



“Authors may post the original unedited, unformatted, peerreviewed versions of their articles on their university or company websites at no charge”
https://apsjournals.apsnet.org/pb-assets/Intellectual_Property-1550840912083.pdf

This document is the original unedited, unformatted, peerreviewed version of an article published in Plant Disease, copyright © The American Phytopathological Society (APS). To access the final edited and published work see <https://doi.org/10.1094/PDIS-07-19-1406-RE>

Full citation reference:

Zuniga, Erick, Joaquin Romero, Andres Ollero-Lara, Maria Lovera, Octavio Arquero, Xavier Miarnau, Laura Torguet, Antonio Trapero, and Jordi Luque. "Inoculum and Infection Dynamics of Polystigma Amygdalinum in Almond Orchards in Spain." 2020. Plant disease 104: 1239-46. <https://apsjournals.apsnet.org/doi/10.1094/PDIS-07-19-1406-RE>.

Document downloaded from:



1 **Inoculum and infection dynamics of *Polystigma amygdalinum* in**
2 **almond orchards in Spain**

3

4 Erick Zúñiga^{a,b}, Joaquín Romero^c, Andrés Ollero-Lara^c, María Lovera^d, Octavio Arquero^d,
5 Xavier Miarnau^e, Laura Torguet^e, Antonio Trapero^c, and Jordi Luque^a

6

7 ^a Plant Pathology, IRTA Cabrils, Carretera de Cabrils km 2, 08348 Cabrils, Spain

8 ^b Plant Physiology Laboratory, Universitat Autònoma de Barcelona, Campus UAB, 08193
9 Bellaterra, Spain

10 ^c Departamento de Agronomía, ETSIAM, Universidad de Córdoba, Campus de Rabanales,
11 Edif. C4, 14071 Córdoba, Spain

12 ^d Departamento de Fruticultura Mediterránea, IFAPA, Alameda del Obispo, 14004
13 Córdoba, Spain

14 ^e Fruit Production Program, IRTA Fruitcentre, PCiTAL, Park of Gardeny, Fruitcentre
15 Building, 25003 Lleida, Spain

16

17 **E-mail addresses:**

18 E. Zúñiga: erick_zur@hotmail.com; J. Romero: joaquinromrod@gmail.com; A. Ollero-
19 Lara: ollero13@hotmail.com; M. Lovera: maria.lovera@juntadeandalucia.es; O. Arquero:
20 octavio.arquero@juntadeandalucia.es; X. Miarnau: xavier.miarnau@irta.cat; L. Torguet:
21 laura.torguet@irta.cat; A. Trapero: trapero@uco.es; J. Luque: jordi.luque@irta.cat

22

23 **Author for correspondence**

24 Dr Jordi Luque

25 Phone: +34 937 507 511

26 Fax: +34 937 533 954

27 E-mail: jordi.luque@irta.cat

28 **Abstract**

29 Red leaf blotch (RLB) disease of almond, caused by *Polystigma amygdalinum*, is an
30 important foliar disease in most production regions of the Mediterranean basin and the
31 Middle East, since severe infections may cause a premature defoliation of the tree. Some
32 key aspects on the epidemiology of *P. amygdalinum* were studied in multi-year trials in two
33 almond-growing regions in Spain, which included the seasonal development of perithecia,
34 production and germination of ascospores, along with the disease incubation and plant
35 infectivity periods. Our results showed that primary inoculum was available in extended
36 periods (January to August). Significant differences in ascospore amounts among regions,
37 higher in the southern Andalusia and lower in the northern Catalonia, and years of study
38 were detected. The factors geographical location, sampling period and evaluation year were
39 found significant on the development of *P. amygdalinum* perithecia. Variable ascospore
40 germination rates were observed from April to July, over 15 % but rarely exceeding 30 %.
41 The RLB infectivity period in Catalonia extended from March to mid-June, while in
42 Andalusia from March to May. The incubation period was mainly in a range of 5 to 10
43 weeks in Catalonia. The environmental conditions of October-January influence the
44 available ascospore amounts in the next season. RLB infection occurs in spring to summer,
45 when mean temperatures are in the range 10 to 20°C. These results represent the first step
46 in developing a prediction model of the disease that might serve as a tool for the control of
47 RLB.

48 **Introduction**

49 Almond (*Prunus dulcis* (Mill.) D.A. Webb) is a traditional and widespread crop in the
50 Mediterranean basin and the Middle East. It is generally considered a marginal crop in this
51 area that is currently grown in dry land under limiting soil and climate conditions, which
52 leads to low yields. Spain leads the world in area under almond cultivation, with more than
53 630,000 ha of almonds grown in 2017 (FAOSTAT 2019; MAPA 2019). However, the
54 Spanish annual yield of almonds with shell is slightly over 400 kg ha⁻¹, much lower than
55 those obtained in the USA, the leading producer country in the world with an average
56 production over 2,500 kg ha⁻¹ (FAOSTAT 2019). In recent years, an intensive almond
57 cropping in Spain has been driven by a general increase in world nut consumption (Miarnau
58 et al. 2010, 2018). This intensive cropping is characterized by the use of new almond
59 cultivars planted in higher tree density, and supported with proper irrigation, fertilization
60 and pesticide programs to reach higher yields (Miarnau et al. 2018; Vargas et al. 2010), as
61 comparable to that obtained in the USA (MAPA 2019). However, there is a great concern
62 about an eventual increase in the incidence of almond diseases, which have become the
63 main limiting factor of these new plantations (Ollero-Lara et al. 2016a, 2019; Torguet et al.
64 2016).

65 Red leaf blotch (RLB) of almond, caused by the ascomycete *Polystigma*
66 *amygdalinum* P.F. Cannon, is a foliar disease which is widely extended among most
67 almond production regions of Europe and Asia (Cannon 1996; Farr and Rossman 2019),
68 where it is considered of high economic relevance in several countries (Cannon 1996; Saad
69 and Masannat 1997). The disease is endemic in these regions and to date is not known in
70 other almond-growing areas in the world, such as the USA or Australia (Farr and Rossman

71 2019). Although RLB is well-known in Spain since old times (González-Fragoso 1927), its
72 incidence has increased worryingly during the last years and is currently considered a re-
73 emerging disease in the new intensive almond plantations (Almacellas 2014; Ollero-Lara et
74 al. 2016a; Torguet et al. 2016). It has been hypothesized that increased incidence of RLB
75 could be related to the better growing conditions for almond cropping in the new intensive
76 plantations, as well as the use of new late-flowering cultivars (especially ‘Guara’ in Spain),
77 which are more susceptible to RLB than traditional ones (Ollero-Lara et al. 2016b, 2019).
78 First RLB symptoms appear as pale green to yellowish spots on both leaf sides in spring,
79 turning into yellowish-orange and later dark brown spots with age. Size of leaf spots
80 increases through the spring and summer seasons and cover almost the whole leaf surface
81 in late summer under favorable weather conditions. These spots are commonly associated
82 with hypertrophy and deformation of leaves caused by the development of the fungal
83 stroma on leaves. Occasionally, severe infections under hot and dry conditions can induce
84 an early leaf fall in summer, thus reducing the photosynthetic activity of trees.

