all influence the development of this disease (Horsflied and Wicks 2014). Likewise, the statistical design of trials including different cultivar collections, germplasm banks, and the length of the monitoring periods in the previously cited studies may all have contributed to the contrasting results obtained. Fungicide treatments applied in orchards (Malagón et al. 2017; Miarnau et al. 2010; Ollero-Lara et al. 2019) may also have added undesired variability to the results.

It has been suggested that cultivars with earlier blooming and leafing dates facilitate extended infection periods during ascospore release, causing a potentially higher susceptibility to RLB (Almacellas 2014; Malagón et al. 2017; Ollero-Lara et al. 2019). However, late cultivars such as 'Tarraco' and 'Guara' were found to be susceptible in our study, while 'Mardía', another very late cultivar, was found to be highly tolerant. Regardless of some disagreements relating to differential RLB susceptibility between cultivars reported in this and previous studies, cultivar susceptibility to RLB might be related to the genetic background (i.e. the pedigree) of most of the cultivars studied. Unfortunately, no studies conducted to date have explored this hypothesis.

In our study, RLB symptoms appeared and increased in intensity from the beginning of May to the end of June. Thereafter, the incidence remained high and fluctuating in summer. There were no clear differences between cultivars regarding the timing of symptom appearance, but data suggested that RLB incidence increased faster in the highly susceptible 'Tarraco' than in the tolerant 'Vairo', which points to a differential behavior of cultivars that should be further studied. In Lebanon, Saad and Masannat (1997) reported a maximum incidence of RLB in mid-July and maximum severity in August, as similarly confirmed in our study. We found that fluctuations in RLB incidence detected in summer could be due to early defoliation and new leaves emerging that masked RLB symptoms. It is also important to highlight that differences in the overall susceptibility of cultivars were most evident in the years with the greatest intensity of disease (e.g., 2016). A further conclusion when assessing the susceptibility of cultivars to RLB is that multi-year assessments of RLB incidence are needed to obtain a better characterization of cultivar susceptibility, especially when symptom assessment is performed under natural infection conditions that are changing year to year.

Our study showed several consistent correlations between RLB incidence and rainfall: total rainfall during the April to July period, but especially from April to May, showed positive and highly-significant correlation with RLB incidence. Rainfall seems essential for the release and dispersion of ascospores (Saad and Masannat 1997).

Years with wet springs (e.g. 2013 and 2014) therefore tended to have higher RLB incidence. Additionally, in Spain, most new cultivars reach full bloom in March, and have fully developed leaves during the first RLB infection periods in April. The significances of the correlations between RLB incidence and temperature-related variables were not as frequently detected as those for rain-related variables. It has been reported that temperatures of around 10ºC and below are needed for ascocarp development and maturation in winter (Ghazanfari and Banihashemi 1976). Such low temperatures were consistently recorded in our study area from November to February. In addition, some negative correlations detected in November supported the possibility that low temperatures in autumn-winter were related to a higher incidence of RLB symptoms in summer, when the trees were evaluated. In contrast, temperatures in spring and summer sometimes correlated negatively with RLB indices, albeit not significantly in some cases. This would suggest that disease symptoms are more intense in warm, but not excessively hot, spring and summer seasons; however, additional annual assessments of RLB incidence should be recorded to confirm this hypothesis. Finally, higher temperatures in summer have been related to shorter disease incubation periods (Saad, 2002, Zúñiga et al. 2020). Based on these results, it can be assumed that environmental conditions can have an important role in RLB infections and symptom expression at a given location. Future epidemiological risk models should incorporate this valuable information, and especially that relating to spring rainfall data.

The two highly susceptible cultivars 'Guara' and 'Tuono' are widely grown in Europe and Northern Africa (Gradziel et al. 2017). 'Guara' accounts for 50% of the acreage of new almond orchards planted in recent decades in Spain (Socias i Company et al. 2009, 2012). These orchards are therefore potentially very susceptible to RLB and it is highly likely that they will require additional applications of fungicides. In contrast, 'Mardía' was characterized as highly tolerant. Zúñiga et al. (2019) found that lignin biosynthesis in 'Mardía' leaves may have a role in repressing the severity of disease. The potential inclusion of 'Mardía' as a parent in breeding programs (Batlle et al. 2017), combining its tolerance to RLB with late blooming and self-fertility, would help to obtain promising new cultivars with increased tolerance to the disease.

The ranking of cultivar susceptibility in this study could help breeders and growers to choose the most suitable cultivars for their breeding programs and planting locations, respectively. This could serve as a medium-term strategy for managing the disease in areas that are environmentally favorable and have high endemic populations of the pathogen.

