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Emerging Needle Blight Diseases in Atlantic Pinus Ecosystems of Spain

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Abstract: Red band needle blight caused by *Dothistroma septosporum* and *D. pini*, and brown spot needle blight caused by *Lecanosticta acicola* provoke severe and premature defoliation in *Pinus*, and subsequent reduction of photosynthetic surfaces, vitality, and growth in young and adult trees. The recurrent damage results in branch and tree death. Until recently, pine needle blight diseases have had only minor impacts on native and exotic forest trees in the North of Spain, but in the past five years, these pathogen species have spread widely and caused severe defoliation and mortality in exotic and native plantations of *Pinus* in locations where they were not detected before. In an attempt to understand the main causes of this outbreak and to define the effectiveness of owners' management strategies, four research actions were implemented: a survey of the management activities implemented by the owners to reduce disease impact, the evaluation of specific symptoms and damage associated with infection, and the identification of the causative pathogenic species and their reproductive capacity. Morphological characteristics of the fungus and molecular identification were consistent with those of *Lecanosticta acicola* and *Dothistroma* spp., *D. septosporum*, *D. Pini*, and both mating types were present for the three identified pathogens. The local silvicultural management performed, mainly pruning and thinning, was not resulting in the expected improvement. The results of this study can be applied to establish guidelines for monitoring and controlling the spread of needle blight pathogens.

Keywords: needle blight; *Pinus*; defoliation; *Dothistroma*; *Lecanosticta*

1. Introduction

Red band needle blight caused by *Dothistroma* spp. and brown spot needle blight caused by *Lecanosticta acicola* (Thümen) H. Sydow are serious forest diseases in many countries [1–4], particularly when conifers, mainly *Pinus radiata* D. Don, are planted out of their native American forest regions and in European plantations, although recent evidence suggests that in Scandinavia and other

Northern-European countries native *P. silvestris* L. and *P. nigra* Arnold are also suffering severe defoliation from *Dothistroma* [1].

The symptoms of these two diseases are quite similar, including severe defoliation that results in significant growth loss when more than 25 % of the needles are diseased [2–4]. The diseases have caused major epidemics in *Pinus radiata* (Monterey pine) in the Southern Hemisphere, Central Africa, Chile, New Zealand, and Australia [2,5]. In recent decades, they have also been increasing in incidence and severity in the Northern Hemisphere. Currently, serious epidemics are occurring on *Pinus contorta* var. latifolia Dougl. Loud. (lodgepole pine) in British Columbia, Canada [6,7] on *Pinus nigra* Arnold subsp. laricio (Poiret) Maire (Corsican pine) in Britain [8], and *Pinus radiata* in Spain [9,10]. These pathogens are found in most European countries and their spread coincided with importations and plantations of hosts out of their native areas in Europe, Africa, Australasia, and America [1,11–15].

Although red band and brown spot needle blights occur widely on host species in their native area, plantation monocultures are usually regarded as more susceptible to outbreaks than native ecosystems [16]. They frequently cause the most damage as invasive diseases in exotic plantations and have resulted in the abandonment of planting species such as *P. radiata* in East Africa [3]. In addition to the abundance of host material, possible reasons for the disease increasing in severity and incidence are directional climate changes [7], and the occurrence of both mating types, which would enable sexual reproduction and possible increases in the virulence of these pathogens [17].

These pathogens have spread quickly in Central and Northern Europe [15,18] and their control is difficult due to the large size of the infested areas and the successful adaptation of the fungi to climatic and natural conditions in new areas [19]. Both diseases are listed in the EU Plant Directive as quarantine pests, and controls are focused on seedlings, since this is the only recognized pathway for spreading in regulatory terms [20]. *Dothistroma* needle blight is considered endemic in Britain and Finland [21] and eradication is considered non-viable in Britain [22]. Both mating types are already present in some European countries. Sexual reproduction increases genetic diversity that can promote fungal proliferation in new environments, virulence on native and exotic hosts, and fungicide resistance [15,17,22,23]. It is not yet known if isolates of the pathogen from different countries differ in virulence. Until this is known it is recommended to restrict the transfer of isolates from countries in which disease presence has already been confirmed [24].

The main aim of this study was to evaluate strategies to prevent the spread of these pathogens that are employed in the region of Northern Spain. The incidence and severity of defoliations were evaluated in *Pinus* plantations, the main causal agents of needle blight and their corresponding mating types were identified, and the effectiveness of the disease management activities performed by local owners was analyzed.