85 *Polystigma amygdalinum* is a biotrophic ascomycete specific to almond, which was
86 first described in 1845 in Italy as *Septoria rubra* var. *amygdalina*, being later reclassified
87 within the genus *Polystigma* as *P. ochraceum* or *P. rubrum*, mainly due to the color of the
88 fungal stroma in the leaves (Cannon 1996). The species *P. amygdalinum* differs from other
89 *Polystigma* species which affect numerous species of the *Prunus* genus due to its host
90 specificity, stromal coloration and the morphology of fruiting bodies and spores (Cannon
91 1996). Because its host specificity, the limited geographical distribution, and the inability to
92 grow in artificial media, research on *P. amygdalinum* is scarce (Cannon 1996). A recent
93 phylogenetic study indicated that *P. amygdalinum* might not belong to the Phyllachorales,
94 as had been considered to date, and should be better accommodated in the

95 Xylariomycetidae subclass of Sordariomycetes, close to Xylariales and Trichosphaeriales
96 (Habibi et al. 2015).

97 The RLB disease is monocyclic and the only inoculum sources are the stromata of
98 affected leaves which fall to the ground in autumn (Banihashemi 1990; Ollero et al. 2016b;
99 Saad and Masannat 1997). The sexual stage is developed in winter on the leaf stromata, and
100 later in spring, ascospores are air-spread and can infect new almond leaves (Banihashemi
101 1990). In Iran, Ghazanfari and Banihashemi (1976) reported on the influence of autumn-
102 winter weather conditions in perithecia development. Based on these authors, the start of
103 perithecia maturation occurs below 10°C. Banihashemi (1990) showed that ascospore
104 release in Iran is related with rain periods, beginning at flowering and reaching the
105 maximum at petals fall (late April to early May). In Lebanon, ascospore release can occur
106 between February and mid-May (Saad and Masannat 1997). However, the effect of weather
107 conditions on the infection process is deeply unknown. After infection, leaf spots appear
108 after an incubation period of 30-35 days (Banihashemi 1990; Cannon 1996; Suzuki et al.
109 2008). The occurrence of secondary cycles has not been confirmed (Ollero-Lara et al.
110 2016b; Saad and Masannat 1997; Shabi 1997), since conidia do not have infective ability
111 and their only function appears to act as spermatia in the sexual reproduction of the
112 pathogen (Cannon 1996; Habibi and Banihashemi 2016).

113 As a monocyclic disease, control management of RLB should be aimed at: (i)
114 reducing primary inoculum potential, i.e. ascospores produced in the affected leaves fallen
115 in previous autumn, and (ii) protecting new almond leaves growing in the season. Based on
116 several authors (Almacellas, 2014, Arquero et al. 2013, Lin and Szeinberg, 1992), the
117 control measures to achieve the first objective include: (i) to remove or bury the leaves, (ii)
118 to favor their decomposition through urea applications and (iii) to treat fallen leaves with

119 fungicides. However, none of these measures have been evaluated for almond crop in Spain
120 (Ollero-Lara et al. 2016b). Regarding the second objective, application of fungicides
121 appears to be the most effective measure to protect the new leaves from RLB infections
122 (Almacellas and Marín 2011; Arquero et al. 2013; Bayt-Tork et al. 2014). For all the above
123 control measures to be effective, it is necessary to know the dynamics of the inoculum
124 potential in fallen leaves, and the conditions influencing ascospore production, dispersal
125 and infection. A decision support system considering the monocyclic pattern of the RLB
126 epidemics might be a helpful tool to optimize fungicide applications.

127 The objective of this study was to characterize the dynamics of the production,
128 maturation and potential dispersion of *P. amygdalinum* ascospores and correlate the key
129 aspects of the disease with the environmental conditions in two Spanish almond-growing
130 regions, namely Andalusia and Catalonia. These two areas are highly representative of the
131 main almond growing areas in Spain (South and Ebro Valley, respectively).

132

133 **Materials and Methods**

134 **Geographical locations.** Three experimental sites were located as follows: one location in
135 Córdoba, Andalusia (S Spain; WGS84 coordinates: UTM 30S X = 341069, Y = 4190753),
136 and two locations in Catalonia (NE Spain), namely Gandesa (UTM 31T X = 284000, Y =
137 4549200) and Les Borges Blanques (Borges, hereinafter; UTM 31T X = 320870, Y =
138 4597530). These sites corresponded to experimental almond orchards located at facilities
139 of IFAPA (Andalusia) and IRTA (Catalonia). Orchards consisted in trees of different
140 national and foreign cultivars, variously arranged and managed under local usual practices.

141 Trees in the orchards were not treated with any chemical product to allow natural infection
142 of leaves by *P. amygdalinum*.

143 **Plant material.** For the experiments on primary inoculum monitoring, development of
144 fruiting bodies, and germination of ascospores; fallen leaves of various almond cultivars
145 with distinct RLB symptoms were collected during early autumn (September) in Andalusia
146 and autumn/winter (December to January) in Catalonia before each specific experiment. As
147 the experimental orchards consisted of different almond cultivars, collected fallen leaves
148 from the ground could not be classified according to their cultivar origin. In each season, an
149 additional bulk sample of leaves collected in Gandesa was taken to Borges in order to study
150 the eventual environmental influence in samples from different geographical origins. This
151 sample (hereinafter as Borges/Gandesa) was considered as a third location within the
152 Catalonian sites. In all experimental sites, leaves were placed into nylon mesh bags with 90
153 to 200 leaves per bag. The bags with fewer leaf numbers were processed earlier in the
154 season whereas the bags with higher amounts of leaves were processed later in the season,
155 since natural decomposition of leaves along the time would have left a low sample amount
156 at later stages. The bags were left outdoors in the orchard by nailing them on the ground.
157 Various numbers of bags ($N > 13$) were prepared for each experimental site and year.

158 For the monitoring of natural infections and the estimation of the disease incubation
159 period, 1-year-old plants of the susceptible cultivar ‘Tarraco’ grafted onto ‘GF-677’
160 rootstock were used. Trap plants were kept in greenhouses while not being exposed to
161 natural RLB infections in the experimental sites, while another group of plants (control
162 plants) never were moved out of the greenhouse. Regardless their geographical location,
163 plants kept in the greenhouses were maintained in 3 liters pots with a peat:perlite mixture
164 (3:1, v:v) (Peat: Floratorf TKS1, Floragard, Germany; Perlite: Europerl, Spain). The

165 substrate was amended with Osmocote Pro 3-4M granular fertilizer (Everris, Heerlen, The
166 Netherlands) at 2.5 g l⁻¹. Plants were irrigated as needed to avoid water stress and never
167 treated with fungicides both in the greenhouse and in the experimental sites.

168 **Monitoring of primary inoculum.** The study of primary inoculum was conducted for
169 three seasons in Andalusia (2014 to 2016) and Catalonia (2015 to 2017). Starting from
170 winter in each year, at the stage of dormant trees, the bags of leaf samples were taken
171 fortnightly to the laboratory till the end of the trial. The sample was oven-dried for 24 h at
172 35°C. Each sample was weighted (dry weight) and later subdivided into eight equally-
173 weighted subsamples. In Andalusia, all eight subsamples were treated separately for
174 ascospore extraction in distilled water, by crushing leaves in a mortar until getting a
175 homogeneous suspension (about 10 min operation). In Catalonia, an additional ascospore
176 extraction method was tested, by continuous stirring (18 h, room temperature) of a
177 suspension of leaf fragments in water. Thus, in Catalonia, four subsamples were extracted
178 by crushing and the remaining four by stirring. In all cases, various amounts of distilled
179 water were used according to the sample weight (about 40 ml per g sample). For both
180 extraction methods, final ascospore suspensions were filtered through a 2-folded 60-µm
181 Nylon mesh and subsamples of ascospore suspensions were examined under a microscope
182 (×250) using a hemocytometer (Neubauer chamber). Ascospores of *P. amygdalinum* were
183 unambiguously identified through their morphology features and counted. Each subsample
184 was measured four times and eight replicated measurements were done per subsample
185 measurement. Results were expressed as numbers of ascospores per g (dry weight) of
186 leaves after proper calculations. The experimental period covered from January to August
187 for all combinations of year and location.

188 **Development of fruiting bodies.** This study was conducted with the leaf samples collected
189 in Catalonia in 2016 and 2017. Prior to the oven-drying of leaf samples, four leaves with
190 well-developed fungal stromata were taken from each sample bag. The outer part of the
191 fungal stroma was cut off with a sterile scalpel and five randomly chosen fruiting bodies
192 from each leaf were individually excised with a hypodermic needle. The fruiting bodies
193 were placed in a water droplet on a microscope slide and bisected to unveil their content,
194 then covered with a cover slip and examined under a light microscope ($\times 250$). The fruiting
195 bodies (20 per each location and sampling period) were classified into six different
196 developmental stages by using modified categories described by Toscano-Underwood et al.
197 (2003), as follows: class P (pycnidia, either with conidia or not); class A (perithecium
198 differentiated, asci undifferentiated or beginning differentiation, ascospores
199 undifferentiated); class B (perithecium differentiated, asci differentiated, ascospores
200 undifferentiated); class C (perithecium differentiated, most asci differentiated, some
201 ascospores (< 8) differentiated); class D (fully matured perithecium, asci differentiated,
202 ascospores (8) fully differentiated); class E (perithecium empty, no asci present, ascospores
203 discharged). The percentages of each developmental status at each monitoring period were
204 calculated.

