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Airborne inoculum dynamics of *Polystigma amygdalinum* and progression of almond red leaf blotch disease in Catalonia, NE Spain

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Abstract

The dynamics of airborne ascospores and disease progress of red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, and their correlations with weather variables were studied from 2019 to 2021 in two almond orchards located in Lleida, NE Spain. Airborne ascospores were detected and quantified by real-time qPCR using species-specific primers for *P. amygdalinum*. Ascospores were detected mainly from April to June, with a high variability between the yearly cumulative concentrations. Positive significant correlations were found between the weekly proportion of airborne ascospores and the number of wet and mild days—either combined or separated—accumulated rainfall, number of rainy days, accumulated low temperatures on wet days, and mean and maximum relative humidity. In contrast, several thermal variables (maximum temperature, VPD, and number of warm days) were negatively correlated with ascospore catches. Positive significant correlations were found between the cumulative proportion of ascospores and RLB incidence and severity. Weekly variations in RLB incidence and severity showed significant positive correlations with the number of warm days while negative with the number of mild days. Severity was also positively correlated with several thermal variables (mean, maximum, and minimum temperature, and VPD), and negatively correlated with the number of cold days and wet and mild days. Stronger correlations were generally found with ascospore catches or disease progress when using concurrent weekly weather data. Gompertz, monomolecular, and logistic growth models were evaluated to describe RLB disease progress.

KEYWORDS
almond, disease progress, epidemiology, *Polystigma amygdalinum*, *Prunus dulcis*, quantitative PCR, red leaf blotch disease

1 | INTRODUCTION

Red leaf blotch (RLB), caused by the fungus *Polystigma amygdalinum* P.F. Cannon, is one of the most important foliar diseases affecting almond trees (*Prunus dulcis* (Mill.) D.A. Webb) in the Mediterranean Basin and Middle East regions (Cannon, 1996; Farr & Rossman, 2022). The disease is endemic in these regions and so far not known in other almond-growing areas, such as the United States or Australia (Farr &

Rossman, 2022). RLB is widespread in Spain (Tuset & Portilla, 1987) and its incidence has worryingly increased in recent years (Miarnau et al., 2021). This increase has been associated to the expansion of almond-growing areas to inland regions with climatic conditions more suitable for the disease development, the change towards intensive plantations with irrigation and high tree densities, and the use of some late-flowering cultivars (especially 'Guara') that are more susceptible to RLB than traditional ones (Almacellas, 2014; Miarnau et al., 2021; Ollero-Lara et al., 2019).

Polystigma amygdalinum is a biotrophic ascomycete specific to almond, which was firstly described in 1843 in France as *Septoria rubra* var. *amygdali* Desm. from almond leaves (Desmazières, 1843). The fungus was reclassified within the genus *Polystigma* and named for decades as *P. ochraceum* (Fr.) Sacc. (=*P. fulvum* (Pers.) DC.), until its identity and currently valid name were established by Cannon (1996) as *P. amygdalinum*. This species differs from other *Polystigma* species due to its host specificity, and stromal and ascospore morphological characteristics (Cannon, 1996).

First RLB symptoms usually appear in spring as pale yellowish spots on both leaf sides, turning into orange-reddish and finally becoming dark brown. Spots size increases with time and may cover almost the whole leaf surface in late summer. In the case of severe infections, early defoliation can be observed in summer (Cannon, 1996; Habibi & Banihashemi, 2016; Shabi, 1997), with a subsequent decrease in tree photosynthetic activity and a reduction in yield in following seasons (López-López et al., 2016). The RLB disease is monocyclic and the only inoculum sources are the perithecia located in the stromata of fallen overwintered leaves (Banihashemi, 1990; Ghazanfari & Banihashemi, 1976). The sexual stage is developed in winter and ascospores are released and air-spread mainly in spring to eventually infect new almond leaves under suitable weather conditions. Following infections, leaf spots appear after a relatively long incubation period of 30–40 days (Banihashemi, 1990; Saad & Masannat, 1997), and eventually up to 10 weeks (Zúñiga et al., 2020). The occurrence of secondary infections has not been confirmed since conidia—mostly produced in summer—are not infective and presumedly act as spermatia in the sexual reproduction of *P. amygdalinum* (Habibi & Banihashemi, 2016; Saad & Masannat, 1997).

RLB control is based on three main strategies: cultural practices to reduce the inoculum source, the use of tolerant cultivars, and the application of fungicides. The most applied cultural practices are to remove or burn the leaf litter in autumn or winter, or to favour its decomposition through urea applications (Almacellas, 2014; Cannon, 1996; López-Moral et al., 2022). Regarding RLB control with fungicides, in-season applications from petal fall to the end of summer have been proved to reduce infections, while evaluations on products efficacy have been conducted mostly in Iran (Banihashemi, 1990; Bayt Tork et al., 2014) and Spain (Torguet et al., 2022).

Research on the biology of *P. amygdalinum* and the RLB epidemiology has been conducted since the early 1970 decade in several countries including Iran, Israel, Lebanon, and Spain, among others. The main research topics included the study of the ascocarp development and maturation (Banihashemi, 1990; Ghazanfari & Banihashemi, 1976; Saad & Masannat, 1997; Zúñiga et al., 2020), ascospore germination (Banihashemi, 1990; Habibi & Banihashemi, 2015; Zúñiga et al., 2020) and release (Banihashemi, 1990; Saad & Masannat, 1997; Sahragard et al., 2007), the subsequent infection and latency incubation periods (Banihashemi, 1990; Sahragard et al., 2007; Zúñiga et al., 2020), and the overall disease progress in different almond cultivars (Miarnau et al., 2021). Most of these works were conducted under field conditions and explored the relationships between weather variables and the pathogen-related variables under study.

