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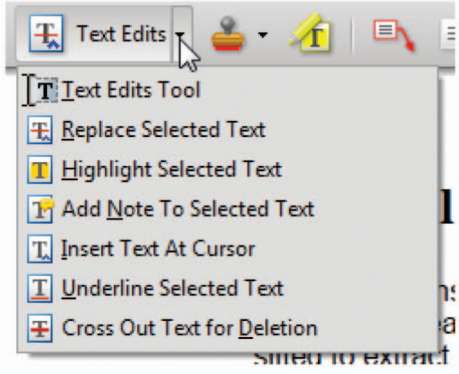

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Effect of water availability and fertilization on water status, growth, vigour and the resistance of Scots pine to fungal mass inoculation with *Ophiostoma ips*

A. ENCINA[1], M. L. VALBUENA[2], J. L. ACEBES[1], F. LIEUTIER[3], & M. M. FERNÁNDEZ[4]*

[1]*A. Fisiología Vegetal, Fac. C.C. Biológicas y Ambientales, Univ. León, 24071 León, Spain,* [2]*A. Ecología, Fac. C.C. Biológicas y Ambientales, Univ. León, 24071 León, Spain,* [3]*Lab. Biologie des Ligneux et des Grandes Cultures, Univ. Orléans, 45067 Orleans, France, and* [4]*A. Zoología, Dpto. C. Agroforestales, ETSIIAA Palencia, Univ. Valladolid, 34071 Palencia, Spain*

Abstract

The effect of water and nutrient availability on the performance of Scots pine (*Pinus sylvestris* L.) against *Ophiostoma ips* (Rumb.), a bark beetle-associated phytopathogenic blue-stain fungus, was investigated. Field-grown trees were subjected for 18 months to water-stress and/or fertilization, and the effects of such treatments on the needle nutrient status, tree vegetative growth and vigour were examined. At the end of the experimental period, the trees were mass-inoculated (800 inocula m^{-2}) with the fungus, and the relationship between resource availability and tree performance against pathogen attack was also tested. Predawn shoot water potential (Ψ_{PD}) of irrigated trees was significantly higher than that of water-stressed trees, and fertilized trees had a significantly lower C/N ratio. The Ψ_{PD} values and needle nitrogen content suggest that resource-limited trees were under moderate stress. Improved nutrient availability significantly increased tree growth and tree vigour. However, no evidence for an effect of improved nutrient availability on tree fungal resistance was found in our study.

Keywords: *mass inoculations, nutrient status,* Ophiostoma ips, *predawn shoot water potential, Scots pine, tree growth, tree vigour, water status*

1 Introduction

Bark beetles (Coleoptera, Scolytidae) are among the most damaging pests of trees in coniferous forests. Endemic populations of bark beetles complete their life cycle on weakened or dying trees, most of them feeding on phloem tissue. Under favourable conditions, bark beetles increase their breeding rate and may become epidemic. Under such conditions, if the establishment of bark beetles is successful, even healthy trees may die within months (Christiansen et al. 1987; Raffa et al. 1993). Most bark beetles associate in a mutualistic relationship with, and function as vectors for, phytopathogenic blue-stain fungi (Ascomycota, Ophiostomatales; Romón et al. 2007; Lu et al. 2009; Masuya et al. 2009; Dobelin 2010; Persiani et al. 2010). During attack by the bark beetles, the fungi are inoculated into the xylem and phloem tissue of host trees. Although the nature of the insect/fungus relationship is not fully understood, fungi would play an active role in the establishment of the bark beetle population. Thus, the fungi may render host carbohydrates more available to the insects as food. Moreover, the spread of fungi through host tissues would contribute to decreasing tree resistance and, hence, would be involved in the death of trees (Christiansen et al. 1987; Paine et al. 1997; Lieutier 2002).

Coniferous trees have developed both constitutive and inducible bark defences against bark beetle/fungal attack. The constitutive defence includes a combination of suberized and lignified tissues (periderm and sclerenchyma) plus the constitutive production of terpenoids (oleoresin) and phenolic compounds in the resin ducts and polyphenolic cells, respectively (Franceschi et al. 2005; Harju et al. 2009). Tree resistance to bark beetle/fungal attack also involves inducible defence systems, such as the

Correspondence: M. M. FERNÁNDEZ, Área de Zoología, Dpto. Ciencias Agroforestales, ETSIIAA de Palencia, Universidad de Valladolid, Avda. Madrid, 44, edif. E. 34071 Palencia, España. Tel: +34 979 108392. Fax: +34 979 108400. Email: mffernan@agro.uva.es

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hypersensitive response consisting of the development of elliptical necrotic areas (induced wound reactions) around the bark beetle entrance points. The induced wound reaction is also characterized by the induced accumulation of oleoresin and phenols together with the formation of a wound peridermis, the activation of polyphenolic cells, and the early lignification of fibres (Franceschi et al. 2005). It has been assumed that induced wound reactions limit beetle activity and fungal spread by the isolation of the damaged or infected tissue (Lieutier et al. 1993; Brignolas et al. 1995; Franceschi et al. 2005). Induced-reaction zones can be located in both the phloem and sapwood. In the latter case, the combination of oleoresin deposition, fungal growth and xylem cavitation is often linked to sapwood damage and xylem dysfunction (Tyrre & Sperry 1989; Paine et al. 1997).