2. Materials and Methods

2.1. Study Area, Field Observations, and Sampling

This study covers forest ecosystems (natural forest and plantations) in the Spanish Atlantic climate region. Field observations and sampling were conducted from spring to late summer in 2015. Surveys were conducted in 311 plots across 1650 km^2 of primarily radiata pine plantations. The plots were randomly located along accessible tracks and averaged 2.25 ha in size. Needle samples from 5 to 10 trees per plot were collected and transported in a cooling box to the laboratory. These were used to identify the causal agents of needle blight. Fungal and foliar samples were maintained in a collection at the technological institute Neiker in Arkaute (Spain).

For each plot, the percentage of trees (scale = 0–100) affected by the disease, disease incidence (S inc), and the severity (scale of 0–1) of the disease (A sev) in affected trees was estimated using the 5% step method. The stand level product of these measurements (S inc × A sev) was used to determine the severity of needle blight within each plot (S sev, scale = 0–100) [25].

Evaluation of damages in the stands was conducted by checking perimeters and interior areas to determine the mean level of damage. For stands with up to 15% overall damage, a general estimation after a tour through the stand was carried out. If the level of infection was 15% or over, an evaluation of 100–200 trees was implemented, following a transect through the longest axis of the area.

Additional information was compiled about age of the trees and management activities implemented in the sampled plantations aimed to reduce the disease impact (high pruning, low pruning, no pruning, thinning, and disposal of pruning waste).

2.2. Laboratory Analyses

Reliable identification of pathogens was performed through evidence of the characteristic conidia in the anamorphic state and by using molecular methods. Needles with brown spot and red band symptoms were sampled and immersed in NaOCl (commercial bleach, 2% active chlorine) for 60 s, and then rinsed in sterile water. Fruiting bodies and spores were observed by optical microscopy of typical conidiospores, which are produced in the conidiomata developed on symptomatic needles that were sampled directly or after incubation in a humid chamber.

In addition, molecular methods were employed to confirm morphological identification of the fungi. DNA samples were obtained from symptomatic and asymptomatic needles using the extraction Kit DNeasy Plant Mini Kit (QIAGEN Gmb, Hilden, Germany).

Blight species were identified using specific primers (LAtef.F, LAtef.R, DStub2-F, DStub2-R, DPtef-F, and DPtef-R) [26]. PCR conditions consisted of PCR buffer (500 mM KCl, 100 mM Tris-HCL pH 8.8, 0.1% Tween-20, 15 mM $MgCl_2$), 200 µM dNTP, 8 pmol of each specific primer, 0.5 U *Taq* DNA Polymerase (BIORON GmbH, Ludwigshafen am Rhein, Germany), and 10–20 ng DNA template in a total volume of 20 µL. Cycling conditions consisted of 10 min denaturation at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 60 °C, 45 s, at 72 °C, and a last extension at 72 °C for 10 min.

Mating types for each detected species were identified using specific primers. Primers specific for *L. acicola* were Md MAT1-1F, Md MAT1-1R, Md MAT1-2F, and Md MAT1-2R [27]. PCR conditions consisted of PCR buffer (500 mM KCl, 100 mM Tris-HCL pH 8.8, 0.1% Tween-20, 15 mM $MgCl_2$), 200 µM dNTP, 6.4 pmol of each specific primer, 0.5 U *Taq* DNA Polymerase (BIORON GmbH, Ludwigshafen am Rhein, Germany), and 10–20 ng DNA template in a total volume of 20 µL. Cycling conditions consisted of 5 min denaturation at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 58 °C, 45 s, at 72 °C, and a last extension at 72 °C for 7 min.

For *D. septosporum* (G. Doroguine) Morelet (as '*septospora*') and *D. pini* Hulbary mating type identification the following specific primers were used: DseptoMat1f *Dothistroma septosporum MAT1-1-1*-specific primer. DpiniMat1f2 *D. pini MAT1-1-1MAT1*-specific primer, DotMat1r *Dothistroma MAT1-1-1*-specific primer, DseptoMat2f *D. septosporum MAT1-2*-specific primer, DpiniMat2f *D. pini MAT1-2*-specific primer, DotMat2r *Dothistroma MAT1-2*-specific primer, for *D. pini*, and *D. septosporum* [17]. The same PCR procedure described for the characterization of *MAT1-1* and *MAT1-2* sequences in *L. acicola* DNA samples were applied. The cycling profile used was: denaturation at 94 °C for 5 min followed by 40 cycles at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min.

