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Effects of various densities of *Ophiostoma ips* inoculations on *Pinus sylvestris* in north-western Spain

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Summary

The aim was to determine the inoculation density above which Scots pine (*Pinus sylvestris* L.) is overcome by the blue-stain fungus *Ophiostoma ips* (Rimb.) Nannf. that is associated with the bark beetle *Ips sexdentatus* Boern. In north-western Spain, stems of 16 Scots pines were inoculated at various densities (0, 400, 800 or 1600 inoculi/m^2) along circumferential 100 or 150 cm wide inoculation belts. Each inoculum consisted of a 5 mm diameter cylinder of malt extract agar colonized by the fungus. Three months later, all trees were harvested and trunk resinosis and foliage colour were visually assessed. The percentage of healthy, desiccated, resin soaked, and blue-stained sapwood, as well as growth productivity indices, were calculated from stem disks cut from within the inoculated zone of each tree. Sapwood-specific hydraulic conductivity (Ks) of each tree was measured in the middle of the inoculated zone. All parameters of tree vigour changed dramatically to the worse when inoculation densities were above 400 inoculi/m^2, and foliage changed from green to yellow-green or yellow when an inoculation density of 800 instead of 400 was used. The percentage loss of sapwood-specific conductivity (PLC) increased from 30 to 90% and the percentage of healthy, conductive sapwood dropped from 85 to 35% at 800 inoculi/m^2. No effect of the width of the inoculation belt was observed, and there was no relationship between tree productivity indices and the level of resistance. A non-linear negative relationship was found between PLC and the percentage of healthy sapwood. It is concluded that tree resistance was overcome and that trees were going to die when the inoculation density was ≥800 inoculi/m^2.

1 Introduction

Bark beetles (Coleoptera, Scolytidae) are the most dangerous pests in coniferous forests. Endemic populations use trees as a substrate to complete their life cycle, feeding on phloem tissues in weak or dead trees. However, under certain conditions, bark beetles populations can become epidemic and attack healthy trees. In this case, successful beetle establishment may lead to death of host trees within a few months (Berryman 1982; Raffa et al. 1993). Most bark beetles carry phytopathogenic blue-stain fungi (Ascomycetes, Ophiostomataceae) that are introduced into the xylem and phloem at the moment of the attack (Berryman 1972; Whitney 1982; Christiansen et al. 1987; Paine et al. 1997). These fungi probably help in the establishment of the beetle populations by contributing to exhausting tree resistance, and may be also involved in the tree killing process (Christiansen et al. 1987; Paine et al. 1997; Lieutier 2002).

Tree resistance to bark beetle/fungus attack involves constitutive and induced defences (Berryman 1972; Raffa and Berryman 1983a; Christiansen et al. 1987; Paine et al. 1997; Lieutier 2002). Under endemic conditions attacks generally fail because tree defences are efficient at low density of attacks. However, each tree has a critical threshold of attack density, above which, tree resistance is overcome and attacks succeed (Berryman

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1976, 1982; Raffa and Berryman 1983a; Christiansen et al. 1987). Such thresholds have been demonstrated for natural attacks of *Dendroctonus ponderosae* Hopk. in *Pinus contorta* Douglas (Waring and Pitman 1983), *Ips typographus* L. in *Picea abies* Karst. (Mulock and Christiansen 1986), *Tomicus piniperda* L. (Långström et al. 1992; Långström and Hellqvist 1993) and *I. acuminatus* Gyll. (Guérard et al. 2000) in *P. sylvestris* L. and *Tomicus* sp. in *P. yunnanensis* Fr. (Lieutier et al. 2003).