Tree resistance has been defined by a critical threshold of attack density above which tree resistance is overcome (Berryman 1982; Raffa & Berryman 1983; Christiansen et al. 1987). Tree resistance may relate to the efficiency of the induced reaction (Lieutier et al. 1993; Krokene & Solheim 1999). In this regard, the length of induced-reaction zones has been used as an indicator of tree resistance or pathogen virulence. Small lesions would be associated with more resistant host trees or, alternatively, with weaker pathogens (Viiri et al. 2001a; Baier et al. 2002; Nagy et al. 2004, 2006; Blodgett et al. 2005; Klepzig et al. 2005). Other results suggest a poor value for the length of induced reactions as a tree resistance index (Croisé et al. 1998a, 1998b; Guérard et al. 2000).

Tree resistance has also been quantified in terms of sapwood dysfunction, expressed either as sapwood damage (sapwood displaying tissue drying, blue-staining or induced resinosis) or as an impairment in water conductivity (losses of hydraulic conductivity; Sperry et al. 1988; Croisé et al. 1998a, 2001; Krokene & Solheim 1998; Guérard et al. 2000; Baier et al. 2002; Sandnes & Solheim 2002; Fernandez et al. 2004; Klepzig et al. 2005; Ben Jamaa et al. 2007).

Environmental conditions, such as nutrient or water availability and temperature, are known to influence the severity and incidence of some woody plant diseases (Schoeneweiss 1981). The results concerning this issue are frequently contradictory or lack statistical significance (Herms 2002). Several authors have studied the susceptibility of conifers to bark beetle/fungal attack and have shown that tree resistance decreases under drought conditions (Raffa 1991; Blodgett et al. 1997; Croisé et al. 2001; Jones et al. 2004; Rouault et al. 2006). In other cases, no relationship between drought stress and disease development has been found (Croisé et al. 1998b; Salle et al. 2008). Regarding fertilization, contrary to the expectation that tree vigour would be associated with increased disease resistance, a considerable body of evidence has indicated a neutral or positive correlation between fertilization and disease susceptibility and herbivory (Herms 2002 and references therein; Jones et al. 2004). However, other works point to a positive correlation between fertilization and a decrease of susceptibility to bark beetles (Knebel et al. 2008).

Our study aimed at gaining information about the effect of water and nutrient availability on the performance of Scots pine (*Pinus sylvestris* L.) against *Ophiostoma ips* (Rumb.), a phytopathogenic blue-stain fungus associated with *Ips sexdentatus* (Boern.). Field-grown trees were subjected to water-stress and/or fertilization and the effects of such treatments on tree growth and vigour were quantified. At the end of the experimental period, the trees were mass-inoculated with the fungus and the relationship between resource availability and tree performance against pathogen attack was also tested. The expectation is that a positive relationship between resource availability and tree defence should be found.

As far as we know, the work presented here is a pioneering experiment in the Iberian Peninsula. The choice of the tree species and pathosystem was mainly based on the fact that *I. sexdentatus* is a very common pest in pine forests (Gil & Pajares 1986), and that Scots pine is one of the most abundant coniferous species, with an evident value in Spain from both an ecological and an economic perspective (Blanco et al. 2005).

Materials and methods

Site description

A field-site (2500 ha) of reforested 40-year-old Scots pines was selected in Riocamba, province of León, north-western Spain. The selected area is fairly flat (1% of slope), 1100 m above sea level. Mean annual precipitation is 1039 mm, and the potential evapotranspiration is 609 mm month^{-1}. During the experimental period, average minimum and maximum temperatures were 2.8°C and 15.5°C, respectively, and the period of drought lasted from mid-June to mid-September. At the end of the experimental period, trees ranged from 8 to 15 m in trunk height, and 6 to 41 cm in diameter at breast height (DBH). Tree density varied from ca. 900 to 2400 trees ha^{-1}, depending on the location.

A humic-acrisol-gleic soil occurs at the site. The phreatic level is located at 45–60 cm, and root depth at about 60–75 cm. The soil has very poor external and internal drainage. Five horizons occur: 0 (0–

5 cm), Au (0–28 cm), AB (28–45 cm), BCg (45–90) and Cg (90–190 cm). Soil analyses indicated a very poor and acidic soil (pH ranged from 4.2 to 5.2) with low productivity and low rates of mineralization (data not shown).

Experimental design

Four 450-m^2 plots were delimited. At each plot, 55 trees with DBH between 24 and 39 cm were selected. From May 1997 to October 1999, a water-stress and a fertilization treatment were applied as follows (Figure 1).

