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Factors Affecting the Distribution of Pine Pitch Canker in Northern Spain

Lior Blank [1,*], Jorge Martín-García [2,3], Diana Bezos [3,4], Anna Maria Vettraino [5], Helena Krasnov [1], José M. Lomba [3,4], Mercedes Fernández [3,6] and Julio J. Diez [3,4]

[1] Department of Plant Pathology and Weed Research, ARO, Volcani Center, Bet Dagan 50250, Israel; hkrasnov@gmail.com

[2] Department of Biology, CESAM (Centre for Environmental and Marine Studies), University of Aveiro, Campus Universitario de Santiago, 3810-193 Aveiro, Portugal; jorge.martin@ua.pt

[3] Sustainable Forest Management Research Institute, University of Valladolid—INIA, Avenida de Madrid 44, 34071 Palencia, Spain; diana.bezos@uva.es (D.B.); josemalomba@gmail.com (J.M.L.); mffernan@agro.uva.es (M.F.); jdcasero@pvs.uva.es (J.J.D.)

[4] Department of Plant Production and Forest Resources, University of Valladolid, Avenida de Madrid 44, 34071 Palencia, Spain

[5] Department for Innovation in Biological, Agro-food and Forest systems (DIBAF) University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy; vettrain@unitus.it

[6] Department of Agroforestry Sciences, University of Valladolid, Avenida de Madrid 44, 34004 Palencia, Spain

* Correspondence: liorb@volcani.agri.gov.il; Tel.: +972-(3)9683581

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Abstract: *Fusarium circinatum* is the causal agent of pine pitch canker disease (PPC), affecting *Pinus* species and other conifers (i.e., *Pseudotsuga menziesii* (Mirb.) Franco.), forming resinous cankers on the main stem and branches and causing dieback in the terminal guide. This pathogen is spreading worldwide, causing economic losses by converting plantations into standing timber without any potential for future production. The disease was recently detected in Northern Spain in plantations of *Pinus radiata* and forest nurseries. The aim of the work reported here was to study the role of climatic and topographic variables, soil properties, and stand characteristics on PPC. For this purpose, we surveyed 50 pine stands in Cantabria and quantified the percentage of trees showing three symptoms in each stand: canker, defoliation, and dieback. We investigated the predictive power of 30 variables using generalized linear models and hierarchical partitioning. Both approaches yielded similar results. We found that the three symptoms correlated with different explanatory variables. In addition, more trees exhibited cankers in the proximity of the coast and the Basque Country. Additionally, our results showed that low canopy cover is related to a high level of the dieback symptom. Overall, this study highlights the important variables affecting the distribution of PPC in Cantabria.

Keywords: forest epidemiology; *Fusarium circinatum*; generalized linear models; hierarchical partitioning; *Pinus radiata*

1. Introduction

Pine pitch canker (PPC) disease is caused by the fungus *Fusarium circinatum* [1] (Teleomorph = *Giberella circinata*), a regulated pathogen under the EU legislations [2]. PPC is wide spread worldwide [3]. The disease was first reported in North Carolina (USA) [4], but since then it was also observed in California (USA) [5], Haiti [6], Chile [7], South Africa [8], Japan [9], Mexico [10], Korea [11], Uruguay [12], Colombia [13], and, more recently, Brazil [14]. In Europe, the first report was in Spain [15] in 2005, and the pathogen has also been reported in France [16], Italy [17], and Portugal [18]. In Italy and France it is now considered eradicated, whereas in Spain and Portugal the disease is established in the forests.

Fusarium circinatum has been found to be pathogenic to over 60 pine species and also to Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.), both in native and non-native forests [3,19–21]. Of these species, Monterey pine (*Pinus radiata* D. Don) is considered to be the most susceptible [22]. *Pinus radiata*, a native species to California (USA), Guadalupe, and Cedros Islands (Mexico), was introduced in the Basque Country (Spain) during the first half of the nineteenth century for commercial purposes because the climatic conditions are similar to its place of origin. Today, this is the most common exotic conifer in northern Spain covering an area of 200,000 ha [23].

Fusarium circinatum causes severe symptoms in mature trees, such as resin bleeding, deformations, and frequent formation of cankers on the trunk or thicker branches. In the crown, symptoms include defoliation, dieback, and presence of red shoots [3] (Figure 1). The increase in the resin production is due to the increment on the number of traumatic resin ducts (TRDs); this could benefit *F.circinatum*, since epithelial cells surrounding the TRDs have starch that the fungus uses for feeding [24]. The fungus can penetrate into the xylem, interrupting the sap flow and girdling the tree or big branches. This girdling can lead to tree or branch disruption due to wind or storms [4]. The fungus also causes damping off in seedlings, leading to mortality rates of up to 100% [20]. Consequently, this pathogen is considered a threat to pine plantations and wood industry productivity throughout the world.

Fusarium circinatum can be naturally disseminated through spores that can be dispersed passively by wind, rain, or different vectors, such as insects [25–27]. However, infection by spores will usually be effective only in open wounds, where the spores can penetrate [28,29]. Generally, injuries are the result of extreme weather conditions (e.g., hail, wind damage, etc.) [30], insects (wood borers), or mechanical injuries [31]. On the other hand, *F. circinatum* can also be spread by human actions, i.e., trade of infected seeds, asymptomatic seedlings and plant products, infected substrates, and tools/machinery.

