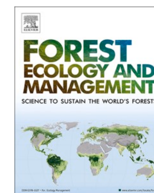
Forest Ecology and Management 536 (2023) 120935

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Cork harvest planning and climate: High air humidity favors availability of airborne inoculum of *Diplodia corticola*

E. Jordán Muñoz-Adalia [a,b,c,*], Anand B. Uppara [d,e], Dalmau Albó [b], Andreu Meijer [b,d], Carlos Colinas [b,d]

[a] *Department of Agroforestry Sciences, University of Valladolid, Av. de Madrid 57, Palencia 34004, Spain*
[b] *Forest Sciences Center of Catalonia (CTFC), Carretera St. Llorenç de Morunys, km.2 Solsona, Catalonia E-25280, Spain*
[c] *Sustainable Forest Management Research Institute, University of Valladolid & INIA, Av. de Madrid 57, Palencia 34004, Spain*
[d] *Department of Forestry and Agricultural Science and Engineering, University of Lleida, Av. de l'Alcalde Rovira Roure 191 Lleida, E-25198, Spain*
[e] *Forest Bioengineering Solutions, S.A., Carretera St. Llorenç de Morunys, km.2 Solsona, Catalonia E-25280, Spain*

ARTICLE INFO

Keywords:
Aerobiology
Botryosphaeria corticola
Emerging forest disease
GAM
Inoculum detection
Nested qPCR

ABSTRACT

The canker disease caused by *Diplodia corticola* is one of the most important emerging pathologies of cork oak (*Quercus suber*) in western Europe. The fungus is dispersed by borer insects, although it is also thought that the spores can be dispersed by wind and rain. The aim of this study was to evaluate the presence of airborne inoculum of *D. corticola* in managed cork oak stands during cork harvesting season. Semi-passive spore traps were set in eight sampling plots in Catalonia (north-eastern Spain) in summer of 2020. Traps were replaced every week and the number of *D. corticolas* spores per sampling event was estimated based on a specific nested-qPCR protocol. Spatial-temporal distribution of airborne inoculum accumulation along sampling areas was analyzed using generalized additive models (GAMs). The availability of airborne inoculum resulted rather low with noticeable accumulation peaks in some of the sampled areas. The fitted GAM revealed a positive effect of high air humidity during the sampling period on the availability of spores. This study represents the first attempt to model the spore release of this emerging pathogen, and it provides insights for developing *D. corticola* canker control strategies based on the precise timing of cork harvesting operations.

1. Introduction

Cork oak (*Quercus suber* L.) silviculture is considered a paradigmatic example of multifunctionality in Mediterranean woodlands. Forest management of this species in the western Mediterranean Basin (mainly Portugal and Spain) is aimed to optimize cork production for the wine tap industry, insulation, synthesis of cork composites, or craft products, among others (Gil, 2015). This forest use is compatible with other productive activities such as livestock rearing, hunting, firewood harvesting, beekeeping, mushroom hunting, and recreation. Cork oak stands are managed both as savanna-like stands in the western Iberian Peninsula (*i. e.*"dehesas" in Spain, "montados" in Portugal) or as middle-density monospecific forests (north-eastern Spain, *e.g.* Catalonia) in order to select the most vigorous trees for productive purposes. These trees are first debarked when they reach a perimeter of 60–65 cm at 1.30 m of height, this initial cork is not useful for the wine stopper industry, and it usually gets low market value. Successive stripping occurs in summer every 9–14 years depending on climate conditions and cork growth, being this regeneration phellem thick enough to reach high market prices (especially after the third stripping) (Montero & Cañellas, 1999).

Quercus suber forests have been facing a challenging situation for the last few decades. Reiterative and persistent abiotic stresses (*i.e.* wildfires and droughts) derived from changes in climate regimes (Olivera and Colinas, 1994), outbreaks of native pests and diseases (*e.g. Lymantria dispar* L., *Coraebus undatus* F.), as well as biological invasions (*e.g. Phytophthora cinnamomi* Rands) (Moricca et al., 2016) dramatically affect the health status of *Q. suber* forests, thus reducing the vigor of the stands and their derived revenues (thinner or lower-quality cork planks). Consequently, forest owners and cork industries have growing concerns about disturbances that threaten the viability of this traditional and sustainable forest use in the middle term.

The disease caused by the ascomycete fungus *Diplodia corticola* Phillips, Alves & Luque, also known as Botryosphaeria canker, is one of the most relevant emerging pathologies of Mediterranean oaks [*i.e.* the

* Corresponding author at: Department of Agroforestry Sciences, University of Valladolid, Av. de Madrid 57, Palencia 34004, Spain.
E-mail address: jordan.munoz@uva.es (E.J. Muñoz-Adalia).

https://doi.org/10.1016/j.foreco.2023.120935
Received 22 December 2022; Received in revised form 9 March 2023; Accepted 11 March 2023
Available online 20 March 2023
0378-1127/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

disease has been reported in woodlands from Algeria, France, Greece, Italy, Morocco, Portugal, Spain and Tunisia (Mahamedi et al., 2020)]. This pathology has been reported in Spain since the early XXth century (Velaz de Medrano and Ugarte, 1922), but outbreaks of this mycosis are gaining attention from forest managers in the last few years. In mature cork oaks, the fungus colonizes the subero-phellodermic layer (*i.e.* phellogen and phelloderm) generating sooty areas clearly visible after debarking. These lesions develop in dark brown bleeding cankers preventing cork regeneration, reducing the vigor of the tree, and making accessible the inner part of the tree to heart rots and wood borers. Moreover, this fungus causes chlorosis, wilting, and plant mortality in *Quercus* spp. seedlings (Luque et al., 2008), and has been described as an exotic pathogen of oaks in North America (Dreaden et al., 2011).

