

Effects of pruning in Monterrey pine plantations affected by *Fusarium circinatum*

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Abstract

Fusarium circinatum Nirenberg and O'Donnell (1998) is the causal agent of Pitch Canker Disease (PCD) in *Pinus* species, producing damage to the main trunk and lateral branches as well as causing branch dieback. The disease has been detected recently in northern Spain in *Pinus* spp. seedlings at nurseries and in *Pinus radiata* D. Don adult trees in plantations. *Fusarium circinatum* seems to require a wound to enter the tree, not only that as caused by insects but also that resulting from damage by humans, i.e. mechanical wounds. However, the effects of pruning on the infection process have yet to be studied. The aim of the present study was to know how the presence of mechanical damage caused by pruning affects PCD occurrence and severity in *P. radiata* plantations. Fifty *P. radiata* plots (pruned and unpruned) distributed throughout 16 sites affected by *F. circinatum* in the Cantabria region (northern Spain) were studied. Symptoms of PCD presence, such as dieback, oozing cankers and trunk deformation were evaluated in 25 trees per plot and related to pruning effect. A significant relationship between pruning and the number of cankers per tree was observed, concluding that wounds caused by pruning increase the chance of pathogen infection. Other trunk symptoms, such as the presence of resin outside the cankers, were also higher in pruned plots. These results should be taken into account for future management of Monterrey Pine plantations.

Key words: pitch canker; Cantabria; Spain; wound; *Pinus radiata*.

Resumen

Efecto de la poda en plantaciones de pino radiata afectadas por *Fusarium circinatum*

Fusarium circinatum Nirenberg and O'Donnell (1998) es el agente causante de la enfermedad del chancro resinoso del pino, que afecta a especies del género *Pinus* y provoca la aparición de chancros resinosos en el tronco y en ramas gruesas, además de puntisechado en la guía terminal. Esta enfermedad fue detectada recientemente en el norte de España asociada a plántulas de coníferas en vivero y a plantaciones de *Pinus radiata* D. Don. *Fusarium circinatum* suele requerir una herida en el árbol para poder infectarlo. Estas heridas pueden estar causadas por insectos o ser de origen antrópico, como las heridas mecánicas. Con la finalidad de conocer cómo las heridas producidas durante la poda afectan a la severidad de la enfermedad del chancro resinoso del pino, se estudiaron 50 parcelas de *P. radiata* (podadas y no podadas) distribuidas a lo largo de la provincia de Cantabria. En cada una de las parcelas fueron evaluados 25 árboles, en los que se estudiaron los síntomas más característicos de la enfermedad, como son puntisechado, presencia de chancros resinosos y deformación del tronco, relacionándolos con la presencia de poda. Se observó una relación significativa entre la poda y el número de chancros presentes en el árbol, lo que indica que la herida producida en este tratamiento selvícola es susceptible de infección por parte del patógeno. Otros síntomas también presentes en el tronco, como los exudados de resina fuera del chancro, aparecieron más frecuentemente en las parcelas podadas. Estos resultados son de gran trascendencia para el futuro manejo de las plantaciones de *P. radiata* afectadas por el chancro resinoso del pino.

Palabras clave: chancro resinoso; Cantabria; España; herida; *Pinus radiata*.

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Abbreviations used: Pitch canker disease (PCD); meters above sea level (m.a.s.l).

Introduction

Pitch Canker Disease of pines is produced by the fungus *Fusarium circinatum* (teleomorph = *Gibberella circinata*) (Nirenberg and O'Donnell, 1998). This fungus poses a threat to pine plantations and forests throughout the world (Wingfield *et al.*, 2008). It was first reported in North Carolina (Hepting and Roth, 1946) but has also been observed in California (Mc Cain *et al.*, 1987), Chile (Wingfield *et al.*, 2002), South Africa (Viljoen and Wingfield, 1994), Japan (Muramoto and Dwinell, 1990), Mexico (Guerra-Santos, 1999), Portugal (Bragança *et al.*, 2009), France (EPPO, 2004) and northern Spain (Landeras *et al.*, 2005). Pitch canker commonly occurs in coastal rather than in inland areas (Wikler *et al.*, 2003), suggesting that the causal agent of this disease has some association with humidity and fog.