According to the EU Plant Health Directive (Directive 2016/2031, in substitution of Directive 2000/29/EC;), *Pinus* spp. cannot be imported as plants for planting, and pine wood and bark should be properly treated. However, pests continue to be intercepted at the EU border on pine tissues (Europhyt database). Eschen et al. (2015) [32] showed that the standard of phytosanitary inspections at the EU border is not homogeneous. Thus, the risk of introductions of *F. circinatum* is still present. Once the pathogen is present in the forest, demarcated areas are delineated to eliminate infected host material and avoid their movement. However, despite environmentally-friendly methods for control [33], sanitation measurements, and a ban on planting susceptible species (*Pinus* spp. and *Pseudotsuga menziesii*) in infected areas (e.g., Spanish Royal Decree 637/2006 and 65/2010), PPC disease is very difficult to eradicate [2].

It is well known that environmental stress [34,35], physiological state of the host [36,37], and forest management [31,38,39] influence the rate of infection and incidence of *F. circinatum* and its pattern of spread. We hypothesized that abiotic factors and forest management play a key role in PPC disease in Spain. The aim of the work reported here was to study the role of climatic and topographic variables, soil properties, and stand characteristics on PPC.

Figure 1. Pine pitch canker (PPC) symptoms of (**a**) cankers, (**b**) defoliation, and (**c**) dieback.

2. Materials and Methods

2.1. Site Description and Sampling Procedure

The study was carried out in the Cantabria province of Northern Spain. This area is west of the Basque Country and east of the province of Asturias (Figure 2). The Cantabrian Sea borders the north of the province, while the Castilla and León regions border the south.

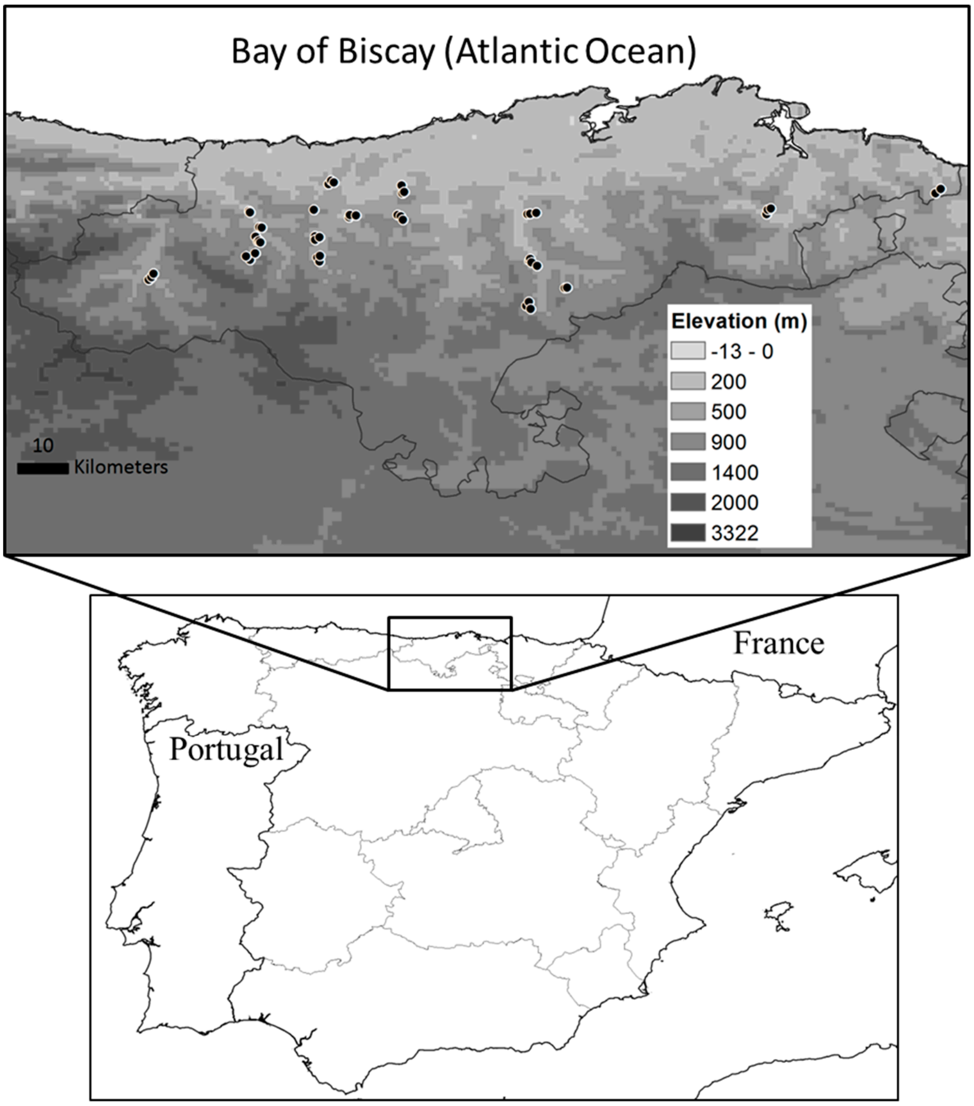

Figure 2. Map of Cantabria (Spain) (**lower** map) and the location of the surveyed sites in Cantabria overlaid on an elevation map (**upper** map).

The ecological and environmental conditions of this area are very conducive to the development of Monterey pine and also PPC: elevation ranges between 0–1000 m asl, warm temperatures (10–14 °C annual average temperature, Atlantic climate) and frequent precipitation (700–2400 mm per year).

A total of 50 plots were selected in areas where the disease was previously detected in order to represent a wide range of environmental and climatic conditions (Figure 2). A minimum distance of 500 m between plots was established. Dendrometric and forest health variables were assessed in 25 trees per plot (i.e., a total of 1250 trees), located near the plot's center. In addition, climatic, soil, topography, and stand characteristic variables of each plot were measured.