PCR products obtained were separated by electrophoresis at 100 V for 30 min on a 1.5% (w/v) agarose gel in 1× Tris-acetate-EDTA buffer (0.4 M Tris, 0.05 M sodium acetate, and 0.01 M EDTA, pH 7.8) and visualized under UV light.

2.3. Statistical Methods

As a preliminary exploratory analysis, multiple correspondence analysis (MCA) was applied on the categorized variables to represent the relationships between the variables. MCA is analogous to principal component analysis (PCA) for categorical (qualitative) variables and allows the projection of samples and variables in a reduced space, facilitating visual interpretation for large datasets. This analysis converts a matrix of data into a graphical display known as factor planes. The rows

and columns of the matrix are plotted (or represented) as points in the factor planes and allow a geometrical representation of the information [28].

In addition, a binary logistic regression model was used to complement the MCA findings. Binary logistic regression is a special type of regression where one dependent binary variable (presence/absence of the pathogen) is related to a set of explanatory variables, listed in Table 1.

Table 1. Characteristics of the plantations (n = 311) included in this study.

Characteristic	Category Codes	Description of Characteristic	Detection of Needle Blight Species Positive	Negative	Number of Plantations
Age (years)	1	<15	28	30	58
	2	15–<20	43	22	65
	3	20–<30	55	39	94
	4	≥30	39	55	94
Pruning	0	no pruning	78	89	167
	1	low	72	39	111
	2	high	17	16	33
Severity (%)	1	<30	28	68	96
	2	30–<60	33	26	59
	3	60–<90	51	18	69
	4	≥90	64	23	87
Defoliation at the base (defb %)	1	<20	16	99	115
	2	20–<40	37	28	65
	3	40–<60	37	6	43
	4	60–<80	46	2	48
	5	≥80	36	4	40
Defoliation at the middle (defi %)	1	<20	24	106	130
	2	20–<40	49	15	74
	3	40–<60	51	8	59
	4	60–<80	18	3	21
	5	≥80	27	0	27
Defoliation at the top (deft %)	1	<20	106	125	231
	2	20–<40	36	7	43
	3	40–<60	14	0	14
	4	60–<80	17	1	18
	5	≥80	5	0	5

3. Results

3.1. Fungal Species, Host Distribution and Mating Type Detection in the Studied Area

Morphological characteristics of the lesions and molecular identification of the fungi obtained from trees displaying symptoms of brown spot and red band needle blights were consistent with those of *L. acicola*, and *D. septosporum* or *D. pini*, respectively (Figure 1); the expected PCR product sizes is indicated for each fungal species.

The frequency of species of pine blight pathogens identified in various host trees is shown in Table 2. *L. acicola* was most common (in 44.7% of samples) and was mainly detected on *P. radiata*. *D. septosporum* was present in 10% of samples and was found predominantly on *P. nigra*. Occasionally two fungal species were detected in the same sample (*D. septosporum* and *L. acicola*, or *D. septosporum* and *D. pini*; in 1.6% of the samples). Detection of *D. pini* was rare (in less than 1% of samples), and it was always detected with *D. septosporum*. In terms of stand age, 81% of the plantations in which needle blight species were found were less than 25 years old.