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## Literature Cited

Almacellas, J. 2014. Síntomas, daños y métodos de control de la mancha ocre del almendro. Vida Rural 389:28–32.

Banihashemi, Z. 1990. Biology and control of *Polystigma ochraceum*, the cause of almond red leaf blotch. Plant Pathol. 39:309–315.

Batlle, I., Dicenta, F., Socias i Company, R., Gradziel, T. M., Wirthensohn, M., Duval, H., and Vargas, F. J. 2017. Classical genetics and breeding. Pages 111–148 in: Almonds, botany, production and uses. R. Socias i Company and T. M. Gradziel, eds. CAB International, Boston, MA.

Bayt Tork, D., Taherian, M., and Divan, R. 2014. Evaluation of some fungicides for controlling almond red leaf blotch (*Polystigma amygdalinum*). Int. J. Adv. Biol. Biomed. Res. 4:1011–1016.

BOE 2002. Real decreto 1201/2002, de 20 de noviembre, por el que se regula la producción integrada de productos agrícolas. Boletin Oficial del Estado, Madrid. Retrieved April 2, 2020 from https://www.boe.es/eli/es/rd/2002/11/20/1201

Cannon, P.F. 1996. Systematics and diversity of the Phyllachoroceae associated with Rosaceae, with a monograph of *Polystigma*. Mycol. Res. 100:1409–1427.

Chiang, K. S., Liu, H. I., and Bock, C. H. 2017. A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy. Ann. Appl. Biol. 171:139–154.

Dicenta, F., Ortega, E., Martínez-Gómez, P., Sánchez-Pérez, R., Martínez-García, P. J., Cremades, T., Gambín, M., and Egea, J. 2008. Almond breeding programme in CEBAS–CSIC, in Murcia (Spain). XIV GREMPA Meeting, Athens, Greece, 30 March-4 April, 2008. Book of abstracts:20.

Dicenta, F., Sánchez-Pérez, R., Rubio, M., Egea, J., Batlle, I., Miarnau, X., Palasciano, M., Lipari, E., Confolent, C., Martínez, P., and Duval, H. 2015. The origin of the self-compatible almond 'Guara’. Sci. Hortic. 197:1–4.

Dicenta, F., Cremades, T., Martínez-García, P. J., Martínez-Gómez, P., Ortega, E., Rubio, M., Sánchez-Pérez, R., López-Alcolea, J., and Egea, J. 2018. Penta and Makako: two extra-late flowering self-compatible almond cultivars from CEBAS-CSIC. Hortscience 53:1700–1702.

Egea, L., García, J. E., Egea, J., and Berenguer, T. 1984. Premières observations sur une collection de 81 variétés d’amandiers située dans le sud-est espagnol. Options Méditerranéennes 84:13–25.

Egea, J., Dicenta, F., Berenguer, T., and García, J. E. 2000. 'Antoñeta' and 'Marta' almonds. Hortscience 35:1358–1359.

Farr, D. F., and Rossman, A. Y. 2019. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved February 11, 2019, from https://nt.ars-grin.gov/fungaldatabases/

Felipe, A. J., and Socias i Company, R. 1987. 'Aylés', 'Guara' and 'Moncayo' almonds. Hortscience 22:961–962.

Felipe, A. J. 2000. El almendro: El material vegetal. Mira Editores, S.A. Zaragoza, Spain.

Ghazanfari, J., and Banihashemi, Z. 1976. Factors influencing ascocarp formation in *Polystigma ochraceum*. Trans. Brit. Mycol. Soc. 66:401–406.

Grasselly, C. H. 1991. Avijor 'Lauranne’. L’Arboriculture Fruitière 436:75.

Grasselly, C. H., and Duval, H. 1997. L’Amandier. CTFIL, Paris, France.

Gradziel, T; Curtis, R., and Socias i Company, R. 2017. Production and growing regions. Pages 70–86 in: Almonds, botany, production and uses. R. Socias i Company and T. M. Gradziel, eds. CAB International, Boston, MA.

Heydarian, A., and Moradi, H. 2005. Relative resistance of selected almond cultivars to the causal agent of red leaf blotch disease, in Chahar Mahal va Bakhtiari Province. Iran. J. Plant Pathol. 41:157–169.

Horsflied, A., and Wicks, T. 2014. Susceptibility of almond cultivars to Tranzschelia discolor. Australasian Plant Pathol. 43:79-87.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., and Rubel, F. 2006. World Map of the Köppen-Geiger climate classification updated. Meteorol. Z. 15:259–263.

Kranz, J. 1962. Plant diseases in Cyrenaica. FAO Plant Prot. Bull. 10:121–125.