Forest health was evaluated by visual assessment of crown and stem conditions following the ICP Forest methodology [40]. We quantified the percentage of trees showing three symptoms in each stand: canker, defoliation, and dieback. At the same time, the diameter at breast height, total height, and canopy cover were measured for all trees. The following stand characteristic variables were quantified: canopy cover (*Canopy*), mean trunk diameter at breast height (*DBH*), stand age (*Age*), trunk perimeter (*Perimeter*), and the tree's mean height (*Height*) (Table 1).

Table 1. Descriptive statistics, across all 50 plots, of the dependent (pathological symptoms) and independent variables used in this study. The proportion of each of the pathological symptoms represents the number of sampled trees out of the 25 in each plot that showed each symptom.

	Variables	Abbreviation	Units	Average	Min	Max
Pathology	Cankers	Canker	proportion	0.20	0.00	0.64
	Defoliation	Defoliation	proportion	0.14	0.00	0.66
	Dieback	Dieback	proportion	0.16	0.00	0.48
Climate	Average annual precipitation	Precipitation	mm	1232	702	1735
	Average annual maximal temperature	Tmax	°C	18.3	17.0	21.0
	Average annual minimal temperature	Tmin	°C	7.7	6.0	9.0
	Average summer temperature	Tm_sum	°C	18.6	17.4	19.8
	Average winter temperature	Tm_win	°C	8.66	7.2	10.2
	Average summer precipitation	Psum	mm	202.1	82.4	251.6
	Average winter precipitation	Pwin	mm	355	165.8	453.3
	Frost period	Frost	Number of months	5.3	3.0	7.0
Topography	Slope	Slope	Degree	15.8	5.0	35.0
	Elevation	Elevation	Meters asl	360.5	92.0	898.0
	Distance from the eastern boarder of Cantabria	distEast	Km	19.3	2.9	39.5
	Distance from the coast	distCoast	Km	19	2.0	39.0
Soil	pH	pH	No units	4.6	3.8	6.6
	Cationic exchange capacity	Conductivity	MS/cm	0.08	0.03	0.6
	Coarse fragments	CF	G/100 gr	5.3	0.0	58.0
	Percentage of sand	Sand	%	56.7	12.6	80.0
	Percentage of silt	Silt	%	17.9	3.3	44.6
	Percentage of clay	Clay	%	24.2	1.4	49.7
	Organic matter	OM	G/100 gr	3.5	0.7	9.4
	Potassium	K	G/100 gr	61.94	13.00	365.00
	Phosphorus	P	Mg/kg	1.2	0.0	5.5
	Calcium	Ca	Meq/100 gr	1.5	0.03	29.1
	Magnesium	Mg	Meq/100 gr	0.25	0.03	0.84
	C/N ratio	CN	No units	11.9	6.2	16.7
	Nitrogen	N	G/100 gr	0.2	0.05	0.44
Stand characteristics	Canopy cover	Canopy	%	44.3	17.7	81.4
	Mean diameter	DBH	cm	25.1	13.8	51.5
	Stand age	Age	years	22.6	5.0	56.0
	Average height	Height	m	16.6	10.5	27.3
	Average perimeter	Perimeter	cm	80.6	47.7	169.3

Five soil samples were collected from the upper 30 cm soil layer in each stand. The first soil sample was taken from the middle of the plot and the rest of the samples were taken two meters away from the first one. The samples were pooled and homogenized to produce one composite sample per plot (Table 1). Particle size distribution was determined by the Bouyoucos method (hydrometer method) (*CF*), and the ISSS (International Society of Soil Science) classification was applied (*Sand*, *Silt*, and *Clay*). The pH was determined potentiometrically with a pH meter in a soil solution (1:2.5, soil:water). Organic matter (*OM*) was determined by the $K_2Cr_2O_7$ method. Total N was determined by Kjeldahl digestion (*N*). Soil available P was extracted by the Olsen procedure and determined photometrically by the molybdenum blue method (*P*). Soil exchangeable cations (K, Ca^{2+} and Mg^{2+}) were extracted with ammonium acetate and determined by atomic absorption/emission spectroscopy (*K*, *Ca*, and *Mg*, respectively). The cationic exchange capacity (*Conductivity*) was determined by Bascomb's method [41].

The topographic and spatial variables studied were: Elevation (*Elevation*), slope (*Slope*), distance from the eastern border of Cantabria (*distEast*) and distance from the sea coast (*distCoast*) (Table 1). The climatic variables include: average annual precipitation (*Precipitation*), average annual minimum (*Tmin*) and maximum (*Tmax*) temperature, mean summer temperature (*Tm_sum*), mean winter temperature (*Tm_win*), mean summer precipitation (*Psum*), mean winter precipitation (*Pwin*), and the number of frost (*Frost*) months. The climatic data were obtained from the Digital Climatic Atlas of the Iberian Peninsula [42]. The maps are based on data from meteorological stations in the Iberian Peninsula. Precipitation values are calculated for at least twenty years and temperatures for at least fifteen years for the 1950–1999 period.

2.2. Statistical Analysis

2.2.1. Spatial Autocorrelation Analyses

Spatial analysis is used to demonstrate that there is no significant spatial autocorrelation at the given sampling scale, in which case classical statistical tests of hypothesis can be used. To assess whether spatial autocorrelation in model residuals could bias statistical testing [43], we calculated global Moran's *I* autocorrelation coefficient to estimate whether the occurrence of the disease exhibits a random spatial pattern. The autocorrelation calculates the Moran's *I* Index value and both the *p* value and Z score, evaluating the significance of the index [44]. The null hypothesis states that there is no spatial clustering of the values associated with the geographic features in the study area. This analysis was implemented using the tool Spatial Autocorrelation Global Moran's *I* in ArcMap10.5.