The etiology of the disease is not completely known. The fungus has been confirmed as carried by some borer insects (*i.e.* Coleoptera: Buprestidae, Curculionidae, and Cerambycidae) but definitely by *Coraebus florentinus* F. (Pinna et al., 2019), *Platypus cylindrus* F. (Muñoz-Adalia et al., 2022b), and *Cerambyx welensii* Küster (Panzavolta et al., 2017), nevertheless its possible role as an endophytic fungus (that is, an organism that colonizes plant tissues without causing apparent symptoms) has been also suggested (Franceschini et al., 2005). One of the most accepted hypotheses about inoculum spreading suggests that spores are dispersed by wind and rain drops as reported in other Botryosphaeriaceae species (Kuntzmann et al., 2009), although this has not been demonstrated yet. In this regard, foresters tend to think that wind spore dissemination could be the key factor for infection development in summer since bark stripping favors effective contact between airborne spores and susceptible plant tissues exposed after debarking.

This study aimed to evaluate whether *D. corticola* airborne inoculum is present during cork harvesting season (traditionally from mid-June to mid-July in north-eastern Spain). In addition, we wanted to study whether spores release is a continuous process or if it occurs in bursts possibly related to weather events. The results of this study will provide information to predict periods of the expected abundance of inoculum, which will allow for planning stripping tasks in managed cork oak stands.

2. Materials and Methods:

2.1. Plot selection and spore trapping

Eight sampling plots were set up in Catalonia (north-eastern Spain) in June 2020 in order to investigate the airborne inoculum of *D. corticola* availability during cork harvesting season. Sampling plots were selected for completely covering the distribution range of *Q. suber* in this region, including different locations, microclimate conditions, stand structures, and *D. corticola* canker infection intensities (Table 1). More specifically, all sampling plots of The European Network on Forest Health (level 1: 16 × 16 km surveillance network) set in *Q. suber* forests in Catalonia were included in the study (*i.e.* three plots; Table 1), three additional stands were added according to Spanish Forest Inventory, following the criteria of Regional Forest Services for sampling all stand structures and climate suitable for *Q. suber* in Catalonia (*i.e.* plots I, III, and VIII). In addition, two plots (*i.e.* plots VI and VII; Table 1) were set in the Municipality of Tordera (Barcelona) where previous field experiments focused on *D. corticola* etiology had been carried out (Muñoz-Adalia and Colinas, 2021). Severity of *D. corticola* canker in each plot was assessed by visual inspection of the plot [*i.e.* 100 m radius around each spore trap (see below)] using a qualitative scale: Low (<20% of cork oaks showing any symptoms of *D. corticola* infection), Medium (20–60% of symptomatic trees), Medium-High (60–80% of symptomatic trees), and High (>80% of symptomatic trees)] (Table 1).

A semi-passive spore trap (Supplementary Material S1) was installed in each plot. The traps consisted of a plastic plate (diameter: 14 cm) attached to a plastic funnel (external diameter: 25 cm) able to rotate in the wind by means of a plastic vane. Each trap was set up in a metal bar fixed to the ground (1.23 ± 0.02 m height, mean value, and standard error; Table 1). A 12 × 5 cm piece of oilcloth tissue was then placed on the plate and fixed using two wooden pegs. The surface of the oilcloth was covered with ~ 1 g of odorless petroleum jelly (Fagron Ibérica, Spain) as an extractant substance. Traps were sampled weekly (from 17.06.2020 to 10.09.2020; first collection date in 26.06.2020; Fig. 1) in order to increase the probability of inoculum detection by spore accumulation, even in areas with expected low prevalence of the pathogen. Accordingly, each oilcloth piece was removed every week and transferred to a sterile 50 mL plastic tube, then a new piece of oilcloth was placed in the trap and covered with petroleum jelly. Samples were stored at 4 °C until processed.

Relative air humidity (%) and air temperature (°C) were recorded each hour during the sampling period in the plots using EL-USB-2 Data Loggers (Lascar Electronics, UK). Data loggers were located on cork oak branches, kept well-ventilated, and covered by a piece of plasticized cardboard to avoid direct sun. Maximum, minimum, and mean records of air relative humidity and temperature were calculated for each period

Table 1
Description of sampling plots.

Plot	Location (Province)	UTM coordinates	Height (m.a.s.l.)	Trap height (m)	Incidence of *Diplodia corticola*[**]	Plot description
I	Arbúcies (Girona)	41.80506 / 2.55770	364.8	1.12	High	Mature mixed stand of cork oaks and *Pinus* spp.
II	Capmany[*] (Girona)	42.37313 / 2.91307	190.2	1.28	Medium-High	Mature mixed stand of cork oaks and *Pinus* spp. Well-developed understory.
III	Fitor (Girona)	41.91067 / 3.09685	226.31	1.16	Medium-High	Mature cork oak stand with sparse understory
IV	Pals[*] (Girona)	41.96365 / 3.15300	41.9	1.25	Low	*Pinus* spp. stand with the presence of *Q. suber* of different ages. Well-developed understory
V	Sant Feliu de Buixalleu[*] (Girona)	41.78989 / 2.58350	375.8	1.26	High	Irregular stand of cork oak with well-developed understory
VI	Tordera 1 (Barcelona)	41.71646/ 2.68003	58.69	1.30	Medium	Low density mature cork oak stand (almost savanna-like distribution of trees)
VII	Tordera 2 (Barcelona)	41.69946/ 2.65492	195.5	1.26	Low	Mature cork oak stand. Well-developed understory
VIII	Tordera 3 (Barcelona)	41.67530 / 2.65820	144.6	1.17	Medium	Young cork oak stands with well-developed understory

[*] Plots included in The European Network on Forest Health (level 1).
[**] Severity of *D. corticola* canker visually assessed according to a qualitative scale [Low (<20% symptomatic cork oaks), Medium (20–60%), Medium-High (60–80%), and High (>80%)].