205 **Germination of ascospores.** This study was conducted with the leaf samples collected in
206 Catalonia in 2017. The viability of ascospores was estimated by determining the
207 germination percentages at each sampling period. From the same leaf samples used in the
208 study on fruiting bodies development, a sufficient amount of perithecia containing mature
209 ascospores was obtained. The perithecia were bisected and their content suspended in a 1.5
210 ml Eppendorf tube containing 1 ml distilled water. A volume (200 μ l) of the ascospore
211 suspension was spread over a potato dextrose agar (PDA, Difco™, Becton, Dickinson &

212 Co., Sparks, MD) plate amended with streptomycin sulphate at 100 IU streptomycin ml⁻¹
213 and incubated at 20°C. Fifty randomly-chosen ascospores per location and sampling date
214 were counted (five replicates, 10 ascospores each) and classified into germinated and non-
215 germinated categories by using a light microscope (250×), at two intervals, 4 h and 24 h
216 after plating. An ascospore was recorded as germinated when the germ tube was greater
217 than half the width of the ascospore, as similarly described by Habibi and Banihashemi
218 (2015).

219 **Disease infectivity and incubation periods.** The disease infectivity and incubation periods
220 were studied in two locations in Andalusia (Córdoba) and Catalonia (Borges), for one
221 (2016) and three (2015 to 2017) years, respectively. For each location and year, a group of
222 130 one-year-old ‘Tarraco’ plants was kept in a greenhouse at IFAPA and RTA facilities,
223 away from an eventual exposure to natural RLB infections. From February to August each
224 year, 10 different plants from the group were brought every two weeks to the experimental
225 orchards and left to be RLB-infected under natural conditions. This resulted in a total of 13
226 recordings per each year and location. After the 2-week exposure to the disease, trap plants
227 were removed from the field and taken back to the greenhouse, where they were monitored
228 weekly from February to October to check for the expression and evolution of RLB
229 symptoms. Numbers of apparently healthy and RLB-affected leaves in each plant were
230 recorded, and the proportion of RLB-affected leaves (incidence) was calculated and
231 averaged for each monitoring period. The infectivity period was determined as the period in
232 which RLB-symptomatic leaves were detected along the experiment. The incubation period
233 was estimated by determining the time (in weeks) between the initial exposure in the
234 orchards and the consistent appearance of disease foliar symptoms minus one week, in
235 order to correct the 2-week exposure interval with its intermediate point.

236 **Weather data.** Main meteorological variables, namely temperature (T), relative humidity
237 (RH) accumulated rainfall were recorded daily in the experimental areas throughout the
238 monitored years. Data from three automatic weather stations included in the weather
239 network services of the regional governments were used. The weather station in Gandesa
240 was located less than 100 m away from the experimental area, about 9 km away in the case
241 of Borges, and about 1 km away in the case of Córdoba. All recorded meteorological data
242 were considered as representative of the weather conditions at the experimental sites. Raw
243 meteorological data were summarized with 14 weather variables: maximum, minimum and
244 mean daily T, maximum and mean RH, accumulated rainfall, number of rainy days (days
245 with rainfall ≥ 0.2 mm), accumulated vapor pressure deficit, number of wet days (see
246 below), accumulated low T in wet days (50-T), and the number of days with mean daily T
247 lower than 10°C, from 10 to 20°C, and equal or higher than 20°C. In addition, number of
248 days considered both wet and with mild T ($10 \leq T < 20^\circ\text{C}$) were also recorded, as those
249 conditions are thought to be potentially optimal for RLB development. Daily vapor
250 pressure deficit (VPD) was calculated from mean daily T and mean RH according to the
251 modified equation of Buck (1981) as described by Rossi et al. (2009):

252

$$253 \quad \text{VPD (hPa)} = (1 - \text{RH}/100) \times 6.11 \times \exp [(17.47 \times T_{\text{mean}})/239 + T_{\text{mean}}]$$

254

255 Days were considered wet when VPD was ≤ 4 hPa or accumulated rainfall ≥ 0.2 mm.
256 The accumulation of low T in wet days was measured as the sum of 50-T only in wet days.
257 The 14 weather variables were calculated for the following time intervals: *i*) from the
258 previous 7- and 14-day periods of each monitoring date, *ii*) from months comprised
259 between June to January, and *iii*) from the subdivisions June-September (stage 1) and

260 October-January (stage 2) in the whole monitoring period indicated in *ii*, and *iv*) from all
261 the infectivity periods of trap plants. This resulted in a total number of 182 weather
262 variables (14 weather variables \times 13 time intervals).

263 **Data analysis.** Data obtained from the primary inoculum monitoring, and incubation and
264 infectivity periods were analyzed using Statistix v.10 (Analytical Software, Tallahassee,
265 FL). Otherwise stated in text and figures, mean values are shown together with their
266 corresponding standard errors. In the primary inoculum monitoring, factorial ANOVA was
267 performed to test main effects and interactions of location and evaluation year on the
268 ascospores amounts recorded periodically. These comparisons were performed by
269 considering only the crushing method of ascospores extraction, as it was only the common
270 ascospore extraction method used in all studied locations. In an exploratory analysis, and to
271 avoid missing data due to differences in the monitoring start and ending dates among
272 locations, only 13 matching data per year and locations were used, i.e. for the period
273 comprised between mid-February and early-August in all years and locations. In further
274 analyses, made separately on the location basis, all recorded data were used. Data were
275 tested for normality, homogeneity of variances and normally-distributed residual patterns
276 using analytical tools of the statistical package. Logarithmic transformations were carried
277 out when necessary. Treatment means were compared using Fisher's protected least
278 significant difference (LSD) at $\alpha = 0.05$.

279 A regression model was fitted to describe the relationship between the ascospore
280 counts from the two different ascospores extraction methods used in this study. Spearman's
281 rank correlation coefficients (ρ) were calculated from the following variables: (*i*) the
282 ascospore counts obtained by the crushing method in each evaluation period, expressed as a
283 proportion of the total accumulated counts during the selected period comprised between

284 mid-February and early-August (ASC_{rate}), and the weather variables observed within the 7-
285 and 14-day periods previous to each ascospore count, (ii) the total ascospore counts during
286 the season, total ascospores number in the previous year, weather variables calculated from
287 months comprised between June to January and stages 1 and 2, and (iii) RLB incidence in
288 trap plants and the weather variables from infectivity periods and ascospores counts at the
289 end of the infectivity period. To avoid misunderstood and random variable associations
290 (Fernández-Escobar et al. 2018), only associations with $\rho > 0.500$ and $P < 0.05$ between
291 variables were considered strong and reliable enough. Therefore, only these relationships
292 were considered in this work.

293 In order to analyze the possible influence of location and sampling period on the
294 development and maturation of *P. amygdalinum* fruiting bodies, an ordinal logistic
295 regression was performed in R v.3.5.1 (<https://www.R-project.org/>) with the *clm*
296 (cumulative link models) function included in the package ‘ordinal’ (Christensen 2018).

297

298 **Results**

299 **Monitoring of primary inoculum.** An exploratory ANOVA test showed that the annual
300 mean amount of RLB potential inoculum, as estimated by the ascospore counts per gram of
301 leaf (hereinafter abbreviated as agl), was significantly influenced by the experimental
302 location ($P = 0.007$), the evaluation year ($P = 0.039$), and their interaction ($P = 0.011$).