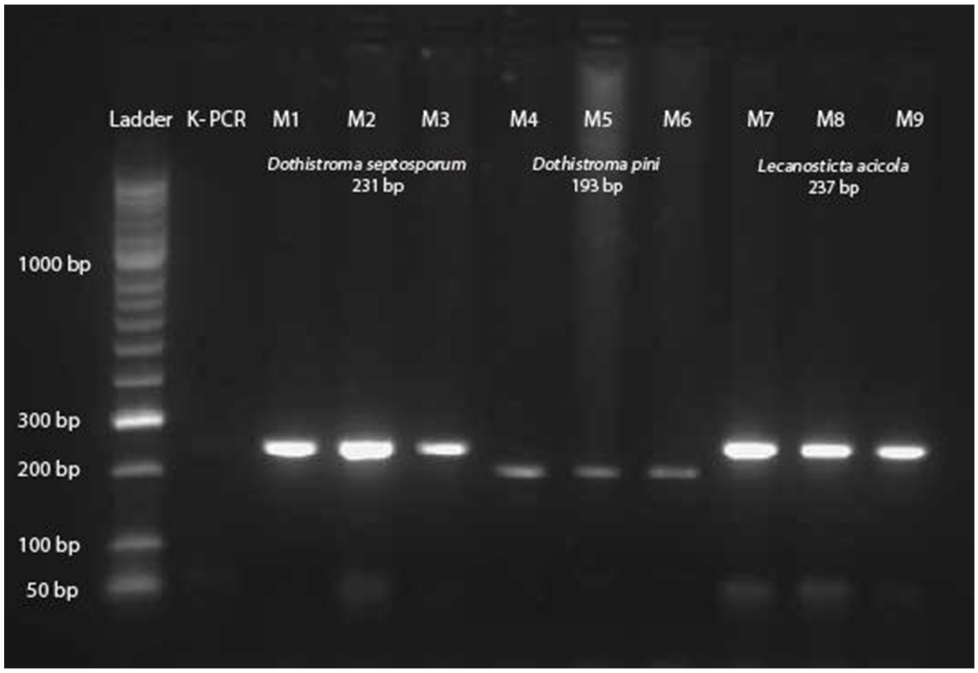

Figure 1. Polymerase chain reaction products using specific primers to detect *Lecanosticta acicola* (LAtef.F, LAtef.R), *Dothistroma septosporum* (DStub2-F, DStub2-R), and *D. pini* (DPtef-F, DPtef-R). The names M1-M9 at the top of the gel refer to sample numbers.

A high diversity of mating types was detected in the study area. Different mating types of *Dothistroma* spp. and *L. acicola* were present in samples from trees exhibiting symptoms of red band and brown spot needle blight (Figure 2). All samples showed either Mat 1, Mat 2, or both Mat 1 and Mat 2. *L. acicola* Mat 1 was detected in 75% of the samples, Mat 2 in 16%, and both Mat 1 and Mat 2 in 8% of the cases. For *D. septosporum,* Mat 2 was most frequent in 63% of samples, Mat 1 appeared in 13% of the samples, and both Mat 1 and Mat 2 were present in 24% of the plantations where this species was detected. *D. pini* was only present in three samples; Mat 2 in two samples, and Mat 1 and Mat 2 together in the third.

Table 2. Frequency of detection of species of *Dothistroma* and *Lecanosticta acicola* in relation to species of hosts.

Fungal spp./Hosts	*nig* [1]	*hal* [2]	*pin* [3]	*pine* [4]	*rad* [5]	*syl* [6]	*men* [7]	*Total*
None detected	44	3	15	1	70	9	1	143
D. septosporum	21	0	2	0	0	2	1	26
D. septosporum and *D. pini*	3	0	0	0	0	0	0	3
D. septosporum and *L. acicola*	0	0	0	0	1	1	0	2
Lecanosticta acicola	3	0	0	0	134	0	0	137
Number of plots	72	3	17	1	205	12	2	311

[1] *Pinus nigra*, [2] *P. halepnesis* Mill., [3] *P. pinaster* Aiton, [4] *P. pinea* L., [5] *P. radiata*, [6] P. sylvestris, [7] *Pseudotsuga menziesii* (Mirb.) Franco.

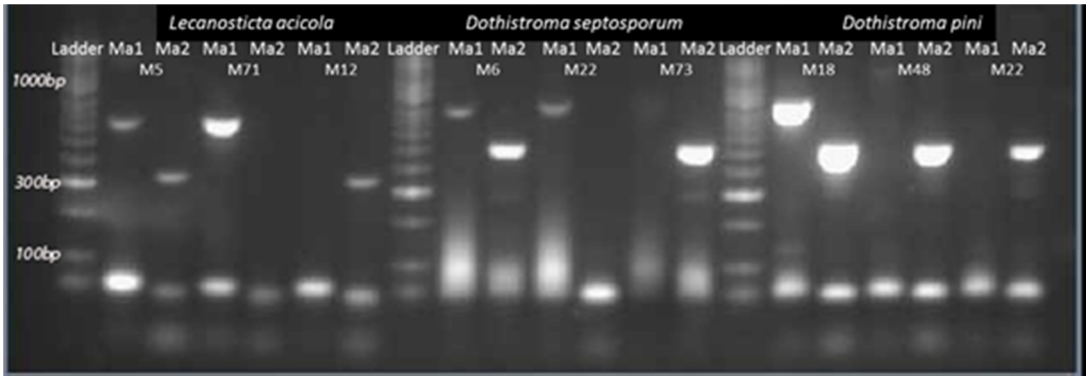

Figure 2. PCR amplicons of *L. acicola*, *D. septosporum*, and *D. pini* obtained using the MAT primers in PCR. The numbers at the top of the gel refer to sample number; Ma 1 Ma 2 refer to mating type MAT 1 and MAT 2, respectively; M5, M71, M12, M6, M22, M73, M18, M48, and M22 refer to sample numbers.