2.2.2. Univariate Analyses

We used non-parametric Spearman correlations to analyze the degree of correlation between variables. We constructed a correlation matrix between all considered variables belonging to the same group, selecting the one with the higher explained variability and subsequently removing all variables that were highly correlated with it, keeping the maximal variance inflation factor (VIF) at 5.84 for the climatic variables and lower than 3 for the other three variable groups. Neter et al. (1989) [45] suggested that multicollinearity is considered severe when VIF > 10. Variance inflation factor was calculated in R using the function 'vif' from the package 'usdm' [46]. In total, 30 variables were evaluated (Table 1). The following variables were excluded from the multivariate analysis duo to collinearity and high VIF: *Precipitation*, *DistEast*, *Sand*, *Ca*, *DBH*, and *OM*.

2.2.3. Multivariate

The 30 variables collected and quantified were classified into four groups of variables: climate, topography, soil, and stand characteristics. Prior to analysis, data normality was checked using the Shapiro–Wilk tests for normality (''Stats" package implemented in R). The datasets for *Defoliation* and *Dieback* were normally distributed without transformation, while *Canker* had to be log transformed. We used logistic regression in the framework of General Linear Models (GLMs). Multi-model inference based on Akaike's information criterion (AIC) was used to rank the importance of variables [47–49]. We used the package ''glmulti" to facilitate multi-model inference based on all first-order combinations of the variables on each scale (128 models for the climate variables, 8 models for the topographic variables, 1024 models for the soil variables, and 16 models for the stand characteristic variables) [50]. Although comparing all possible models is not usually recommended for model selection [47], we decided not to formulate models based on previous knowledge of the PPC disease. As this is the first intensive work aiming to understand the factors affecting this disease in this part of the world, we did not want to constrain the models to previous findings stemming from works in different regions. The estimated coefficients related to each variable and their relative importance were evaluated using multi-model averages. The importance weight for a variable is the sum of Akaike weights of the models in which the variable was present. Model assumptions were verified, following Zuur and Ieno [51], by plotting Pearson residual versus fitted values (using the function 'residualPlots' in the package 'car' [52]) and space coordinates (using the function 'spline.correlog' in the 'ncf' package [53]). In order to better understand the independent contributions of each variable, we used hierarchical partitioning [54,55] in the package 'Hier.part' [56]. Statistical significances of the independent contributions of the variables were tested using a randomization with 500 repetitions by using the function 'rand.HP'. We calculated R_N^2 values [57] using the package 'fmsb' [58]. All statistical analyses were carried out using R 3.1.0 [59].

3. Results

The presence of the symptoms on trees was high across the study area and each of the symptoms was present in about 90% of the surveyed stands. Only one of the forest stands did not show any of the three studied symptoms (canker, defoliation, or dieback). According to the results of the spatial autocorrelation analysis, there was no significant spatial autocorrelation for the three dependent variables (*Canker*: Moran's $I = -0.18$, p value = 0.25; *Defoliation*: Moran's $I = 0.17$, p value = 0.15; and *Dieback*: Moran's $I = 0.07$, p value = 0.52).

Dieback and *Defoliation* were correlated (r = 0.5; p value = 0.0002) (Table 2). The percentage of trees with *Dieback* was significantly correlated with *Precipitation*, *Pwin*, and *Psum* (r = −0.36, −0.35, and −0.32; p value = 0.01, 0.013, and 0.025, respectively). More trees exhibited the *Cankers* near the sea (r = −0.29; p value = 0.04) and in Eastern Cantabria (close to the Basque Country) compared to the areas in the west (near Asturias) (r = −0.35; p value = 0.014) (Table 2). In addition, *Defoliation* was negatively correlated with *Pwin* and *Psum* (r = −0.3, −0.28; p value = 0.031, 0.047, respectively) (Table 2).

Table 2. Spearman correlation coefficient between the independent variables and the three symptomatic variables. Significant correlations are in bold letters (p value < 0.05). Variables marked with * were removed from further analysis due to collinearity.

Independante Variable	Canker	Defoliation	Dieback
Tm_sum	−0.19	0.15	0.03
Tm_win	0.13	0.09	0.03
Psum	0.08	**−0.28**	**−0.32**
Pwin	−0.14	**−0.3**	**−0.35**
Precipitation *	−0.05	−0.27	**−0.36**
Tmax	−0.08	0.26	0.25
Tmin	0.19	−0.03	0.13
Frost	−0.11	0.09	0.02
Slope	0.11	−0.1	0.08
Elevation	−0.1	−0.12	−0.01
DistEast *	**−0.35**	0.09	−0.12
DistCoast	**−0.29**	0.07	−0.12
pH	0.09	−0.02	0.08
Conductivity	−0.07	0.02	0.17
CF	0	−0.14	0.18
Sand *	−0.24	−0.06	−0.1
Silt	0.11	−0.06	0.05
Clay	0.12	0.14	0.09
OM *	−0.16	−0.01	0.13
P	−0.2	0.1	0.1
K	0.06	−0.09	0.02
Ca *	−0.17	−0.17	0.04
Mg	−0.07	−0.24	0.05
N	−0.19	−0.07	0.13
CN	0.03	0.16	0.09
Canopy	0.03	−0.06	−0.28
DBH *	0.08	−0.07	0.06
Age	0	0.09	0.25
Perimeter	0.05	−0.15	−0.05
Height	−0.01	−0.16	−0.13
Canker			
Defoliation	0.04		
Dieback	0.13	**0.5**	

The best models selected for each symptom in each of the four variable groups are shown in Supplementary Materials (Table S1). According to the validation process, these models did not show spatial correlation. In addition, we found that the relationship between Pearson residuals versus fitted explanatory variables showed no clear violations of the model assumptions (Table S2).