Kuiper, F. K., and Fisher, L. 1975. 391: A Monte Carlo comparison of six clustering procedures. Biometrics 31:777–783.

Lin, A., and Szteinberg, A. 1992. Control of the almond disease *Polystigma* by urea treatments. Hassadeh (Israel) 73:62 (abstract).

López-López, M., Calderón, R., González-Dugo, V., Zarco-Tejada, P., and Fereres, E. 2016. Early detection and quantification of almond red leaf blotch using high-resolution hyperspectral and thermal imagery. Remote Sens. 8 (276), 23 pp.

Malagón, J., Velázquez, L., Carot, M., and Felipe, C. 2017. Comportamiento de las variedades de almendro en zonas frías. Revista de Fruticultura 53:6–23

MAPA, 2019a. Avances de superficies y producciones anuales de cultivos, año 2018. Retrieved August 12, 2019, from https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/

MAPA, 2019b. Registro de productos fitosanitarios. Retrieved August 12, 2019, from https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/productos-fitosanitarios/fitos.asp

Miarnau, X., Vargas, F. J., Montserrat, R., and Alegre, S. 2010. Aspectos importantes en las nuevas plantaciones de almendro en regadío. Revista de Fruticultura 10:94–103.

Miarnau, X., and Vargas, F. J. 2013. Susceptibilidad varietal a dos de las principales enfermedades del almendro, “fusicoccum” y “mancha ocre”. Boletín Agrícola El Arbolar. S.A.T. Arboreto y Crisol de frutos secos S.A.T., Lleida, Spain.

Miarnau, X., Montserrat, R., Batlle, I., Alegre, S., and Vargas, F. J. 2013. High density planting in almond orchards. VI International Symposium on Almonds and Pistachios. ISHS. Spain, Murcia, May 27–31, 2013.

Miarnau, X., Torguet, L., Batlle, I., and Alegre, S. 2016. El cultivo del almendro en alta densidad. Revista de Fruticultura 49:68–87.

Ollero-Lara, A., Agustí-Brisach, C., Lovera, M., Roca, L., Arquero, O., and Trapero, A. 2019. Field susceptibility of almond cultivars to the four most common aerial fungal diseases in southern Spain. Crop Prot. 121:18–27.

Saad, A. T. 2002. Red leaf blotch. Pages 28–29 in: Compendium of nut crop diseases in temperate zones. B. L. Teviotdale, T. J. Michailides and J. W. Pscheidt, eds. APS Press, Saint Paul, MN.

Saad, A. T., and Masannat, K. 1997. Economic importance and cycle of *Polystigma ochraceum*, causing red leaf blotch disease of almond, in Lebanon. EPPO Bull. 27:481–485.

Sahragard, N., Eshaghi, R., Aflaki, M. R., and Banihashemi, Z. 2007. Time of fungicide application against *Polystigma amygdalinum* in almond based on ascospore discharge in Chahar Mahal va Bakhtiari province. Iran J. Plant Pathol. 43:219–239.

Socias i Company, R., and Felipe, A. J. 1999. 'Blanquerna', 'Cambra' y 'Felisia’. Tres nuevos cultivares autógamos de almendro. ITEA Producción Vegetal 95V:111–117.

Socias i Company, R., and Felipe, A. J. 2006. 'Belona' and 'Soleta', two new autogamous almonds. Nucis 13:9–12.

Socias i Company, R., Kodad, O., Alonso, J. M., and Felipe, A. J. 2008. 'Mardía' almond. Hortscience 43:2240–2242.

Socias i Company, R., Gómez Aparisi, J., Alonso, J. M., Rubio-Cabetas, M. J.; and Kodad, O. 2009. Retos y perspectivas de los nuevos cultivares y patrones de almendro para un cultivo sostenible. ITEA 105:99–116.

Socias i Company, R., Kodad, O., Alonso, J. M., Espada, J. L., Chomé, P., and Martínez-Treceño, A. 2012. La incidencia de las nuevas variedades auto-compatibles en el cultivo del almendro en España. Revista de Fruticultura 18:26–32.

Vargas F. J., and Romero, M. A. 1994. 'Masbovera', 'Glorieta', and 'Francolí', three new almond varieties from IRTA. Acta Hortic. 373:75–82.

Vargas, F. J., Romero, M. A., Clavé, J., Vergés, J., Santos, J., and Batlle, I. 2008. 'Vayro', 'Marinada', 'Constantí' and 'Tarraco' almonds. Hortscience 43:535–537.

Ward Jr., J. H. 1963. Hierarchical groupings to optimise an objective function. J. Am. Stat. Assoc. 58:236–244.