3.2. Disease Impact and Its Connection with Implemented Silvicultural Management

Field characteristics of the studied plantations were recorded including the age and disease status of the plots as well as silvicultural practices implemented by the owners (Table 1). In most plantations trees were not pruned. Where they were pruned the pruning was directed to remove live or dead branches for further improvement of crops to produce knot free wood.

The estimated severity of needle blight in plantations ranged from 5% to 95%. Defoliation was greater at the base of the diseased trees (55.6% $\pm$ 27.8%), and less in the middle (43.5% $\pm$ 26.1%) and at the top (18.2% $\pm$ 21.7%) of the diseased trees. In comparison, in plantations where the disease was not present 16.6% $\pm$ 11.6%, 12.4% $\pm$ 7.8%, and 5.8% $\pm$ 12% of the trees were defoliated at the base, middle, and top, respectively. Defoliation at the base was three times greater in infested plantations. Defoliation at the base in healthy plantations can be associated with self-pruning due to the lack of light.

In MCA (Figure 3), the spread of the category quantifications for every variable is represented, and reflects the relationships between variables in each dimension. MCA revealed that the first horizontal dimension explained 45.6% of the total inertia (variance), as the first factor plane represents the largest inertia, while the second vertical dimension explained 26.3%. Additional dimensions explained less than 1.5% each and hence had no practical significance (Table 3). With respect to the damage caused by these diseases, the first dimension is related to detection and disease symptoms (mainly defoliation at the base and in the middle part of the trees) and the second dimension is related also to defoliation at the base and intermediate part of the trees. Figure 4 shows, for each variable, a measure of its importance, which can be regarded as a squared component loading that is computed for each dimension. This measure is also the variance of the quantified variable in that dimension. Variables such plantation age and pruning, located very close to the origin, do not highlight correspondence in any dimension. With regard to management practices in the plantations, statistically significant differences were not observed in the disease severity of unhealthy plots subjected to different management activities such as extent of thinning or removal of diseased branches after pruning ($p > 0.05$; χ^2 test).