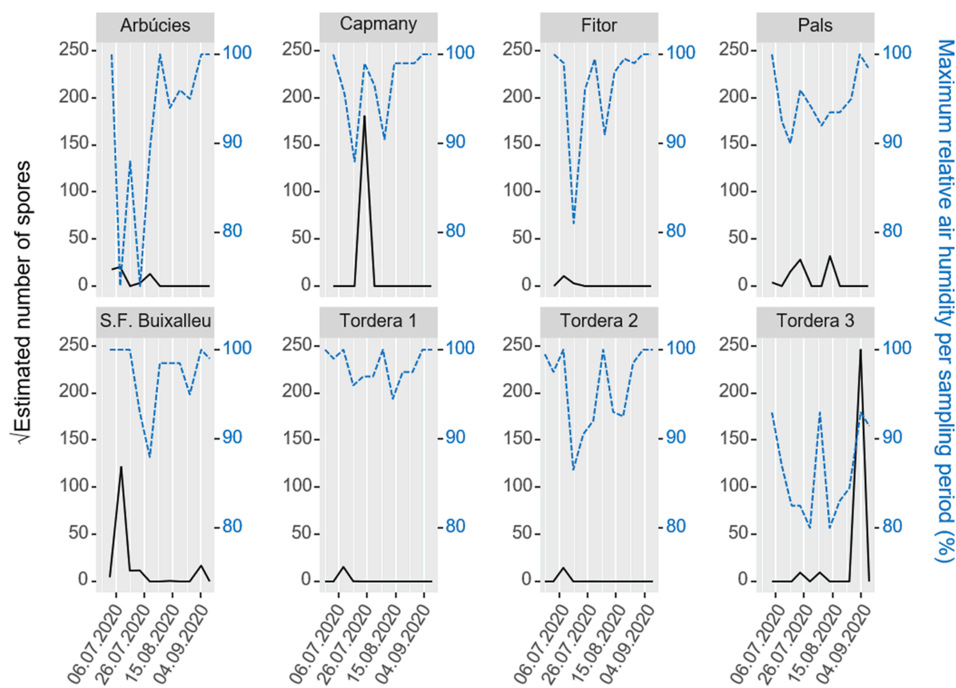


Fig. 1. Airborne inoculum availability (square root of estimated number of spores) per sampling plot in the studied period. Abbreviation: S.F. Buixalleu: Sant Feliu de Buixalleu.

between consecutive sampling events (Hrpmax, Hrpmin, Hrpm, Tpmax, Tpmin, and Tpm, respectively), any measurement gap because of datalogger failure was estimated using records from the nearest meteorological station (Servei Meteorològic de Catalunya; https://www.meteo.cat/).

2.2. DNA extraction and D. corticola diagnosis

Total genomic DNA was extracted from 250 mg of each petroleum jelly sample using DNeasyPowerSoil Kit (Qiagen, Germany) following the manufacturer's instructions and kept at −20 °C until being processed. The quality and quantity of extracted DNA were estimated using a spectrophotometer (NanoDrop 1000 Thermo Scientific, USA). The presence of *D. corticola* in the samples was assessed following the nested-qPCR protocol described by Muñoz-Adalia et al. (2022b). Firstly, a 495 bp region of mitochondrial DNA helicase gene was amplified by PCR using MyTaq DNA Polymerase (meridian Bioscience Inc., USA) [50 μL of reaction volume: 0.8 μL of each primer (25 μM; Table 2), 10 μL of 5x buffer, 35.4 μL of double sterilized MiliQ water, 1 μL of polymerase, and 2 μL of template DNA] in an Eppendorf Master cycler nexus X2 (Eppendorf, Germany). Reaction conditions consisted of 1 min at 95 °C of denaturation, followed by 35 cycles of 15 s at 95 °C, 15 s at 54 °C, and 10 s at 72 °C; final elongation: 10 min at 72 °C. The second step was performed by quantitative real-time PCR (qPCR) using a specific primer pair and TaqMan© probe (Table 2) targeting a 71 bp fragment. This second step was performed in a StepOnePlus real time PCR thermocycler (Applied Biosystems, USA) using TaqMan© universal PCR Master Mix (Applied Biosystems, USA). The reaction volume was fixed to 20 μL, including 0.2 μL of each primer and probe (25 μM), 10 μL of PCR master mix, 2 μL of template DNA (PCR product from step 1 diluted 1:10), and 7.4 μL double sterilized MiliQ water. The qPCR conditions consisted of 10 min at 95 °C of denaturation, followed by 40 cycles of 15 s at 95 °C and 1 min at 55 °C.