Zúñiga, E., Luque, J., and Martos, S. 2019. Lignin biosynthesis as a key mechanism to repress *Polystigma amygdalinum*, the causal agent of the red leaf blotch disease in almond. J. Plant Physiol. 236:96–104.

Zúñiga, E., Luque, J., Torguet, L., and Miarnau, X. 2017. Biología y epidemiología de la mancha ocre del almendro en Cataluña. Revista de Fruticultura 57:6–15.

Zúñiga, E., Romero, J., Ollero-Lara, A., Lovera, M., Arquero, O., Miarnau, X., Torguet, L., Trapero, A., and Luque, J. 2020. Inoculum and infection dynamics of *Polystigma amygdalinum* in almond orchards in Spain. Plant Dis. 104:1239–1246.

## Figures

Fig. 1. Disease severity classes used in the evaluation of red leaf blotch of almond: class 0 (0% affected leaf surface, no disease); class 1 (1-10%), class 2 (11-20%), class 3 (21-50%) and class 4 (>50%)

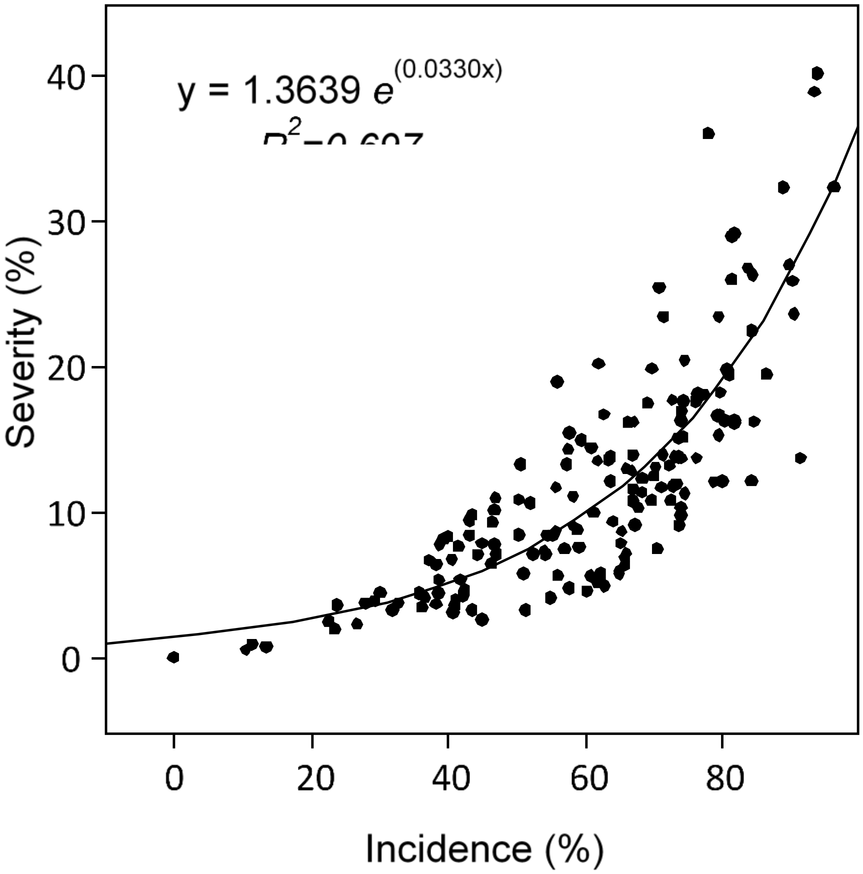

Fig. 2. Exponential regression describing the relationship between the annual incidence and severity of twenty-one almond cultivars naturally infected with red leaf blotch in a multi-year (2011-2018) trial to evaluate cultivar susceptibility. Each point represents the average incidence and severity values for each year and cultivar combination