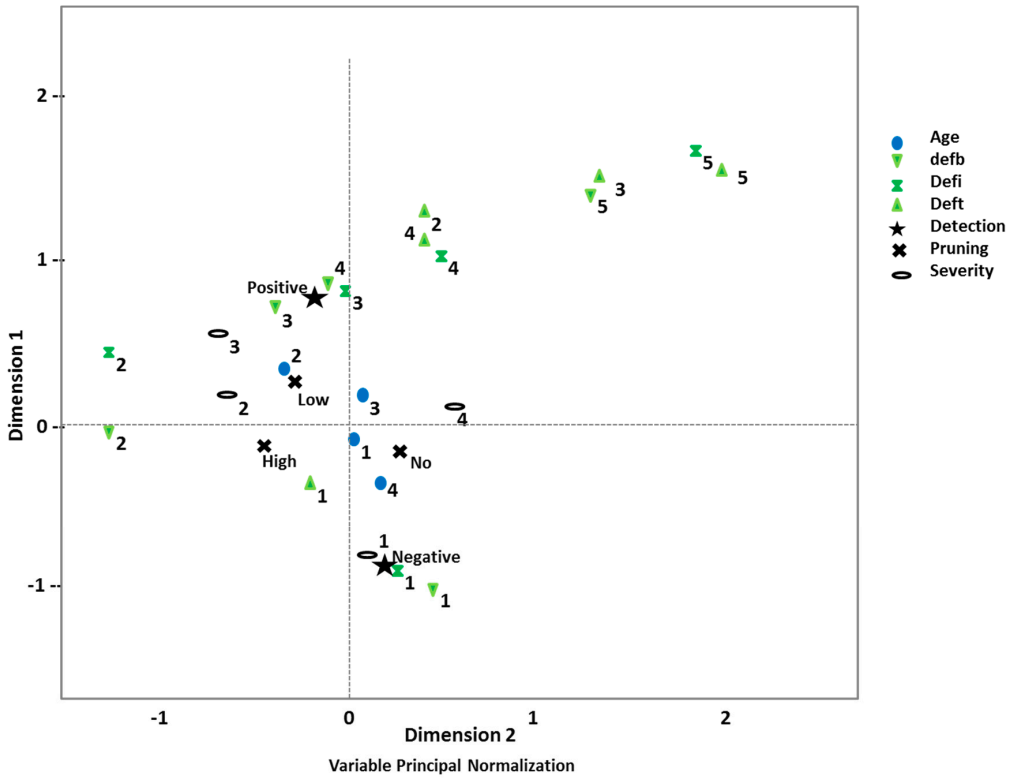

Figure 3. Relationship among fungal infection severity, symptoms, and tree characteristics in the surveyed plantations. Variety and dimensions of dichotomized presence absence of needle pathogenic species, healthy vs. unhealthy, visualized with multiple correspondence analysis and the rest of the variables: Age, defb = defoliation of the tree at the base; defi = defoliation at the middle of the tree; deft = defoliation at the top of the tree; Pruning, severity = severity of the disease.

Table 3. Logistic Regression Analysis of disease detection: Factors explaining disease development in plantations of *Pinus* (n = 311).

Variables	B (S.E.)	Wald	ODDS (C.I. 95%)
Age		5.37	
Age (1)	1.27 (0.70) *	3.24	3.54 (0.89–14.08)
Age (2)	1.50 (0.71) **	4.35	4.47 (1.09–18.23)
Age (3)	0.64 (0.60)	0.98	1.9 (0.53–6.75)
Pruning	ns	0.91	
Waste	ns	0.001	
Thining	ns	0.002	
Severity		15.98	
Severity (1)	−1.69 (0.76) **	4.96	0.18 (0.04–0.81)
Severity (2)	−0.81 (0.61)	1.74	0.44 (0.13–1.48)
Severity (3)	1.11 (0.69)	2.52	3.03 (0.77–11.89)
defb		16.07	
defb (1)	−2.89 (1.33) **	4.73	0.06 (0.00–0.75)
defb (2)	−1.57 (1.14)	1.89	0.21 (0.02–1.95)
defb (3)	0.53 (0.97)	0.3	1.71 (0.25–11.53)
defb (4)	3.28 (1.42) **	5.28	26.56 (1.62–43.53)
defi	ns	5.71	
deft	ns	0.82	

p values: ns, ≥0.10; *, <0.10; **, <0.05. Model Chi^2 (1) = 168.50, $p < 0.001$; Nagelkerke R^2 = 0.70; B, Coefficient for the constant or "intercept"; S.E., standard error around the coefficient for the constant; Wald, Wald criterion; ODDS and 95.0% C.I., Exponentiation of the B coefficient, which is an odds ratio (this value is given by default because odds ratios can be easier to interpret than the coefficient, which is in log-odds units); C.I., Confidence Interval.

The logistical analysis was conducted to predict detection of blight needle disease using as predictors the coded variable of age, defoliation at the base, in the middle, and at the top of the trees, plot severity, and management activities. A test of the full model against a constant only was statistically significant, indicating that the predictors as a set reliably distinguished between positive and negative detection of needle blight (chi square = 168.551, $p < 001$ with $df = 21$). Nagelkerke's R^2 of 0.70 indicated a moderately strong relationship between prediction and grouping. Prediction success overall was 87.4% (81.2% for negative detection and 90.8% for positive detection of the pathogens). The Wald criterion demonstrated the significant contribution of predictors to the model ($p < 0.05$). The most significant factor was the highest defoliation on the base defb (4), $p < 0.05$ and ODD = 26.56, followed by Age (1) and Age (2) ($p < 0.05$; ODDs 3.54 and 4.46, respectively) and Severity (1) ($p < 0.05$; ODD = 0.18). Management activities such as pruning and removal of pruning debris "waste" did not have a significant effect on the disease severity ($p > 0.05$) (Table 3).