From May to November, the soil surface of water-stressed plots was covered with PVC sheets. Additionally, water-stressed plots were delimited by a 1 m × 1 m-trench lined with PVC sheets. On water-stressed plots, borderline trees were not used owing to their proximity to the trench. Irrigated plots were watered weekly, by dripping 50 l of water per tree, from mid-June to mid-September. Water dosage and frequency were determined on the basis of site soil and climatic conditions to avoid an excessively long period of soil water drought.

In order to modify the tree nutrient status, a soil fertilization treatment was applied from June 1997 till May 1999: N (131 kg ha^{-1}; NH_4NO_3), P (96 kg ha^{-1}; P_2O_5), K (166 kg ha^{-1}; K_2O); Mg (60 kg ha^{-1}; MgO) and Ca (400 kg ha^{-1}; $CaCO_3$) were spread on the soil surface.

The water-stress and soil fertilization treatments were applied to the selected plots as indicated in the following distribution: Plot 1: water-stressed + fertilized (WSF); Plot 2: watered + fertilized (WF); Plot 3: watered (W) and Plot 4: water-stressed (WS). The watered and fertilized plot (WF) was regarded as control.

Predawn shoot water potential measurement

The predawn shoot water potential (Ψ_{PD}, MPa) was measured monthly (from May to October) using a Scholander–Hammel pressure chamber (PMS Instrument). Ψ_{PD} was assayed in 30 shoots per treatment collected from the upper third of the crown (6 trees × 5 shoots per tree). Measurements were made immediately after the shoots had been sampled between 3:00 and 6:00 am.

Needle growth parameters and nutrient status

Needles were sampled yearly at the end of the growing season (October) in sunlight-exposed shoots collected from the top of the trees. When possible, needles were collected from the same whorl. Needle dry weight was calculated by drying needles at 60°C until a constant weight was reached and then stored in a dry atmosphere. Leaf area was calculated assuming the needle as a hemi-cylinder. After measuring the length and calculating average needle diameter ($n = 5$), the needle area was estimated as: needle area = 1/2Π(× average diameter × length). Needle area and dry weight were calculated for 80 needles per treatment (8 trees × 10 needles per tree).

Tree nutrient status was addressed by analysing the needle C/N ratio of eight trees per treatment. After drying at 60°C for 72 h, the needles were mill-powdered (Softonic.com) and total C and N needle contents were assayed according to the methods of Dumas and Kjeldahl.

Tree growth and vigour measurement

DBH was measured at the end of each growing season (October). Based on the DBH measurements, the annual increase in DBH was expressed as the current year DBH increase/DBH ratio.

Increases in shoot/branch length and the vigour index were calculated after the trees had been felled at the end of the experimental period. The increase in shoot and branch length were expressed as the current year shoot internode length/total tree length ratio, and the current year branch internode length/total branch length ratio, respectively.

The vigour index was calculated as BA_1/SA, where BA_1 is the cross-sectional area of the annual ring and SA is the sapwood basal area (Waring et al. 1980). For this purpose, transverse sections (3 mm) were sliced from the tree trunk 120 to 160 cm above the ground. Each slice was computed, after digitization, using image-analysis software (Windendro, Chicoutimi, Canada). The annual rings and total sapwood

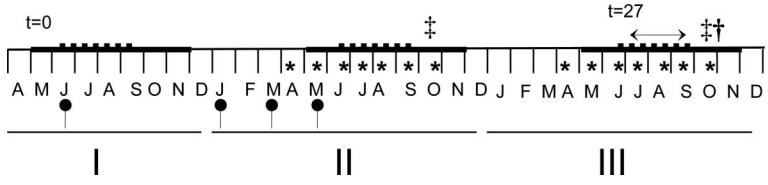

Figure 1. Experimental design followed in this work. (↑) fertilization treatment; (—) water-stress treatment; (- - -) irrigation; (↔) mass inoculation experiment; (I–III) year; (*t*) time in months; (†) shoot/branch internode increase and vigour index measurements; (‡) needle growth and nutrient status measurements; (*) Ψ_{PD} measurement.

areas were the means of four measurements taken at four different positions along the radii, 90° apart. In all cases the heartwood–sapwood boundary was clearly visible.

Fungal mass inoculation

At the end of the experimental period, a fungal mass inoculation experiment was carried out (mid-June 1999; Figure 1). Four trees (7–10 cm in DBH) were belt-inoculated with *O. ips* on the main stem, 0.3–1.3 m above ground, at a density of 800 inocula m^{-2} (Fernandez et al. 2004). In order to prevent contamination by other organisms, monospore fungal cultures were used. *O. ips* isolation and tree inoculation were carried out following the method described by Fernandez et al. (2004). Inoculations were made by introducing a 5-mm-diameter disc of fungal culture into a cambium-deep hole made with a cork-borer. The hole was then closed with the previously removed bark disc. Non-bored trees were regarded as controls.