2.3. Inoculum quantification

Genomic DNA was extracted using E.Z.N.A.® Plant DNA Kit from ~ 100 mg of lyophilized mycelium of *D. corticola* strain CAA007-1 (NCBI GenBank accession numbers: MW699645/MW699639) powdered in a Bead Mill 24 (Fisherbrand, USA) for 20 s at 3.2 m/s. Once DNA was extracted and quantified using a nanodrop, serial dilutions were prepared using double sterilized MiliQ water (*i.e.* 1:1, 1:10, 1:10^2, and 1:10^3). Serial dilutions were amplified as positive controls as previously described for spore trap samples. Resulting ct-values of serial dilutions and their corresponding DNA concentrations were used for fitting a standard curve in the R environment (R core team, 2022) whose equation was used for estimating the relative biomass of *D. corticola* (pg DNA/trap) in each sampling event and location. The number of spores (S) corresponding to the calculated biomass of each sample was estimated using the size of haploid nuclear genome (C-value) provided by Muñoz-Adalia et al. (2022b) as a reference for expected DNA quantity in a single conidium of *D. corticola.*

2.4. Data analysis

The variation of spore release during the summer was analyzed by computing generalized additive models (GAMs) using the package "mgcv" (Wood, 2021) in R. These models are useful tools for modelling

Table 2
Primer pairs and probe used in nested-qPCR protocol. Source: Muñoz-Adalia et al. (2022b).

Step	Method	Primer pair	TaqMan© probe
1	PCR	NestDQF1/NestDQR2 (5́-ACGGTGCATGAGAGACTTGT-3́ / 5́-TGCTTGATTTCCACGGCTTC-3́)	–
2	Quantitative real-time PCR	DcorQ1/DcorQ2 (5́-GATCTGCGAAGCAAGAGGAC-3́ / 5́-GTGGGGAGTGGATTGGAGTA-3́)	QsubHyb (5́ FAM-GCCATCATCTCAAATGGCTT-TAMRA 3́)

not linear tendencies using smooth functions for predictor variables (Pedersen et al., 2019). Each model was fitted for the estimated number of spores as response variable (S), while sampling date (*i.e.* Julian date, that is correlative numbering from 17th June as day 1 to 10th September as day 86) and plot as explicative variables. Microclimate data (*i.e.* Hrpmax, Hrpmin, Hrpm, Tpmax, Tpmin, and Tpm) were also included as explicative variables after evaluating its possible correlation using the package "corrplot" in R (Wei and Simko, 2021) (Supplementary material S2). A total of 30 GAMS (Gaussian distribution for errors) were computed considering Julian date as an explicative smooth variable (*i.e.* low rank isotropic smoother). The sampling plot was considered in 25 of these models as an explicative factor. More specifically, 18 GAMs considered Julian as a smooth variable separated by plot, whereas the rest of the models include the plot as an independent factor. Climate variables were included in 28 models as smooth predictors or tensor product smooths when the interaction of variables was considered. The model included the Julian date by the plot as a unique explicative variable (*i.e.* Model2) and the four models with lower AIC values (Akaikes Information Criteria) were selected for further analysis (Table 3). Selection of the most explicative model was performed using three complementary indicators: (i) AIC value, (ii) percentage of explained deviance, and (iii) R^2. The two models with lower AIC were compared using the χ^2 test. The R package "gratia" (Simpson, 2021) was used for evaluating model fitness and visualizing predictor functions.

3. Results

The spore-trap survey performed in this study revealed air-borne inoculum availability during summer including cork harvesting season (Fig. 1). Specifically, we found 44.44% of positive amplifications (N = 90 samples for an 86-days sampling period). Otherwise, 23.33% of total samples resulted in ct values < 17, the calculated ct threshold for a theoretical sample load with a single conidium, according to the standard curve (R^2: 0.614; p-value < 0.01; Fig. 2) and C-value. The biomass of *D. corticola* estimated per sample (complete dataset) was 46.79 ± 29.10 pg DNA/sample, which corresponds to 1245.57 ± 774.76 estimated spores/sample. Accordingly, the biomass for the subset of positive samples was 105.29 ± 64.75 pg DNA/sample (2802.54 ± 1723.42 estimated spores/sample), and 200.56 ± 120.90 pg DNA/sample (5338.14 ± 3217.97 estimated spores/sample) for the subset of samples with ct < 17.

Spore availability was not constant during the sampling period thus describing a peak-based pattern throughout sampling areas (Fig. 1). This observation was supported by the GAM Model2y, which showed the highest fitting indicators and significantly differed from the following model with lower AIC (Model2z4; p-value <0.01) (Table 3). Specifically, four of the analyzed plots (*i.e.* Arbúcies, Fitor, Tordera 1, and Tordera 2; Fig. 1) showed scarce airborne inoculum availability throughout the sampling period that did not significantly vary by date (Table 4). Spore availability in Pals did not significantly change by date, although two narrow peaks (three sampling events) of less than 1050 estimated spores/samples were recorded (Fig. 1). Julian date was significantly related to spore availability in Capmany, Sant Feliu de Buixalleu, and Tordera 3 (Table 4), where 1–3 sharp peaks were detected per plot (Fig. 1). The unique noticeable record of spores in the cork oak stand of Capmany corresponded to a marked peak between 17th and 24th July (32600 spores/sample approx.). A later peak was detected in Tordera 3 (60540 spores/sample approximately between 27th August and 4th September), whereas Sant Feliu de Buixalleu exhibited a higher availability of inoculum in general terms with moderate-long periods with 100–200 estimated spores/sample in July and September. An earlier accumulation peak was recorded in this latter plot between 2nd-10th July when the estimated inoculum load reached 14754 spores/sample.

The relative air humidity between sampling dates (maximum records; Hrpmax) recorded in the sampling plots resulted in a significant explicative variable for inoculum availability according to Model2y (Table 3). Accordingly, the predictor smooth function highlights a positive response in the estimated number of spores for Hprmax comprised between 81% and 91% (Fig. 3).