Regarding the inoculum dynamics of *P. amygdalinum*, ascospore production and release have been studied in Iran (Banihashemi, 1990; Ghazanfari & Banihashemi, 1976; Sahragard et al., 2007) and Lebanon (Saad & Masannat, 1997). According to Ghazanfari and Banihashemi (1976), perithecia maturation start at around 10°C in winter. Banihashemi (1990) showed that ascospore release began at blooming (early March in the studied cultivar) and continued for 4–5 weeks, reaching the maximum at petal fall. In Lebanon, ascospore release occurs between February and mid-May (Saad & Masannat, 1997). Little information is available on *P. amygdalinum* ascospore production and release in Spain. Zúñiga et al. (2020) reported on the seasonal evolution of ascocarp maturation and production of ascospores, but no information on ascospore release and dispersal is available. Therefore, quantitative information on the dynamics of the airborne inoculum of *P. amygdalinum* remains to be investigated.

Thus, the main objectives of this study were: (i) to describe the dynamics of *P. amygdalinum* airborne ascospores over a 3-year period in NE Spain using spore traps coupled with qPCR-based methods, (ii) to explore the correlations between ascospore catches and weather variables, (iii) to model the RLB disease progress over time, and (iv) to explore the correlations of disease progress with weather variables and ascospore catches.

2 | MATERIALS AND METHODS

2.1 | Experimental orchards

Experiments were conducted from 2019 to 2021 in two almond orchards located in Lleida region, NE Spain: Les Borges Blanques (Borges hereafter; UTM coordinates: WGS84 Datum, 31T $x = 320{,}870$, $y = 4{,}597{,}530$), and Vilagrassa ($x = 341{,}313$, $y = 4{,}612{,}125$). The orchard at Borges is an experimental plot planted in 2009 as bare root trees with 21 cultivars grafted onto 'INRA GF-677' rootstock, pruned as a central axis, and with a tree spacing of 4 m × 2 m. The orchard at Vilagrassa is a commercial plot of 'Tarraco', a late-blooming cultivar (Vargas et al., 2008), grafted onto 'INRA GF 677' rootstock, planted in 2007, pruned as open-vase, and with a tree spacing of 7 m × 6 m. In both orchards, RLB disease occurs naturally (Miarnau et al., 2021). Orchards were 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 in either of the two orchards.

2.2 | Airborne inoculum dynamics

A Hirst 7-day recording volumetric spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) was placed in a central position in each orchard, under 'Tarraco' trees, a highly susceptible cultivar to RLB (Miarnau et al., 2021). Spore traps operated with an airflow of 10 L min^{-1} from mid-February (weeks 7–8) to mid-September (weeks 36–37) 2019–2021. This experimental period corresponds, in the case of 'Tarraco' cultivar, from BBCH phenological stage 51–81 (Sakar et al., 2019).

Airborne particles were captured on a Melinex® 200 gauge (TEKRA, New Berlin, WI, USA) clear plastic tape, rotating at 2 mm h^{-1}, which was previously coated with a layer of silicone solution (Lanzoni, Bologna, Italy). Tapes were replaced weekly and cut into 1-day sections to estimate the daily airborne ascospore concentration of *P. amygdalinum*, according to the molecular methods described by Zúñiga et al. (2018). Total DNA was extracted from each daily tape section using the E.Z.N.A® Plant DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Next, a real-time quantitative PCR (qPCR) in a Stepone™ Real-Time PCR System thermal cycler (Life Technologies, Carlsbad, CA, USA) was conducted, using the *P. amygdalinum* specific primer pair PamyI2F4/PamyI2R2 and the optimized reaction conditions described by Zúñiga et al. (2018). Three technical replicates for each biological sample were analysed, and a sterile HPLC-quality water template was used as a negative control. The ascospore quantification was done by fitting the obtained C_q values to a standard curve formula (with intercept = 34.26 and slope = 3.349), obtained previously by Zúñiga et al. (2018) from a dilution series of a *P. amygdalinum* ascospore suspension placed on plastic tapes. Daily concentration of ascospores was then calculated considering the daily air volume sunk by the spore trap and expressed as ascospores m^{-3} day^{-1}. For the statistical analysis, we calculated the weekly proportion (0–1) of ascospores based on the cumulative concentration of ascospores caught by the spore traps in each orchard and year.

2.3 | Disease progress

Disease symptom progression was evaluated on three 'Tarraco' trees located nearby the spore trap in each orchard. RLB incidence and severity were evaluated on a weekly basis, from March to September 2021, corresponding from BBCH phenological stage 59 to 81 in the studied cultivar. On each tree, 50 fully expanded leaves were arbitrarily selected from new shoots, at different heights and orientations on the outer canopy, and visually assessed. Disease incidence and severity were evaluated according to procedures described by Miarnau et al. (2021). RLB incidence was recorded as the percentage of leaves showing at least one identifiable RLB lesion regardless of its size, whereas RLB severity was estimated from the mean percentage of affected leaf surface, by classifying leaves into the following category classes: class 0 (0% affected leaf surface), 1 (1%–10%), 2 (11%–20%), 3 (21%–50%), and 4 (>50%).

2.4 | Weather data

Daily weather data (accumulated rainfall, mean and maximum relative humidity, and mean, minimum, and maximum temperature) were monitored during the experimental period in both orchards by automatic weather stations belonging to the Meteorological Service of Catalonia (MeteoCat; https://ruralcat.gencat.cat/web/guest/agrometeo.estacions). The meteorological station at Borges was located about 250 m away from the orchard. The nearest weather station to the Vilagrassa orchard was located about 6 km away, in Tàrrega (x = 347,015, y = 4,614,430). Additional derived variables were calculated, coming to a total of 14 weather variables (Table 1). These variables were summarized on a weekly basis (Table S1).