Similarly, critical thresholds of inoculation density have been defined and experimentally measured after artificial mass inoculations with fungi isolated from bark beetles, such as *Ophiostoma clavigerum* (Robinson-Jeffrey and Davids) Harrington isolated from *D. ponderosae* (Raffa and Berryman 1983b), *Ceratocystis polonica* (Siem.) Moreau isolated from *I. typographus* (Horntvedt et al. 1983; Christiansen 1985b), *Leptographium wingfieldii* Morelet and *O. minus* Hedgcock isolated from *T. piniperda* (Solheim et al. 1993; Croisé et al. 1998), and *O. brunneo-ciliatum* Matth.-Käärik from *I. acuminatus* (Guérard et al. 2000). Knowledge of critical thresholds of attack and inoculation density is valuable in forest pest management, as it can be used to estimate tree resistance and fungal pathogenicity, and ultimately to predict the risk of tree mortality in the case of bark beetle outbreaks. In the present paper, we determine the critical threshold of inoculation density in Scots pine (*P. sylvestris*) inoculated with *O. ips* (Rumb.) Nannf., a fungus associated with *I. sexdentatus* (Boern).

2 Material and methods

In June 1998, 16 Scots pines (25 years old and 8.4 ± 1.0 m tall; Table 1) were selected in a reforested area in the south-eastern of the province of León (Northwest Spain) and subjected to four treatments. Four trees were inoculated with *O. ips* at a density of 400 inoculi/m^2, four at 800 inoculi/m^2, and four others at 1600 inoculi/m^2. The remaining four trees were not inoculated to serve as control trees. Inoculations were performed on the main stem from 0.3 m above ground upwards on a 1 m wide circumferential belt of two trees per inoculation density, and on a 1.5 m wide belt of another two trees per inoculation density (Table 1). Mass inoculations were performed with 3-week-old sporulating monospore malt agar cultures (30 g malt extract with 15 g agar-agar in 1 l distilled water). The fungus originated from *I. sexdentatus* galleries and was collected from naturally attacked *P. pinaster* Aiton logs in the forest of Tabuyo del Monte (province of León, Spain). Inoculations where made by introducing a 5 mm diameter disk of fungal culture into a cambium-deep hole made with a cork-borer. The hole was then closed with the previously removed bark disk. To express the inoculum pressure as a percentage of tree surface, the number of inoculi was counted and the total of the inoculated surface was estimated based on the dimensions of the inoculi. The whole tree-bole surface was calculated assuming that the bole was a cone.

Three months after inoculation, foliage colour (green, yellow-green or yellow) and resin exudation (presence of stripes of resin) on the bark surface (N = none exudation, L = low exudation, M = medium exudation) were estimated visually for each tree (Table 1). Trees were then harvested and two stem disks were taken from the inoculation belt of each tree at 20 cm from the upper and lower end of the belt (or at similar distances from the ground in control trees). Dried, resin soaked, blue stained, and total area of sapwood on the disks was redrawn on transparent paper. These areas were then computed after digitization with image analysis software (Windendro, Chicoutimi-Canadá). In each case, functional sapwood was estimated by subtracting heartwood, resin-soaked sapwood, dried sapwood and blue-stained sapwood, from total wood cross-section area. For a non-damaged tree, functional sapwood was estimated by subtracting heartwood from total wood cross-sectional area. The stem disks were also used to calculate productivity indices, as the ratios between, the cross-sectional sapwood area of the 1997 ring (P1), or the

Table 1. **Morphological characteristics of the trees used in the experiment, with results on resin exudation and foliage colour 3 months after inoculation with *Ophiostoma ips***

