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Climate, host ontogeny and pathogen structural specificity determine forest disease distribution at a regional scale

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Predicting forest health at a regional level is challenging as forests are simultaneously attacked by multiple pathogens. Usually, the impacts of each pathogen are studied separately, however, interactions between them can affect disease dynamics. Pathogens can interact directly by competing for the same niche, but also facilitate or suppress each other via indirect effects through the host. We studied 66 native Mediterranean *Pinus nigra* stands located in the Pyrenees which were affected by two pathogens with different structural specificity: *Dothistroma pini* causing Dothistroma needle blight and *Diplodia sapinea* causing Diplodia shoot blight. We explored the ecology of both pathogens and whether the diseases they caused had an impact on trees and recruits. No signs of competition were found on adult trees. Diplodia shoot blight was restricted to the warmest and driest areas, while no climatic restrictions were identified for Dothistroma needle blight. Both diseases caused additive effects on crown defoliation and defoliated trees showed stagnated growth. In the regeneration layer, signs of disease suppression were found. In the warmest and driest areas, seedling mortality was mainly associated with Diplodia shoot blight, even though both pathogens were detected. Clear signs of *D. pini* spillover from canopy trees to recruits were found. However, seedling mortality caused by Dothistroma needle blight was only restricted to the coldest and wettest sites where *D. sapinea* could not survive. Large crowns in adult trees probably allow both pathogens to co-exist and cause additive impacts. The smaller size of recruits and a higher susceptibility to environmental stress compared to adult trees probably facilitates the effects of Diplodia shoot blight which masked those caused by Dothistroma needle blight. By considering climatic constraints, host ontogeny and structural specificity, we could dissect the disease impacts of two different pathogens and successfully explain forest health at a regional scale.

Keywords: Climate, *Diplodia sapinea*, *Dothistroma pini*, forest health, growth, *Pinus nigra*

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Introduction

Forests are exposed to several biotic and abiotic stressors that affect their health. However, our capacity to predict the outcome of such interactions is still limited. Understanding how different pathogens interact with each other has received little attention. Earlier theoretical frameworks and studies to understand how different biotic and abiotic agents interact with each other have mainly focused on predicting tree death (Manion 1981, Oliva et al. 2014). The most accepted theory of forest decline is where tree death is predicted to be a result of a combination of predisposing, inducing and contributing factors (Manion 1981). In general, it is assumed that adverse conditions may predispose tree hosts and make them more susceptible to disease, as it has been shown for drought and hail (Stanosz et al. 2001, Desprez-Loustau et al. 2006, Oliva et al. 2021). However, tree decline processes can also result from a combination of predisposing and inciting events caused solely by pathogens (Oliva et al. 2016). Interactions between pathogens have been rarely studied in depth and in general, we lack the ecological insights to predict their outcome.

The ecological impact of each pathogen is often analysed separately. The impact caused by co-occurring pathogens may differ from the one produced by a single pathogen since interactions between pathogens can lead to changes in disease dynamics and severity (Tollenaere et al. 2016). The interaction between pathogens present on the same host may result in competition for the same niche when one pathogen inhibits or reduces the development of the other pathogen, an additive effect when the development of one pathogen is not altered by the presence of another, or a synergy when one pathogen promotes the development of the other one (Marçais et al. 2011, 2017, Jesus Junior et al. 2014, Dutt et al. 2022). Studies from agricultural systems have shown that synergistic pathogen–pathogen infection resulted in an increased disease severity and a severe decline of crops (reviewed by Lamichhane and Venturi 2015). In addition, it has been shown that the presence of a pest increased the probability of infection by a second pathogen (Meyer et al. 2015, Stemmelen et al. 2023). On the other hand, competitive interactions were found as a major factor determining the prevalence of pathogens (Kozanitas et al. 2017) and their distribution (Desprez-Loustau et al. 2018).

Pathogen coexistence can be influenced by priority effects in which earlier-arriving species can promote or inhibit the establishment of later-arriving species (Connell and Slatyer 1977). Other factors including differences in host ontogeny and climate can also shape the interactions between pathogens (Kozanitas et al. 2017, Desprez-Loustau et al. 2018). Moreover, the outcome of multiple infections can vary depending on the pathogen niche specialization. Pathogens often infect a specific plant organ and tissue, thus exhibiting structural specificity and occupying separate niches facilitating the coexistence between them (Gause 1934). In reality, most studied pathogen–pathogen combinations in forest systems refer to cases in which pathogens seem to co-occur (Oliva and Colinas 2010, Madsen et al. 2021), but little theoretical work has been placed on the ecological mechanisms governing such interactions.

Diplodia shoot blight and Dothistroma needle blight are forest diseases caused by two pathogens with different structural specificities. Shoot blight is a widespread forest disease in Europe caused by the fungus *Diplodia sapinea* (Fabre et al. 2011, Luchi et al. 2014). *Diplodia sapinea* is a very thermophilic fungus whose distribution is thought to be limited by cold temperatures (Keen and Smits 1989, Fabre et al. 2011, Brodde et al. 2019). The fungus is a tree endophyte of shoots that becomes pathogenic when the host becomes stressed (Stanosz et al. 2001). Shoot blight affects current-year shoots of pines leading to shoot death and decreased tree growth (Desprez-Loustau et al. 2006, Brodde et al. 2019). On the other hand, Dothistroma needle blight is one of the most important diseases of pines worldwide (Drenkhan et al. 2016). The disease is caused by two different pathogens, *Dothistroma septosporum* which has been recorded in both the Northern and Southern Hemispheres, and *D. pini* which is known only in Europe and the United States and Canada (Barnes et al. 2016, 2022, van der Nest et al. 2023). *Dothistroma* has shown a wide ecological amplitude as its distribution ranges from areas with tropical to subarctic climates (Drenkhan et al. 2016). Needle blight affects previous-year needles of pines resulting in severe crown defoliation and tree growth arrest (Bulman et al. 2013).

In this work, we studied native Mediterranean *Pinus nigra* stands affected by two pathogens (*Dothistroma* sp. and *Diplodia sapinea*) affecting both canopy trees and recruits, but with a different structural specificity. In the studied area, forest health is generally poor and canopy defoliation and lack of regeneration are very frequent. We explored the ecology of the two pathogens in 66 pine stands located along an elevation gradient in the eastern Spanish Pyrenees and how their respective diseases were having an impact on canopy pines and recruits. In each stand, we assessed Dothistroma needle blight and Diplodia shoot blight severity in canopy pines and recruits, and the biomass of the respective pathogen in recruits. We estimated canopy spore load based on the combined variables of canopy disease severity, spore survival and symptoms on needles. Dendrochronological analyses were conducted to assess radial growth responses to diseases in canopy pines with different defoliation levels. Canopy defoliation and conspecific mortality were also assessed.