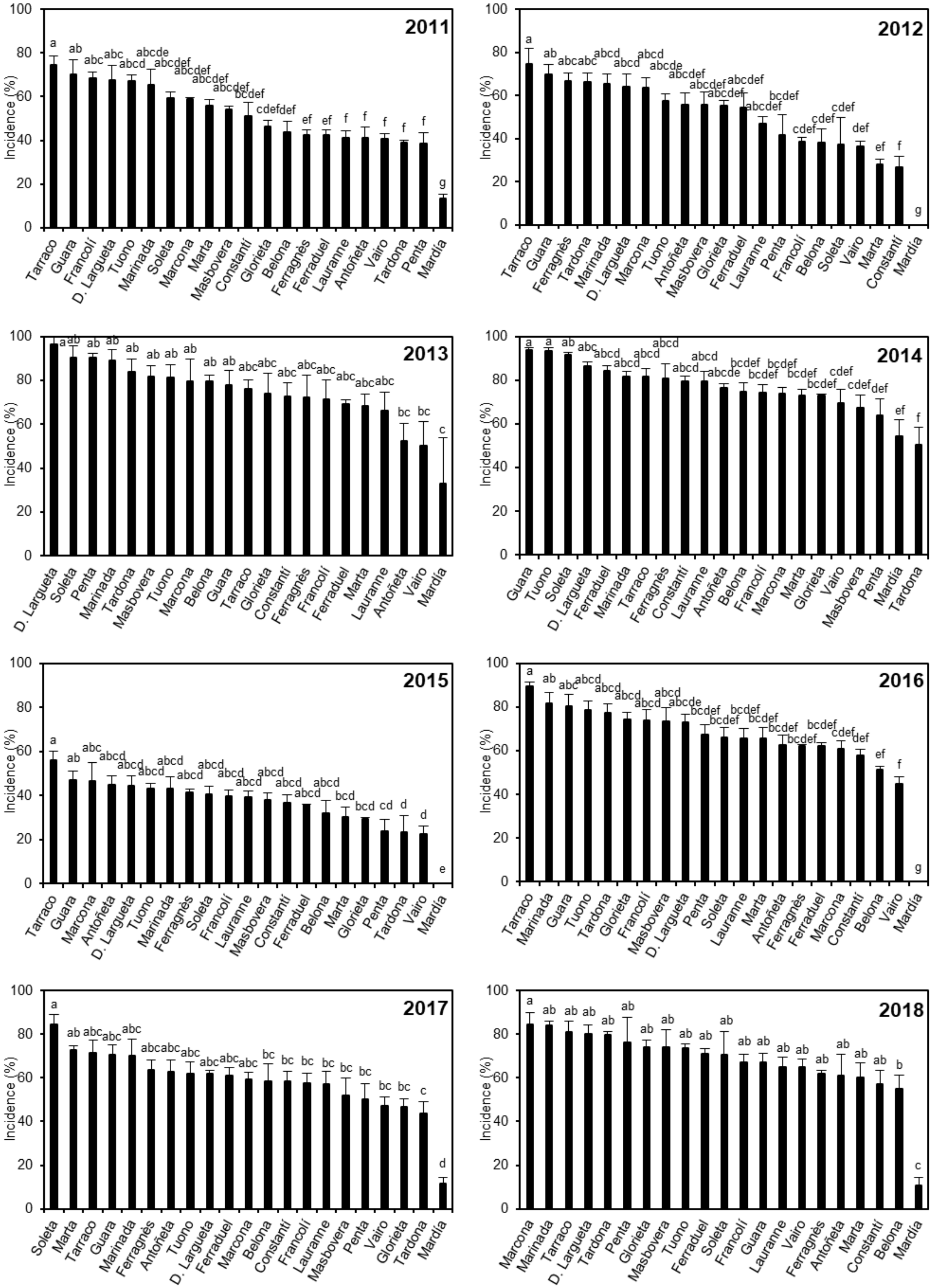
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Fig. 3. Mean disease incidence (± standard error), expressed as the percentage of infected leaves, of twenty-one almond cultivars naturally infected with red leaf blotch in 2011 to 2018. Within each graph (i.e., year), vertical bars with different letters are statistically different according to a repeated-measures design model and mean separation using Tukey-Kramer’s test ($P < 0.05$)