2.5 | Data analysis

The correlations of current and past weather conditions with the ascospore catches of *P. amygdalinum* in the study period 2019–2021 were evaluated. Spearman's correlation coefficients (ρ) were calculated between the weekly proportion of ascospores caught by the spore trap and the values of the 14 weather variables for the same concurrent week, as well as for 1 and 2 weeks before. Spearman's correlation coefficients were also calculated between these weather variables and the weekly increase in RLB incidence and severity in 2021, as well as between RLB incidence and severity levels and the cumulative proportion of ascospores. Additionally, Spearman's correlation coefficients were calculated among weather variables

TABLE 1 Name and description of the studied weather variables.

Variable	Description
Temp.mean	Mean daily temperature (°C)
Temp.max	Mean maximum daily temperature (°C)
Temp.min	Mean minimum daily temperature (°C)
Rh.mean	Mean daily relative humidity (%)
Rh.max	Mean maximum daily relative humidity (%)
Rain	Accumulated rainfall (mm)
Rain.days	Number of rainy days (with *Rain* ≥0.2 mm)
VPD	Accumulated vapour pressure deficit (hPa), calculated as described by Rossi et al. (2009): $VPD = (1 - Rh.mean/100) \times 6.11 \times \exp[(17.47 \times Temp.mean)/(239 + Temp.mean)]$
Wet.days	Number of wet days (with *VPD* ≤0.4 hPa or *Rain* ≥0.2 mm)
Low.temp.wet	Accumulated low temperatures in wet days, measured as the sum of (50 – *Temp.mean*) only in wet days
Cold.days	Number of cold days (with *Temp.mean* < 10°C)
Mild.days	Number of mild days (with *Temp.mean* ≥ 10°C and < 20°C)
Warm.days	Number of warm days (with *Temp.mean* ≥ 20°C)
Wet.mild.days	Number of days both wet and mild

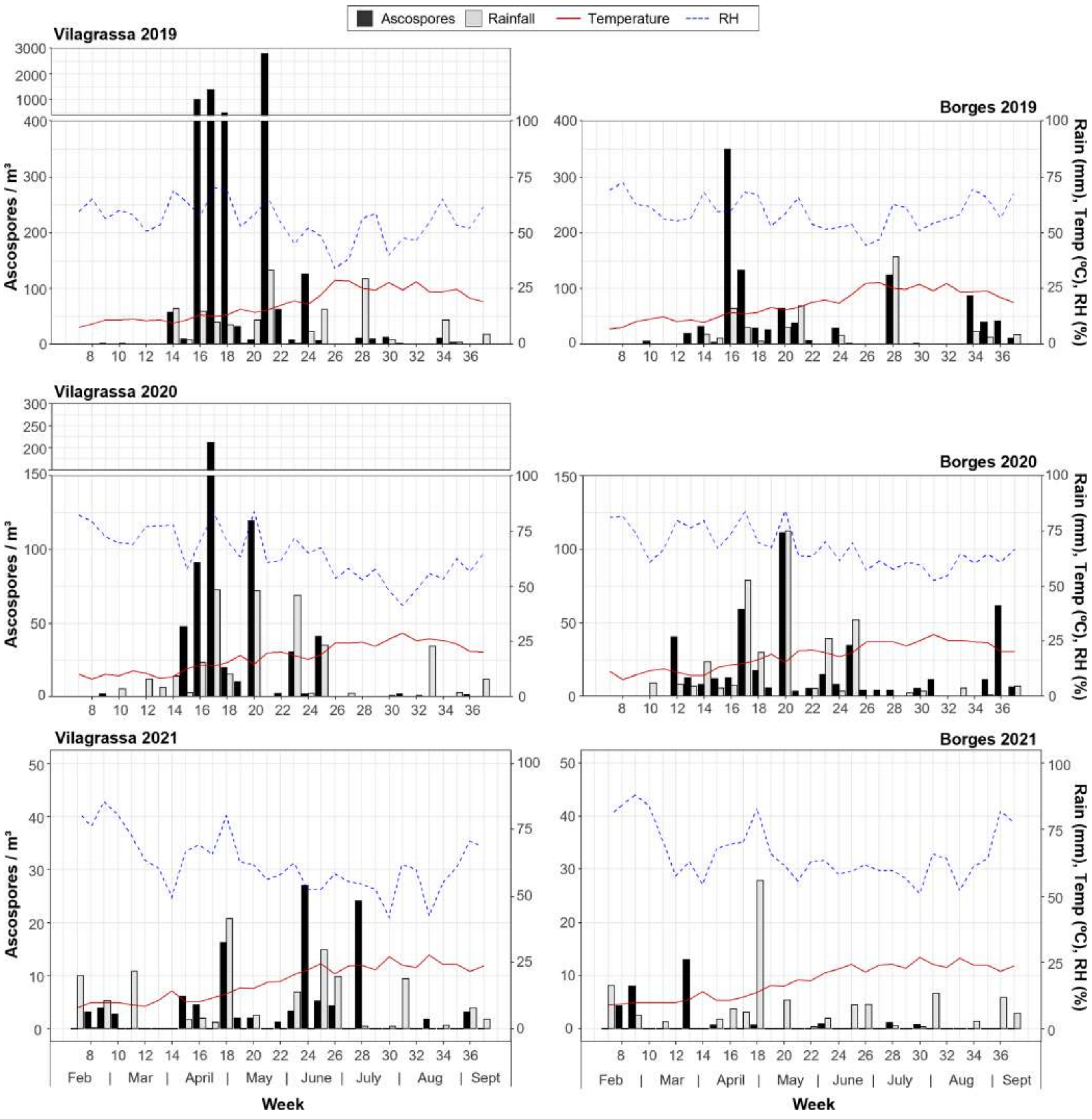

FIGURE 1 Weekly quantifications of airborne ascospores of *Polystigma amygdalinum* (black bars) in Vilagrassa and Borges orchards from 2019 to 2021. Grey bars, solid lines, and dashed lines depict the weekly accumulated rainfall, mean temperature, and mean relative humidity, respectively.