Number of trees	Inoculation density (inoculi/m^2)	Inoculation belt width (cm)	Tree height (m)[1]	Tree diameter (cm, at 130 cm above ground)[1]	Dimensions of stem sections used to measure Ks		Resinosis (%)	Foliage colour 3 months after inoculation (in % needles)
					Length (cm)[1]	Diameter (cm)[1]		
2	400	100	8.2 ± 1.1	7.3 ± 1.1	19.8 ± 0.4	6.8 ± 1.0	100 (L)[2]	100 G[3]
2	400	150	8.9 ± 0.1	7.5 ± 0.7	17.5 ± 2.8	7.4 ± 0.5	100 (L)	100 G
2	800	100	8.6 ± 1.5	8.8 ± 1.1	19.3 ± 3.0	7.4 ± 0.8	50 (N), 50 (L)	50 G, 25 Y-G, 25 Y
2	800	150	9.0 ± 0.4	8.3 ± 0.4	19.0 ± 1.4	8.2 ± 0.7	100 (L)	50 G, 25 Y-G, 25 Y
2	1600	100	8.7 ± 0.4	7.3 ± 1.8	18.5 ± 2.1	6.9 ± 0.9	50 (L), 50 (M)	100 Y
2	1600	150	7.2 ± 1,1	7.5 ± 0.0	19.0 ± 2.1	6.5 ± 0.1	50 (N), 50 (M)	100 Y
2	0	0	7.5 ± 1.3	7.3 ± 1.1	19.8 ± 0.4	7.0 ± 1.1	100 (N)	100 G
2	0	0	9.9 ± 0.3	7.8 ± 1.1	19.3 ± 0.4	7.0 ± 0.7	100 (N)	100 G

[1]Values are mean ± SD.
[2]N, none; L, low; M, medium.
[3]G, green; T-G, yellow-green; Y, yellow.

1993–1997 rings (P5), respectively, and the total cross-sectional sapwood area (WARING and PITMAN 1980, 1983). For each individual tree, productivity indices and dried sapwood, resin-soaked sapwood or blue-stained sapwood percentage were expressed as the average of the two stem disks.

Sapwood-specific hydraulic conductivity (Ks) was measured on 17.5–19.8 cm long stem sections taken from the middle of the inoculated belt of each tree (Table 1). For the control trees, the logs were taken on a similar height on the trunk. The methods and techniques to measure Ks were those developed by SPERRY et al. (1988) and previously used and described in detail in experiments similar to ours (GUÉRARD et al. 2000; CROISÉ et al. 2001). Briefly, deionized, degassed and acidified water (due to the use of 0.2 g/l of Phlosine B as colorant) was used at a pressure of 5 kPa obtained from a water tank placed exactly 0.5 m above the sample. The flow through the log segment was recorded as the weighted amount of water recovered at the open end of the segment after 10 min circulation. Hydraulic conductivity (K) was calculated as: $K = F \times L/P$, where F is the flow of water per time (kg/s), L the length of the segment (m) and P the pressure applied at segment entry. Values of K were standardized to Ks (in Kg/m/s/Mpa) by dividing K by the total sapwood cross-sectional area of the sample. The dysfunction in xylem hydraulic properties was expressed as percentage loss of conductivity (PLC), relative to maximal hydraulic conductivity (Km), with $PLC = [1 - (Ks/Km)] \times 100$. In our study, the potential occurrence of tracheid occlusion made it impossible to use restoration of xylem tracheid under high pressure (SPERRY et al. 1988). We, thus, estimated Km from the relationship between diameter and actual Ks from non-inoculated trees. In addition to the four control trees used in 1998, Ks was determined for 12 non-inoculated healthy trees that were cut 1 year later in October 1999 to estimate Km (Fig. 1). Data collected in 1998 and 1999 were tested for homogeneity.

Analysis of variance (using STATISTICA software) or Student's t-test was used to compare treatments, at a significance level of 0.05. Multiple regression followed by ANOVA was used to compare the two groups of control trees (1998/1999).

3 Results

No significant influence of the size of the inoculation belts on any of the parameters was found. Results were, thus, combined for the two inoculation belt sizes.