Topographic variables explained the highest percentage of the variation for *Canker* compared to *Defoliation*, and *Dieback* (12%, 1.5%, and 4%) (Figure 3), while the stand characteristics explained 20% of the variation for *Dieback* and only 9% and ~1% for *Defoliation* and *Canker*, respectively (Figures 3–5). The variations of the three symptoms were equally explained by the climatic variables (~20%) (Figures 3–5). Soil variables explained more of the variation in cankers compared to the variation in *Dieback* and *Defoliation* (Figures 3–5).

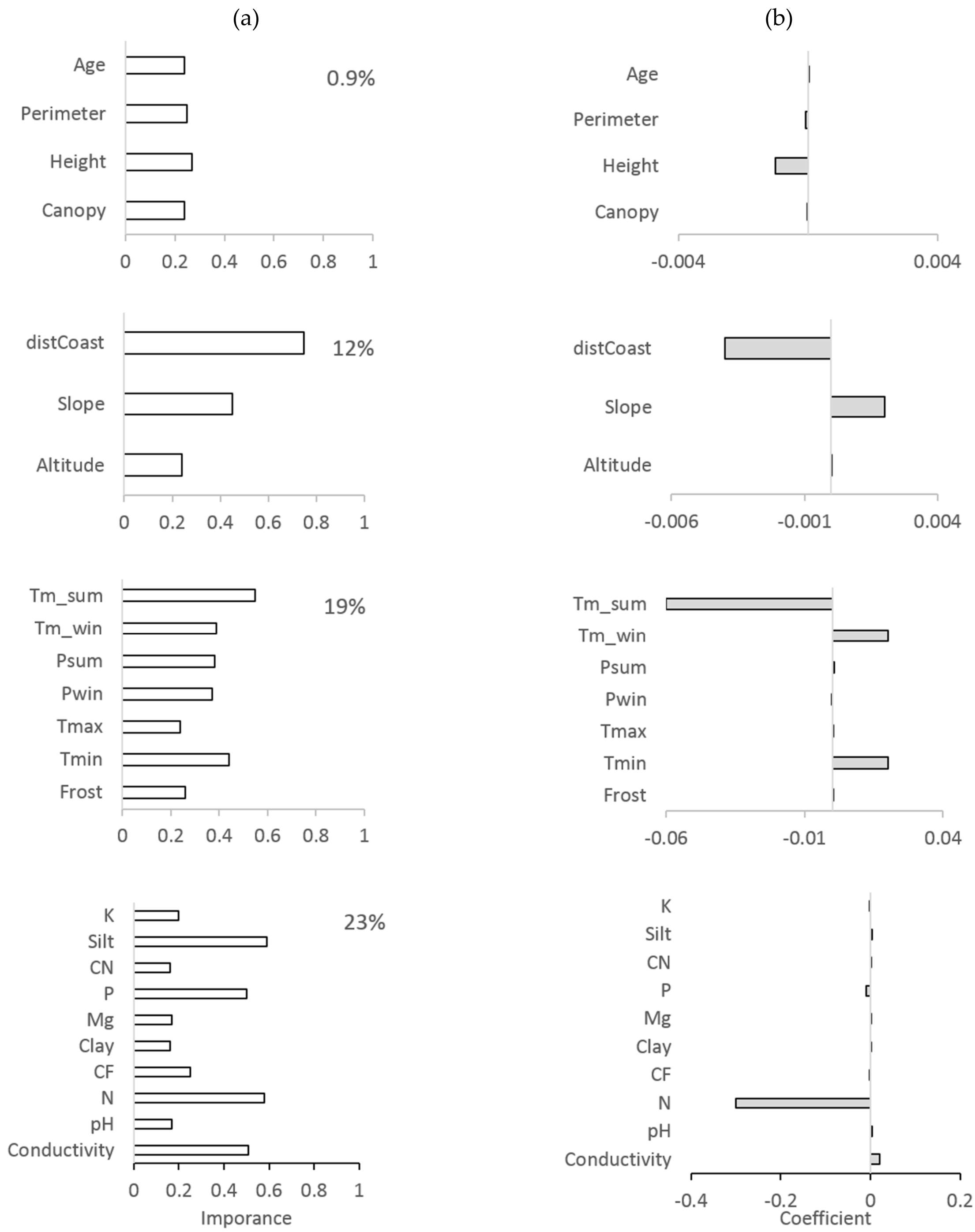

Figure 3. The relative importance (**a**) and the estimated coefficients (**b**) of variables estimated across all fitted GLM models using a multi-model average approach for the *Canker* symptom. We also indicate the percentage of deviance explained by each complete model.