Sapwood water conductivity estimation

Sapwood specific hydraulic conductivity (Ks) was measured on stem sections (17.5–19.8 cm long) taken from the middle of the inoculated belt. For control trees, the sections were taken at a similar height on the trunk. The methods and techniques for measuring Ks were those developed by Sperry et al. (1988) and described in detail by Guérard et al. (2000) and Croisé et al. (2001). The degree of embolism was expressed as the percentage loss of conductivity (PLC), relative to maximal hydraulic conductivity (Km), following the equation $PLC = [1 - Ks/Km] \times 100$. As previously described (Fernandez et al. 2004), Km was estimated based on the relationship between the diameter (D) and the Ks, which is obtained from non-inoculated and healthy trees ($n = 12$) as follows: $Ks = 2.6 \times 10^{-6} \times D^{5.87}$ ($r^2 = 0.87$).

Assessment of sapwood damage and induced-reaction zone

Based on the method described by Fernandez et al. (2004), inoculated trees were felled and two transverse discs (5-mm thick) were sliced inside the inoculation belt (at 20 cm from the ends of the belt or at a similar height in control trees). Dried, resin-soaked, blue-stained and total sapwood areas on the discs were redrawn on transparent paper. These areas were then digitized and computed using image-analysis software (Windendro, Chicoutimi, Canada). In each case, functional sapwood was estimated by subtracting heartwood, resin-soaked sapwood, dried sapwood and blue-stained sapwood from the total wood cross-sectional area. The percentage of functional, dried, resin-soaked or blue-stained sapwood was expressed as the mean of the two stem discs sliced from each individual tree. For control trees (not damaged), functional sapwood was estimated by subtracting the heartwood from the total wood cross-sectional area.

The total length of the induced-reaction zone around the inoculation point was measured with a ruler.

Data analysis

Statistics were performed with Statistica software after the data had been tested for normality. A two-way analysis of variance (ANOVA) followed by Tukey's test was used to compare treatments, and the level of significance was tested at $p < 0.05$. Data are expressed as means ± standard deviation.

Results

Time-course of predawn shoot water potential

During the irrigation period (mid-June to mid-September), the Ψ_{PD} measured in watered trees (WF and W) was significantly higher ($p < 0.05$) than that of water-stressed trees (WS and WSF; Figure 2) with the exception of the results obtained in August 1998 (Figure 2A) where differences between treatments were not found. The lowest mean Ψ_{PD} recorded in the water-stressed trees (WS and WSF) ranged between −1.15 MPa (August 1998) and −1.20 MPa (September 1999). The lowest mean Ψ_{PD} of the watered tress (WF and W) was always above −1.15 MPa.

ANOVA of the data obtained for the irrigation period revealed that water-stress was the main factor accounting for differences in Ψ_{PD} ($F_{1,19} = 10.10$, $p < 0.05$ in July 1998; $F_{1,20} = 31.07$, $p < 0.05$ in September 1998; $F_{1,20} = 5.85$, $p < 0.05$ in July 1999; $F_{1,20} = 13.77$, $p < 0.05$ in August 1999; $F_{1,20} = 11.56$, $p < 0.05$ in September 1999). No interaction between water-stress and fertilization was found.

Needle nutrient status

Soil fertilization significantly modified needle nutrient status. As expected, average needle nitrogen (N) content was higher in the fertilized trees (WSF and WF) than in non-fertilized ones (W and WS): 17.2 mg g^{-1} dry weight vs. 12.6–15.0 mg g^{-1} dry weight in 1998 and 13.8–14.7 mg g^{-1} dry weight vs. 12.9–11.8 mg g^{-1} dry weight in 1999. Fertilization had only a slight effect on needle carbon (C) contents. The mean C contents measured in 1998

were 527.2–521.4 mg g^{-1} dry needle in fertilized trees vs. 515.7–516.1 mg g^{-1} dry needle in non-fertilized trees. Differences in C content were even smaller in 1999: 505.2–500.9 mg g^{-1} dry needle in fertilized trees vs. 506.8–505.2 mg g^{-1} dry needle in non-fertilized trees.

Based on these results, fertilization significantly lowered the needle C/N ratio ($F_{1,28} = 18.43$, $p < 0.05$ in 1998; $F_{1,28} = 8.34$, $p < 0.05$ in 1999, Figure 3) The ANOVA did not show a significant influence of water-stress on needle nutrient status ($F_{1,28} = 4.17$, $p > 0.05$ in 1998; $F_{1,28} = 1.51$, $p > 0.05$ in 1999) nor any interaction between soil fertilization and water-stress ($F_{1,28} = 4.01$, $p > 0.05$ in 1998; $F_{1,28} = 0.02$, $p > 0.05$ in 1999).