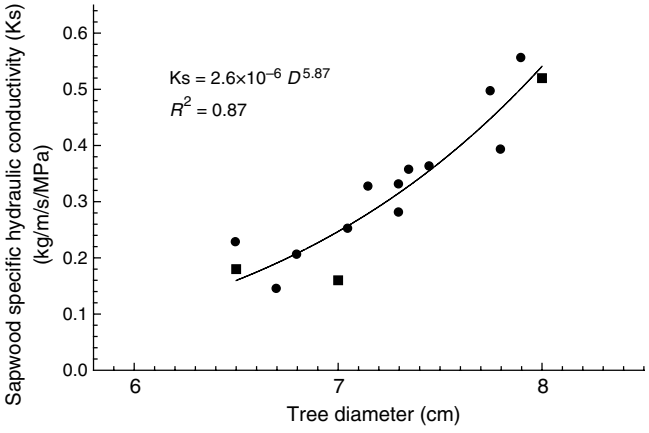

Fig. 1. Relationship between tree diameter at breast height (d.b.h.) and sapwood-specific hydraulic conductivity (Ks) in stems of non-inoculated Scots pine trees in 1998 (■) and 1999 (●). Each value represents one stem segment of one tree. D, tree diameter (cm)

3.1 Resinosis and foliage colour

Control trees and all trees that received 400 inoculi/m^2 had green needles 3 months after inoculation, while trees that received 800 inoculi/m^2 had a high percentage of yellow-green or yellow needles, and the needles of all trees inoculated at 1600 inoculi/m^2 were yellow (Table 1).

3.2 Effect of treatments on sapwood-specific hydraulic conductivity

The PLC increased as inoculation density increased, but the relationship was not linear (Fig. 2a) The inoculation density I_{50} that decreased the hydraulic conductivity by 50% in three months was evaluated to be about 500 inoculi/m^2 based on a logistic function fitted to the data. Maximum PLC (close to 90%) was reached between 800 and 1000 inoculi/m^2. Expressing the inoculum pressure in terms of percentage of inoculated tree surface produced a similar curve (Fig. 2b). The PLC reached values near 90% for ≥0.45% inoculated surface area.

3.3 Effect of treatments on sapwood damage

When the inoculation density increased, the percentage of healthy sapwood decreased (Fig. 3), with a particularly dramatic drop between 400 and 800 inoculi/m^2, and reaching values close to 10% at 1600 inoculi/m^2. However, the percentage of healthy sapwood did not differ significantly between the two highest inoculation densities. The loss of healthy sapwood was mainly due to tissue drying close to the wounds. The percentage of blue-stained sapwood did not differ significantly between inoculation densities, and the percentage of sapwood soaked with resin was also quite similar.

3.4 Relationship between vitality parameters

The PLC was significantly and negatively correlated with the percentage of healthy sapwood in a non-linear relationship (R^2 = 0.83; Fig. 4). It was significantly and

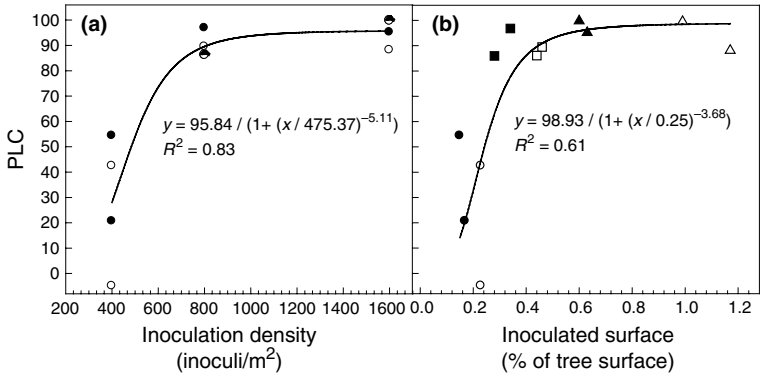

Fig. 2. Effect of mass inoculations with *Ophiostoma ips* on the percentage loss of sapwood-specific hydraulic conductivity (PLC) in Scots pines. The inoculum pressure was expressed as number of inoculi/m^2 (a) or as percentage of trunk surface inoculated (b). Filled symbols = 100 cm wide inoculation belt; empty symbols = 150 cm wide inoculation belt. (b) ○ and ● = 400 inoculi/m^2; □ and ■ = 800 inoculi/m^2; △ and ▲ = 1600 inoculi/m^2