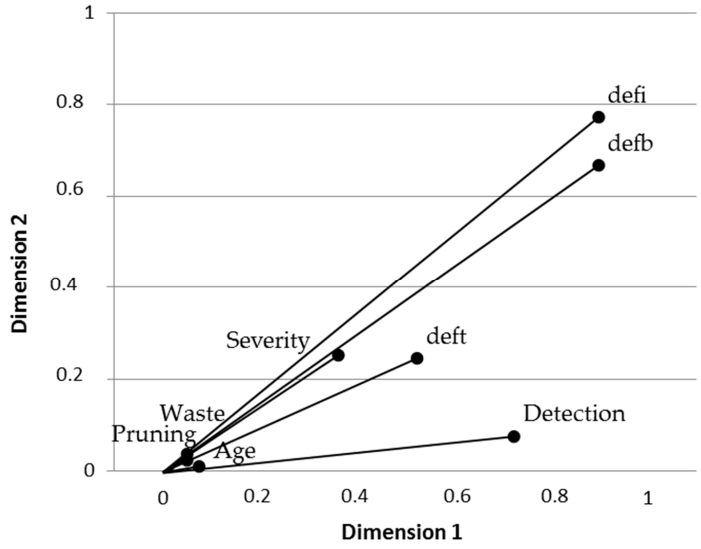

Figure 4. Multiple correspondence analysis (MCA) dimensions contain variances, indicating which variables are related along which dimension.

This study revealed two distinctive and significant dimensions of disease presence. There was a clear relationship among age (≤2), disease severity (1), high defoliation at the tree base (4), and damage to trees from the pathogens. On the other hand, the management activities carried out in infested plantations in an attempt to reduce the negative effect of the diseases had a low or null effect on the extent of damage. There were no differences in detection or severity of the disease associated with the traditional management activities that are normally recommended to reduce the disease impact.

4. Discussion

The purpose of this work was to determine the extent of needle blight diseases caused by pathogenic fungi in susceptible forest ecosystems in the North of Spain and the impact of management activities in disease development. The fungal species causing needle blight and their corresponding mating types were identified, their severity was evaluated, and the effect of the main management activities performed by local owners in infested plantations was analyzed. Assessment methods of the disease were selected to identify factors correlated with disease incidence in areas where management practices are possible. The study was not aimed at comparing presence vs. absence of disease, but rather it was designed to discriminate between factors associated with high or low disease levels where disease was confirmed.

In this study we discovered that three needle blight pathogens, *L. acicola*, *D. septosporum*, and *D. pini* are present in Spain and, moreover, that both mating types for each are present, in some cases in the same plantation. The presence of both mating types of the three species in the same plantations could indicate the presence of sexual crossing and the potential to increase the development and spread of the diseases. In this context of increased genetic diversity of the pathogens, the implementation of successful control measures becomes even more complicated. It has been reported that the introduction of the second mating type of a pathogen can aggravate the disease severity and it may increase the resistance of the pathogens to chemical or biological treatments due to a rapid increase in virulence as a consequence of genetic exchange [29].

The symptoms of both red band and brown spot needle blights are most severe at the lower and middle part of the crowns and in trees less than 25 years old. This is the standard rotation age for *P. radiata* in New Zealand and Europe and it could be the reason why the symptoms are more prevalent in these plantations because in contrast to other *Pinus* species, there is some evidence that *P. radiata* trees develop resistance to the fungus gradually as they mature [11,30–32].

Two very different forest management strategies are currently in place in the plantations in the study area. Although some owners are still undertaking major pruning, a large part of the collective is managed using low cost methods that do not involve pruning and thinning regimes. On plantations where pruning and thinning are implemented, only low pruning (to about 2.2 m when the trees are 8–10 years old) or high pruning (to about 5.5 m at the age of 13–15 years) are employed, and usually only a single thinning. The pruning height does not exceed half of the tree height [33].

Silvicultural practices such as thinning, pruning, and removal of pruning debris are conducted in these plantations mainly for two objectives. Firstly, they increase the value of the wood products. These silvicultural practices encourage trees to develop a strong structure and produce knot-free wood. Knots are the primary reason for reduction in lumber value. Secondly, they improve tree health by increasing the airflow through the stands, making the microclimate conditions less favorable to disease development. Removing broken or damaged branches encourages wound closure and prevents diseases from entering the tree [34,35].