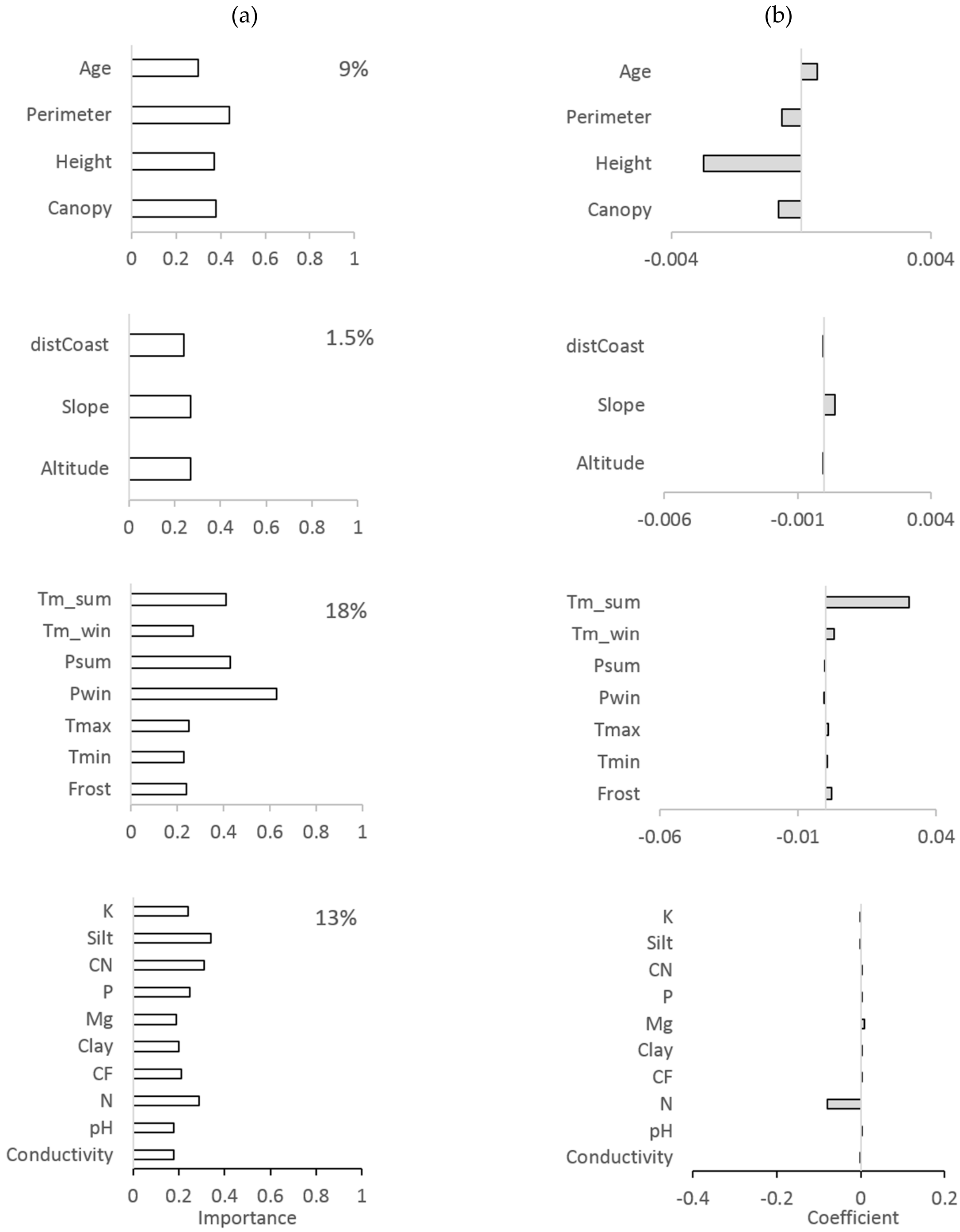

Figure 4. The relative importance (**a**) and the estimated coefficients (**b**) of variables estimated across all fitted GLM models using a multi-model average approach for the *Defoliation* symptom. We also indicate the percentage of deviance explained by each complete model.