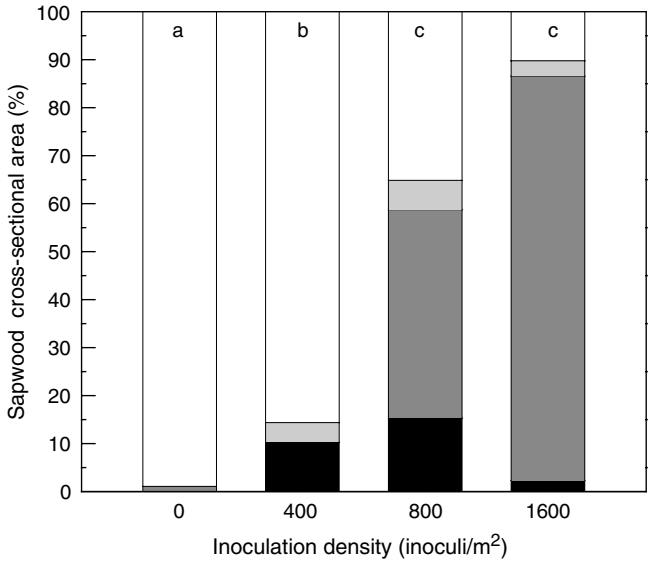

Fig. 3. Effect of mass inoculation of Scots pine trees with *Ophiostoma ips* on the percentage of healthy (□), resin soaked (▫), dried (▪), and blue-stained (■) sapwood cross-sectional area (n = 4 for each column). Different letters indicate statistically significantly different sizes of the healthy sapwood area by Student's t-test, $p < 0.05$

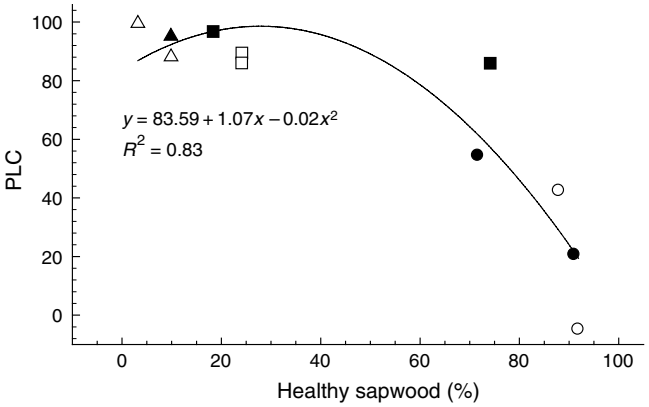

Fig. 4. Relationship between percentage loss of conductivity (PLC) and the percentage of functional sapwood after inoculation of Scots pines with *Ophiostoma ips* at different densities. Each value represents one stem segment. ○ and ● = 400 inoculi/m^2; □ and ■ = 800 inoculi/m^2; △ and ▲ = 1600 inoculi/m^2; filled symbols = 100 cm wide inoculation belt; empty symbols = 150 cm wide inoculation belt, x = percentage of functional sapwood; y = PLC

positively correlated with the percentage of dried sapwood (R^2 = 0.87). Resin soaking and blue staining did not show any correlation with PLC (data not shown). No relationship was found between productivity indices and PLC (Fig. 5).