Pinus species like *P. pinaster*, *P. radiata* and *P. sylvestris* (Landeras *et al.*, 2005, Pérez-Sierra *et al.*, 2007) as well as *Pseudotsuga menziesii* (Gordon *et al.*, 1996) show disease susceptibility. *Pinus radiata*, planted widely worldwide, is a species extremely sensitive to pitch canker disease (Viljoen *et al.*, 1995) and symptoms observed on these trees are very severe (Gordon *et al.*, 2001). Several *Pinus* species like Japanese black pine (*P. thunbergiana*) and Italian stone pine (*P. pinea*) are known for suffering little or no damage from the disease (Gordon *et al.*, 2001).

Fusarium circinatum symptoms include bleeding, resinous cankers with tree trunk deformation. The wood beneath the sunken bark of cankers is usually pitch-soaked. As these cankers grow they may girdle the larger shoots producing dieback (Blakeslee *et al.*, 1980). Defoliation and trickles of resin can also be found on diseased trees. These symptoms spoil the trees, and together with the premature trees' death, result in economic loss to the affected regions. The pathogen also causes damping-off and mortality in seedlings. Consequently, this fungus may be considered a threat to pine plantations and wood industry productivity throughout the world.

Fusarium circinatum seems to require fresh wounds on trees as infection court (Dwinell *et al.*, 1985), such as those caused by insects from the subfamily *Scolytinae* that have been found to be not only wounding agents but also vectors in California (Storer *et al.*, 2004). Dwinell *et al.* (1985) suggested that *F. circinatum* inoculum could infect wounds produced by

pruning, mowing and harvesting, although no study was carried out on this issue. Wounds caused by hurricanes or those resulting from wind-thrown needles are also thought to provide an infection court for the pathogen to infect the trees (Kelley and Williams, 1982). Notwithstanding, the susceptibility of these wounds to infection could decrease significantly with wound age (Sakamoto and Gordon, 2006). Nonetheless, other studies as Correl *et al.* (1991) suggest that branches with mechanical wounds are not susceptible to infection even if airborne inoculum is present, postulating that airborne spores are unable to infect wounds. On the other hand, pruning could be considered for removing diseased branches, though this approach is not effective in eradicating the disease (Gordon *et al.*, 2001). Attempts to remove disease causing fungi have been made via tree pruning, though it was shown that this treatment does not completely eliminate the disease from the tree (Moorman and Lease, 1999). As such, forest management should be considered as an important factor for decreasing disease establishment and spread (Waring and O'Hara, 2005). The effect of pruning has never been studied in Monterrey pine plantations where the disease is destroying the trees. Some reports regarding the effect of the presence of wounding agents have been made in the United States, where most Monterrey pine appears in native stands (Gordon *et al.*, 2001).

The objective of this study was to check out the effect of pruning on *P. radiata* plantations infected with PCD to determine whether pruning wounds provide an infection court for *F. circinatum*, increasing the disease severity.

Material and Methods

Plots selection

Data were collected from *P. radiata* plantations distributed throughout 16 sites affected by *F. circinatum* in the region of Cantabria (northern Spain) with high occurrence of severe PCD affected stands. From June to October, 2010, several factors related to the disease were measured.

Fifty plots (pruned and unpruned) were selected among 16 sites (Figure 1) affected by *F. circinatum*, maintaining a distance of at least 500 meters between them. The location where the plot was set in each stand was randomly selected (Table 1).