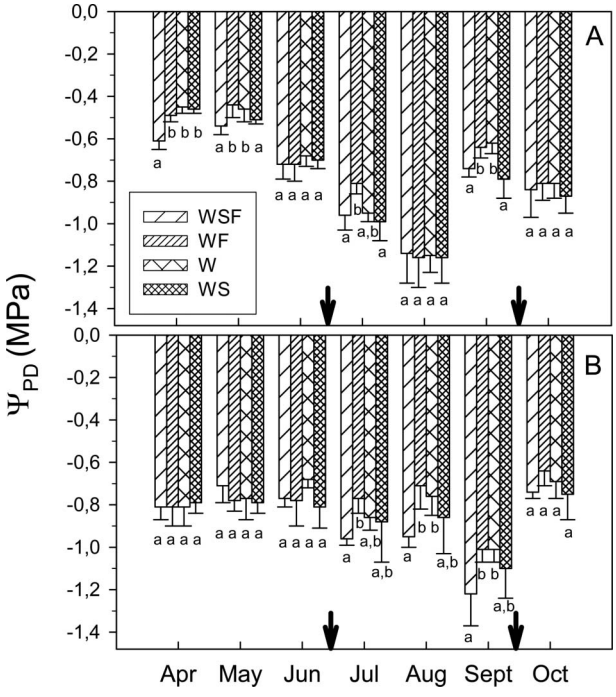

Figure 2. Predawn shoot water potential variation of Scots pine trees subjected to water-stress and/or fertilization. The data were recorded from April to October of (A) 1998 and (B) 1999. Values are the means ± SD ($n = 6$). Within a month, asterisk indicates significant differences between treatments ($p < 0.05$). Treatments: WSF = water-stressed + fertilized; WF (control) = watered + fertilized; W = watered; WS = water-stressed. Within a month, bars with different letters indicate significant differences ($p < 0.05$; ANOVA followed by Tukey's test). Watering treatment lasted the in-between arrows period.

Needle growth

Although needles from fertilized trees (WSF and WF) were on average larger and heavier than those from non-fertilized ones (W and WS), needle growth parameters did not significantly vary upon treatment (Table I).

Tree growth and vigour index

Significant differences in growth parameters were only found ca. 30 months after the start of the experiment (1999 data; Table II). Control trees (WF) showed the highest growth values. This effect was especially noticeable when compared with WS trees, where significant differences ($p < 0.05$) in all growth parameters were detected.

ANOVA of the 1999 data indicated that soil fertilization was the main factor accounting for differences in DBH increase ($F_{1,103} = 12.05$,

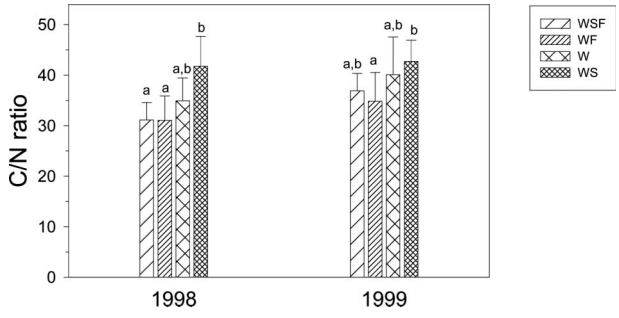

Figure 3. Needle C/N ratio of Scots pine trees subjected to water-stress and/or fertilization. Data correspond to needles collected in October 1998 and 1999. Values are the means ± SD ($n = 8$). Within a year, bars with different letters indicate significant differences ($p < 0.05$; ANOVA followed by Tukey's test). Treatments as in Figure 2.

Table I. Effects of drought stress and fertilization on Scots pine needle growth.

Year	Parameter	Treatment			
		WSF	WF	W	WS
1998	Needle area (cm^2)[1]	0.80 ± 0.25^{a}	1.03 ± 0.32^{a}	0.73 ± 0.26^{a}	0.67 ± 0.13^{a}
	Needle dry weight (mg)	$10.95 \pm 3.55^{a,b}$	15.30 ± 625^{a}	$10.12 \pm 4.05^{a,b}$	9.48 ± 3.25^{b}
1999	Needle area (cm^2)	0.63 ± 0.09^{a}	0.66 ± 0.13^{a}	0.56 ± 0.14^{a}	0.54 ± 0.08^{a}
	Needle dry weight (mg)	7.86 ± 1.23^{a}	8.34 ± 2.35^{a}	8.17 ± 2.47^{a}	7.48 ± 1.30^{a}

Values are means ± SD of eight measurements. Within a year, values with different letters indicate significant differences ($p < 0.05$). Treatments: WSF = water-stressed + fertilized; WF (control) = watered + fertilized; W = watered; WS = water-stressed. [1]Needle area was calculated on fresh needles as follows: 1/2(Π × average diameter × length).

Table II. Effects of drought stress and fertilization on Scots pine shoot growth and vigour index.