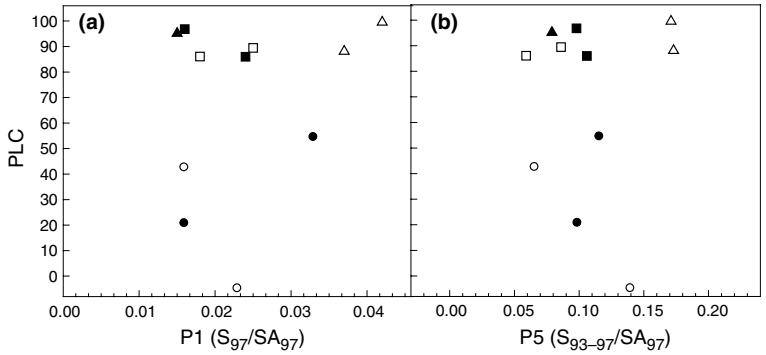

Fig. 5. Relationship between productivity indices and percentage loss of conductivity (PLC) in Scots pine trees mass inoculated with *Ophiostoma ips* at different densities. Each value represents one stem segment. ○ and ● = 400 inoculi/m^2; □ and ■ = 800 inoculi/m^2; △ and ▲ = 1600 inoculi/m^2; filled symbols = 100 cm wide inoculation belt; empty symbols = 150 cm wide inoculation belt. P1 and P5 = ratio between cross-sectional sapwood area of the 1997 or the 1993–1997 rings and total cross-sectional sapwood area in autumn 1997. S_{97} = area of transversal section of the 1997 sapwood ring. $S_{93–97}$ = area of the transversal section of the 1993–1997 sapwood rings. SA_{97} = area of sapwood transversal section up to 1997

4 Discussion

Although re-isolation of *O. ips* was not tried, effects observed in inoculated trees are attributable to *O. ips*, because no adverse effects were observed in the non-inoculated controls. In addition, great care was taken to avoid contamination by other organisms. For example, the inoculations were undergone with pure monospore cultures.

The number of trees used per category is similar or even higher than that used in other experiments concerned with comparable topics (Christiansen and Hornтvedt 1983; Christiansen and Solheim 1985; Christiansen and Berryman 1995; Christiansen and Glosli 1996; Bois and Lieutier 1997; Krokene et al. 2000 among others). Such low numbers can be explained by the considerable amount of work that mass inoculation of trees represents.

The lack of effect of the size of the inoculation belt is in agreement with the results of Guérard et al. (2000) after mass inoculation with *O. brunneo-ciliatum* on the same tree species. Certainly differences in belt sizes higher than those used in our experiment are necessary to induce such an effect.

All parameters related to tree survival 3 months after mass inoculation with *O. ips* dramatically changed to the worse when inoculation densities were above 400 inoculi/m^2. Tree resistance seemed to be fully overcome and trees were going to die at 800 inoculi/m^2, indicating that the critical threshold of inoculum density was a little bit lower than this value. In a similar experiment with *O. brunneo-ciliatum* on the same tree species, Guérard et al. (2000) observed a gradual decrease of tree vitality parameters with increasing inoculation density, and all trees survived up to 1000 inoculi/m^2. They consequently questioned the existence of a threshold above, which trees die inevitably. Our results on the same tree species with a fungus very closely related to *O. brunneo-ciliatum* show that such a threshold exists, but that it probably lies higher than 1000 inoculi/m^2. *Ophiostoma ips*, thus, appears to be more virulent than *O. brunneo-ciliatum*. It should also be considered that the trees used by Guérard et al. (2000) were only 7–8 years old. Both *Ophiostoma* species are closely associated

with both *I. acuminatus* and *I. sexdentatus* at a frequency higher than 95% (Lieutier et al. 1989a, 1991) and have been demonstrated to play a role in beetle establishment (Lieutier et al. 1989b, 1995). Consequently, although the virulence of *O. ips* may be higher than that of *O. brunneo-ciliatum*, we can hypothesize that the aggressiveness of the two beetle–fungus associations is comparable, and that the critical threshold of attack density by *I. sexdentatus* may be around 850 attacks/m^2 as for *I. acuminatus* (Guérard et al. 2000). Direct measurements of this threshold level with insects themselves are, however, needed to confirm this hypothesis. Such a value is quite high compared with 300–500 for *I. typographus* (Christiansen 1985a) and 60 for *D. ponderosae* (Raffa and Berryman 1983a), and confirms the low aggressiveness of these two bark beetle species.