Table 1. Characterization and location of the *P. radiata* surveyed plots

Plot number	Municipality	Orientation	Altitud (m.a.s.l. ^a)	Coast Dist (Km)	P ^b /UP ^c
1	Luena	S	92	39.52	UP
2	Luena	E	359	38.25	UP
3	Luena	N	370	38.89	P
4	Rionansa	E	300	36.79	P
5	Rionansa	SW	290	36.19	P
6	Rionansa	N	270	31.38	P
7	Rionansa	S	268	30.09	UP
8	San Pedro del Romeral	SW	517	30.66	P
9	San Pedro del Romeral	W	448	30.55	P
10	Villafufre	W	413	15.28	UP
11	Villafufre	NW	476	15.48	UP
12	Villafufre	NW	487	10.26	UP
13	Rionansa	NW	600	10.52	P
14	Rionansa	SE	519	9.05	P
15	Rionansa	E	322	20.93	P
16	Udías	NW	269	21	P
17	Udías	N	130	21.24	P
18	Udías	NW	219	15.14	P
19	Corvera de Toranzo	NW	469	15.63	P
20	Corvera de Toranzo	NW	476	14.92	UP
21	Corvera de Toranzo	S	359	12.9	P
22	Corvera de Toranzo	S	359	13.35	UP
23	Ruesga	W	406	12.95	P
24	Ruesga	SE	404	19.49	P
25	Ruesga	N	481	20.34	P
26	Mazcuerras	SW	147	19.3	P
27	Mazcuerras	E	170	17.08	UP
28	Mazcuerras	NE	131	16.75	P
29	Los Corrales de Buelna	Flat	470	17.45	UP
30	Los Corrales de Buelna	SE	460	23.43	UP
31	Los Corrales de Buelna	SE	426	22.4	P
32	Cabezón de la Sal	N	343	23.12	P
33	Cabezón de la Sal	N	214	15.21	P
34	Cabezón de la Sal	W	417	12.2	UP
35	Cillórigo de Liébana	NW	387	15.04	P
36	Cillórigo de Liébana	E	374	6.61	UP
37	Cillórigo de Liébana	NW	370	6.17	P
38	Castro Urdiales	N	533	5.63	UP
39	Castro Urdiales	W	297	2.94	P
40	Rionansa	S	271	3.73	P
41	Rionansa	S	251	20.57	P
42	Rionansa	SE	225	21.29	P
43	Cabuérniga	S	403	20.26	UP
44	Cabuérniga	NW	376	21.52	UP
45	Cabuérniga	S	321	20.76	UP
46	Rionansa	S	285	20.49	P
47	Rionansa	W	208	18.29	P
48	Cabuérniga	N	431	18.47	P
49	Cabuérniga	E	898	18.11	UP
50	Cabuérniga	SE	313	17.08	P

^aMeters above sea level. ^bPruned. ^cUnpruned.