Year	Parameter	Treatment			
		WSF	WF	W	WS
1998	DBH increase[1]	0.019 ± 0.009^{b}	0.011 ± 0.008^{a}	0.008 ± 0.006^{a}	0.009 ± 0.006^{a}
	Branch length increase[2]	0.34 ± 0.05^{a}	0.33 ± 0.05^{a}	0.35 ± 0.04^{a}	0.25 ± 0.04^{a}
	Shoot length increase[3]	0.030 ± 0.007^{a}	0.030 ± 0.009^{a}	0.029 ± 0.007^{a}	0.026 ± 0.006^{a}
	Vigour index[4]	0.035 ± 0.008^{a}	0.033 ± 0.012^{a}	0.027 ± 0.009^{a}	0.028 ± 0.007^{a}
1999	DBH increase	0.017 ± 0.008^{a}	0.018 ± 0.009^{a}	$0.013 \pm 0.006^{a,b}$	0.012 ± 0.006^{b}
	Branch length increase	$0.21 \pm 0.03^{a,b}$	0.24 ± 0.07^{b}	0.33 ± 0.07^{b}	0.20 ± 0.04^{a}
	Shoot length increase	$0.022 \pm 0.005^{a,c}$	0.030 ± 0.009^{a}	0.024 ± 0.005^{b}	$0.020 \pm 0.006^{b,c}$
	Vigour index	$0.033 \pm 0.008^{a,b}$	0.035 ± 0.017^{a}	$0.027 \pm 0.007^{a,b}$	0.024 ± 0.006^{b}

Values are means $\pm$ SD of 24 (DBH increment) or 14 (shoot length increase, branch length increase and vigour index) measurements. Within each line, values with different letters indicate significant differences ($p < 0.05$). Treatments: WSF = water-stressed + fertilized; WF (control) = watered + fertilized; W = watered; WS = water-stressed. [1]DBH increase was expressed as current year DBH increase to total DBH ratio. [2]Branch length increase was expressed as current year branch internode length/total branch length ratio. [3]Shoot length increase was expressed as current year shoot internode length/total tree length ratio. [4]Vigour index was expressed as BA_1/SA, where BA_1 is the cross-sectional area of the annual ring and SA is the sapwood basal area.

$p < 0.05$) and vigour index ($F_{1,52} = 9.10$, $p < 0.05$), whereas differences in branch length increase ($F_{1,52} = 13.63$, $p < 0.05$) and shoot length increase ($F_{1,49} = 9.09$, $p < 0.05$) can be attributed to the water-stress treatment. With the exception of increases in shoot length ($F_{1,49} = 4.16$, $p < 0.05$), no soil fertilization/water-stress interaction was detected ($p < 0.05$).

Embolism in stem and sapwood damage

Inoculation with *O. ips* caused both sapwood damage (Figure 4A–D) and impairment of sapwood water conductivity (Figure 4E). In comparison with non-inoculated trees (Figure 4A), a gradual decrease in the percentage of healthy sapwood in all the inoculated trees was recorded along time. The decrease in functional sapwood was paralleled by an increase in dried (Figure 4B), blue-stained (Figure 4C) and resin-soaked (Figure 4D) sapwood. Regardless of treatment, no significant differences in Ψ_{PD} between inoculated and non-inoculated trees were detected 90 days after mass inoculation (data not shown).

In those trees in which the loss of healthy sapwood was more marked (ca. 50% on average for WSF and WS trees), this effect was accompanied by tissue drying around wounds (Figure 4B). Fungal proliferation and resin-soaking were low and similar in all the treatments (Figure 4C, D).

At 90 days after tree inoculation, the percentage losses of specific conductivity (Figure 4E) ranged from ca. 70% in the WF trees to 85% in the WS trees. A significant positive non-linear relationship ($r^2 = 0.75$) between the percentage loss of specific conductivity and the loss of healthy sapwood was found (Figure 5).

Because of high variability, significant between-treatment differences were not found. Regardless of treatment, no evident symptoms of tree mortality were detected after mass inoculation. Based on visual estimation, resin exudation ranged from low to medium, and foliage colour varied from green to green-yellow.

Length of induced reaction

Induced-reaction zones around inoculation points were recorded in all the inoculated trees. The length of induced-reaction zones increased with time (Figure 6), reaching 12–15 cm in length 90 days after fungal inoculation. No effect of treatment on the length of induced-reaction zones was detected.

Discussion

Effect of water-stress and fertilization on growth and nutrient status of trees

As expected, water-stress modified the tree shoot water potential by decreasing Ψ_{PD} (Figure 2). As long as the Ψ_{PD} values of the water-stressed trees ranged from -0.71 to -1.21 MPa during the drought period (mid-June to mid-September), trees can be regarded as being under a moderate water-stress (Croisé et al. 2001). In any case, the average Ψ_{PD} values obtained in this work were below the drought stress levels shown to induce stomatal closure ($\Psi_{PD} = -1.3$ MPa) or embolism ($\Psi_{PD} = -2.0$ MPa) in Scots pine (Cochard 1992; Jackson et al. 1995; Croisé et al. 1998b).