Table 3
Generalized additive model (GAM) selection. Selected model in bold. S, estimated spore load per trap; Hrp/Tp, relative air humidity / temperature recorded between sampling events in each plot (m = mean; min = minimum; max = maximum), respectively; s, Low rank isotropic smoother. Abbreviation: AIC, Akaike's information criteria.

Model name	Description	AIC	Explained deviance	R^2
Model2	S ~ s(Julian, by plot)	1738.630	84.8%	0.793
Model2d	S ~ s(Julian, by plot) + s (Tpm)	1737.288	85.5%	0.798
Model2x	S ~ s(Julian, by plot) + s (Hrpmin)	1730.048	88.5%	0.821
Model2y	**S ~ s(Julian, by plot) + s (Hrpmax)**	**1572.441**	**98.4%**	**0.970**
Model2z4	S ~ s(Julian, by plot) + s (Hrpmin, by plot)	1602.166	97.3%	0.957

4. Discussion

The canker disease caused by *D. corticola* is the main concern for cork oak forest owners in north-eastern Spain since it threats sustainable and profitable management of cork oak stands in the middle term. One of the most intriguing aspects of its infection procedure is the way the fungus propagates in the forest as well as how it colonizes mature oaks.

This study confirmed the presence of airborne inoculum of *D. corticola* during the summer using a highly specific molecular method. The availability of spores described a peak-based pattern that seems to be the most common dissemination strategy among forest pathogens (Iturritxa et al., 2007; Wyka et al., 2018), although some exceptions of this pattern have been described [*e.g.* almost permanent spore spreading with periods of high sporulation, as reported for *Fusarium circinatum* Nirenberg & O'Donnell (Dvořák et al., 2017)]. Inoculum detection reported in our eight sampling plots supports that *D. corticola* airborne spores are released in bursts. In parallel, the spore load of *D. corticola* estimated in subcortical carrier insects (*i.e. P. cylindrus*) using the same molecular protocol also resulted quite variable throughout the summer (Muñoz-Adalia et al., 2022b). This suggests that *D. corticola* takes advantage of specific environmental conditions for spore dissemination (see below) at least during the driest season when weather could be less favorable for pycnidia formation or/and spore survival. Similar patterns have been reported in other Botryosphaeriaceae members. Kuntzmann et al. (2009) performed a long-period monitoring program of spore release in vineyards. These authors found that 90% of *Diplodia seriata* De Not. and *Diplodia mutila* (Fr.) Mont. spores were released during the growing season (*i.e.* April-October) with some noticeable spore accumulation peaks throughout the year. More specifically, *D. mutila* exhibited more narrow peaks than *D. seriata* with an accumulation of released spores during the summer. This tendency completely agrees with the results provided here for *D. corticola*; besides, Kuntzmann et al. (2009) found a positive effect of rainy days on the increase of spore availability recorded by passive jelly traps (see below). The maximal records of accumulated spores in the aforementioned study were 1800–3300 propagules, which is similar to the average estimated number of spores per sample we found for *D. corticola* and represents <23% of total spores estimated for the peaks reported in Capmany, Tordera 3, and Sant Feliu de Buixalleu (Fig. 1).

Temperature and humidity drive fungal growth and sporulation, and they should be therefore considered as key factors in the etiology of forest diseases. The results reported here link peak-based spore release patterns with microclimatic conditions. So, our GAM revealed maximal relative air humidity as a triggering factor for *D. corticola* spore

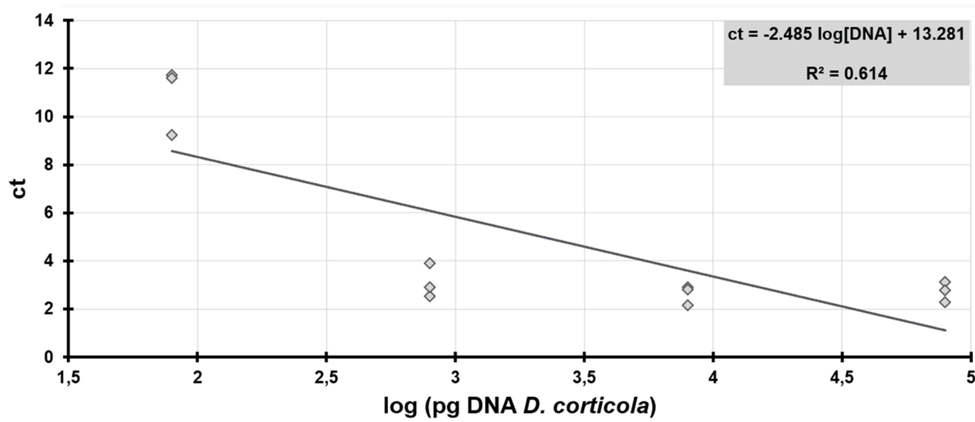


Fig. 2. Standard curve fitted for concentration of *Diplodia corticola* DNA and ct values from nested qPCR.

Table 4
Predictors summary of GAM fitted for spore production (Model2y). S, estimated spore load per trap; Hrpmax, Maximum relative air humidity recorded between sampling events in each plot; s, Low rank isotropic smoother. Abbreviation: edf, effective degrees of freedom.