(Figure S1). All correlation analyses were implemented using the function *rcorr* of the 'Hmisc' package in the R software version 4.0.2 (R Core Team, 2020).

Disease growth models (namely, Gompertz, monomolecular, and logistic) were fitted separately for each orchard using nonlinear regression for RLB incidence mean data against days after disease onset. Models' equations were $y_t = K \times \exp[-y_0 \times \exp(-r \times t)]$ for Gompertz, $y_t = K[1 - y_0 \times \exp(-r \times t)]$ for monomolecular, and $y_t = K/[1 + y_0 \times \exp(-r \times t)]$ for logistic, in which y_t is disease incidence at a given time, y_0 is initial disease incidence, r is disease increase rate, K is disease maximum incidence, and t is time in days. To check models' goodness-of-fit, the pseudo-coefficient of determination (pseudo-R^2), the residual standard error (RSE), and Lin's concordance correlation coefficient (CCC) (Lin, 1989) were calculated. All analyses were implemented using the function *fit_nlin2* of the R package 'epifitter' (Alves & Del Ponte, 2021).

3 | RESULTS

3.1 | Airborne inoculum dynamics

We detected and quantified *P. amygdalinum* airborne ascospores in both orchards for the three monitored years (Figure 1). The earliest seasonal detections were on 17 February 2021 in both orchards. The latest seasonal detections were on 15 September 2019 and 3 September 2021 for Borges and Vilagrassa, respectively. In Borges, the yearly cumulative concentrations of ascospores in 2019, 2020, and 2021 were 1042.5, 505.9, and 29.5 ascospores m^{-3}, respectively; while in Vilagrassa were 6043.8, 580.5, and 110.5 ascospores m^{-3}. Considering the weekly cumulative proportion of ascospores (Figure 2), in Vilagrassa most ascospores (>75%) were collected between weeks 20 and 28, that is, from May to July, in the monitored years. In Borges, the period of major ascospore catches was more extended than in Vilagrassa, and the 75% of ascospore catches was reached between weeks 28 and 35 (from July to August) in 2019 and 2020. However, in Borges in 2021, 85% of ascospores were already caught in week 13, corresponding to the end of March.

In Borges, mean weekly temperatures of the experimental period each year ranged from 7.5 to 27.5°C in 2019, from 7.7 to 27.9°C in 2020, and from 9.2 to 26.8°C in 2021; mean weekly relative humidity ranged from 44.3% to 72.6% in 2019, from 52.4% to 84.3% in 2020, and from 50.9% to 87.9% in 2021; and accumulated rainfall in the experimental period was 110.3 mm in 2019, 263.9 mm in 2020, and 143.8 mm in 2021. In Vilagrassa, mean weekly temperatures of the experimental period each year ranged from 9.0 to 28.7°C in 2019, from 7.9 to 29.0°C in 2020, and from 8.3 to 27.6°C in 2021; mean weekly relative humidity ranged from 34.0% to 70.3% in 2019, from 41.6% to 83.6% in 2020, and from 42.0% to 85.7% in 2021; and accumulated rainfall in the experimental period was 162.7 mm in 2019, 372.3 mm in 2020, and 183.6 mm in 2021 (Table S1).

We generally found stronger correlations with the weekly proportion of ascospores when considering the concurrent weather data, as compared to those involving past weather data from 1 or 2 weeks before (Figure 3a and Table S2). We observed that ascospore catches were significantly correlated ($p < .05$) with 11 of the 14 studied weather variables with concurrent data. Positive significant correlations were found between the weekly proportion of airborne ascospores and the number of wet and mild days (either combined or separated), the accumulated rainfall, the number of rainy days, accumulated low temperatures on wet days, and the relative humidity (mean and maximum), in decreasing order of strength ($0.46 \geq \rho(df = 172) \geq 0.26$, all $p < .01$). Negative significant correlations were found for accumulated vapour pressure deficit (VPD), the number of warm days, and maximum temperature, in decreasing order of strength ($-0.24 \leq \rho(172) \leq -0.17$, all $p < .05$). Several thermal variables (mean, maximum, and minimum temperature, VPD, and number of warm days) were highly positively intercorrelated (Figure S1).