Material and methods

Study sites and field measurements

The field study was conducted in 66 *P. nigra* stands along an elevation and climatic gradient covering the distribution of *P. nigra* in the eastern Spanish Pyrenees during March and April 2022. Sampled forests consisted of pure *P. nigra* stands (with > 90% of canopy trees) with *P. nigra* and other tree species such as *P. halepensis*, *P. sylvestris*, *Quercus faginea*, *Q. humilis* and *Q. ilex* also growing in the regeneration layer.

The gradient ranged from an elevation of 363 to 1380 m above sea level, covering a high breadth of climatic conditions with annual mean temperatures ranging from 7.7 to 13.6°C and total annual precipitation ranging from 494 to 918 mm. In each plot, shrub cover was visually estimated, tree density was determined based on the distance to the kth tree (Prodan 1968), and the basal area was measured using a chain relascope. In addition, the relative location of each sampled stand from the crest to the riverbank was used as a proxy for the influence of the river on the stand. In each stand, six mature trees (ca 75-year-old trees) closer to the centre of the plot were selected to measure diameter at breast height (DBH), needle retention, canopy defoliation and disease severity in the canopy. For Dothistroma needle blight, disease severity was measured as the percentage of the crown showing needle blight symptoms, i.e. pine needles with more than one third of a needle showing necrosis. For Diplodia shoot blight, disease severity was measured as the percentage of the current year's shoots showing shoot blight symptoms, i.e. necrotic shoots with entire needles showing a brown colour. Canopy defoliation was visually assessed as the percentage of the crown showing defoliation. Needle retention was measured as the number of whorls with needles. At plot level, incidence was calculated as the percentage of the six mature trees with disease severity of ≥ 20%. To assess *Dothistroma* sp. survival, needles from three different canopy pines were collected from the ground, placed in three bags and stored at 4°C. In each plot, a linear transect of 20 × 4 m was carried out in which the number of seedlings (up to 2 m in height) of *P. nigra* and other conspecific and heterospecific tree species was recorded to obtain the density of the regeneration. Moreover, the height and disease severity of Dothistroma needle blight and Diplodia shoot blight were measured. Incidence in the regeneration layer at the plot level was assessed as the proportion of seedlings with disease severity of ≥ 20%. To quantify *D. sapinea* and *Dothistroma* sp. in the seedlings, three seedlings were randomly selected from the transect. When seedlings were not present in the transect, three seedlings were randomly selected outside the transect but within the plot. From each seedling, three branches were collected and stored at −20°C until processed for DNA extractions.

Pathogen and disease quantification

Fallen needles collected from the ground were used to estimate Dothistroma needle blight symptoms in canopy pines. Fallen needles were collected at random from three different sites in the plot. Needles were inspected under the microscope and the presence of characteristic red bands was used to further confirm that symptoms observed in the canopy indeed corresponded to Dothistroma needle blight.

For Dothistroma needle blight pathogen quantification in seedlings, three needles from each branch of the three seedlings were pooled together, surface sterilised with 4% NaOCl and then immersed in distilled water. For Diplodia shoot blight pathogen quantification, one shoot from each branch of the three seedlings were pooled. Needles were removed from shoots and the bark was scraped to reduce contamination. Needles and shoots were cut into small pieces, lyophilised at −54°C for 72 h and ground using liquid nitrogen. DNA was extracted with the NucleoSpin© Plant II kit (Macherey-Nagel) as described in Oliva et al. (2017). *Dothistroma septosporum* and *D. pini* were quantified by TaqMan® qPCR using the DStub2 primers and probe and DPtef primers and probe, respectively as in Ioos et al. (2010). The abundance of *D. sapinea* was quantified by TaqMan® qPCR as described by Luchi et al. (2005). Standard curves were obtained using serial dilutions ranging from 10^6 to 10 copies.

Dothistroma spore survival

Fallen needles collected from the ground were also used for germination tests to assess spore survival. Up to ten needles showing characteristic red bands and erumpent conidiomata were selected from each site for pathogen isolation. Needles were first wiped clean with distilled water and carton towel to remove any contaminating material. Erumpent conidiomata were excised from the needle and plated onto 2% Dothistroma sporulating media (DSM: 20 g malt extract, 5 g yeast extract and 15 g agar). These were then further rolled across the media to release spores. After 2–4 days, spore survival was assessed by the successful germination of spores and single germinating spores were transferred to clean plates to allow the cultures to grow. To confirm the species of the isolated fungi, a few isolates were randomly selected from a few sites to sequence. DNA extraction, and ITS PCR and sequencing were carried out as described in Barnes et al. (2016).

Dendrochronological analyses

Radial growth responses to diseases in canopy pines were assessed by using dendrochronology. Two cores per tree were taken at 1.3 m using 5-mm Pressler increment borers. A total of 74 trees were sampled from three sites (42°03′50″N, 1°10′15″E; 42°01′30″N, 1°22′35″E and 41°59′40″N, 1°35′35″E). At each site, defoliated (mean canopy defoliation of 64%) and non-defoliated (mean canopy defoliation of 3%) trees with a similar DBH (22.6 cm and 25.8 cm for defoliated and non-defoliated, respectively) were selected. Cores were air-dried, glued into wooden supports and sanded until tree-ring boundaries were clearly visible (Fritts 1976). Cores were visually cross-dated. Then, cross-dating was checked using the COFECHA software (Holmes 1983), which calculates moving correlations between the individual series and the mean site series. Cores were scanned at a resolution of 1200 dpi (EPSON XL 10000) and ring widths were measured to a 0.01 mm precision using the CooRecorder-CDendro software (Larsson and Larsson 2018). Individual ring-width series were transformed into basal area increment (BAI) series assuming a circular stem shape and using the dplR package (Bunn 2008). Ring widths were not detrended but transformed into BAI series. When assessing radial growth, BAI reflects better changes in stemwood production, thus, allowing the detection of changes between defoliated and non-defoliated trees.

Basal area increment individual series were averaged considering defoliated and non-defoliated trees for the best-replicated period 1983–2021. Radial growth data of the three sampled sites was combined as similar differences between defoliated and non-defoliated trees and a high growth coherence between sites was found (correlations of individual series and mean site series ranged from 0.69 to 0.77).