Response variable	Smooth term	edf	p-value
S (n = 90)	Hrpmax	8.97	< 0.01
	Julian (Arbúcies)	1.00	0.38
	Julian (Capmany)	9.89	< 0.01
	Julian (Fitor)	1.00	0.39
	Julian (Pals)	1.00	0.98
	Julian (Sant Feliu de Buixalleu)	8.94	< 0.01
	Julian (Tordera 1)	1.00	0.96
	Julian (Tordera 2)	1.00	0.73
	Julian (Tordera 3)	8.99	< 0.01

availability. Similarly, spore peaks were reported for *D. seriata*, *Spencermartinsia viticola* (Phillips & Luque) Phillips, Alves & Crous, and *Neofusicoccum* sp. in Chilean vineyards in a two-year long monitoring program (Valencia et al., 2015). In that study, the spore abundance in passive traps revealed accumulation peaks during the rainiest period of the year without spore records during summer (semiarid Mediterranean climate). A similar weather-spore release association was found by Úrbez-Torres et al. (2010) who reported sporulation peaks of Botryosphaeriaceae members [*e.g. D. seriata*, *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & Phillips, and *Botryosphaeria dothidea* (Moug.) Ces. & De Not., among other taxa] coinciding with rainy and overhead irrigation events. Duration of wet conditions seems to be another crucial factor in the sporulation process, Shutong et al. (2012) found maximal spore accumulation of *Botryosphaeria berengeriana* f.sp. *piricola* Kogan. & Sakuma infecting the trunk of *Malus domestica* Borkh. when rain events extended >2 h. These authors experimentally evaluated how moisture retention on the surface of infected plant tissue could affect spore release thus reporting a minimal 2 h-period of high moisture for initiating sporulation (high level of spore release within 12 h of high moisture maintenance). Otherwise, the local temperature did not show any significant effect on the occurrence of spore peaks according to the results provided here (Table 3). In this regard, Botryosphaeriaceae members tend to exhibit rather wide ranges of suitable temperatures for sporulation and spores germination (Copes and Hendrix, 2004; Liu et al., 2022). Regarding vegetative growth, a previous study estimated optimum growth rates *in vitro* between 21-27 °C (lower growth inhibition threshold: ≤5 °C; upper: ≥30 °C) for *D. mutila, B. dothidea,* and *Dothiorella sarmentorum* (Fr.) Phillips, Alves & Luque (Sánchez et al., 2003). Suitable temperature for *in planta* growth seems to be also wide for this taxonomic group since Sánchez et al. (2003) reported the three

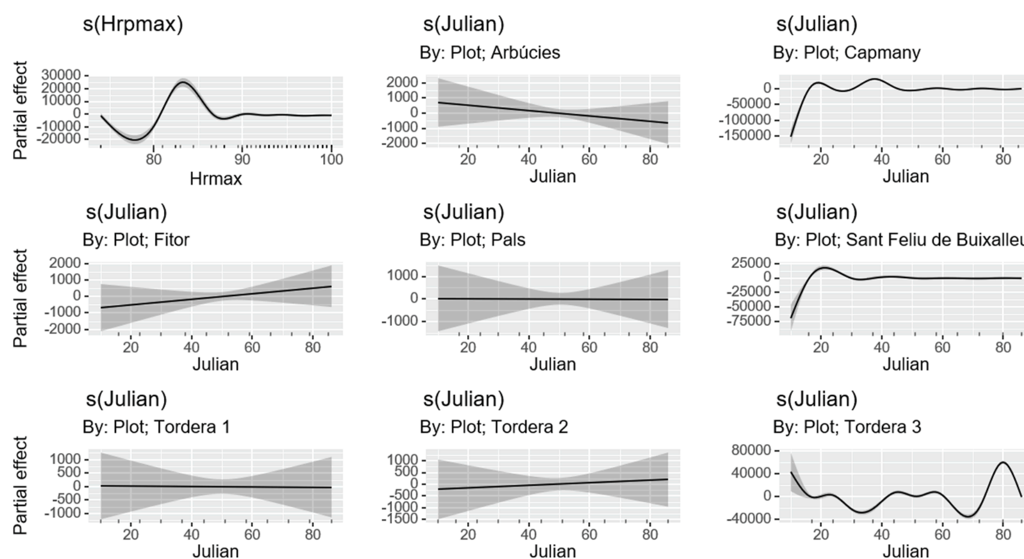


Fig. 3. Estimated effect of smooth coefficient function of maximum relative air humidity per sampling period (Hrpmax), and Julian date by experimental plot. Grey shaded areas represent 95% confidence intervals. Positive values of predictor effect indicate positive effects of the corresponding smooth variable in the estimated number of spores of *Diplodia corticola* per sampling period. Julian date: 20 (06.07.2020), 40 (26.07.2020), 60 (15.08.2020), and 80 (04.09.2020).

mentioned fungal species being able to colonize plant tissues at >25 °C, whereas Luque et al. (2002) reported not clear growth inhibition of *D. mutila* at 5–12 °C infecting cork oak seedlings. Accordingly, humidity could act as a more limiting factor for fungal development and spore release than the temperature for *D. corticola*, although a positive correlation between air temperature and spore peak occurrence has been found for instance for *Diplodia sapinea* (Fr.) Fuckel in northern Spain (Iturritxa et al., 2007). In consequence, we consider that spore release of *D. corticola* could be considered as humidity-dependent during the summer since sporulation peaks tend to take place when relative air humidity becomes high (in some noticeable cases when maximal air humidity reached high values after dry periods, see for instance the peak of Capmany or the two last peaks of Tordera 3; Fig. 1). Future long-term studies should investigate sporulation patterns also outside of the summer season as well as the effect of wetness duration on the host's surface to completely characterize the infective microclimatic window suitable for *D. corticola* in cork oak stands.