In our study, fertilization significantly decreased the needle C/N ratio. The changes in the C/N ratio after fertilization were caused by an increase in

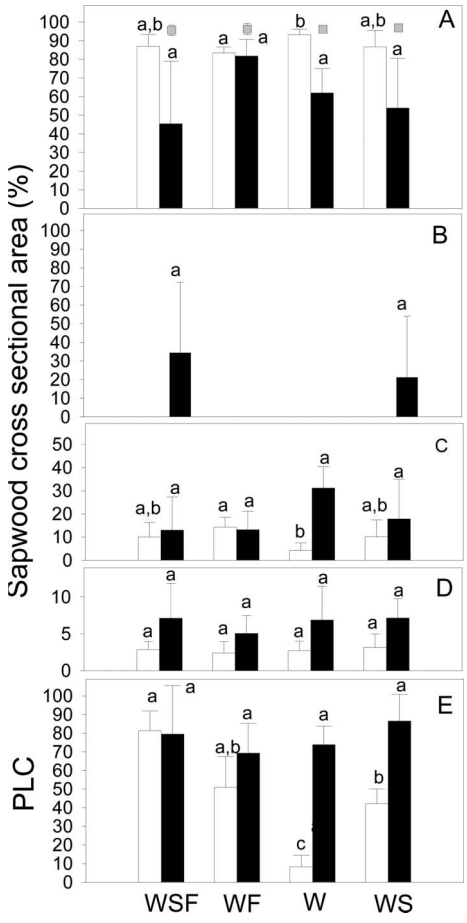

Figure 4. Effect of mass inoculation with *Ophiostoma ips* on: (A) percentage of sapwood remaining functional after inoculation; (B) percentage of dried sapwood after inoculation; (C) percentage of sapwood affected by resinosis; (D) percentage of blue-stained sapwood and (E) loss of sapwood specific hydraulic conductivity (PLC) of Scots pine trees subjected to water-stress and/or fertilization. Trees were mass-inoculated in mid-June 1999 and data recorded 14 (white bars) and 90 (black bars) days after mass inoculation. The fraction of functional sapwood in non-inoculated trees is represented as grey squares in (A). Values are means ± SD ($n = 4$). Within sampling date, bars with different letters indicate significant differences ($p < 0.05$). Treatments as in Figure 2.

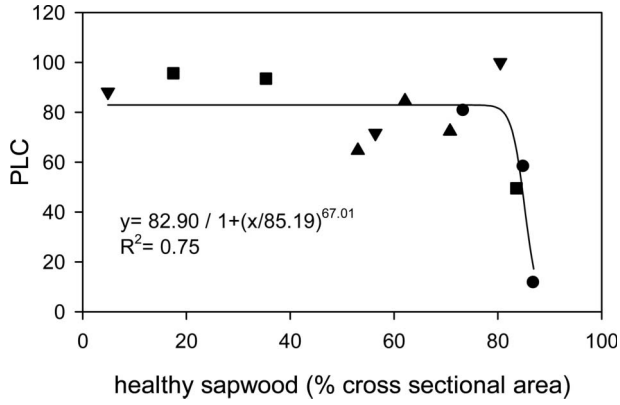

Figure 5. Relationship between loss of sapwood specific hydraulic conductivity (PLC) and the percentage of functional sapwood of Scots pine trees subjected to water-stress and/or fertilization after mass inoculation with *Ophiostoma ips*. Each value represents one stem segment inoculated at a density of 800 inocula m^{-2} in mid-June 1999. Treatments: (■) WSF = water-stressed + fertilized; (●) WF (control) = watered + fertilized; (▲) W = watered; (▼) WS = water-stressed.

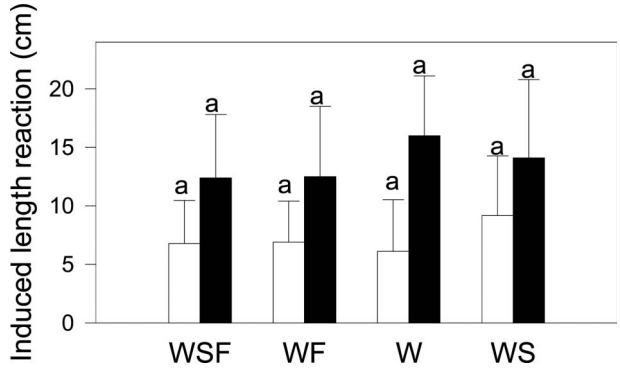

Figure 6. Length of reaction zones recorded on phloem from Scots pine trees subjected to water-stress and/or fertilization after mass inoculation with *Ophiostoma ips*. Trees were mass-inoculated in mid-June 1999 and samples were collected 14 (white bars) and 90 (black bars) days after mass inoculation. Values are means ± SD ($n = 30$). Within sampling date, means with different letters indicate significant difference among plots ($p < 0.05$). Treatments as in Figure 2.

needle N content as the C content hardly varied among treatments. Optimal needle N levels for Scots pine stands have been estimated to be in the 14–20 mg g^{-1} range (Sikström et al. 1998; Tamm et al. 1999; Nielsen (Abrahansem 2003), while a deficiency limit of 12 mg g^{-1} has been established (Pietiläinen et al. 2005). Thus, our results would indicate (1) that the needle N content of non-fertilized trees was suboptimal, as expected for trees growing in a very acidic and poor soil, and (2) that N fertilization increased needle N content to reach the optimum range. Needle C/N ratios reported by Viiri et al. (2001b) and Blodgett et al. (2005) in related studies were in the range of those reported here.