Climatic data

Climatic data were obtained from the E-OBS 0.25° gridded dataset ver. 25.0e (Cornes et al. 2018) using the KNMI Climate Explorer (https://climexp.knmi.nl/start.cgi). For each plot, we obtained monthly mean temperature and precipitation data from 1992 to 2022. We computed the mean temperature and precipitation for each month over the available period. Seasonal climatic data were obtained by computing the average temperature and precipitation for three months. Autumn included data from September to November, winter from December to February, spring from March to May and summer from June to August. Annual mean temperature was obtained by averaging the temperature of all months and annual total precipitation from the sum of monthly precipitation data.

Statistical analyses

For each plot, the disease severity in canopy pines was calculated as the mean disease severity of the six pines selected during the sampling. Disease severity in seedlings was calculated as the mean disease severity of the seedlings present in the transect of each plot. Pathogen abundance and the density of regenerating seedlings were log-transformed to meet the normality assumption of regression residuals. The association of severity of diseases (i.e. mean disease severity), pathogen abundance (i.e. quantity detected by qPCR), needle symptoms (i.e. percentage of fallen needles with the presence of characteristic red bands) and spore survival (i.e. percentage of spore germination from conidiomata of fallen needles) with topographical variables (i.e. altitude, orientation, river proximity), climatic variables (temperature and precipitation) and stand variables (i.e. density, structure, basal area and DBH) was evaluated. Logistic regressions assuming a quasibinomial distribution were used to evaluate correlations including disease severity as a response variable while linear regressions were used when pathogen abundance, needle symptoms and spore survival were the response variables. The association of canopy defoliation, conspecific mortality, shrub cover and density of heterospecific seedlings with both diseases' measurements and topographical, climatic and stand variables was evaluated by linear regressions. Linear and logistic regression analyses were performed using the *lm* and *glm* functions in R ver. 4.2.3 (www.r-project.org), respectively. Differences in needle retention between stands depending on Dothistroma needle blight severity were tested using post hoc Tukey tests with JMP Pro ver. 17.0.0 (SAS Institute Inc.). Differences in annual growth rates between defoliated and non-defoliated trees were assessed using Mann–Whitney tests.

Structural equation modelling

We used structural equation models (SEMs) to evaluate the interaction between Dothistroma needle blight and Diplodia shoot blight and their impact on the canopy and regeneration layer. For the first SEM, we evaluated how diseases in the canopy had an impact on canopy defoliation and the regeneration layer. Moreover, temperature was included in the model to test its influence on all the interactions. In the second SEM, we tested the influence of diseases both at canopy level and at the regeneration layer in the conspecific mortality. In addition, we evaluated the influence of temperature in these interactions. In the third SEM, we studied the influence of river proximity and temperature in the canopy spore load and the pathogen abundance in seedlings. To do so, spore load was considered a latent variable used as an estimate of the viable spores falling from the canopy to the regeneration layer. We assumed that the quantity of spores could be related to the quantity of fruiting bodies and the quantity of viable spores. Thus, spore load was inferred from Dothistroma needle blight severity in the canopy, needle symptoms (as an estimate of the quantity of needles with fruiting bodies) and spore survival (measured from germinating spores from fruiting bodies of needles on the ground and used as an estimate of the viability of spores in the canopy). Initially, SEMs included correlations between all variables. Then, non-significant paths were removed. To assess the goodness of fit of the reduced model versus the full model we used the chi-square value (χ^2), the Akaike information criterion (AIC), the root mean square error of approximation (RMSEA) and the p-value. For SEM analysis, a p-value > 0.05 indicates an improvement of the reduced model versus the full model. SEM analyses were performed with JMP.

Results

Distribution of diseases

In the two studied pathogens, host ontogeny played a different role in disease severity. In the recruits, the incidence of Dothistroma needle blight was similar to that observed in canopy pines (17.7 vs 19.8%, respectively). By contrast, Diplodia shoot blight incidence in seedlings was ca three times higher than in canopy pines (16.5 vs 5.3%, respectively).

The association between disease severity and climatic conditions was also different for the two studied diseases. In canopy pines, no association between climate and Dothistroma needle blight severity at canopy level was found (Table 1, Fig. 1a). By contrast, Diplodia shoot blight was most severe in stands located in the warmest and driest sites where both diseases co-occurred (Table 1, Fig. 1a). In the regeneration layer, strong regional segregation was found between Dothistroma needle blight and Diplodia shoot blight (Fig. 1b). Diplodia shoot blight was found in warm and dry areas as seen for canopy pines (Table 1). For Dothistroma needle blight, contrary to canopy pines, the disease showed an association with climate and greater disease severity was found in cold and rainy areas (Table 1).

Table 1.Association between disease and pathogen measurements and topographical, climatic and stand variables for Dothistroma needle blight and Diplodia shoot blight at canopy, regeneration and litter layers. Bold values indicate a significant association at $p < 0.05$. 'Coef' columns show the coefficient estimate value of the association. 'p' columns show the p-value of the association.