303 Because of interactions, further ANOVA tests were performed on data subsets according to
304 their location origin. In general, the ascospore production period extended from January to
305 August among the studied areas and seasons (Fig. 1). Highest amounts of ascospores were
306 recorded in Córdoba, whereas those values of Borges and Gandesa were about a tenth of

307 the amounts recorded in Córdoba. The Borges/Gandesa group did not differ from Borges
308 and Gandesa ($P = 0.922$) in terms of mean annual ascospore amounts. In Córdoba, higher
309 amounts of ascospores were observed in 2014 and 2015 in comparison to 2016, whereas in
310 Borges and Gandesa a higher amount of ascospores was observed in 2017 than in previous
311 years (all $P < 0.001$).

312 In Córdoba, mean ascospore amounts of *P. amygdalinum* obtained by leaf crushing
313 were in the range from 1×10^6 to 6×10^6 agl (in 2014 and 2016), whereas about a 10-fold
314 higher annual mean, i.e. 2×10^7 agl, was estimated in 2015 (Fig. 1A). In 2014, ascospores in
315 Córdoba were recorded early at the beginning of the season in January and remained quite
316 stable at about 1×10^7 agl thereafter. In 2015, higher amounts of ascospores were mainly
317 detected in a six-month interval, i.e. March to August, and ranged between 1×10^6 and
318 1×10^8 agl within this period. In 2016, ascospores were detected between March and June,
319 and peaked up to 8×10^6 agl in April (Fig. 1A). In the Catalan locations, Borges and
320 Gandesa, the presence of ascospores was detected from January to August, with some
321 exceptions: no recordings after mid-June in 2015 in Borges, and none or occasional low
322 recordings at the beginning of the season in 2016 in Gandesa and Borges/Gandesa (Fig.
323 1B,C,D). Mean annual ascospore amounts obtained from crushed leaves were mostly in the
324 range from 1×10^5 to 1×10^6 in 2015 and 2017, and between 1×10^4 and 1×10^5 in 2016, with
325 several occasional peaks along the seasons. Occurrence of those peak values in ascospore
326 amounts was variable among locations and years, and a pattern of peak occurrence was not
327 clearly observed (Fig. 1). Dynamics of the RLB inoculum potentials along the season were
328 similar in the cases of Gandesa and Borges/Gandesa in 2015 and 2016, and slightly
329 different from Borges within the same years. However, dynamics of ascospore amounts in
330 2017 were similar for the three Catalan leaf sources (Fig. 1B,C,D).

331 Ascospores amounts obtained through the stirring-bath technique were consistently
332 lower than those obtained by crushing (Fig. 1B,C,D). A significant linear relationship
333 ($P < 0.001$, $R^2 = 0.6721$) between the log-transformed data of the two ascospore extraction
334 methods was found as follows: $\log(\text{Ascospores}_{\text{stirring}}) = 1.1619 + 0.7128 \times$
335 $\log(\text{Ascospores}_{\text{crushing}})$. The equivalent power function was therefore: $\text{Ascospores}_{\text{stirring}} =$
336 $14.5180 \times (\text{Ascospores}_{\text{crushing}})^{0.7128}$. Moreover, there were no significant differences
337 between samples from different origin (Borges and Borges/Gandesá) which were obtained
338 with either extraction method ($P = 0.621$ and $P = 0.497$ for crushing and stirring,
339 respectively).

340 Associations with $\rho > 0.500$ and $P < 0.05$ between the rate of ascospore amounts per
341 season (ASC_{rate}) and any tested weather variable were not found. On the other hand, the
342 total amount of ascospores per season was only significantly correlated with weather-
343 derived variables of stage 2 (October-January), which resulted in significant ($P < 0.05$) ρ
344 values higher than 0.700 in the following cases: mean RH ($\rho = 0.767$), accumulated rainfall
345 ($\rho = 0.717$), number of raining days ($\rho = 0.728$) and number of days with mean daily T
346 higher than 20°C ($\rho = 0.706$). Moreover, in October, the mean of maximum RH ($\rho = 0.783$),
347 the accumulated rainfall ($\rho = 0.733$), and the number of days with mean daily T equal or
348 higher than 20°C ($\rho = 0.706$) were positively correlated with the total amount of ascospores
349 per season. On the other side, only the number of days with daily mean T from 10 to 20°C
350 in October was negatively correlated ($\rho = -0.792$) with the total amount of ascospores. In
351 January, accumulated rainfall also showed a positive correlation with the total amount of
352 ascospores per season ($\rho = 0.783$). No more associations were found between the seasonal
353 total ascospore amounts and weather variables for the remaining months of the stage 2.

354 **Development of fruiting bodies.** The development and maturation of perithecia along the
355 season was confirmed through the observation that immature stages (A, B) were prevalent
356 at the beginning of the experimental period whereas mature stages (C, D, E) were mainly
357 recorded later in the season (Fig. 2). Results from the ordinal logistic regression analysis on
358 the whole dataset showed that all analyzed factors and their interactions, except for the
359 interaction location \times week, were significant (*results not shown*). Because of interactions,
360 further ordinal logistic regressions were performed on data subsets according to the location
361 of sampled leaves. In addition, datasets from Borges and Borges/Gandeses were combined
362 into a single dataset to evaluate the influence of the geographical origin of samples.
363 Separate analyses of each location subsets showed the significance of factors sampling
364 period, year, and their interaction (all $P < 0.001$). However, the origin of leaf samples in the
365 Borges and Borges/Gandeses subset was not found significant ($P = 0.262$), nor the
366 interactions: origin \times sampling period ($P = 0.618$), origin \times evaluation year ($P = 0.262$), and
367 origin \times sampling period \times evaluation year ($P = 0.618$).

368 In Gandesa, fully mature perithecia (D) were observed rarely in 2016, and the
369 percentages of this class never exceeded 20% throughout the assay. However, mature
370 perithecia with percentages equal or higher than 40% were detected in 2017 from early-
371 May (week 18) until the end of the experiment (Fig. 2A). In Borges, proportion of mature
372 ascocarps were more frequently detected than in Gandesa. Thus, class D ascocarps reached
373 80% at mid-May 2016 (week 20), and remained in the range 15 to 45% until August. In
374 2017, the percentage of mature perithecia observed in Borges prevailed above 50% from
375 late-February (week 8) to the end of the experiment (Fig. 2B). Regarding the
376 Borges/Gandeses samples, maturation of ascocarps behaved similarly as those from Borges,
377 as shown earlier by the non-significance of the geographical origin factor and its

378 interactions in the statistical analyses. Thus, percentages above 50% in class D perithecia in
379 2017 prevailed in almost every week from mid-February on (Fig. 2C).

380 **Ascospore germination.** Ascospores of *P. amygdalinum* germinated on PDA as earlier as 4
381 h after plating, but highest percentages of ascospore germination were observed at 24 h
382 incubation (Fig. 3). In general, germination percentages at 24 h ranged 12 to 44% for all
383 leaf samples origins, with mean values for each leaf origin as follows: Gandesa, 16.6 ± 3.8
384 %; Borges, 19.2 ± 1.5 %; Borges/Gandesa, 20.9 ± 1.2 %. In Gandesa, the highest
385 germination percentage (44.0 ± 8.0 %) was observed in mid-July (week 28), whereas in
386 Borges the maximum (28.0 ± 2.0 %) occurred in mid-April (week 16). Ascospores from the
387 Borges group showed consistently 20% and above of germination during the first half of
388 the monitoring period (Fig. 3). Similarly, ascospores from the Borges/Gandesa group
389 showed the higher germination percentage (30.0 ± 2.0 %) in April, just as the samples from
390 Borges did.

391 **Disease infectivity and incubation periods.** Trap plants exposed in Córdoba and Borges
392 showed that RLB infections occurred from March (week 9) to late July (week 30), although
393 higher infection percentages were mainly detected from week 9 to week 18, i.e. from
394 March to early May (Fig. 4). Moreover, the incidence of RLB in trap plants was positively
395 correlated with the number of days with mean daily T from 10 to 20°C ($10 \leq T < 20$ °C)
396 ($\rho = 0.526$, $P = 0.001$) and the number of days both wet and mild T ($VPD \leq 4$ hPa or
397 $R \geq 0.2$ mm, and $10 \leq T < 20$ °C) ($\rho = 0.632$, $P < 0.001$).