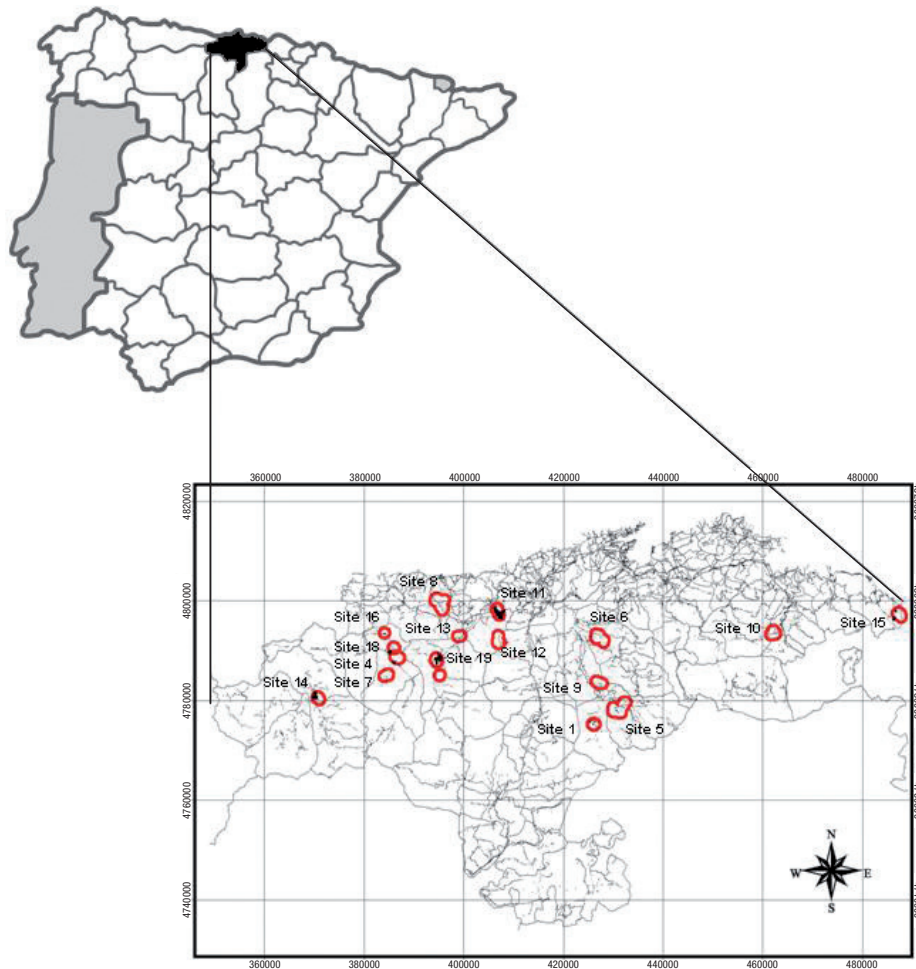


Figure 1. Geographical situation of the study sites in Cantabria (Spain).

Field work

Twenty five trees per plot were evaluated, considering dendrometric and forest health variables. A total of 1250 trees were measured against the below variables. Within the dendrometric variables, tree diameter, total height, first living branch height and pruning height were measured. The plant health variables included number and location (internodes or whorl) of cankers, flow of resin on the cankers (from 1 to 3 where 1 = light, 2 = medium, 3 = abundant), percentage of trunk perimeter affected by the canker (< 33%, 33-66% or > 66%), five degrees of defoliation (1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100%), presence of trickles of resin outside the cankers (from 0 to 3, where 0 = absence, 1 = light, 2 = medium, 3 = abundant), presence of red shoots in the crown

(from 0 to 3, where 0 = absence, 1 = on 1/3 of the crown, 2 = on 2/3 of the crown, 3 = on all the crown), dieback (from 0 to 3, where 0 = absence, 1 = on 1/3 of the crown, 2 = on 2/3 of the crown, 3 = on all the crown) and mortality.

Distance of each plot to the coast was calculated with Arc View 3.0 using UTM coordinates recorded in the plots centre.

Statistical analysis

The effect of pruning on the disease occurrence was analyzed using both univariate and multivariate analyses. Data were subjected to analysis of variance (ANOVA) to assess the significance of differences in symptomatology between pruned and unpruned plots. Correlation analysis

was used to establish the linear relationship between selected variables. The non-parametric Kruskal-Wallis test (data could not be transformed to fit a normal distribution) was used to determine whether significant differences existed between pruned and unpruned plots.

Non-parametric multivariate analysis, recommended for non-normal data, was performed to assess in detail the influence of pruning on plant health variables. A non-parametric multidimensional scaling (NMDS) analysis was executed including different forest health variables (number of cankers, trickles of resin, red shoots, defoliation and dieback). NMDS was carried out using Bray-Curtis distance.

Multiple response permutation procedure (MRPP) was used to tests whether there were differences between pruned and unpruned plots. Ordination diagrams, Ordihull combined with Ordispider were used to represent the items on a class and to combine the items to their class centroid (Oksanen, 2005).

All statistical analyses were carried out at the 0.05 level of significance. Data analyses were run on VEGAN package 2.0-2 of R version 2.14.1.

Results

The average number of cankers was significantly higher ($n = 50$, $F = 5.232$, $p = 0.026$) in pruned than in unpruned plots (Table 2). It was also observed that the number of cankers present on whorls was significantly higher in pruned plots ($n = 50$, $F = 4.256$, $p = 0.044$) than in unpruned ones while the number of cankers present in internodes showed no significant relationship to pruning. Pruned plots also showed a level of resin trickles significantly higher than unpruned plots ($n = 50$, $F = 5.064$, $p = 0.029$). The NMDS shows the distribution of most pruned plots in the area of the graph where cankers and trickles of resin are present (Fig. 2). MRPP test also reflected a relation between health variables and prune ($A = 0.0181$, $p = 0.031$).