3.2 | Disease progress

First RLB symptoms in 2021 were observed on 2 June (week 23, Figure 4) in both orchards. The main increase in disease incidence was observed between weeks 25 and 29 (mid-June to mid-July, BBCH phenological stage 81). RLB incidence and severity reached higher values in Vilagrassa, with maximum values of 95% and 46% respectively, than in Borges, with maximum values of 83% and 24%. In both orchards, we occasionally registered a decrease in RLB incidence and severity from mid-July on. Positive significant

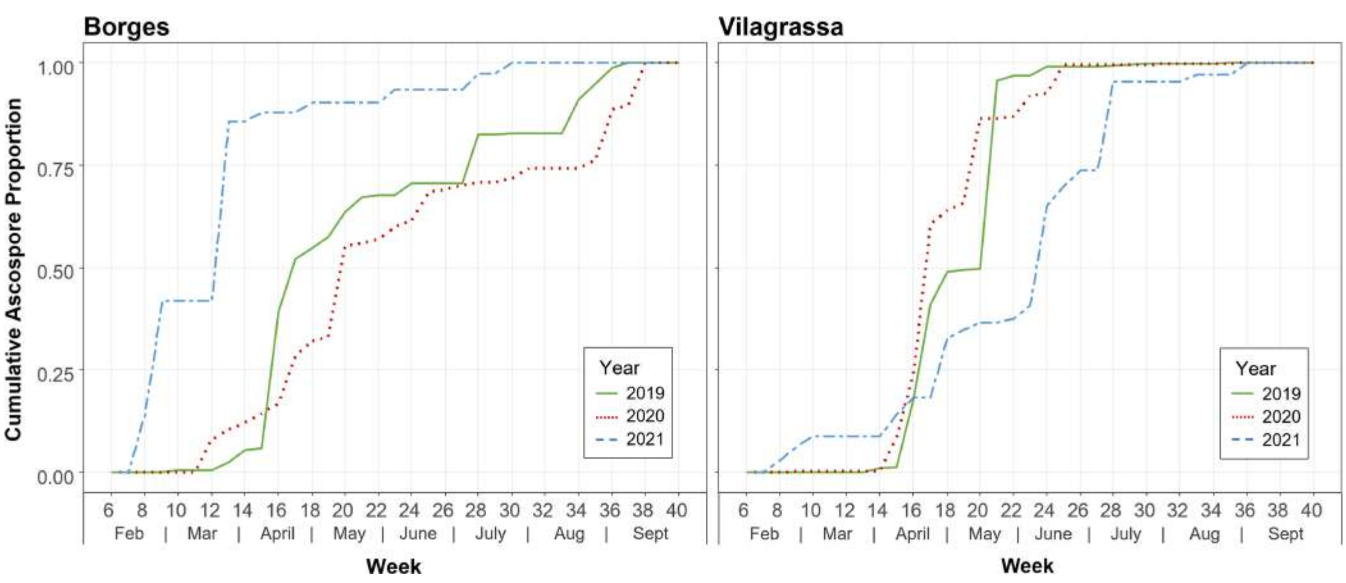

FIGURE 2 Weekly cumulative proportion of *Polystigma amygdalinum* airborne ascospores in Borges and Vilagrassa orchards within the season's growth periods of 2019–2021.