398 Overall incidence in Córdoba was higher than in Borges in 2016, as infections in
399 Córdoba were well ranging 30 to 70% within the weeks 9 to 14, whereas equivalent values
400 in Borges were ranging between 5 and 20% (Fig. 4). However, no differences in mean RLB
401 incidence of trap plants ($P = 0.064$) between Borges (5.54 ± 5.60 %) and Córdoba ($26.55 \pm$

402 6.19%) were detected. Data collected in Borges in three consecutive years (2015 to 2017)
403 indicated that infections decreased drastically in June and only sporadic infections were
404 detected later, coinciding with daily mean T over 20 °C in this period (Fig. 4). In addition,
405 no differences in RLB incidence of trap plants ($P = 0.167$) were detected in Borges when
406 comparing all three monitored years.

407 The incubation periods estimated from the data recorded in 2015 to 2017 in Borges
408 were mostly between 6 and 10 weeks, but extreme values (from 2 to 12) were occasionally
409 recorded (Fig. 5). The duration of the incubation period tended to decrease from week 20 in
410 2016 (Fig. 5), but this trend was not observed in 2015 and 2017, since correlations were not
411 significant (*data not shown*).

412

413 **Discussion**

414 Some key aspects of the *P. amygdalinum* epidemiology have been studied from 2013
415 to 2017 in two almond-growing regions in Spain, namely Andalusia and Catalonia, which
416 included the potential primary inoculum development, and the incubation and infectivity
417 periods. Correlation analyses between biological and meteorological data contributed to a
418 better understanding of the pathogen life cycle on almond.

419 Previous data about the RLB epidemiology and strategies to control this disease at
420 worldwide level were based on studies conducted in Iran and Lebanon, which reported on
421 the production of ascospores, the disease infection period and the control of RLB using
422 fungicides (Ashkan and Assadi 1974; Banihashemi 1990; Bayt-Tork et al. 2014; Ghazanfari
423 and Banihashemi, 1976; Saad and Masannat 1997). In Spain, previous knowledge on the
424 RLB disease include some field observations about symptom incidence and severity

425 (Almacellas 2014; Ollero-Lara et al. 2016a; Ollero-Lara et al. 2016b), and cultivar
426 susceptibility (Ollero-Lara et al. 2019). Thus, the current work aimed to increase the
427 knowledge on the dynamics of major aspects of the RLB disease in our country, where
428 environmental conditions for almond-growing areas could be different from those of
429 previously studied cases in Iran and Lebanon.

430 In previous research conducted in Iran and Lebanon, a main period from April to May
431 was reported for the potential primary inoculum availability (Ashkan and Assadi 1974;
432 Banihashemi 1990; Saad and Masannat 1997), which coincides with that observed in
433 Córdoba and Borges in 2014 and 2015, respectively. However, an extended period of
434 ascospore availability, i.e. from February to August, was repeatedly observed in later
435 seasons in our study. This suggests a larger period where primary inoculum of RLB can be
436 present in Spain, thus increasing the potential risk of infections during favorable periods.
437 The amounts of ascospores recorded in this study cannot be compared with data on
438 ascospore counts reported by Banihashemi (1990) and Saad and Masannat (1997), since
439 methods in those latter studies were based on the quantification of captured airborne
440 ascospores. In our study, the ascospore extraction methods from leaves may have
441 overestimated the amounts of available ascospores, especially when extracting ascospores
442 by crushing. Fruiting bodies can be physically broken when leaves were crushed so that the
443 whole perithecia content is released to the medium and higher ascospore amounts can be
444 therefore recorded.

445 Banihashemi (1990) suggested that changing environmental conditions could
446 influence ascospore release and RLB infections. In this study we observed that total amount
447 of ascospores per season were correlated positively with environmental conditions of
448 previous fall and winter seasons (October to January), especially with variables related to

449 water availability and, to a lesser extent, to temperature. Thus, *P. amygdalinum* benefits
450 from the hydration of fallen leaves and T above 20°C during fall, mainly in October, to
451 produce higher inoculum potentials during the next season. However, Ghazanfari and
452 Banihashemi (1976) reported that *P. amygdalinum* requires T below 10°C in fall and winter
453 to favor ascocarp development in the next season, which is in contrast with our results.
454 Further research is therefore needed to clarify the influence of fall and winter
455 environmental conditions on the seasonal dynamics of the disease. However, correlations
456 must be treated with caution to avoid spurious associations (Fernández-Escobar et al.
457 2018). Geographical conditions may also play a major role in the primary inoculum
458 development, as confirmed by the sharp differences in annual potential inoculum amounts
459 observed in Andalusia and Catalonia. These results confirm the idea that RLB of almond
460 needs to be studied in each region where it is reported. Moreover, it is advisable to conduct
461 a multi-year monitoring of the primary inoculum since, as reported here, highly variable
462 ascospore amounts can be recorded among seasons.

463 The maturation of *P. amygdalinum* perithecia and ascospores was rather related to
464 seasonal weather conditions than to the geographical origin of samples. However, a
465 disparity between maturity of fruiting bodies and primary inoculum dynamics was detected
466 in some cases. Thus, perithecia reached maturation late in the season in 2016 in all
467 locations while free ascospores were detected from the first weeks of the year until the end
468 of the experiment. We hypothesize that this might have been due to the sample size of the
469 analyzed leaves, which could have been insufficient to adequately represent how fruiting
470 bodies developed in the leaf litter along the season. Gadoury et al. (1992) reported a
471 disparity between morphological maturity of ascospores and physiological maturity of asci
472 in the apple scab fungus, *Venturia inaequalis*, which could be comparable to our results.

473 The authors found that discharge of ascospores was recorded as early as asci were rated as
474 mature in approximately 10 to 15% of full maturity.

475 In our study, ascospores were able to germinate but failed to grow further, in
476 agreement with data reported by Habibi and Banihashemi (2015). However, germination
477 percentages after 24 h incubation were consistently low, well below 30% in most cases.
478 These low germination percentages could be related to the biotrophic nature of the
479 pathogen (Cannon 1996; Habibi and Banihashemi 2015) or even to unknown
480 environmental factors. Data on ascospore germination could be useful in a first stage for
481 testing fungicides *in vitro* against *P. amygdalinum*, as well as in the development of
482 mechanistic predictive models on RLB epidemiology.

483 The natural RLB infections observed in trap plants occurred between week 9 and 22
484 (February to May), despite the geographical location of almond orchards. Moreover, we
485 found T between 10 °C and 20 °C promoted infection by *P. amygdalinum*, particularly
486 when associated with wetness conditions. Rainfall and high RH could provide adequate
487 moisture for ascospore dispersal and subsequent infection. The importance of T and
488 moisture (hydrothermal variables) is well-characterized in many pathosystems (Agrios,
489 2005), and are generally known to explain plant disease development (Lowell et al. 2004),
490 such as in the *Plasmopara viticola*-grape (Rossi et al. 2007) and the *Venturia pirina*-pear
491 (Rossi et al. 2009) pathosystems. Moreover, infections in Andalusia and Catalonia declined
492 when T raised above 20 °C, thus suggesting that warmer temperatures were inhibiting
493 RLB infections. It is known that optimum temperature for ascospore germination and
494 appressorium formation among *Phyllachora* species is 10 °C to 20 °C (Banihashemi 1990;
495 Dittrich et al. 1991; Parbery 1963), and that T above 25 °C inhibits appressorium formation
496 (Habibi and Banihashemi 2015). These data would be compatible with fewer infections of

497 *P. amygdalinum* being detected in summer. Our results showed that the incubation period
498 mostly ranged between 5 and 10 weeks, but can be as long as 12 weeks in spring and as
499 short as 2 weeks in summer. These results differ clearly from those reported by Ashkan and
500 Assadi (1974), who estimated a narrower incubation period of 30 to 35 days, and those by
501 Banihashemi (1990), who reported a similar period (30 to 40 days).