	Canopy				Regeneration								Litter			
	Disease				Disease				Pathogen				Disease		Spore survival	
	Needle blight		Shoot blight		Needle blight		Shoot blight		*D. pini*		*D. sapinea*		Needle blight		*D. pini*	
	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p
Topographical variables																
Altitude	−0.003	**0.0029**	−0.001	0.1424	−0.001	0.2944	-0.003	0.0555	−0.003	**0.0495**	−0.003	**0.0051**	−0.003	**0.0107**	−0.006	**0.0001**
Orientation (South)	0.000	0.9569	0.002	0.2345	−0.001	0.7521	-0.002	0.6628	0.000	0.9785	−0.001	0.8590	−0.001	0.7597	0.009	0.0941
River proximity	1.543	**0.0490**	0.642	0.0667	−0.226	0.7601	0.341	0.7431	2.824	**0.0100**	0.115	0.8578	2.093	**0.0105**	2.980	**0.0065**
Climatic variables																
Mean temperature																
Annual	−0.103	0.5114	0.280	**0.0001**	−0.275	**0.0445**	0.717	**0.0007**	−0.331	0.1756	0.108	0.4109	−0.244	0.0884	0.459	0.0543
Autumn	−0.108	0.4865	0.271	**0.0001**	−0.272	**0.0475**	0.698	**0.0007**	−0.316	0.1942	0.122	0.3501	−0.238	0.0980	0.479	**0.0408**
Winter	−0.173	0.3590	0.280	**0.0009**	−0.320	0.0624	0.761	**0.0008**	−0.315	0.2836	0.212	0.1765	−0.251	0.1622	0.659	**0.0123**
Spring	−0.080	0.5860	0.271	**0.0001**	−0.261	**0.0405**	0.687	**0.0010**	−0.333	0.1478	0.078	0.5328	−0.234	0.0798	0.372	0.0974
Summer	−0.072	0.6020	0.261	**<0.0001**	−0.247	**0.0392**	0.653	**0.0011**	−0.323	0.1361	0.059	0.6175	−0.233	0.0615	0.318	0.1324
Precipitation																
Annual	0.001	0.7642	−0.004	**<0.0001**	0.004	**0.0389**	-0.010	**0.0014**	0.005	0.1260	0.000	0.8314	0.004	**0.0304**	−0.003	0.2894
Autumn	0.003	0.9152	−0.045	**0.0001**	0.046	**0.0369**	-0.100	**0.0072**	0.068	0.0881	0.007	0.7452	0.053	**0.0194**	−0.015	0.6903
Winter	0.012	0.6312	−0.050	**<0.0001**	0.045	**0.0344**	-0.136	**0.0008**	0.064	0.1071	−0.001	0.9580	0.054	**0.0138**	−0.044	0.2691
Spring	0.010	0.6739	−0.044	**0.0001**	0.043	**0.0425**	-0.117	**0.0006**	0.052	0.1701	−0.012	0.5477	0.044	**0.0482**	−0.059	0.1045
Summer	0.004	0.8545	−0.044	**<0.0001**	0.041	**0.0494**	-0.105	**0.0018**	0.052	0.1637	−0.009	0.6368	0.040	0.0671	−0.038	0.2788
Stand variables																
Density	−0.009	0.5758	0.013	**0.0138**	−0.023	0.2186	0.040	0.0574	0.004	0.8791	−0.008	0.6157	−0.006	0.6936	−0.057	**0.0445**
Structure (Even-aged)	−0.352	0.3835	−0.187	0.3130	−0.717	0.0509	0.658	0.2049	−0.301	0.6112	0.288	0.3722	−0.234	0.5623	−1.363	**0.0141**
Basal area	0.001	0.9622	−0.011	0.1826	−0.018	0.2270	0.032	0.0868	0.013	0.6094	0.007	0.6072	0.016	0.3529	−0.003	0.8857
DBH	−0.019	0.6432	−0.022	0.2306	−0.018	0.6178	0.005	0.9096	0.075	0.1668	0.071	**0.0119**	0.035	0.3672	0.038	0.5416

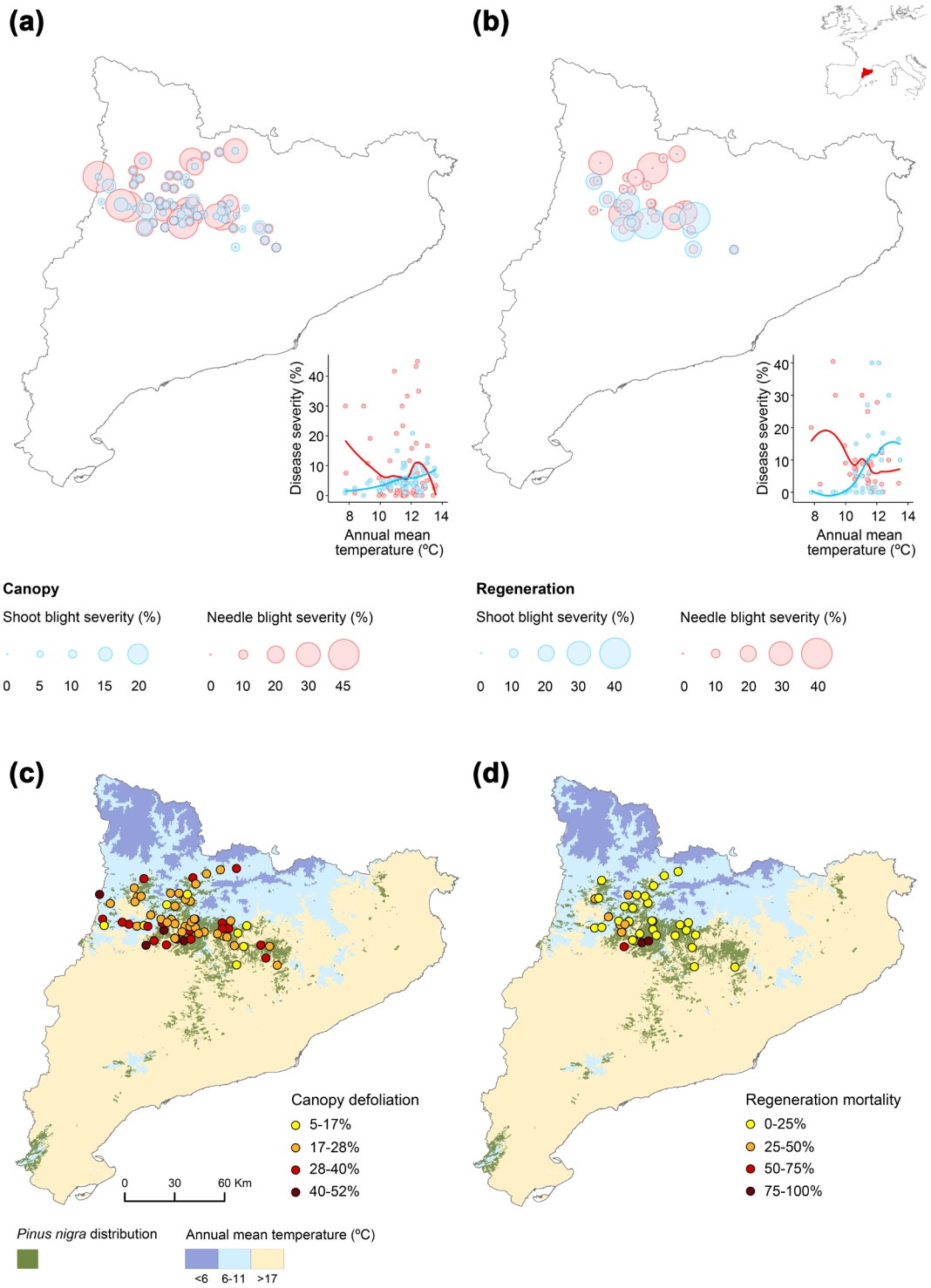

Figure 1. Severity of Dothistroma needle blight and Diplodia shoot blight in (a) canopy pines and (b) the regeneration layer. The inset plots show severity of diseases (red for Dothistroma needle blight and blue for Diplodia shoot blight) depending on the annual mean temperature. Impacts of diseases in terms of (c) canopy defoliation and (d) *P. nigra* mortality.