Our study revealed that silvicultural practices did not significantly reduce needle blight disease severity in infested plantations, in contrast to expectations. Although they did not eliminate the pathogens, these practices have previously been reported to reduce the inoculum and the disease level. Bulman et al., Gadgil, and Mullet et al. [4,36,37] showed evidence for a reduction in disease levels from thinning and pruning of the lower branches in at least one season. However, other authors [38–40] did not report positive effects of low pruning on disease reduction, which is consistent with our observations. In two cases [39,40] the lack of an observed effect was attributed to the size of the blocks used in the trials. On the other hand, Gibson et al. [41] reported some evidence that pruning may accelerate the onset of mortality in affected stands. In one highly infested plantation, unpruned plots had 2.8% mortality compared with 8.8% mortality in pruned plots one year after treatment.

The plantations evaluated in this study implemented management practices at different times; some plantations were recently pruned and thinned and others were pruned and thinned several years ago (>5 years). This may explain the observed effect on needle blight disease severity of these practices. In addition, the strong influence of climate on the incidence of needle blight diseases may mask the effect of thinning and pruning since the infested plantations in this study are located for the most part (84%) in a region with the highest climate risk factors which may influence the development of needle blight disease [7,42–44]. Climate change may impact tree health and place managed plantations at high risk by altering the disturbance dynamics of native forest insect pests and microbial pathogens, as well as facilitating the establishment and spread of nonindigenous species [45]. Changes in the patterns of disturbance by forest pests are expected under a changing climate as a result of warmer temperatures, changes in precipitation, increased drought frequency, and higher carbon dioxide concentrations [46].

In the case of the studied area in the North of Spain, climate projections under greenhouse gas emission scenarios indicate that this area will experience changes in climate throughout the 21st century,

including warming of surface air (especially heat wave episodes) and intensification of extreme daily rainfall (10%). Observations made in the studied area throughout the 20th century indicate increases (albeit slight) in air temperature and mean sea level that are in agreement with these projections. The result may be changes in the regime of flood events and the torrential character of the draining rivers [47].

In recent years there has been a drastic intensification in the severity of red band needle blight caused by *D. septosporum* and *D. pini* and brown spot needle blight caused by *L. acicola* in western Canada, the United States, and Europe [6,7,19,48]. The decline in forest health in these countries could be explained by a combination of factors including the presence and high density of the host, the cosmopolitan nature of the fungal species present in the regions with tropical, subtropical, temperate, Mediterranean, Atlantic, continental, and subarctic climates, climate conditions suitable for pathogen growth, and directional climate change that improves growth conditions [7,43,49].

To minimize the environmental and economic impact of needle blight disease, and encourage the sustainability of the most susceptible forest ecosystems, cultural practices and control strategies may require the combination of several methods [4,10,50]. Cultivation of alternative tolerant, resistant, or non-host forest species adapted to the local growing conditions is recommended in areas with the highest disease risk. However, since disease resistance is believed to be associated with tree maturation [32], prevalence of natural biological control agents, and genetic diversity of fungal populations [17], application of biological and copper-containing fungicides, and the use of fertilizers could encourage optimal growth of the trees and contribute to the recovery from fungal damage and other stressful factors, especially in seedlings and young plantations [51]. These measures do not eradicate the causal pathogens, and the application of chemical treatments in these ecosystems is not common, especially in adult plantations. In addition, there is a reluctance to apply aerial fungicides in forest ecosystems in EU and in New Zealand [44]. Exploration of new host species and provenances, breeding for increased resistance, and forest diversification have been reported as the key options to improve disease management in the future [10].

5. Conclusions

Needle blight is an economically important disease in many parts of the world and estimates of the economic injury level made by different researchers are consistent. Growth losses have been demonstrated when disease levels reach about 20% of the crown. The disease impact and fungal diversity of the pathogens revealed in this study suggest that implementation of successful control measures is not an easy task. The wide spread of the disease, its severity in plantations in which silvicultural management practices were implemented, the presence of three different pathogens identified as species causing severe defoliation, and the report of two mating types in the tree species complicate efficient control strategies.

Despite the fact that there are several options for disease control, each option has to be weighed against the availability of resources, cost of implementation, and political, environmental, and market restrictions. Substitution of a non-susceptible tree species for one that is highly susceptible may seem a reasonable measure but, in the studied area, widespread planting of a new species may not be feasible, especially when processing plants and markets are set up for the susceptible species. In addition, it is difficult to guarantee the future health of substitute forest species in the context of a changing climate and the global trade in wood products that is conducive to the introduction of pathogens.

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