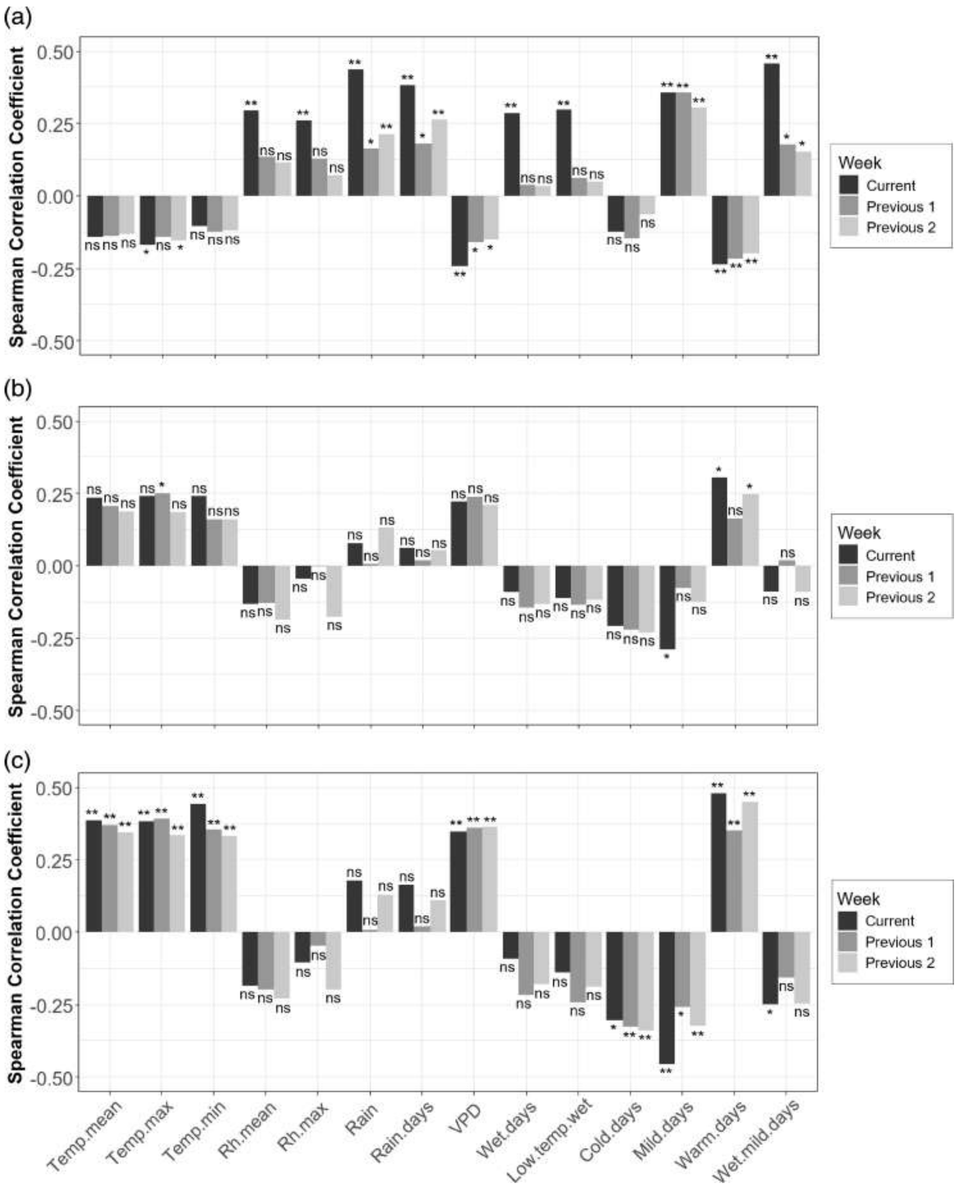

FIGURE 3 Spearman's rank correlation coefficients between the values of 14 weather variables for the current week, 1 week before (Previous 1), and 2 weeks before (Previous 2) and (a) the weekly proportion of *Polystigma amygdalinum* ascospores caught within season's growth periods of 2019–2021, (b) the weekly increase in the red leaf blotch incidence, and (c) severity in 2021. ** and * indicate significance at $p < .01$ and 0.05, respectively; *ns* indicates no significance.

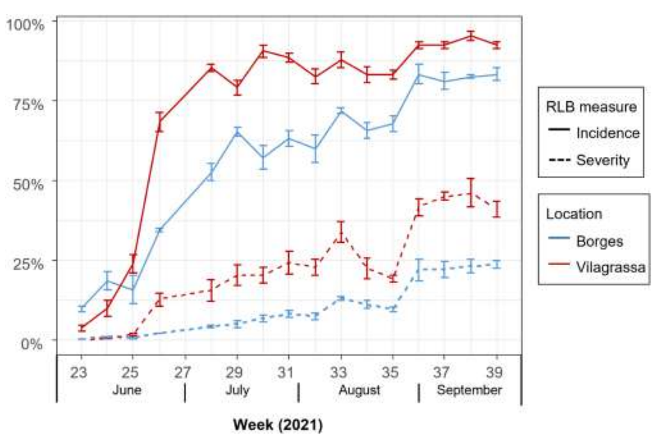

FIGURE 4 Seasonal disease progress of the almond red leaf blotch from June (detection of the first symptoms) to September 2021 in Borges and Vilagrassa, expressed as the mean incidence and severity values (±standard errors).

correlations were found between the cumulative proportion of ascospores, and RLB incidence and severity ($\rho(62) = 0.81$ and $\rho(62) = 0.82$, respectively, both $p < .01$).

Regarding the association between either current and past weather conditions and the weekly increase in RLB incidence (Figure 3b and Table S2) or severity (Figure 3c, Table S2), we generally found stronger correlations when using the concurrent weather data in the calculations. We observed that the weekly increase in RLB incidence and severity showed significant positive correlations with the number of warm days ($\rho(62) = 0.31$ and $\rho(62) = 0.48$, respectively, all $p < .05$), and negative correlations with the number of mild days ($\rho(62) = -0.29$ and $\rho(62) = -0.46$, respectively, all $p < .05$). Significant correlations were stronger for the increase in RLB severity than for RLB incidence. Additionally, the weekly increase in RLB severity was positively correlated with temperature (minimum, mean, and maximum) and accumulated VPD, in decreasing order of strength ($0.44 \geq \rho(62) \geq 0.35$, all $p < .01$), being all

TABLE 2 Parameter values and goodness-of-fit indexes of the models used to describe the progress of the red leaf blotch disease incidence in Borges and Vilagrassa almond orchards in 2021.

Orchard	Models	Estimated parameters[a]			Goodness of fit[b]		
		y_0	r	K	CCC	pseudo-R^2	RSE
Borges	Gompertz	0.105 (0.034)	0.038 (0.007)	0.825 (0.046)	0.972	0.946	0.061
	Monomolecular	0.071 (0.045)	0.018 (0.005)	0.938 (0.096)	0.974	0.948	0.060
	Logistic	0.205 (0.044)	0.031 (0.008)	1 (0.152)	0.945	0.899	0.085
Vilagrassa	Gompertz	0.000 (0.000)	0.217 (0.049)	0.881 (0.016)	0.987	0.977	0.055
	Monomolecular	−0.061 (0.080)	0.047 (0.009)	0.930 (0.040)	0.961	0.925	0.090
	Logistic	0.096 (0.054)	0.107 (0.030)	1 (0.042)	0.939	0.931	0.120

[a] y_0: initial disease incidence; r: rate of disease increase; K: maximum disease incidence. Standard errors of the estimated parameters are in parentheses.
[b] CCC: Lin's concordance correlation coefficient; pseudo-R^2: pseudo-coefficient of determination; RSE: residual standard error.