Microsite conditions affected the two diseases differently. Dothistroma needle blight severity tended to be more prevalent near valley bottoms (Table 1). By contrast, no effects of microsite conditions were found for Diplodia shoot blight (Table 1).

Impacts of diseases

The presence of both diseases was associated with additive impacts of diseases at canopy level (Fig. 2). In the studied area, high levels of canopy defoliation were found along the elevation gradient (Fig. 1c, 3a). Dothistroma needle blight was the main disease associated with canopy defoliation, however, in warm areas, a minor but significant contribution of Diplodia shoot blight in canopy defoliation was also observed (Fig. 2a). No interaction between both diseases contributing to defoliation was found ($p = 0.6$), rejecting a putative indirect effect of Dothistroma needle blight on defoliation due to the presence of Diplodia shoot blight and vice versa (Fig. 2a).

In the regeneration layer, *P. nigra* mortality was all over the studied area (Fig. 1d), however, the particular disease associated with mortality depended on climatic conditions. In the warmer areas, higher regeneration mortality was associated with Diplodia shoot blight (Fig. 2b). By contrast, in cold areas, mortality was associated with Dothistroma needle blight (Fig. 2b).

For Dothistroma needle blight, climate affected the disease and the pathogen differently. While climate restricted the disease to cold and rainy regions, it did not appear to be a limiting factor for the pathogen. Temperature was not associated with the abundance of the needle blight pathogen in the seedlings which was found indistinctly in warm and cold areas (Fig. 4a). Pathogen abundance in the seedlings was associated with a higher spore load in the canopy, i.e. with a higher estimate of spores falling from the canopy (Fig. 4a–b). A higher abundance of *D. pini* was found in those seedlings showing more severe symptoms of Dothistroma needle blight. As for canopy needle blight, pathogen abundance was related to microsite conditions. SEMs suggested that a greater pathogen spillover from canopy pines to seedlings was mediated by the influence of river proximity (Fig. 4b). In addition, spore survival was higher in more open stands (Table 1). In all cases, with the sequencing of cultures and qPCR analyses, only *D. pini* was present in the affected areas.

In the studied area, predicting forest health directly from site variables was not possible. No associations between canopy defoliation, heterospecific density and shrub cover and topographical, climatic and stand variables were found (Supporting information). However, considering the role of climate, host ontogeny and pathogen structural specificity allowed disentangling individual and combined impacts of both diseases on *P. nigra* stands and explained regional forest health. Canopy defoliation was mainly associated with Dothistroma needle blight and it was associated with an increased bush cover (Fig. 2a, 3c). A lower density of conspecific seedlings was found in stands with higher disease

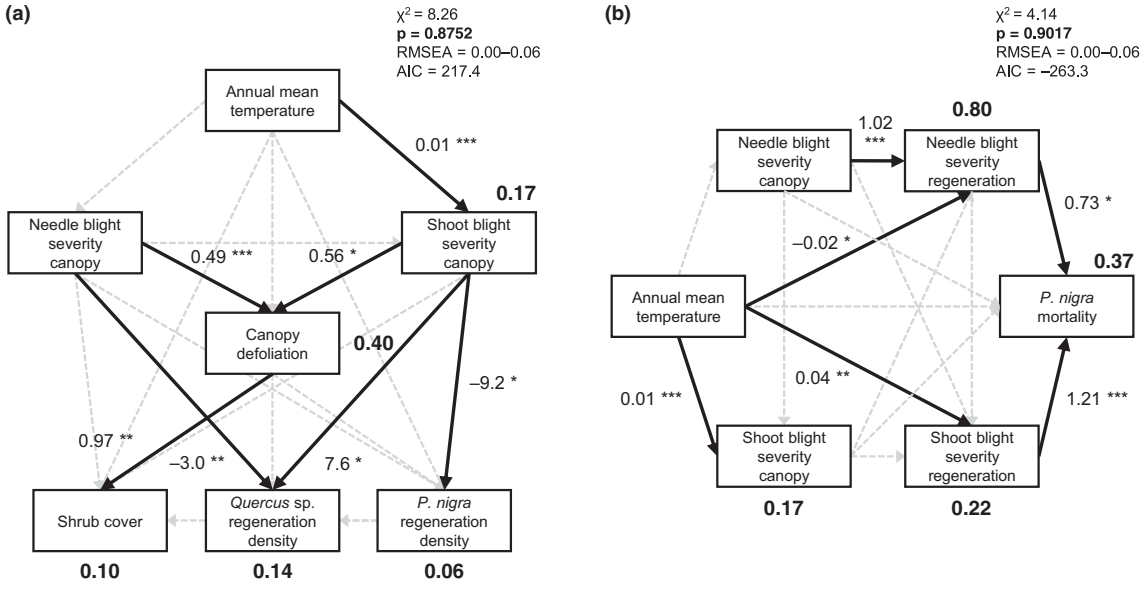

Figure 2. Structural equation model (SEM) analyses of the impact of diseases. (a) Impact of temperature and canopy diseases severities on canopy defoliation and the regeneration layer. (b) Impact of temperature, severity of diseases both at canopy and regeneration levels on conspecific mortality. Values shown next to the pathways represent coefficient correlation estimates, and the R^2 of each variable is shown in bold face. The chi-square value (χ^2) and the Akaike information criterion (AIC) of each model are shown. The root mean square error of approximation (RMSEA) is expressed on its lower and upper limits at 90%. p-values higher than 0.05 and shown in bold face indicate a better fit of the reduced SEM (only black pathways) over the full model (both black and grey pathways). Grey dashed lines indicate non-significant correlations ($p \geq 0.05$). '*', '**' and '***' indicate significant associations at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