502 Although *P. amygdalinum* has been considered as a biotrophic pathogen (Cannon,
503 1996), other plant pathogens with a similar multistage development as *P. amygdalinum* are
504 classified as hemibiotrophic pathogens, such as *Mycosphaerella graminicola* (Fuckel) J.
505 Schröt., *Pyricularia oryzae* Cavara, and *Colletotrichum* spp. (Marshall et al. 2011; Mentlak
506 et al. 2012; O'Connell et al. 2012). This hemibiotrophic lifestyle can be easily recognized
507 in *P. amygdalinum*: (i) a long initial biotrophic phase, with the pathogen spreading inside
508 living host cells without causing noticeable host cell damage in spring and summer, and (ii)
509 a short necrotrophic phase in which pathogen growth causes multiple host cell death and
510 the darkening of leaf stroma (Saad and Masannat 1997; Zúñiga et al. 2019). Lastly, *P.*
511 *amygdalinum* continues the necrotic phase on fallen leaves (from October to January),
512 which is previous to the final development and maturation of perithecia and ascospores.

513 In this work we have studied the primary inoculum dynamics, the development of the
514 fruiting bodies, the germination of ascospores as well as the natural infections of the RLB.
515 The results reported here can help in building a future prediction model, which would
516 integrate some key biological aspects of *P. amygdalinum* with the environmental conditions
517 met in each almond-growing area. Thus, predicting risk events for RLB infection could
518 help in taking more effective decisions on management programs and control strategies.

519 **Acknowledgments**

520 Research funded by the Instituto Nacional de Investigación y Tecnología Agraria y
521 Alimentaria (INIA), grants RTA2013-00004-C03-01 and RTA2017-00009-C04-01, and
522 with matching funds from the European Regional Development Fund (ERDF). E. Zúñiga
523 was supported by CONACYT (Mexico) with a predoctoral grant. J. Luque, L. Torguet and
524 X. Miarnau were partially supported by CERCA programme, Generalitat de Catalunya
525 (Spain). The authors thank F. Luque and A. López-Moral for their skillful technical
526 assistance

527

528 **Literature cited**

529 Agrios, G.N., 2005. Plant Pathology, 5th ed. Elsevier Academic Press, San Diego. 952 pp.
530 Almacellas, J., and Marín, J. P. 2011. Control de plagas y enfermedades en el cultivo del
531 almendro [in Spanish]. Vida Rural 334:68–74.
532 Almacellas, J. 2014. Síntomas, daños y métodos de control de la mancha ocre [in Spanish].
533 Vida Rural 389:28–32.
534 Arquero, O. 2013. Manual del almendro [in Spanish]. Junta de Andalucía-Consejería de
535 Agricultura, Pesca y Desarrollo Rural, Sevilla. 80 pp.
536 Ashkan M. and P. Assadi, 1974. Red blotch of almond (*Polystigma ochraceum*) in Iran.
537 Iranian J. Plant Pathol. 10:49–63.
538 Banihashemi, Z. 1990. Biology and control of *Polystigma ochraceum*, the cause of almond
539 red leaf blotch. Plant Pathol. 39:309–315.

540 Bayt-Tork, D., Taherian, M., and Divan, R. 2014. Evaluation of some fungicides for
541 controlling almond red leaf blotch (*Polystigma amygdalinum*). Int. J. Adv. Biol.
542 Biomed. Res. 2:1011–1016.

543 Buck, A. L. 1981. New equations for computing vapour pressure and enhancement factor.
544 J. Appl. Meteorol. 20:1527–1532

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

547 Christensen, R.H.B. 2018. Ordinal - Regression models for ordinal data. R package version
548 2018.8-25. URL: <http://www.cran.r-project.org/package=ordinal/>.

549 Dittrich, U., Hock, J., Kranz, J., and Renfro, B. 1991. Germination of *Phyllachora maydis*
550 ascospores and conidia of *Monographella maydis*. Cryptogamic Bot. 2:214–218.

551 FAOSTAT 2019. Food and Agriculture data. Retrieved January 11, 2019, from
552 <http://www.fao.org/faostat/en/#data>

553 Farr, D.F., and Rossman, A.Y. 2019. Fungal Databases, U.S. National Fungus Collections,
554 ARS, USDA. Retrieved January 11, 2019, from [https://nt.ars-](https://nt.ars-grin.gov/fungaldatabases/)
555 [grin.gov/fungaldatabases/](https://nt.ars-grin.gov/fungaldatabases/)

556 Fernández-Escobar, R., Trapero, A., and Domínguez, J. 2018. Experimentación en
557 Agricultura [in Spanish]. Consejería de Agricultura y Pesca, Junta de Andalucía,
558 Sevilla.

559 Gadoury, D. M., Seem, R. C., Rosenberger, D. A., Machardy, W. E., and Berkett, L. P.
560 1992. Disparity between morphological maturity of ascospores and physiological
561 maturity of asci in *Venturia inaequalis*. Plant Dis. 76:277–282.

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

564 González-Fragoso, R. 1927. Botánica Criptogámica Agrícola. Espasa-Calpe, Madrid,
565 Spain. 321 pp.

566 Habibi, A., and Banihashemi, Z. 2015. Ascospore germination and appressorium formation
567 in vitro of *Polystigma amygdalinum* and its survival period. Iranian J. Plant Pathol.
568 51:461–469.

569 Habibi, A., and Banihashemi, Z. 2016. Mating system and role of pycnidiospores in
570 biology of *Polystigma amygdalinum*, the causal agent of almond red leaf blotch.
571 Phytopathol. Mediterr. 55:98–108.

572 Habibi, A., Banihashemi, Z., and Mostowfizadeh-Ghalamfarsa, R., 2015. Phylogenetic
573 analysis of *Polystigma* and its relationship to *Phyllachorales*. Phytopathol. Mediterr.
574 54:45–54.

575 Lin, A., and Szteinberg, A. 1992. Control of the almond disease *Polystigma* by urea
576 treatments. Alon Hanotea 47:15–21.

577 Lowell, D.J., Powers, S.J., Welham, S.J., and Parker, S.R. 2004. A perspective on the
578 measurement of time in plant disease epidemiology. Plant Pathol. 53:705–712.

579 MAPA 2019. Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente.
580 Retrieved January 11, 2019, from
581 [http://www.mapama.gob.es/es/estadistica/temas/estadisticas-](http://www.mapama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/)
582 [agrarias/agricultura/superficies-producciones-anuales-cultivos/](http://www.mapama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/).

583 Marshall, R., Kombrink, A., Motteram, J., Loza-Reyes, E., Lucas, J., Hammond-Kosack, K.
584 E., Thomma, B. P. H. J., and Rudd, J. J. 2011. Analysis of two in planta expressed
585 LysM effector homologs from the fungus *Mycosphaerella graminicola* reveals
586 novel functional properties and varying contributions to virulence on wheat. Plant
587 Physiol. 156:756–769.

588 Mentlak, T.A., Kombrink, A., Shinya, T., Ryder, L.S., Otomo, I., Saitoh, H., Terauchi, R.,
589 Nishizawa, Y., Shibuya, N., Thomma, B. P. H. J., and Talbot, N. J. 2012. Effector-
590 mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is
591 necessary for rice blast disease. *Plant Cell*. 24:322–335.

592 Miarnau, X., Torguet, L., Zazurca, L., Maldonado, M., Girabet, R., Batlle, I., and Rovira,
593 M. 2018. El futuro del almendro en España: ¿Será posible producir 4.000 kg de
594 grano/ha? [in Spanish]. *Horticultura* 337:16–26.

595 Miarnau, X., Vargas, F.J., Montserrat, R., and Alegre, S. 2010. Aspectos importantes en las
596 nuevas plantaciones de almendro en regadío – Almendro [in Spanish]. *Revista de*
597 *Fruticultura – Especial Almendro*:94–103.