Table 2. ANOVA results for pruning

Source	d.f	Mean Sq	F-value	p-value
Whorl	1	0.93	4.256	0.044
Tricles of resin	1	1.09	5.064	0.029
Dieback	1	0.005	0.023	0.878
Defoliation	1	0.92	4.209	0.045
Cankers	1	1.13	5.232	0.026
Red shoots	1	0.02	0.108	0.743

Regarding symptoms affecting tree crown, like defoliation, dieback and presence of red shoots, it was observed that these variables showed apparently higher mean levels in unpruned plots than in pruned ones (Table 3). Defoliation showed significant differences between unpruned plots and pruned ones ($n = 50$, $F = 4.209$, $p = 0.045$). On the other hand, no significant differences were found for dieback and red shoots ($p > 0.05$).

The non-parametric Kruskal-Wallis test showed that the presence of dead trees was not significantly related to prune ($n = 50$, $\chi^2 = 0.4774$, $p = 0.489$), though higher number of dead trees appeared in unpruned plots (11.1%) than in pruned ones (7.5%).

Correlation between mean number of cankers per plot and the distance of each plot from the coast showed a significant relationship ($r = -0.30$, $F = 4.726$, $p = 0.0347$), thus those plots nearest to the coast presented a higher number of cankers. Nonetheless, a significantly higher number of red shoots was observed when the distance from the coast increased ($n = 50$, $r = 0.64$, $F = 0.108$, $p = 0.743$).

Discussion

Fusarium circinatum's capacity for infection seems to depend on the presence of biotic and/or abiotic

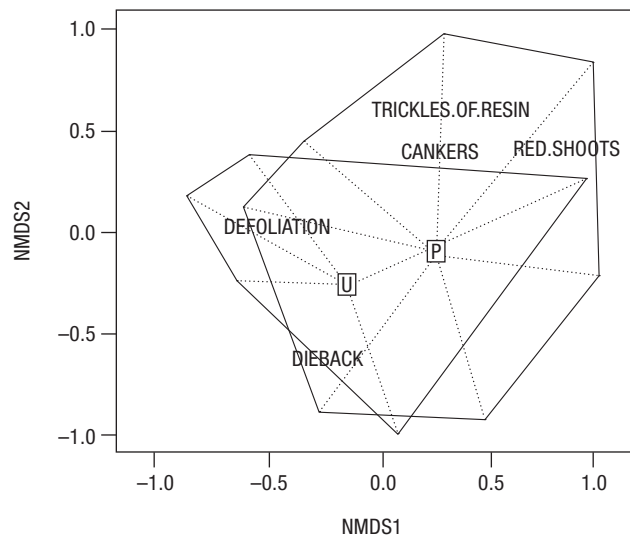


Figure 2. Ordination diagram diagram NMDS with methodology Ordihull combined with Ordispider. Pruned (P) and Unpruned (UP) plots are ordered in the centroids, forest health variables (dieback, defoliation, cankers, trickles of resin, red shoots) are also represented.

Table 3. Mean values of PCD symptoms in pruned and unpruned plots

	N cankers/ tree	N cankers on whorl/tree	N cankers on internode/tree	Defoliation level (0-5)/tree	Dieback level (0-3)/tree	Resin trickle level (0-3)/tree	Red shoots level (0-3)/tree
Pruned plots	0,43±0,39a	0,33±0,35a	0,10±0,13a	0,65±0,61b	0,27±0,37a	0,28±0,25a	0,07±0,15a
Unpruned plots	0,21±0,16b	0,14±0,14b	0,06±0,07a	1,14±1,04a	0,29±0,19a	0,13±0,13b	0,09± 0,14a
Pruned range	0,00-1,44	0,00-1,44	0,00-0,52	0,00-2,32	0,00-2,00	0,00-1,32	0,00-0,64
Unpruned range	0,00-0,60	0,00-0,56	0,00-0,24	0,00-3,68	0,04-0,84	0,00-0,56	0,00-0,48

Variables with the same letter showed no significant differences.

wounding agents (Gordon, 2006). As noted during this survey, symptoms of PCD that largely appear in the main stem, such as cankers or resin drops, become more frequent in pruned trees. This could indicate that pruning wounds in the trunk have an increased chance of becoming infected by *F. circinatum* as well as increasing the severity of the disease. This is also supported by the relationship found between the number of cankers on whorls and pruning. According to Gordon (2006), mechanical wounds in a PCD infected area sustained infection at a very low rate, and this rate would decrease if the wound size decreases.