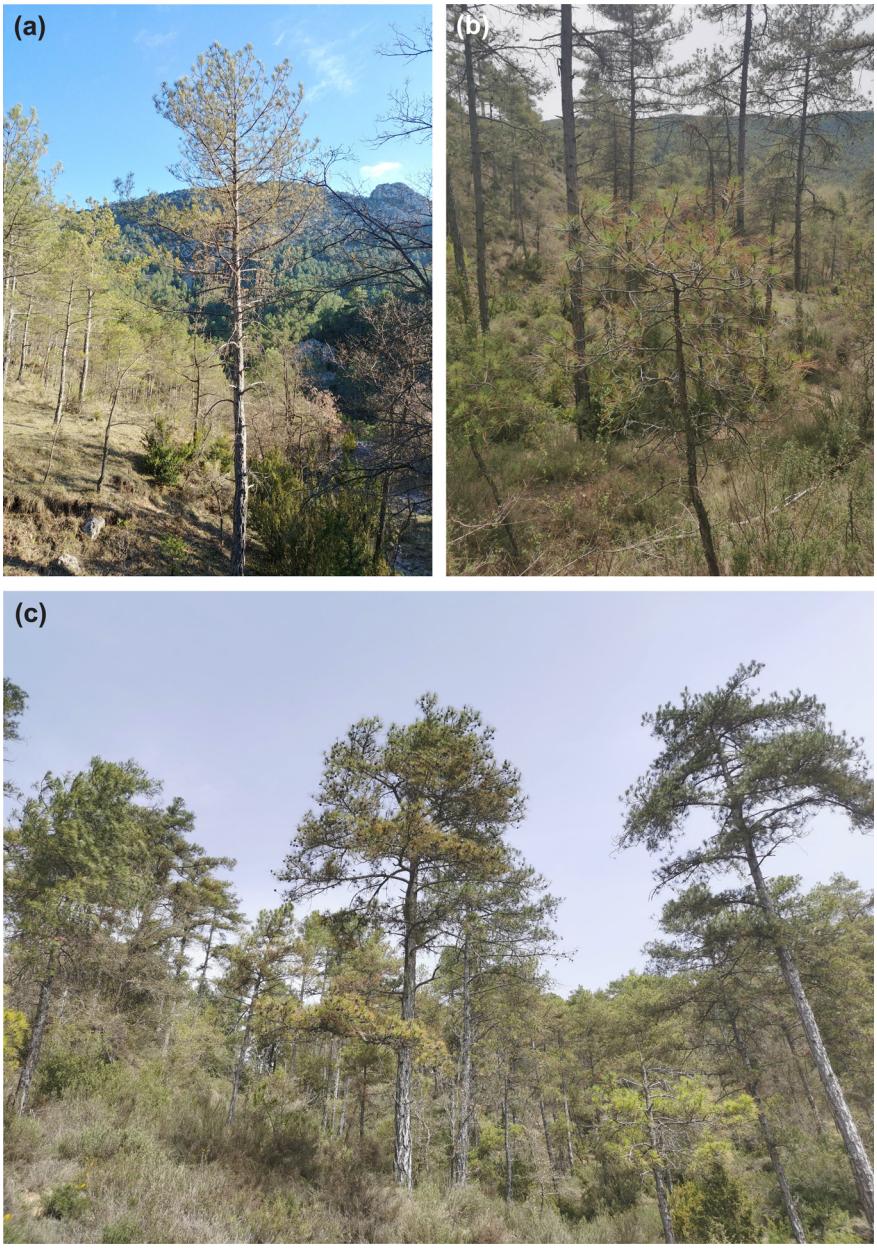

Figure 3. Impact of diseases on pine stands. Strong canopy defoliation on (a) adult trees and (b) recruits. (c) Pine stand with defoliated trees and an increased bush cover.

levels of Diplodia shoot blight (Fig. 2a). Moreover, Diplodia shoot blight severity favoured density of heterospecific seedlings (Fig. 2a). Lower needle retention was found in canopy pines with greater levels of Dothistroma needle blight severity (Fig. 5a). Furthermore, lower radial growth for the past 22 years (period 2000-2021) was found in defoliated canopy pines compared to non-defoliated canopy pines (Fig. 5b, Supporting information). From 2000 to 2021, the mean ± SE basal area increment values of defoliated and non-defoliated trees were 4.01 ± 0.43 cm^2 $year^{-1}$ and 9.20 ± 0.58 cm^2 $year^{-1}$, respectively. Growth reductions were also evident in both groups of trees during dry years such as 2005 and 2012.

Discussion

Predicting forest health at a regional level is a challenging task. Forests are exposed to several abiotic and biotic factors that affect their health. The ecological impact of each pathogen is often analysed separately, thus omitting possible interactions between them that can lead to changes in their respective disease dynamics and severity. Here, we studied native Mediterranean *P. nigra* stands affected by two pathogens, *D. pini* and *D. sapinea*, with different climatic requirements and structural specificities which cause different impacts depending on host ontogeny. The studied stands show rather poor health condition with conspicuous defoliation levels in the