In a previous study, we evaluated symptoms development of *D. corticola* cankers in mature *Q. suber* after stripping (Muñoz-Adalia and Colinas, 2021). In that study, we found that cork oaks are susceptible at least within the first 35 days after peeling being air humidity an associated factor to canker appearance. Serrano et al. (2015) also reported more intense damage caused by *D. corticola* in cork oaks in more humid areas, supporting the idea of air humidity as a predisposing factor of this disease. The results provided here show a positive effect of wet periods on airborne inoculum availability as mentioned above. This phenomenon could take place in parallel with carrier insects' dissemination since a previous study showed higher captures of *P. cylindrus* related to middle-high humidity periods (Muñoz-Adalia et al., 2022a). Altogether, summer wet episodes emerge as plausible crucial events in disease development since they take place in a favorable period of the year that provides suitable conditions for the co-occurrence of the three main elements in the pathosystem: susceptible hosts (debarked trees), vector insects *sensu lato* performing dissemination flight (that is, colonizing new hosts for breeding), and airborne inoculum of the pathogen.

5. Conclusions

This study investigated for the first time the airborne inoculum availability of *D. corticola* during the summer. According to our results, *D. corticola*'s spore availability seems to be low during this season, with some intense and local events of spore abundance triggered by wet episodes. Consequently, a higher density of airborne inoculum seems to be concentrated in meteorologically predictable peaks. This is very important for cork oak stand managers since stripping after high-humidity/rainy days could severely increase the risk of effective contact between spores and susceptible hosts. This information makes it possible to issue guidelines for cork harvest planning.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

E. Jordán Muñoz-Adalia: Conceptualization, Metodology, Investigation, Data curation, Formal analysis, Visualization, Supervision, Original draft writing, Review and editing. **Anand B. Uppara:** Data curation, Investigation, Writing, Review and editing. **Dalmau Albó:** Data curation, Investigation, Review and editing. **Andreu Meijer:** Data curation, Investigation, Review and editing. **Carlos Colinas:** Conceptualization, Funding acquisition, Project administration, Resources, Methodology, Supervision, Visualizarion, Writing, Review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was partially funded by the Generalitat de Catalunya, Departament d'Acció Climàtica, Alimentació i Agenda Rural (Spain). Anand B. Uppara is granted by an Industrial Pre-Doctoral Fellowship at Forest Bioengineering solutions and the Forest Sciences Center of Catalonia (CTFC) (reference: E-25-2020-0115755). Authors thank J. Heras (Generalitat de Catalunya), O. Plana (Diputació de Barcelona), A. Torrell (Forestal Catalana), and J.M. Riba for their helpful advice and support in plot selection and sampling development. Fungal strain CAA007-1 used in molecular procedures was provided by Dr. A. Alves (Universidade de Aveiro, Portugal). Authors especially thank Ajuntament de Tordera, Agents Rurals, the staff of Forestal Catalana, and forest owners for giving support in fieldwork and trap monitoring.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.120935.

References

Copes, W.E., Hendrix, F., 2004. Effect of Temperature on Sporulation of *Botryosphaeria dothidea*, *B. obtusa*, and *B. rhodina*. Plant Dis. 88, 292–296. https://doi.org/10.1094/PDIS.2004.88.3.292.

Dreaden, T.J., Shin, K., Smith, J.A., 2011. First Report of *Diplodia corticola* Causing Branch Cankers on Live Oak (*Quercus virginiana*) in Florida. Plant Dis. 95, 1027. https://doi.org/10.1094/PDIS-02-11-0123.

Dvořák, M., Janoš, P., Botella, L., Rotková, G., Zas, R., 2017. Spore dispersal patterns of *Fusarium circinatum* on an infested monterey pine forest in North-Western Spain. Forests 8, 432. https://doi.org/10.3390/f8110432.

Franceschini, A., Linaldeddu, B.T., Marras, F., 2005. Occurrence and Distribution of Fungal Endophytes in Declining Cork Oak Forests in Sardinia (Italy). Bulletin IOBC 28, 67–74.

Gil, L., 2015. New cork-based materials and applications. Mater. 8, 625–637. https://doi.org/10.3390/MA8020625.

Iturritxa, E., Ganley, R., Wart, S., 2007. Dispersión por vía aérea de esporas de *Diplodia pinea* en tres localidades de la Cornisa Cantábrica. Bol. San. Veg. Plagas 33, 383–390.

Kuntzmann, P., Villaume, S., Bertsch, C., 2009. Conidia dispersal of *Diplodia* species in a French vineyard on JSTOR. PhytopatholMediterr 48, 150–154.

Liu, J., Zhang, L.Y., Wang, H.Y., Liu, N., Lian, S., Xu, X.M., Li, B.H., 2022. The effect of temperature and moisture on colonization of apple fruit and branches by *Botryosphaeria dothidea*. Phytopathology 112, 1698–1709. https://doi.org/10.1094/PHYTO-11-21-0487-R.

Luque, J., Parladé, J., Pera, J., 2002. Seasonal changes in susceptibility of *Quercus suber* to *Botryosphaeria stevensii* and *Phytophthora cinnamomi*. Plant Path. 51, 338–345. https://doi.org/10.1046/j.1365-3059.2002.00713.x.

Luque, J., Pera, J., Parladé, J., 2008. Evaluation of fungicides for the control of *Botryosphaeria corticola* on cork oak in Catalonia (NE Spain). For. Pathol. 38, 147–155. https://doi.org/10.1111/j.1439-0329.2007.00526.x.

Mahamedi, A.E., Phillips, A.J.L., Lopes, A., Djellid, Y., Arkam, M., Eichmeier, A., Zitouni, A., Alves, A., Berraf-Tebbal, A., 2020. Diversity, distribution and host association of Botryosphaeriaceae species causing oak decline across different forest ecosystems in Algeria. Eur J Plant Pathol. 158, 745–765. https://doi.org/10.1007/s10658-020-02116-4.