598 O’Connell, R. J., Thon, M. R., Hacquard, S., Amyotte, S. G., Kleemann, J., et al. 2012.
599 Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome
600 and transcriptome analyses. *Nat. Genet.* 44:1060–1065.

601 Ollero-Lara, A., López-Moral, A., Lovera, M., Raya, M. C., Roca, L. F., Arquero, O., and
602 Trapero, A. 2016a. Las enfermedades del almendro en Andalucía [in Spanish].
603 *Revista de Fruticultura* 49:166–183.

604 Ollero-Lara, A., Lovera, M., Roca, L. F., Arquero, O., and Trapero, A. 2016b.
605 Susceptibilidad varietal del almendro a la mancha ocre en Andalucía [in Spanish].
606 *Vida Rural* 412:14–22.

607 Ollero-Lara, A., Agustí-Brisach, C., Lovera, M., Roca, L. F., Arquero, O., and Trapero, A.
608 2019. Field susceptibility of almond cultivars to the four most common aerial fungal
609 diseases in southern Spain. *Crop Prot.* 121:18–27.

610 Parbery, D. 1963. Studies on graminicolous species of *Phyllachora* Fekl. I. Ascospores-
611 their liberation and germination. *Austral. J. Bot.* 11:117–130.

- 612 Rossi, V., Caffi, T., Bugiani, R., Spanna, F., and Dellavalle, D. 2007. Estimating the
613 germination dynamics of *Plasmopara viticola* oospores using the hydro-thermal
614 time. *Plant Pathol.* 57:216–226.
- 615 Rossi, V., Salinari, F., Patteri, E., Giosuè, S., and Bugiani, R. 2009. Predicting the
616 dynamics of ascospore maturation of *Venturia pirina* based on environmental
617 factors. *Phytopathology* 99:453–461.
- 618 Saad, A. T., and Masannat, K. 1997. Economic importance and cycle of *Polystigma*
619 *ochraceum*, causing red leaf blotch disease of almond in Lebanon. *OEPP/EPPO*
620 *Bull.* 27:481–485.
- 621 Shabi, E. 1997. Disease management of the almond pathogens *Glomerella cingulata*,
622 *Polystigma ochraceum* and *Tranzschelia pruni-spinosae*. *OEPP/EPPO Bull.*
623 27:479–480.
- 624 Suzuki, Y., Hatakeyama, S., Harada, Y., and Tanaka, K. 2008. *Polystigma fulvum*, a red
625 leaf blotch pathogen on leaves of *Prunus* spp., has the *Polystigmia pallescens*
626 anamorph/andromorph. *Mycoscience* 49:395–398.
- 627 Torguet, L., Batlle, I., Alegre, S., and Miarnau, X. 2016. Nuevas plagas y enfermedades
628 emergentes, una amenaza para el cultivo del almendro en España [in Spanish].
629 *Revista de Fruticultura* 49:152–165.
- 630 Toscano-Underwood C., Huang, Y. J., Fitt, B. D. L., and Hall, A.M. 2003. Effects of
631 temperature on maturation of pseudothecia of *Leptosphaeria maculans* and *L.*
632 *biglobosa* on oilseed rapeseed debris. *Plant Pathol.* 52:726–736.
- 633 Vargas, F. J., Romero, M., Batlle, I., Rovira, M., Gispert, J. R., Romero, A., Alegre, S., and
634 Miarnau, X. 2010. El programa de mejora de variedad de almendro del IRTA [in
635 Spanish]. *Revista de Fruticultura – Especial Almendro*:10–23.

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

639 **Figure captions**

640 **Fig. 1.** Dynamics of *Polystigma amygdalinum* ascospores extracted from infected almond
641 leaves during a 3-year monitoring period conducted in two almond-growing regions in
642 Spain. Specific locations and monitoring periods: A) Córdoba (2014-16), B) Gandesa
643 (2015-17), Les Borges Blanques (2015-17), and D) leaves taken from Gandesa to Les
644 Borges Blanques (2015-17)

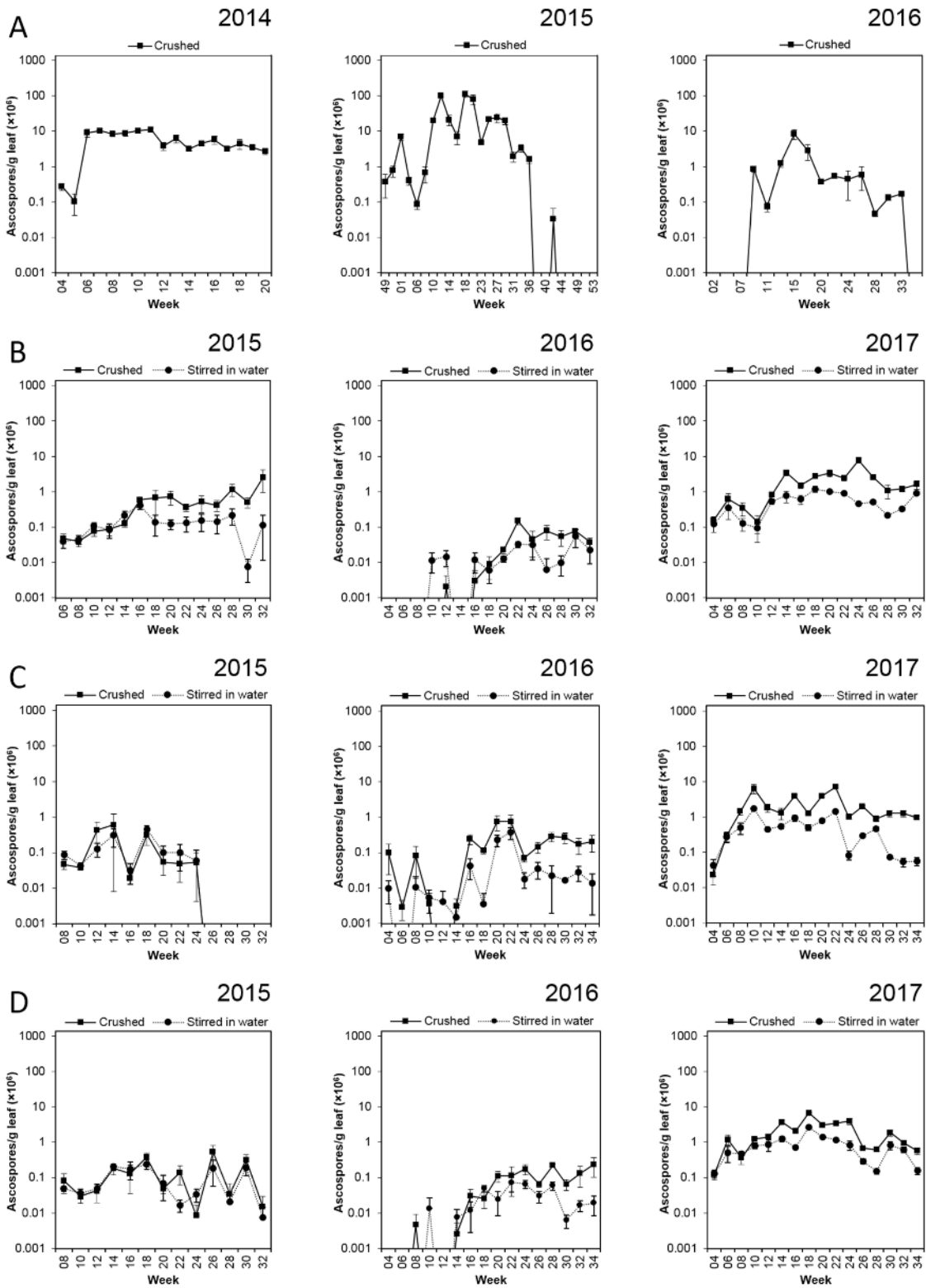
645 **Fig. 2.** Development of *Polystigma amygdalinum* fruiting bodies on almond leaves in two
646 consecutive years and for three sample origins (Gandesa, Les Borges Blanques and
647 Borges/Gandesa). Results are shown in a gray scale as percentages of fruiting bodies (N =
648 20) for each sampling period. Abbreviations: (P) Pycnidia, no perithecia present; (A)
649 Differentiated immature perithecium, with undifferentiated asci and ascospores; (B)
650 Immature perithecium with differentiated asci and undifferentiated ascospores; (C)
651 Immature perithecium and differentiated asci with < 8 ascospores/ascus; (D) Mature
652 perithecium and asci with 8 ascospores/ascus; (E) Empty perithecium, without asci and
653 ascospores

654 **Fig. 3.** Germination percentages of *Polystigma amygdalinum* ascospores (N = 50) recorded
655 from three leaf sample origins (Gandesa, Les Borges Blanques and Borges/Gandesa).