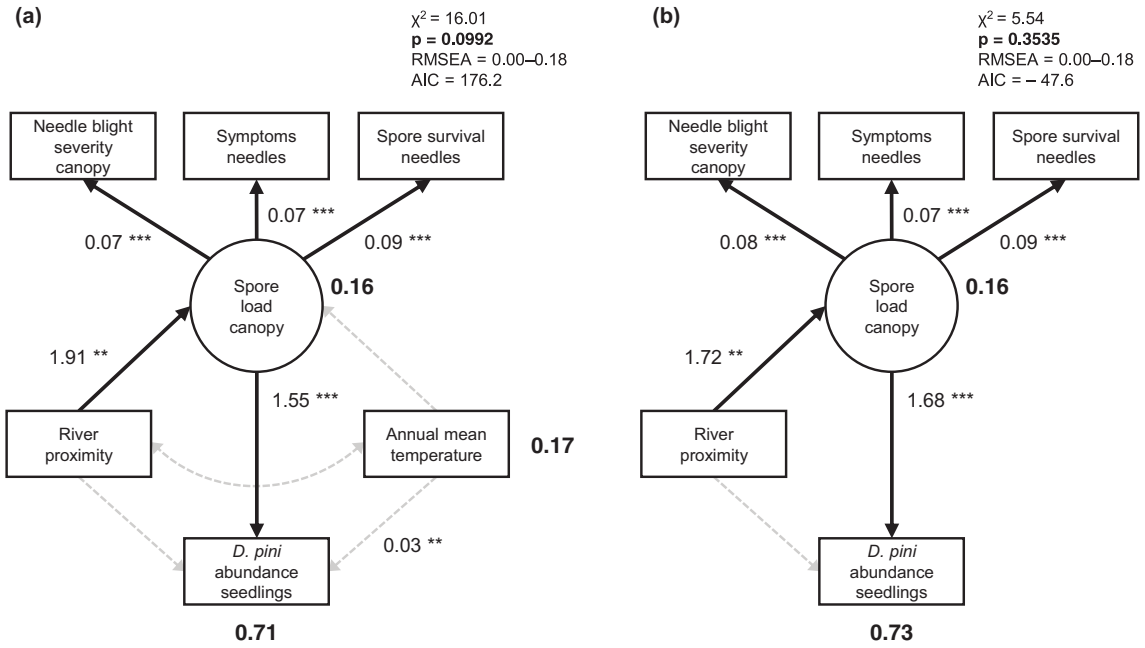

Figure 4. Structural equation model (SEM) analysis of the influence of (a) temperature and (b) river proximity in the spore load in canopy pines and pathogen abundance in seedlings. Spore load is a latent variable inferred from Dothistroma needle blight severity in the canopy, symptoms from fallen needles and spore survival on needles. The latent variable is used as an estimate of viable *Dothistroma* spores falling from the canopy to the regeneration layer and is indicated in the figure with a circle. Values shown next to the pathways represent coefficient correlation estimates, and the R^2 of each variable is shown in bold face. The chi-square value (χ^2) and the Akaike information criterion (AIC) of each model are shown. The root mean square error of approximation (RMSEA) is expressed on its lower and upper limits at 90%. p-values higher than 0.05 indicate a better fit of the reduced SEM (only black pathways) over the full model (both black and grey pathways). Grey dashed lines indicate non-significant correlations ($p \geq 0.05$). '**' and '***' indicate significant associations at $p < 0.01$ and $p < 0.001$, respectively.

canopy and conspecific mortality in the regeneration layer. We explored the ecology of the two pathogens along an elevation gradient and how their diseases were having an impact on canopy trees and conspecific and heterospecific recruits.

In the studied area, attempts to predict forest health directly from site variables did not prove successful. Neither defoliation nor lack of regeneration could be satisfactorily explained by either climate or stand conditions. However, by considering the presence of the two pathogens, their climatic constraints, host ontogeny preferences and their structural specificity we were able to disentangle the individual and combined impacts of the two different diseases and successfully explain health at a regional scale. At canopy level, Diplodia shoot blight was restricted to warm and dry areas, while no climatic restrictions were found for Dothistroma needle blight. The higher disease levels of Diplodia shoot blight found in areas with higher temperatures are in accordance with the distribution pattern of shoot blight found in other regions of Europe (Fabre et al. 2011, Luchi et al. 2014, Brodde et al. 2019). We found that Dothistroma needle blight was related to microsite conditions. Greater damage in canopy pines tended to be more prevalent near valley

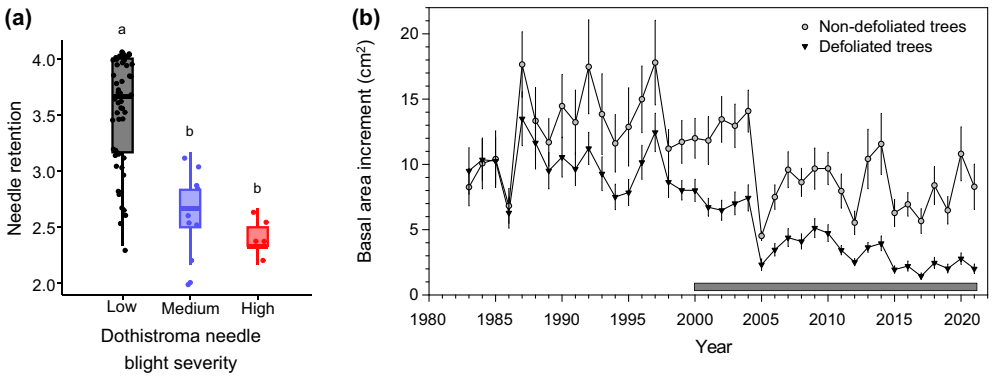

Figure 5. Impact of Dothistroma needle blight on (a) needle retention and (b) radial growth (basal area increment, means ± SE) of canopy pines. In (b), the grey bar indicates the period when growth of defoliated trees was significantly ($p < 0.05$) lower than that of non-defoliated trees.

bottoms, possibly due to the presence of humid conditions conducive to disease (reviewed by Drenkhan et al. 2016, Woods et al. 2016). Temperature and precipitation have been linked with Dothistroma needle blight severity (Fabre et al. 2012, Welsh et al. 2014, Woods et al. 2016), but in the studied area neither temperature nor precipitation was associated with Dothistroma needle blight severity.

The ecology of both diseases together with their structural specificity explained the regional distribution of defoliation. Both diseases caused an additive impact on canopy pines. Dothistroma needle blight was possibly the main disease associated with crown defoliation, however, a significant contribution of Diplodia shoot blight was also observed in warm areas. The stronger contribution of Dothistroma needle blight on canopy defoliation could be the result of the higher Dothistroma needle blight severity observed compared to Diplodia shoot blight severity in the studied area. Therefore, it could be possible to observe a higher contribution of shoot blight causing crown defoliation in areas with a higher prevalence of shoot blight, for instance in connection with hail storms or drought spells (Caballol et al. 2022a). The lack of a climatic signal for defoliation arose from the lack of a climatic signal in the main causal agent. Even though both Dothistroma needle blight and Diplodia shoot blight were found in canopy pines, no facilitation between diseases was observed. Reports of interactions between needle diseases and other types of diseases are meagre, but in the case of needle diseases, interactive effects seem to be rare, and it rather looks as the disease affecting a larger organ benefits more than the one affecting the smaller organ than vice versa, i.e. root rot pathogens will benefit more from foliar diseases than vice versa. For instance, a greater growth reduction caused by *Armillaria* was found when trees were also infected by Dothistroma needle blight than when attacked by *Armillaria* alone (Shaw and Toes 1977). Shoot pathogens such as *Gremmeniella abietina* also benefit from previous attacks of the same pathogen (Oliva et al. 2016), again indicating that impacts of pathogens affecting larger organs such as stems, branches or roots may benefit more from interactions with other diseases than diseases affecting smaller organs such as needles.