Montero, G., Cañellas, I.M., 1999. Manual de reforestación y cultivo de alcornoque (*Quercus suber* L.). Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain. ISBN: 84-7498-475-0. 103 pp.

Moricca, S., Linaldeddu, B.T., Ginetti, B., Scanu, B., Franceschini, A., Ragazzi, A., 2016. Endemic and emerging pathogens threatening cork oak trees: Management options for conserving a unique forest ecosystem. Plant Dis. 100, 2184–2193. https://doi.org/10.1094/PDIS-03-16-0408-FE.

Muñoz-Adalia, E.J., Ahmed, J., Colinas, C., 2022a. Microclimatic Conditions Drive Summer Flight Phenology of *Platypus cylindrus* in Managed Cork Oak Stands. J. Appl. Entomol. 146, 964–974. https://doi.org/10.1111/JEN.13025.

Muñoz-Adalia, E.J., Colinas, C., 2021. Susceptibility of Cork Oak (*Quercus suber*) to Canker disease caused by *Diplodia corticola*: When time is of the essence. New For 52, 863–873. https://doi.org/10.1007/s11056-020-09829-8.

Muñoz-Adalia, E.J., Meijer, A., Colinas, C., 2022b. New QPCR Protocol to Detect *Diplodia corticola* Shows Phoretic Association with the Oak Pinhole Borer *Platypus cylindrus*. Pest Manag. Sci. 78, 3534–3539. https://doi.org/10.1002/PS.6994.

Olivera, A.; Colinas, C., 1994. Observaciones sobre la posible correspondencia entre la evolucion climatica y el decaimiento de los alcornocales, in: VII Congreso Nacional de La Sociedad Española de Fitopatología. Barcelona, p. 52.

Panzavolta, T., Panichi, A., Bracalini, M., Croci, F., Ginetti, B., Ragazzi, A., Tiberi, R., Moricca, S., 2017. Dispersal and propagule pressure of Botryosphaeriaceae species in a declining oak stand is affected by insect vectors. Forests. https://doi.org/10.3390/f8070228.

Pedersen, E.J., Miller, D.L., Simpson, G.L., Ross, N., 2019. Hierarchical generalized additive models in ecology: An introduction with mgcv. PeerJ, e6876. https://doi.org/10.7717/peerj.6876.

Pinna, C., Linaldeddu, B.T., Deiana, V., Maddau, L., Montecchio, L., Lentini, A., 2019. Plant Pathogenic Fungi Associated with *Coraebus florentinus* (Coleoptera: Buprestidae) Attacks in Declining Oak Forests. For. 10, 488. https://doi.org/10.3390/F10060488.

R core team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Sánchez, M.E., Venegas, J., Romero, M.A., Phillips, A.J.L., Trapero, A., 2003. *Botryosphaeria* and Related Taxa Causing Oak Canker in Southwestern Spain. Plant Dis. 87, 1515–1521. https://doi.org/10.1094/PDIS.2003.87.12.1515.

Serrano, M.S., Romero, M.A., Jiménez, J.J., DeVita, P., Ávila, A., Trapero, A., Sánchez, M.E., 2015. Preventive control of Botryosphaeria canker afecting Quercus suber in southern Spain. Forestry 88, 500–507. https://doi.org/10.1093/forestry/cpv016.

Shutong, W., Wang, Y., Hu, T., Cao, K., 2012. Crucial Weather Conditions for Conidia Release of *Botryosphaeria berengeriana* de Not. f. Sp. Piricola on Apple Stems. Acta Hortic. 940, 701–706. https://doi.org/10.17660/ActaHortic.2012.940.98.

Simpson, G. 2021. gratia: Graceful ggplot-Based Graphics and Other Functions for GAMs Fitted using mgcv. https://gavinsimpson.github.io/gratia/.

Úrbez-Torres, J.R., Battany, M., Bettiga, L.J., Gispert, C., McGourty, G., Roncoroni, J., Smith, R.J., Verdegaal, P., Gubler, W.D., 2010. Botryosphaeriaceae Species Spore-Trapping Studies in California Vineyards. Plant Dis. 94, 717–724. https://doi.org/10.1094/PDIS-94-6-0717.

Valencia, D., Torres, C., Camps, R., López, E., Celis-Diez, J., Besoain, X., 2015. Dissemination of Botryosphaeriaceae conidia in vineyards in the semiarid Mediterranean climate of the Valparaíso Region of Chile. Phytopathol. Mediterr. 54, 394–402. https://doi.org/10.14601/Phytopathol_Mediterr-16055.

Velaz de Medrano, L., Ugarte, J., 1922. El alcornoque y el corcho: Cultivo, aprovechamiento e industrias derivadas. Ed. Calpe, Spain, 234 – pp.

Wei, T., Simko, V. package "corrplot": Visualization of a Correlation Matrix. (Version 0.92). https://github.com/taiyun/corrplot.

Wood, S. GAM computation vehicle with automatic smoothness estimation. https://cran.r-project.org/web/packages/mgcv/mgcv.pdf.

Wyka, S.A., Mcintire, C.D., Smith, C., Munck, I.A., Rock, B.N., Asbjornsen, H., Broders, K. D., 2018. Effect of Climatic Variables on Abundance and Dispersal of *Lecanosticta acicola* Spores and Their Impact on Defoliation on Eastern White Pine. Phytopathology 108, 374–383. https://doi.org/10.1094/PHYTO-02-17-0065-R.