Moderate nutrient or water deficits can inhibit shoot growth, either directly or indirectly, by affecting bud formation, cambial growth, stem and branch elongation and leaf expansion and by promoting leaf abscission (Kozlowsky & Pallardy 1997). In terms of leaf growth, it has been demonstrated that essential mineral deficiencies and/or water deficit inhibit leaf growth by reducing both leaf number and size even before other symptoms become visible (Kozlowsky & Pallardy 1997).

The results obtained in this study show that leaf growth did not respond positively to water and/or nutrient supply. Although average values indicate that control trees (WF) had larger needles that accumulated more dry weight in comparison with non-fertilized and/or water-stressed trees (Table I),

differences were not statistically significant. However, an increase in resource availability had a positive impact on shoot growth (Table II). In general, at the end of the experimental period (1999), increases in diameter, shoot length, branch length and vigour index were higher in control trees (WF) than in non-fertilized and/or water-stressed trees. These findings are in accordance with the results reported for comparable experiments (Kytö et al. 1996; Croisé et al. 2001; Viiri et al. 2001b). With few exceptions, differences in growth parameters were statistically significant, especially when control trees (WF) were compared with water-stressed and non-fertilized trees (WS). The lack of significance in the tree growth parameters corresponding to the data for 1998 (Table II) could be due to the fact that any growth efficiency parameter needs about 2 years after treatment before a response is seen (Waring et al. 1992).

Therefore, results obtained in this work indicate that improved resource availability increases tree vegetative growth and vigour. According to the effect of resource availability on the parameters analysed, a scale of stress could be established as follows: WS > W = WSF > WF (control). The Ψ_{PD} values (Figure 2) and needle N content (Figure 3) of the WS trees suggest that resource-limited trees were under moderate stress.

Impact of resource availability on tree performance against pathogen attack

An inoculation density of 800 inocula m^{-2}, expected to overcome the resistance of Scots pine (Fernandez et al. 2004), was used in the current experiment. No data about the effect of mechanical wounding on sapwood damage are available. In a comparable experiment, Guérard et al. (2000) demonstrated that even in the case of higher inoculation densities, aseptic mechanical wounding yielded a very moderate response compared with fungal inoculation. Following these observations, it may be concluded that the responses recorded on our mass-inoculated trees would be associated to *O. ips* colonization.

The results obtained clearly show that inoculation with *O. ips* caused both sapwood damage and impairment in sapwood water conductivity. The inoculated trees showed a significant reduction in functional sapwood and a marked drop in sapwood hydraulic conductivity in comparison with the non-inoculated trees. After inoculation, functional sapwood and PLC were correlated in a negative non-linear relationship. The observed relationship is consistent with the assumption that moderate reductions in functional sapwood can cause dramatic increases in PLC as previously reported by Guérard et al. (2000) and Fernandez et al. (2004). Three months after the trees had been inoculated, PLC values were in the range of those previously reported by our group in a similar field experiment (Fernandez et al. 2004).

Similar to Croisé et al. (2001), no effect of fungal mass inoculation on tree Ψ_{PD} was detected. In our experiment, Ψ_{PD} measured at the crown top did not differ between non-inoculated trees and mass-inoculated trees with high PLC values (>70%). This result would reflect the ability of trees to compensate the loss of conductive sapwood by increasing the capacity of healthy xylem to maintain the supply of water and minerals to leaves (Tyrre & Sperry 1989).

The relationship among drought, xylem embolism and disease susceptibility is well documented. It has been demonstrated for Scots pine that embolism and loss of hydraulic conductivity start when the xylem water potential reaches −2.0 MPa (Cochard 1992). On the other hand, water-stress causing a drop in Ψ_{PD} below −2.0 MPa has been demonstrated to decrease Scots pine resistance to *Leptographium wingfieldii* (Croisé et al. 2001). Although the Ψ_{PD} values of the water-stressed trees studied here were always above those levels, we anticipated a positive interaction between water-stress and fungal mass inoculation. In agreement with this assumption, the water-stressed trees (WSF and WS) had the highest values of sapwood damage (including tissue drying) and PLC. We suggest that water-stress would be the main factor modulating the tree's response to mass inoculation. In this respect, it is interesting to note that fertilization of water-stressed trees (WS vs. WSF) did not cause any reduction either in sapwood damage or in the impairment of sapwood hydraulic conductivity.

Results show that moderate water-stress and fertilization had no influence on the response of trees to fungal mass inoculation. However, some interesting trends can be outlined. Control trees (WF) were less impaired than stressed trees in terms of losses of sapwood specific conductivity and sapwood damage. Moreover, the fraction of sapwood affected by resinosis and blue staining was lower than for other groups of trees, and no sapwood drying was recorded.

In conclusion, the results obtained in this work indicate that watering and fertilization significantly increased tree growth and vigour, and decreased the needle C/N ratio of trees growing at a nutrient-deficient site. However, no statistical evidence for an effect of improved nutrient availability on tree fungal resistance could be found.

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