Defoliated canopy pines showed arrested radial growth. Thus, the impact of *D. pini* on needles probably reduced the photosynthetic capacity explaining the long-term reduction in growth rates and stem wood production (Gibson 1972). Indeed, we found lower needle retention in canopy pines with greater levels of Dothistroma needle blight. However, we found interesting potential impacts of disease beyond the actual host. In the studied area, canopy defoliation was associated with an increased bush cover, which could in turn increase the risk of forest fires. The higher bush cover was likely a result of the higher amount of light going through the canopy. Other factors such as climate, topographical or stand variables that could alternatively explain bush cover were ruled out.

Contrary to canopy pines, signs of disease suppression of Diplodia shoot blight over Dothistroma needle blight were found in the regeneration layer under warm and dry conditions. Seedling mortality was associated with Dothistroma needle blight only in cold and moist areas where *D. sapinea* was mostly absent. In warm and dry areas, where both pathogens were present, mortality was mainly associated with *D. sapinea*, suggesting that Dothistroma needle blight was somehow suppressed. Competition has been shown to occur between pathogens occupying the same niche (Al-Naimi et al. 2005, Kozanitas et al. 2017). *Diplodia sapinea* and *Dothistroma pini* have different structural specificities, i.e. occupy different niches, however it is possible that because *D. sapinea* affects the entire shoot it is able to mask the expression of the needle blight caused by *D. pini*. It is also possible that microsite and ontogenic conditions contribute to the dominance of Diplodia shoot blight over Dothistroma needle blight in the regeneration layer in the warmest sites. On the one hand, a higher susceptibility to water deficit of recruits compared to adult trees possibly favoured the effects of shoot blight (Stanosz et al. 2001). On the other hand, the moisture requirements for *D. pini* may be more difficult to achieve in the warmer areas than in colder stands. Likewise, we speculate that the lack of disease suppression in canopy crowns could arise from the fact that large crowns in adult trees probably allowed the co-existence of both diseases and also due to a higher drought resistance of adult trees compared to conspecific recruits. Whether a higher severity of one of the diseases over the other could benefit one or the other at organism level should be further investigated.

The higher *D. pini* abundance in seedlings was a result of a higher spore load in the canopy. River proximity seemed to play a role in the canopy spore load. Spore survival was also higher in more open stands and low-density stands. It seems that those conditions could favour spore survival since a similar pattern was observed for the production and survival of *D. sapinea* spores, in which tree density and temperature played a role (Brodde et al. 2019, Caballol et al. 2022b). Even though *D. pini* spillover from canopy pines to recruits was found all over the gradient, conspecific mortality caused by Dothistroma needle blight was only restricted to cold and rainy areas. In warm and dry areas, temperature favoured *D. sapinea* spillover from the canopy resulting in negative canopy-understory feedbacks (Caballol et al. 2022b). As reported earlier, lower densities of conspecific seedlings were observed in stands with higher disease levels of Diplodia shoot blight in the canopy whereas an increased heterospecific regeneration was found. Changes in density of both conspecific and heterospecific seedlings were not associated with topographical, climatic or stand variables ruling out a possible effect of the environment on regeneration density. It seems likely that heterospecific regeneration could be favoured by the negative feedback of diseased canopy pines on conspecific regeneration.

In the studied area, only *D. pini* was detected. This result is in contrast with previous observations of Dothistroma needle blight in Europe and elsewhere where red band needle blight has been linked with *D. septosporum*, and where *D. pini* has always shown a low frequency (Drenkhan et al. 2016, Ortíz de Urbina et al. 2016). Nevertheless, the predominance of *D. pini* in the studied area is in line with its predominance in the southern half of France where the ratio between *D. pini* and *D. septosporum* increased with increasing temperature (Fabre et al. 2012). The distribution of *D.*

pini is also patchy with reports in central France and eastern Europe (Drenkhan et al. 2016) but absent in the Italian peninsula (Ghelardini and de Groot 2020, Aglietti et al. 2021). In general, until now little was known about this pathogen and its epidemiology, but our results suggest that the disease caused by either *D. pini* or *D. septosporum* displays a similar behaviour, i.e. no climatic constraints for the pathogen, and the disease favoured by moist conditions. Whether *D. pini* is native or exotic in Europe is currently under debate. The high genetic diversity in native *P. nigra* forests could indicate that sexual reproduction is taking place in the studied area (van der Nest et al. 2023), however, the sexual state of *D. pini* has not been observed so far.

We obtained mechanistic insights on the regional variation of forest health by accounting for the ecology of two pathogens and their associated impacts. Our findings showed that defoliation occurred mainly due to Dothistroma needle blight caused by *D. pini* even though *D. sapinea* could cause defoliation in warm and dry areas where climatic conditions were suitable for its survival. Because defoliation was caused by Dothistroma needle blight, a causal link between disease and impact allowed assigning the observed symptoms of stagnated growth to disease and not vice versa. By contrast, in the regeneration layer, signs of disease suppression were found. Symptoms of disease and mortality showed that Diplodia shoot blight could eventually mask Dothistroma needle blight in warm and dry areas even though seedlings were subjected to spillover and infection of both pathogens from the canopy. By understanding the canopy-regeneration feedback, we were able to understand that the lack of regeneration was not due to the growth of competing tree species nor due to the expansion of the bush cover, but rather to a direct effect of pathogen spillover from the canopy. No signs of facilitation were observed for the studied diseases, i.e. indirect effects of one disease causing an impact through the other disease were not supported in either canopy pines or in recruits. Our results suggest that climate posed a strong constraint on forest disease and modulates their interaction directly by affecting pathogen distribution rather than via host susceptibility. In conclusion, the impacts caused by two different pathogens could be dissected by considering climatic constraints, host ontogeny and structural specificity, thus explaining forest health at a regional scale.

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Author contributions

Maria Caballol: Conceptualization (lead); Formal analysis (lead); Investigation (lead); Writing – original draft (lead); Writing – review and editing (lead). **Francesc Serradó**: Investigation (supporting); Writing – review and editing (supporting). **Irene Barnes**: Investigation (supporting); Writing – review and editing (supporting). **J. Julio Camarero**: Investigation (supporting); Writing – review and editing (supporting). **Cristina Valeriano**: Investigation (supporting); Writing – review and editing (supporting). **Michele Colangelo**: Investigation (supporting); Writing – review and editing (supporting). **Jonàs Oliva**: Conceptualization (lead); Investigation (lead); Writing – review and editing (lead).

Data availability statement

Data are available from Figshare: https://doi.org/10.6084/m9.figshare.24211398 (Caballol et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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