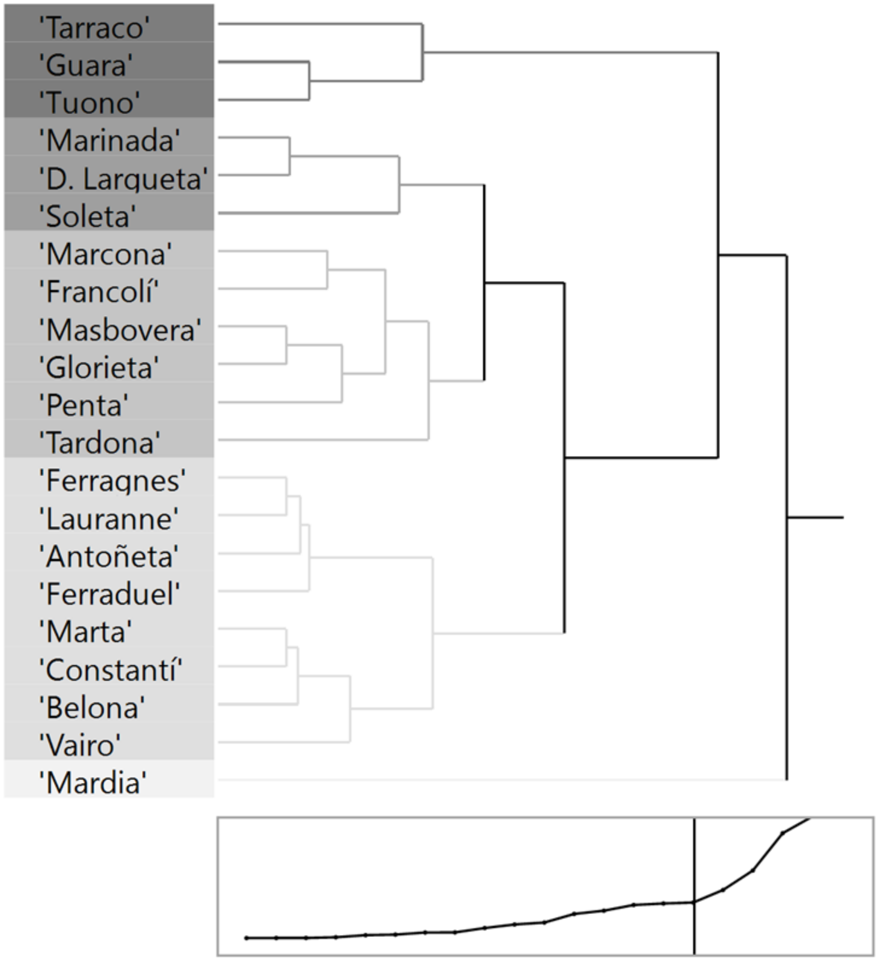


Fig. 4. Dendrogram and scree plot (bottom) for a hierarchical cluster analysis of the incidence and severity of red leaf blotch affecting twenty-one naturally infected almond cultivars during the period 2011-2018. Five categories of susceptibility were defined as follows: highly susceptible (dark gray), susceptible (medium gray), moderately susceptible (gray), tolerant (light gray) and highly tolerant (very light gray). In the scree plot, the X axis represents the number of clusters (in increasing order), the Y axis represents the distance between clusters, and the vertical dashed line represents the inflection point that defines the five clusters to be retained

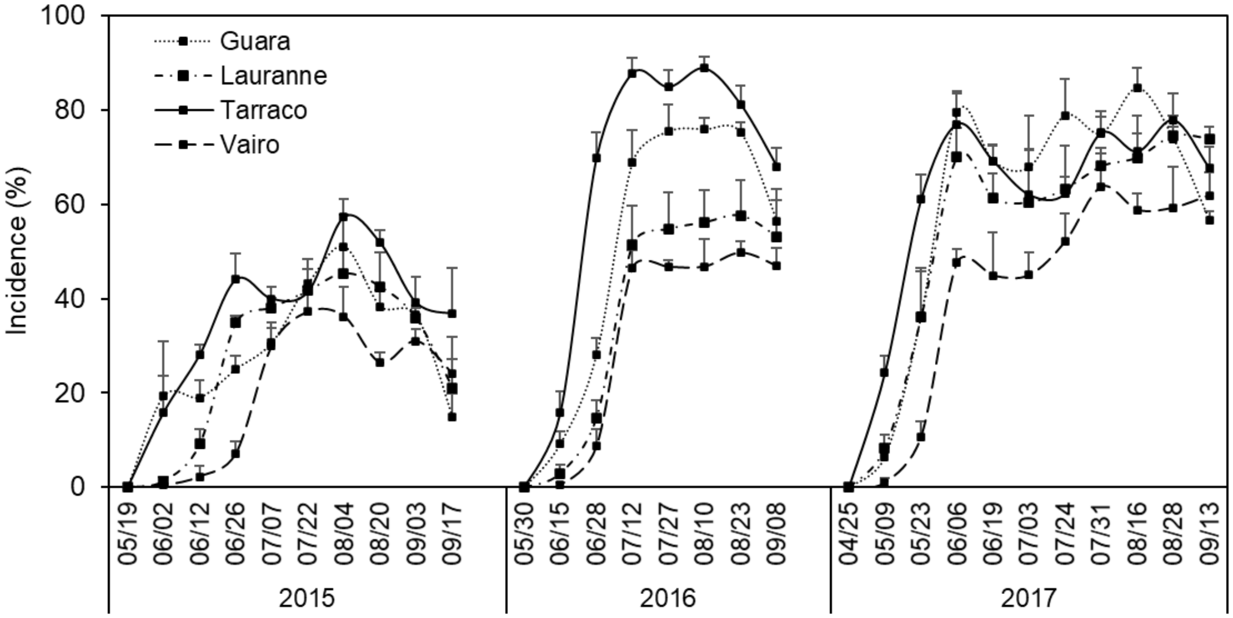


Fig. 5. Intra-seasonal disease progress curves from April to September 2015-2017 based on the incidence (± standard error) of red leaf blotch for four almond cultivars