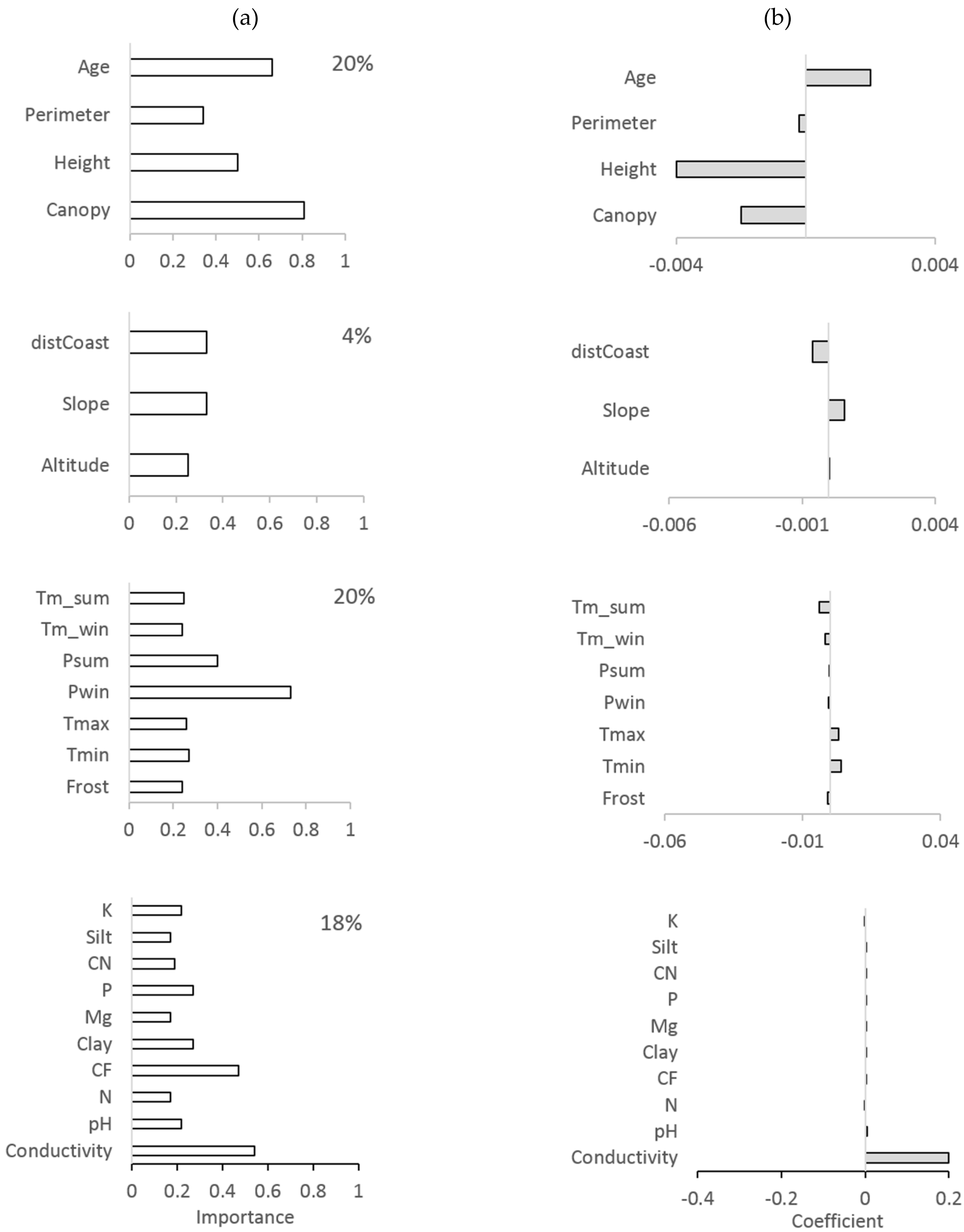

Figure 5. The relative importance (**a**) and the estimated coefficients (**b**) of variables estimated across all fitted GLM models using a multi-model average approach for the *Dieback* symptom. We also indicate the percentage of deviance explained by each complete model.

The variable *DistCoast* was the only variable from the topographic group of variables found to be important and only for the *Canker* symptom (Figures 2 and 6a). This variable had a negative relationship with high and significant independent contribution with *Canker* (Table 3), indicating that plantations close to the sea were more severely affected by PPC.

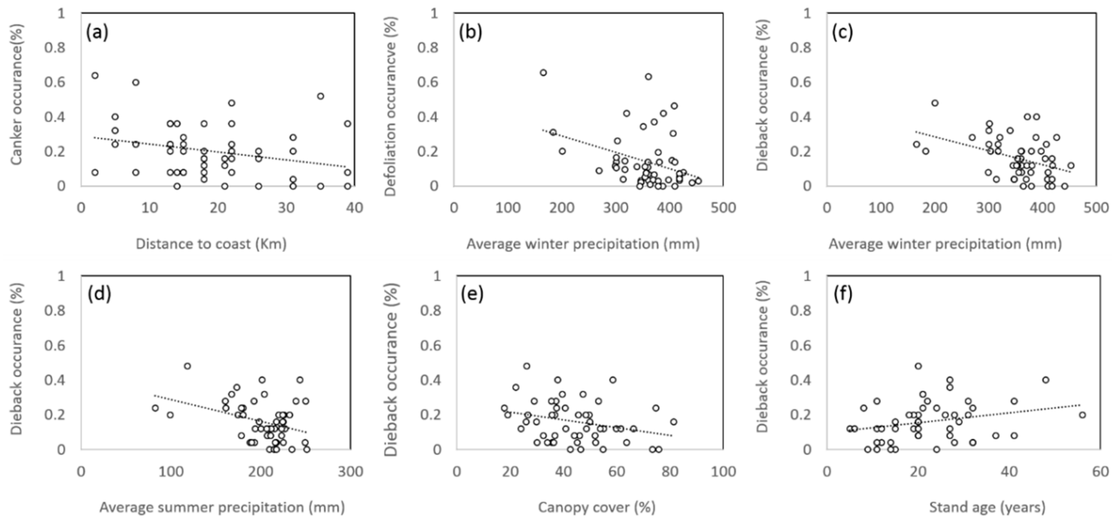

Figure 6. Plots of the key predictor variables, for *Canker*: (**a**) distance to coast; for *Defoliation*: (**b**) average winter precipitation; and for *Dieback*: (**c**) average winter precipitation, (**d**) average summer precipitation, (**e**) canopy cover, and (**f**) stand age.

Table 3. The variables with a significant (bold letters: p value < 0.05) and highest independent contribution as determined by hierarchical partitioning analysis and results of the randomization tests.

Group of Variables	Canker	Defoliation	Dieback
Climatic			
Pwin	-	**39**	**45**
Psum	-	-	**31**
Topography and spatial			
distCoast	**73**	-	-
Soil			
-	-	-	-
Stand characteristics			
Canopy	-	-	**49**
Age	-	-	**35**

Canopy and *Age* were the only stand characteristic variables with high (49% and 35%, respectively) and significant independent contribution to *Dieback* (Table 3 and Figure 6). This relationship was negative for *Canopy* implying that increased canopy cover is related to decrease in dieback symptoms (Figure 6e) and positive for *Age* indicating that in older stands there were more trees with dieback (Figure 6f). Precipitation was found to have a high and significant independent contribution with a negative association with *Defoliation* (*Pwin*) (Figure 6b) and *Dieback* (*Pwin* and *Psum*) (Table 3; Figure 4, Figure 5, and Figure 6c,d, respectively).

4. Discussion

In this study, we focused on the effects of climatic, topographic variables, soil properties, and stands characteristics on the PPC disease. This study on PPC, which includes 50 surveyed plots and 30 variables, is the first of its kind. However, it is important to stress that the results of this study are based on a single year's survey. This means that generalization should be undertaken with caution.