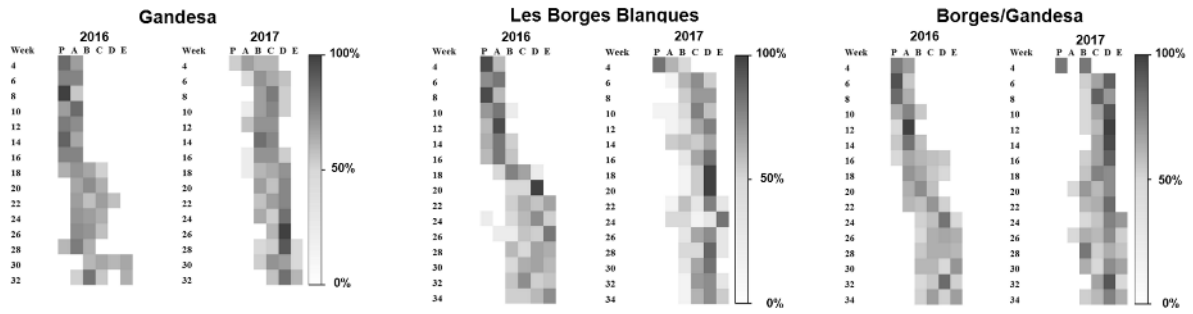
656 **Fig. 4.** Red leaf blotch incidence (%) in ‘Tarraco’ susceptible almond trees exposed to
657 natural infection periods in Córdoba (2016) and Les Borges Blanques (2015–17). Mean
658 temperatures shown in the secondary (right) axe

659 **Fig. 5.** Incubation periods of almond red leaf blotch in ‘Tarraco’ susceptible almond trees
660 exposed to natural infections in Les Borges Blanques (2015–17)

661



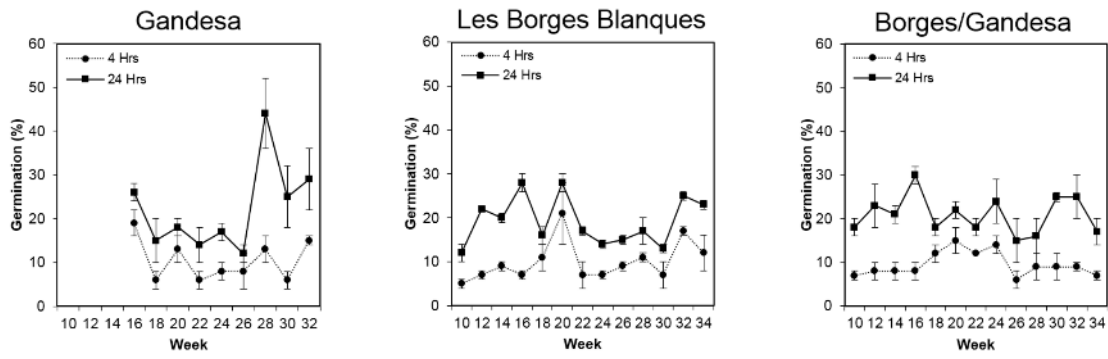
664 **Fig. 2.**



665

666

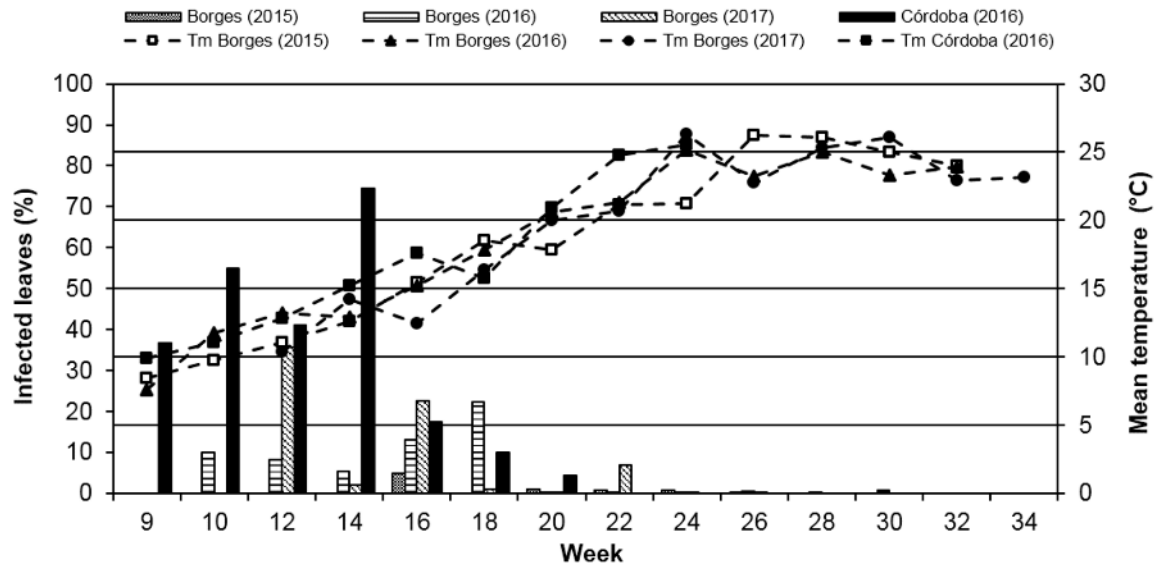
667 **Fig. 3.**



668

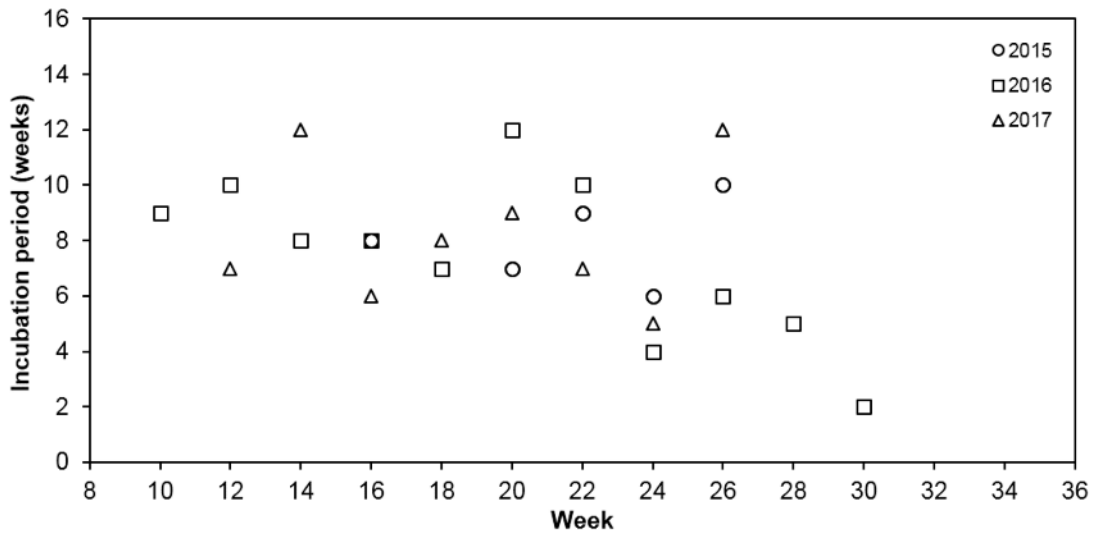
669

670 **Fig. 4.**



671

672 **Fig. 5.**



673