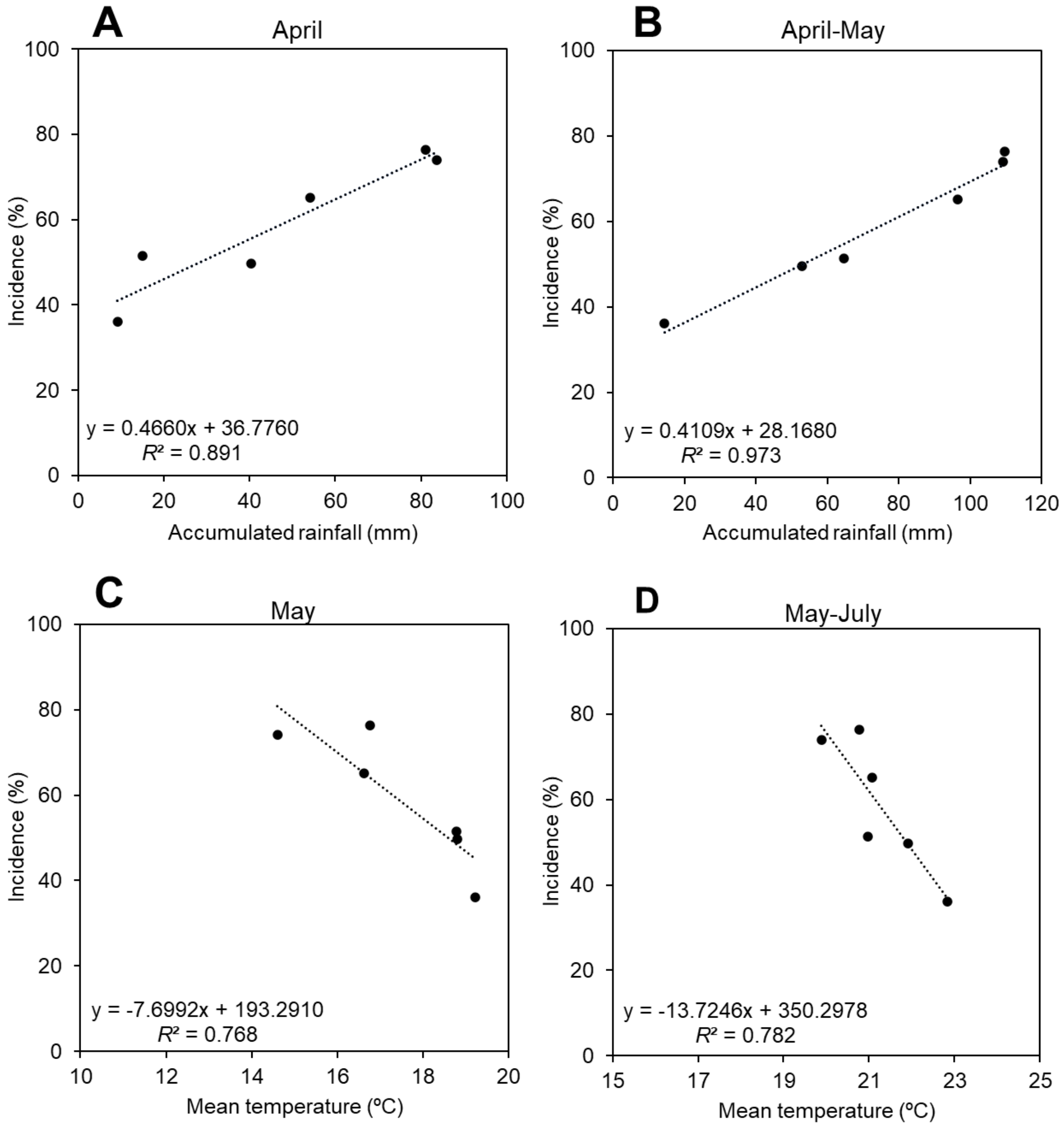
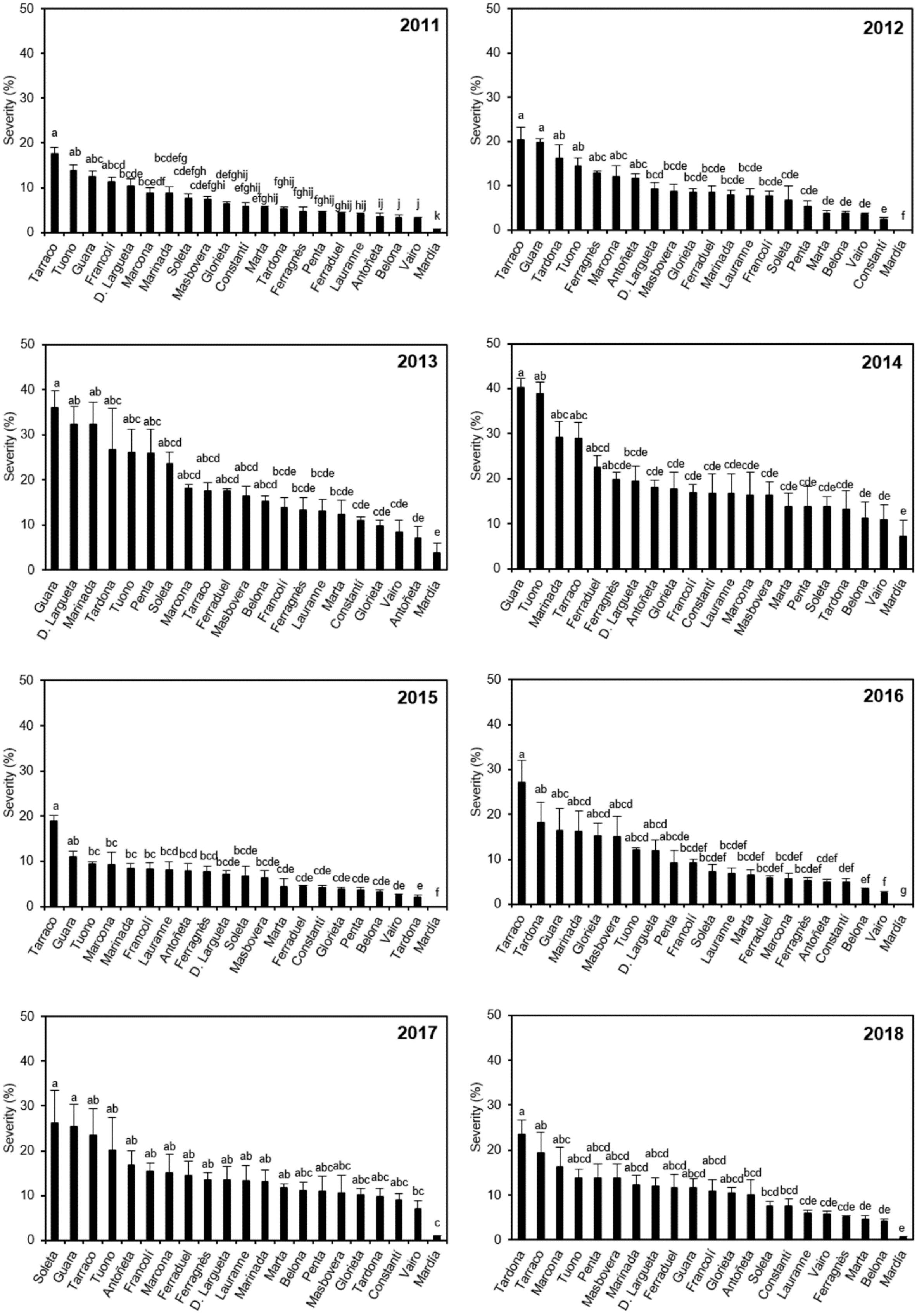


Fig. 6. Linear regressions between the average annual incidence of red leaf blotch on twenty-one almond cultivars and accumulated rainfall in A, April, and B, April-May, and mean temperature in C, May, and D, May-July in 2010-2016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Antoñeta
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2016
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Supplementary Figure S1. Mean disease severity (± standard error), expressed as the percentage of infected leaf surface, of twenty-one almond cultivars naturally infected with red leaf blotch in 2011 to 2018. Within each graph (i.e., year), vertical bars with different letters are statistically different according to a repeated-measures design model and mean separation using Tukey-Kramer's test ($P < 0.05$)

Supplementary_Table__S2_Miarnau_RLB

Supplementary Table S2. Summary of environmental factors monitored in 2010-2016 during the assessment of susceptibility of twenty-one almond cultivars.

Supplementary_Tab
le__S3_Miarnau_RLB

Supplementary Table S3. Correlation coefficients between annual incidence of red leaf blotch on twenty-one almond cultivars and environmental factors monitored in 2010-2016.