The relationship between inoculation density and damage to the hydraulic system (xylem) has already been demonstrated (Christiansen and Berryman 1995; Croisé et al. 1998). These authors used the percentage of inoculated cambial surface of the trunk as another way to express the inoculum pressure. In our experiment, the critical threshold is reached for an inoculated bark surface representing 0.4% of the total bark area of the trunk. Although we did not find any effect of the size of the inoculation belt, certainly, if the same number of inoculi would have been regularly distributed over the bole, the effect on the tree would have been weaker. In our experiment, the local density of inoculation very likely played a more important role than the percentage of inoculated bark surface. However, nothing was known regarding these aspects or about the role of the location of the inoculation belt on the trunk, although this may be of importance to mimic and compare aggressiveness of bark beetle species that attack at different levels on the bole.

Our results suggest a negative non-linear relationship between PLC and the percentage of healthy sapwood. The PLC increased much faster than the percentage of healthy sapwood decreased. This confirms the results of Guérard et al. (2000) who concluded that PLC seems a more sensitive parameter for detecting changes in sapwood conductivity than other parameters-like the reaction zone length. In our experiment, tree mortality occurred only after very severe PLC (≥60–70%), i.e. when conductive sapwood area decreased to ≥50–60%. Guérard et al. (2000) did not observe any tree mortality, as Ks in their experiment stayed below 60%. The tree could, thus, have been able to compensate the loss of conductive sapwood area by increasing the water flow through the still conductive part of this tissue. Measurements of conductance would clarify this point.

We observed only little blue-stained sapwood, and the percentage of this area did not correlate to PLC. Similar observations were reported for Scots pine after mass inoculations with other species of fungi. Well above the threshold, blue stain becomes dominant (Solheim et al. 1993; Croisé et al. 1998, 2001). Below the threshold, Guérard et al. (2000) with *O. brunneo-ciliatum* also noted very little blue stain but abundant resinosis and desiccation. In our experiment, the density of 800 inoculi/m^2 was just above the threshold while 1600 inoculi/m^2 was largely above. Nevertheless, both treatments exhibited little blue stain 3 months after inoculation. An explanation could be the speed at which the fungus overcomes tree resistance. Three months may be just time enough for this relatively non-virulent fungus to overcome tree resistance. The validity of such an explanation would confirm that sapwood invasion by the fungus becomes important, and that tree death occurs, only after the critical threshold is reached and tree resistance is exhausted (Croisé et al. 1998; Lieutier 2002). In Norway spruce inoculated with *Ceratocytis polonica*, blue stain always appears long before the critical threshold is reached and even in trees that resist attacks (e.g. Christiansen 1985b; Christiansen and Berryman 1995; Krokene et al. 1999). An effect of the nature of the species (tree and fungus) on the invasion process itself can thus be suspected.