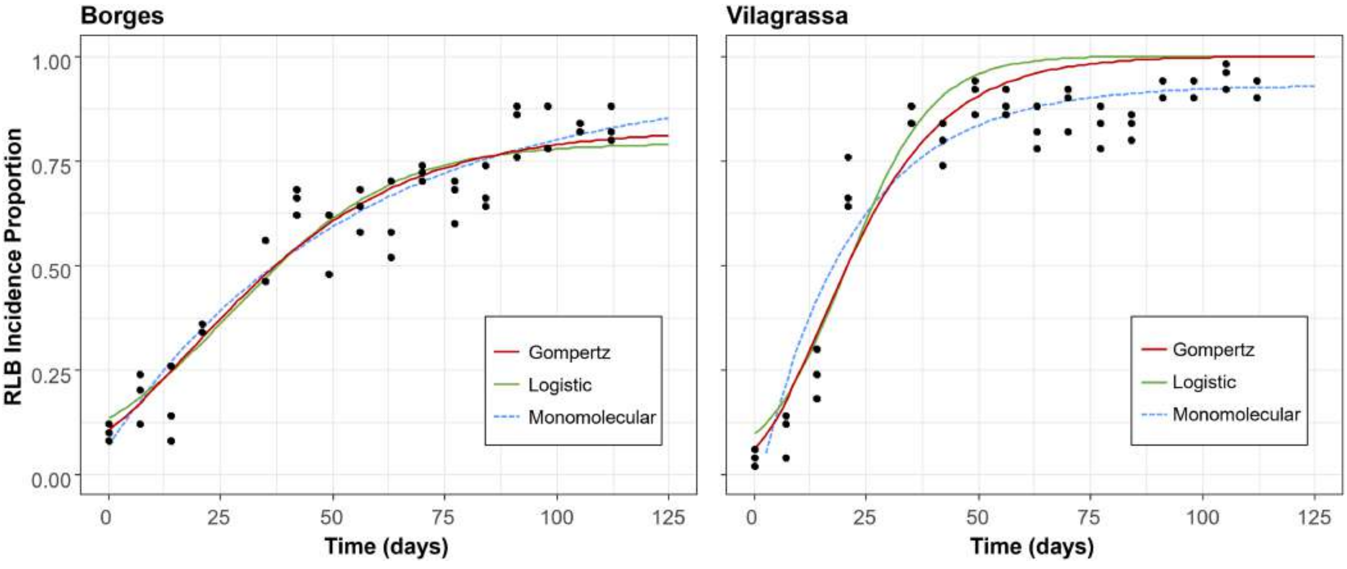

FIGURE 5 Gompertz, logistic and monomolecular curves (lines) fitted to the red leaf blotch disease progress data (dots) after disease onset in Borges and Vilagrassa in 2021.

these thermal variables highly intercorrelated (Figure S1); and negatively correlated with the number of cold days and the number of wet and mild days ($\rho(62) = -0.31$ and $\rho(62) = -0.25$, respectively, both $p < .05$).

Regarding disease growth models, parameter values and model fit statistics are shown in Table 2, whereas disease progress and equation fitting are plotted in Figure 5. We found that Gompertz, monomolecular, and logistic growth models presented high goodness-of-fit when describing disease progress in both orchards, with Lin's concordance correlation coefficients (CCC) higher than 0.94, residual standard errors (RSE) lower than 0.12, and pseudo-R^2 higher than 0.90 in all cases (Table 2). In these models, growth epidemic rate (r) was higher in Vilagrassa ($0.05 \le r \le 0.22$) than in Borges ($0.02 \le r \le 0.04$), while initial disease incidence (y_0) was lower in Vilagrassa than in Borges ($-0.06 \le y_0 \le 0.10$, as compared to $0.07 \le y_0 \le 0.21$, respectively) (Table 2, Figure 5).

4 | DISCUSSION

We have studied the dynamics of airborne ascospores of *P. amygdalinum* and the red leaf blotch disease progression in northeastern Spain. To the best of our knowledge, this is the first time that the specific qPCR protocol developed by Zúñiga et al. (2018) to detect and quantify *P. amygdalinum* ascospores is implemented in a field study using volumetric air samplers. This molecular method offers advantages over the traditional microscopic morphological quantification of airborne plant pathogens, including shorter processing times and higher specificity and sensitivity for fungal identification and quantification (Dung et al., 2018; González-Domínguez et al., 2020; Marimon et al., 2020).

Previous data about *P. amygdalinum* inoculum development in Spain showed that ascospores were potentially available from January to August (Zúñiga et al., 2020). Consistent with this finding, our results showed that ascospores of *P. amygdalinum* can be released, depending on the location and the year, from February to mid-September. In other studies conducted in Iran and Lebanon, where ascospores were identified and counted by microscopy techniques, a main period from March to April was reported for ascospore release (Banihashemi, 1990; Saad & Masannat, 1997). Our results describe a wider period of airborne ascospore detection, which could be explained by the different geographical conditions but also by the more sensitive qPCR-based methods used in our study. Most of the ascospores caught in our study (>75%) were already detected from May to July in Vilagrassa, and from July to August in Borges, which in both cases is later than reported in Iran (Banihashemi, 1990; Saad & Masannat, 1997). The wider period of ascospore detection in Borges, compared to Vilagrassa, could be due to the higher mean relative humidity recorded between weeks 27 and 35, corresponding to July and August, in the period 2019–2021 (about 10%–15% higher than in Vilagrassa), that could have extended the period for inoculum depletion to the end of summer. Nevertheless, most of the ascospores in Borges in 2021 were detected before mid-April, which is still consistent with previous findings (Banihashemi, 1990; Saad & Masannat, 1997).

High variability was observed between the yearly cumulative concentration of ascospores across the studied orchards and years, with decreasing values from 2019 to 2021. This could be due to multiple factors, including the RLB incidence in preceding years, the amount of fallen infected leaves from the previous season, or the weather conditions recorded within the period from October to January in the previous season, which influence ascospore production and maturation (Zúñiga et al., 2020), among other factors. No information has been found on the relationship between the amount of *P. amygdalinum* inoculum and the ascospore production and release in the following year. Regarding the weather conditions, the accumulated rainfall in 2020, from April to June, was well over the average records (146% higher than the equivalent average for the decade 2010–2019; own elaboration from MeteoCat database at: https://ruralcat.gencat.cat/web/guest/agrometeo.estacions). It is known that convective rain episodes may wash-out airborne particles (Gatz & Dingle, 1970; Rodríguez-Solà et al., 2022), thus resulting in lower ascospore captures in 2020. This might consequently result in less infections in 2020 (not evaluated in this study) and reduce the inoculum and ascospore captures for the next season (2021). It is clear from this study that the relationship between the potential inoculum (i.e. infected fallen leaves) and the airborne ascospore dynamics of the following season should be investigated in further research. Nevertheless, in the correlation analyses we addressed the variability in the yearly cumulative concentration of ascospores by calculating the proportion (0–1) of ascospores at weekly intervals.