In this work, we measured three known pathological symptoms of the PPC disease—cankers, dieback, and defoliation [7]. The dieback symptom in the PPC disease usually occurs at the tips of the branches due to the physical block of water flow by the girdling cankers that develop at the *F. circinatum* infection site [22]. Loss of foliage can be attributed to shoot dieback and water stress and this applies when the disease state is advanced.

In this work, we found low correlation between the three studied pathological variables. In addition, these three symptoms correlated with different explanatory variables, suggesting that the occurrence of each symptom stems from different conditions. It seems that topography and soil affected mainly the canker symptoms, while stand characteristics rather affected the dieback symptoms. It is important to consider the different symptoms and their causes, as many symptoms can be the result of different interactions among tree–soil–environment and different pathogens. This is the case, for example, for defoliation in pines, which can be due to many causes independently or combined: the presence of *F. circinatum* or other pathogens, such as *Dothistroma septosporum*, *Dothistroma pini*, and *Lecanosticta acicula* [60], or the lack of secondary macronutrients [61]. This is an important aspect to consider when studying and monitoring this and other diseases.

PPC is a globally spread disease but is found predominantly in areas with relatively warm and moist climates [62], indicating that climatic conditions influence the growth of *F. circinatum*. Thus, climatic conditions are thought to play a key role in PPC disease establishment and severity. Temperature is known to influence *F. circinatum* growth, spore germination, and infection [62]. *Fusarium circinatum* growth pace and spore germination were found to decrease with decreasing temperature and were found to be minimal at 10 °C [63]. In our work, temperature was not found to be important to any of the disease symptoms, probably because the temperature in this relatively small region was relatively constant in both the summer and winter in all 50 surveyed stands.

Humidity, in addition to temperature, affects the establishment of *F. circinatum* [3]. In California, PPC is found mainly in warm and moist areas near the coast [22]. Additionally, Sakamoto and Gordon [63] found a significant effect of humidity on frequency of infection in wounds caused by insects. Wikler et al. (2003) [38] found that stands near the coast had higher levels of disease compared to inland stands. They speculated that this is due to microclimate conditions that might affect the success of fungus colonizing the host or due to differences in the distribution of insects vectoring the pathogen. Our results corroborate these observations, as we found that the occurrence of the trees with canker symptoms increased towards the coast. We also found that the occurrence of *Defoliation* and *Dieback* was high in stands that received low amounts of precipitation. *Fusarium circinatum* spore dispersal is facilitated, among other factors, by wind and rain. In addition, the incidence of PPC tends to increase in foggy, humid, and rainy regions [38,64]. Thus, we argue that these two symptoms, in addition to being affected by *F. circinatum*, might also be the result of other conditions.

Our results demonstrate that the disease symptoms also had geographical aspects, by showing that the occurrence of the trees with canker symptoms was much greater in Eastern Cantabria, near the Basque Country, which was the first place were the disease was detected in Spain [65]. This might be the result of efficient *F. circinatum* dispersion by the wind or by insect vectors [25]. Alternatively, as Wikler et al. (2003) [38] suggested, the differences in disease occurrence of PPC might be the result of differences between regions within the study area that have different abundances of insects that vector the disease or alternatively act as wounding agents. Several bark beetle species that can vector *F. circinatum* have been identified in Northern Spain [25,66].

Soil nutrients are important factors affecting plant growth, development, and resistance against different pathogens [67]. It was found that nutrient levels can affect susceptibility to the PPC pathogen. Specifically, high levels of nutrients in the soil, have been found to increase PPC severity [67]. Fertilized trees have been found to have a significantly higher rate of disease incidence than unfertilized trees [68]. In our work, out of the studied 13 soil variables, there was no significant variable explaining the variation in the three studied pathological symptoms, possibly because the level of the nutrients in these unfertilized stands did not affect the disease.

The variables in the fourth variables group, stand characteristics, were not correlated with *Canker* and *Defoliation*. However, our results show that low canopy cover is related to a high level of the dieback symptom. This result somewhat coincides with the significant relationship between crown height and PPC occurrence that was reported in California. Wikler et al. (2003) [38] found that trees that had larger crown heights were less likely to get infected than those with small crown heights.

Although *F. circinatum* affects trees of all ages, we found that in older trees the severity of *Dieback* was higher. Aging is known to affect the capacity of trees to recover following periodic damages. The young, vigorous tree replaces damaged tissues and resumes growth, while the older tree has a slower metabolism and a slower rate of wound recovery, which increases the tree's susceptibility to pathogens [69].

5. Conclusions

The effect of an infection with *F. circinatum* on many species of pine is known, but their interactions with environmental, climatic, topographic, and stand characteristics are yet to be studied. This study demonstrates that the known symptoms of the disease were affected by different variables and that they are probably the results of a combination of conditions. In addition, we also found that PPC disease occurrence was affected by the location of the stands. Stands further away from the coast and from the Basque Country were less severely infected. Among other reasons, this could be the result of abiotic differences along the Cantabria province, such as the climate conditions, or biotic characteristics, such as the distribution of insect vectors. The complexity of these interactions requires further studies to clearly establish the environmental factors affecting *F. circinatum* spread.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/4/305/s1, Table S1: Summary of the selected GLM models for each of the three symptoms in each of the four variables groups, according to AIC. Only models with a difference between their AIC and the best fitting model lower than two are shown, Table S2: Summary of the Pearson residual against the fitted model variables.

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Abbreviations

PPC	Pine Pitch Canker
Asl	above sea level

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