5

Résumé

L'objectif était de déterminer le seuil critique de densité d'inoculation du Pin sylvestre (*Pinus sylvetris* L.) après inoculation massive par le champignon *Ophiostoma ips* (Rumb.) Nannf. isolé du Scolyte *Ips sexdentatus* Boern. L'expérience s'est déroulée dans le Nord-Ouest de l'Espagne. Seize pins sylvestres ont été inoculés à des densités variées (0, 400, 800 or 1600 inoculations/m^2) sur des ceintures d'inoculation de 100 ou 150 cm de large. Chaque inoculum est un cilindre de 5 mm de diamètre de malt agar colonisé par le champigon. Trois mois plus tard, tous les arbres ont été abattus. Les écoulements de résine sur le tronc et la couleur du feuillage ont été estimés visuellement. Le pourcentage d'aubier sain, imprégné de résine, sec, ou bleui, de même que des indices de productivité ont été calculés sur des sections transversales de chaque arbre au niveau de la zone d'inoculation. La conductivité hydraulique spécifique de l'aubier (Ks) de chaque arbre a été mesurée au milieu de cette zone. Tous ces paramètres de survie décroissent quand la densité d'inoculation est superior à 400 inoculi/m^2, tandis que la couleur du feuillage vire du vert au jaune-vert ou jaune quand on utililise une densité d'inoculation de 800 inoculi/m^2 tandis que une densité de 400 inoculi/m^2. La perte de Ks (calculée en comparant le Ks mesuré à la conductivité maximale) passe de 30% à 90% et le pourcentage d'aubier conducteur chute de 85% à 35%. On en conclue que la résistance des arbres est vaincue et que ceux-ci sont condamnés à mourir quand la pression d'inoculum atteint une densité de 800 inoculations/m^2, mais aucun effet de la largeur de la ceinture d'inoculation n'a été noté. Aucune relation n'a été observée entre la productivité et le niveau de résistance. Une relation négative non-linéaire a été trouvée entre la perte de Ks et le pourcentage d'aubier sain mesuré sur les sections transversales.

Zusammenfassung

Bestimmung der Schadensschwelle für Ohiostoma ips an Pinus sylvestris im Nordwesten Spaniens mittels Variation des Infektionsdruckes

Das Ziel der Arbeit war die Bestimmung derjenigen Inokulationsdichte, oberhalb welcher der Bläuerreger *Ohiostoma ips*, der mit dem Grosser Kiefernborkenkäfer (*Ips sexdentatus*) vergesellschaftet ist, zum Absterben der Gemeinen Kiefer (*Pinus sylvestris*) führt. Im Nordwesten Spaniens wurden die Stämme von 16 Kiefern stammumfassend auf einer Länge von 100 oder 150 cm unterschiedlich dicht (0, 400, 800 oder 1600 Inokuli/m^2) mit *O. ips* beimpft. Jedes Inokulum bestand aus einem vom Pilz bewachsenen Zylinder (Druchmesser 5 mm) aus Malzager. Die Bäume wurden drei Monate nach der Inokulation gefällt und auf Harzfluss untersucht. Zudem wurde die Nadelfarbe beurteilt. Die Anteile von gesundem, vertrocknetem, harzgetränktem und verbläutem Splintholz sowie Zuwachsindices wurden für Stammscheiben bestimmt, die vom inokulierten Stammabschnitt jeden Baumes stammten. In der Mitte des inokulierten Stammabschnitts jeden Baumes wurde die spezifische hydraulische Leitfähigkeit des Splints (Ks) gemessen. Alle gemessenen Wert verschlechterten sich dramatisch, wenn die Inokulationsdichte 400 Inokuli/m^2 überstieg. Die Nadeln verfärbten sich von grün nach gelbgrün oder gelb bei 800 Inokuli/m^2 im Unterschied zu den sich nicht verfärbenden Nadeln von Bäumen, die nur mit 400 Inokuli/m^2 beimpft wurden. Der prozentuale Verlust an spezifischer hydraulischer Leitfähigkeit (PLC) stieg bei einer Inokulationsdichte von 800 Inokuli/m^2 von 30 auf 90% an und der Prozentsatz von gesundem, leitendem Splint nahm von 85 auf 35% ab. Dagegen hatte die Länge des inokulierten Stammabschnittes keinen Einfluss. Zudem konnte zwischen der Zuwachsleistung und dem Grad der Resistenz keine Beziehung festgestellt werden. Eine nichtlineare Beziehung zeigte sich dagegen zwischen der PLC und dem Anteil gesunden Splints. Mit dieser Arbeit konnte gezeigt werden, dass die Resistenz bei einer Inokulationsdichte von 800 Inokuli/m^2 oder mehr zusammenbricht und die Bäume absterben.

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