Few studies have addressed the relationship between weather conditions and inoculum availability of *P. amygdalinum*. Ghazanfari and Banihashemi (1976) reported that temperatures <10°C in fall and winter are a prerequisite for ascocarp formation and development in *P. amygdalinum*, while Zúñiga et al. (2020) found that seasonal accumulated amounts of available ascospores were positively correlated with variables related to water availability (maximum relative humidity and accumulated rainfall) and temperatures ≥20°C during the previous fall, mainly in October, and the accumulated rainfall in January. In the present study, we found that the weekly proportion of caught ascospores was correlated with several weather variables of the concurrent week. Some thermal variables (maximum temperature, VPD, and the number of warm days) were negatively correlated with ascospore catches, while the number of mild days (with mean temperatures ≥10 and <20°C) and the variables related to humidity and rain were positively correlated. Thus, in general terms, *P. amygdalinum* inoculum release may benefit from mild temperatures and the hydration of fallen leaves. This is consistent with Torguet et al. (2022) observations, who found that when fungicide applications were conducted after >15 mm of rain with 10–15°C as minimum temperature, their efficacy against RLB was comparable to calendar-based treatments, thus suggesting that these conditions are favourable for infections. As similar to our findings, mild temperatures and moisture have been pointed as beneficial for ascospore release in other monocyclic ascomycete pathogens, such as *Plurivorosphaerella nawae* (Hiura & Ikata) O. Hassan & T. Chang (Vicent et al., 2011) or *Taphrina deformans* (Berk.) Tul. (Rossi et al., 2007), causal agents of the persimmon circular leaf spot and the peach leaf curl, respectively.

Regarding RLB disease progress, in 2021 the first symptoms were observed at the beginning of June. This is consistent with previous studies in the same region and with the same cultivar 'Tarraco' where, depending on the year, first RLB symptoms developed between the beginning of May and the first half of June (Miarnau et al., 2021), and also coincident with additional previous reports on the disease progress (Banihashemi, 1990; Saad & Masannat, 1997). In our study, RLB incidence and severity progressively increased between mid-June to mid-July. Afterwards, disease intensity remained relatively stable, with some fluctuations that could be due to an early defoliation associated with the disease and the emergence of new leaves (Miarnau et al., 2021). The positive correlation ($\rho(62) > 0.80$) between RLB incidence/severity and the accumulated proportion of ascospores confirms the relationship between airborne ascospore catches and disease progress.

Our study showed positive correlations between the weekly increase in RLB incidence/severity and the number of warm days (with mean temperatures ≥20°C) in the concurrent week, while negative correlations were found with the number of mild days (with mean temperatures ≥10 and <20°C). Disease severity was also positively correlated with the thermal variables mean, maximum, and minimum temperature, and VPD, and negatively correlated with the number of cold days and both wet and mild days. Thus, our results suggest that RLB symptom expression would benefit from concurrent warm temperatures in the studied region, in contrast with ascospore release, that would depend on mild temperatures ($10 \le T < 20$°C) and water-related variables. Miarnau et al. (2021) found that the annual RLB incidence was positively correlated with accumulated rainfall in spring, while it was negatively correlated with high temperatures in spring and summer. Zúñiga et al. (2020) suggested that infections occur in spring and summer when mean temperatures are in the range of 10–

20°C. These previous studies are consistent with our results, as we found similar correlations of the same weather variables but with concurrent ascospore catches, which determine subsequent infections and consequently, after the incubation period, the disease expression.

Gompertz, monomolecular and logistic growth models presented all high goodness-of-fit when describing disease progress in both orchards. The monomolecular model has been often used to describe monocyclic diseases (Madden et al., 2007; Pfender, 1982; Vicent et al., 2012), as RLB is. Both Gompertz and logistic models have similar sigmoidal curve shapes and are often used to describe polycyclic epidemics (Madden et al., 2007). Nevertheless, it is not possible to infer the nature of a disease from disease progress curve fitting (Pfender, 1982; Xu, 2006). In the case of the RLB, the only inoculum sources are the perithecia located in the stromata of fallen leaves (Banihashemi, 1990; Zúñiga et al., 2020). Using Gompertz, monomolecular or logistic equations, growth epidemic rate (r) was higher in Vilagrassa than in Borges, while initial disease incidence (y_0) was lower, regardless of the fitted model. This can be explained because the first RLB register in Borges had a higher incidence than in Vilagrassa, although the progression of the disease was later slower in the case of Borges. Considering RLB as a monocyclic disease, we can then emphasize the importance of RLB control by managing the inoculum source (e.g. through cultural practices) or by predicting its production, release and dispersal dynamics, as the seasonal disease progress depends on tree infections coming from inoculum produced in previous epidemics or in epidemics at other nearby locations (Bergamin-Filho & Amorim, 2002; Madden et al., 2007).

The results of the current research increase the knowledge of *P. amygdalinum* biology and the epidemiology of RLB of almond. The results reported here will contribute to the development of a predictive model for the periods of airborne inoculum availability in the region of Catalonia (NE Spain), that could be incorporated into a decision support system to make RLB management programs more efficient and sustainable.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in CORA (Catalan Open Research Area. Repositori de Dades de Recerca) at https://doi.org/10.34810/data237

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