Volatiles generated by trees after pruning also increase the likelihood of infection, primarily because some insects carrying the fungus feel attracted by these volatiles (Gordon, 2011). Thus, the pine shoot beetle, *Tomicus piniperda*, seemed to attack *P. sylvestris* pruned trees more frequently than unpruned ones (Långström and Hellqvist, 1992). Several bark beetles are also attracted by resin odours from damaged bolts after pruning allowing them to later also attack neighbouring healthy trees (Jactel *et al.*, 2009).

Another factor that could possibly increase the disease incidence in relation to pruning could be the lack of disinfections of forestry machinery between and following cuttings, spreading the infection among stands. This is one of the main ways for *Cryphonectria parasitica* (the causal agent of chestnut blight) spread (Gouveia, 2001). In order to reduce chestnut blight risk accurate disinfection of pruning tools must be performed. Moreover, pruning should be carried out only during periods of lowest host receptiveness and susceptibility or when infection risk is lower, i.e. when spores inoculum is minimum (Guérin and Robin, 2003). For *C. parasitica*, these two conditions happen in winter. However, the most suitable period for pruning Monterey pine regarding PCD, is not well established. Further studies regarding the *F. circinatum* cycle and spore dispersal in this region should be

conducted to develop a better management of Monterey pine plantations.

The number of dead trees was apparently higher in unpruned plots than in pruned ones, which could be related to the decrease of the quantity of inoculum in the air and surrounding trees due to pruning and wood removal. Bernhold *et al.* (2006) reported the effects of clear-cutting and the effect of removing infected slash in *P. sylvestris* stands affected by *G. abietina*, concluding that it reduces the risk of infection but does not eradicate the infection source. Laflamme (1999) demonstrated a reduction in scleroderris canker in red pine (*P. resinosa*) caused by *G. abietina* from 67% to 22% one year following pruning. Furthermore, the effect of pruning apple trees affected by the causal agent of sooty blotch *Gloeodes pomigena* was observed to reduce the incidence and the severity of the disease (Ocamb-Basu *et al.*, 1988). Pruning diseased trees could have different effects depending on the specific behaviour of the pathogen. Thus, for avoiding forest health related problems, Waring and O'Hara (2005) expressed the need of a combined solution among management tools and the knowledge of their effect on each disease. The apparent contradiction between an evident increase in the number of cankers and a decrease in the mortality, both following pruning, requires a detail analysis in further studies.

Another critical component allowing *F. circinatum* to survive and infect is environmental moisture (Gordon, 2006). Stand moisture content can be decreased by pruning through increased light and surface wind speed within the stand (Pollet and Omi, 2002; Jactel *et al.*, 2009), which could reduce successful pathogen survival. To assess the importance of environmental moisture, proximity to the coast and symptomatology were correlated. Thus, it was found that plot distance from the coast is an important factor influencing the disease occurrence, further underscoring the importance of environmental moisture. This influence of the coast, where the environmental conditions are more favourable for

the infection (Wingfield *et al.*, 2008), has been previously noted in California (Wikler *et al.*, 2003) with one exception in Sierra Nevada (Vogler *et al.*, 2004). The effect of the coast proximity is also clear in Spain, where more *P. radiata* plantations are affected by the disease on the northern coast. However, experiments carried out by Sakamoto and Gordon (2006) under both controlled and field conditions showed no significant relationship between infection rate and relative humidity.

In conclusion, wounds caused by pruning have an increased chance of becoming infected by the pathogen which could increase cankers and deformation. On the other hand, pruning could improve crown aspect decreasing defoliation. Notwithstanding, pruning in Monterey pine diseased plantations is not desirable as a result of stem deformation caused by cankers, making them useless for the wood industry. For better understanding of the impact of pruning and other silvicultural treatments on pitch canker affected trees, further research including tree pruning relative to seasonal and fungal cycle should be done.

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