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Fungal infection and mechanical wounding induce disease resistance in Scots pine

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Abstract

Scots pine trees (*Pinus sylvestris*) recovering from a 90–100% defoliation 2–3 years previously were pretreated with small mechanical wounds or inoculations with the blue-stain fungi *Leptographium wingfieldii* and *Ophiostoma canum*. Pretreated trees were less susceptible to a subsequent massive inoculation with *L. wingfieldii* than untreated control trees, which were extensively colonised by the mass-inoculation. A low pretreatment dosage of *L. wingfieldii* was somewhat more effective in inducing disease resistance than a higher dosage. Pretreatment with *L. wingfieldii*, *O. canum*, and mechanical wounding were about equally effective inducers of resistance in Scots pine, even though *L. wingfieldii* is known to produce much more extensive phloem necrosis than the other pretreatments. Thus, the strength of the induced resistance response did not depend on the amount of host tissues that was destroyed by the pretreatment. Previously, induced disease resistance has been demonstrated in Norway spruce (*Picea abies*), and the present study shows that similar responses can be activated in Scots pine.

Introduction

Early in last century it was observed that plants with previous exposure to pathogen infection developed enhanced resistance to subsequent infections (Chester, 1933). More recent studies have shown that several processes are responsible for such induced disease resistance, including cross-protection, antagonism between pathogens, and systemic acquired resistance (SAR) (Kessmann et al., 1994; Ryals et al., 1994; Hammerschmidt and Smith Becker, 1997). The only well-documented examples of induced disease resistance in conifers are the response of Norway spruce trees (*Picea abies* (L.) Karsten) after pretreatment inoculations with the bark beetle-associated blue-stain fungus *Ceratocystis polonica* (Siem.) C. Moreau (Christiansen et al., 1999; Krokene et al., 1999), and Monterey pine seedlings (*Pinus radiata* D. Don) after foliar treatment with 5-chlorosalicylic acid (Reglinski et al., 1998). Resistance responses have also been induced in cell suspension cultures of different pine species treated with biological and biochemical elicitors (Lesney, 1989; Campbell and Ellis, 1992; Hotter, 1997).

Unlike Norway spruce and many other conifers, Scots pine (*Pinus sylvestris* L.) is not subjected to large-scale outbreaks by aggressive, tree-killing bark beetles. The major bark beetle pest of Scots pine under Nordic conditions is the pine shoot beetle *Tomicus piniperda* (L.), a non-aggressive beetle that normally attacks dying trees or fresh timber, but whose shoot feeding behaviour in healthy trees is causing extensive economical losses (Långström, 1983; Långström and Hellqvist, 1991). Pine trees weakened by defoliation or other stress factors may, however, be successfully colonised and killed by the beetle

(Annila et al., 1999). Even though *T. piniperda* rarely attacks and kills vigorous trees, it is associated with phytopathogenic blue-stain fungi that are introduced into the host tree (Lieutier et al., 1989; Solheim and Långström, 1991). The most virulent of these fungi, *Leptographium wingfieldii* Morelet, is able to kill healthy trees when it is inoculated under the bark at a sufficient dosage, causing extensive necrosis in the phloem, colonising the sapwood and disrupting water transport (Långström et al., 1993; Solheim et al., 1993).

In this study, we tested whether induced resistance responses similar to those demonstrated in Norway spruce could be activated in stressed Scots pine trees that are potential hosts for the pine shoot beetle. Trees recovering from a severe defoliation episode were preinoculated with sterile wounds or different fungi and subjected to a massive *L. wingfieldii*-infection to test (1) whether induced disease resistance could be elicited, and (2) whether this response was specific to the pretreatment or not, i.e. whether different types of pretreatment could protect trees against *L. wingfieldii*.

Materials and methods

Fifty Scots pine trees of similar size (diameter at 1.3 m height: 84.5 ± 2.7 mm (mean $\pm$ SD)) were selected from a single stand at Hökensås, SW Sweden. The stand had been severely defoliated (ca. 90–100% of needle biomass removed) by an extensive outbreak of the pine looper, *Bupalus piniaria* (Lepidoptera, Geometridae) in 1996 (Långström et al., 1999).

On 5 June 1998, three groups of six trees were randomly assigned to different pretreatments: (1) high dosages (100 inoculations/m^2, 15–16 inoculations/tree) or (2) low dosages (50 inoculations/m^2, 8 inoculations/tree) with *L. wingfieldii*-inoculations, and (3) no pretreatment. The trees were estimated to have less than 30% of full foliage, with needles only on the short shoots developed in 1997 and on the expanding shoots of the current year. Inoculations were done by removing a bark plug with a 5-mm cork borer, inserting inoculum in the wound, and replacing the plug. Inoculum consisted of actively growing mycelium of *L. wingfieldii* (isolate NISK 97-849/1) on malt agar (2% malt, 1.5% agar). Four weeks after pretreatment, all 18 trees were mass-inoculated with *L. wingfieldii* (800 inoculations/m^2, $\sim$127 inoculations/tree) to determine tree resistance. Both pretreatment and mass-inoculations were evenly spaced over a 0.6 m section of the stem from about 1.0–1.6 m height. The trees were felled on 11 November, 1998.

On 25–26 May 1999, four groups of eight trees were randomly assigned to pretreatments consisting of a low dosage (50 inoculations/m^2) of cork borer inoculations with (1) *L. wingfieldii*, (2) *Ophiostoma canum* (Münch) H. and P. Sydow (isolate NISK 97-33/47), and (3) sterile agar, and (4) no pretreatment as a control. *O. canum* is an avirulent blue-stain fungus that is associated with the pine shoot beetle *Tomicus minor* (Hart.) (Francke-Grosmann, 1967; Solheim et al., 2000). The trees were estimated to carry about 30–50% of full foliage, with smaller-than-normal shoots of age classes 1997 and 1998. Four weeks after pretreatment all 32 trees were mass-inoculated with *L. wingfieldii* (800 inoculations/m^2) to determine tree resistance. The trees were felled on 27 September, 1999.

Before felling, the vertical extension of six phloem necroses on each tree was measured. In 1998, three of the uppermost and three of the lowermost necroses were measured. In 1999, six of the uppermost necroses were measured. Because necroses tended to coalesce within mass-inoculated sections in susceptible trees, their lengths were measured upwards from upper inoculation points, and downwards from lower inoculation points. After felling a thin disc (5 mm) was cut midway through the inoculated stem section, and the areas of heartwood, desiccated, fresh and blue-stained sapwood was out-lined on the upper surface of the discs based on translucency. The surface areas were later determined with a computer-connected planimeter (Krokene and Solheim, 1998). Along the disc circumference, the proportion of dead and live cambium was measured. Re-isolation of fungus was made from three challenge inoculation points per tree.

Data were subjected to ANOVA, using the general linear models procedure on SAS (SAS Institute, 1987). If treatments were significantly different ($p < 0.05$), means were separated using LSD at $p = 0.05$. Percentage data were arcsin-transformed and phloem necrosis data were log-transformed before analysis to correct for unequal variance and departures from normality.

Results

In 1998, control trees and trees pretreated with a high or low dosage of *L. wingfieldii* showed significantly

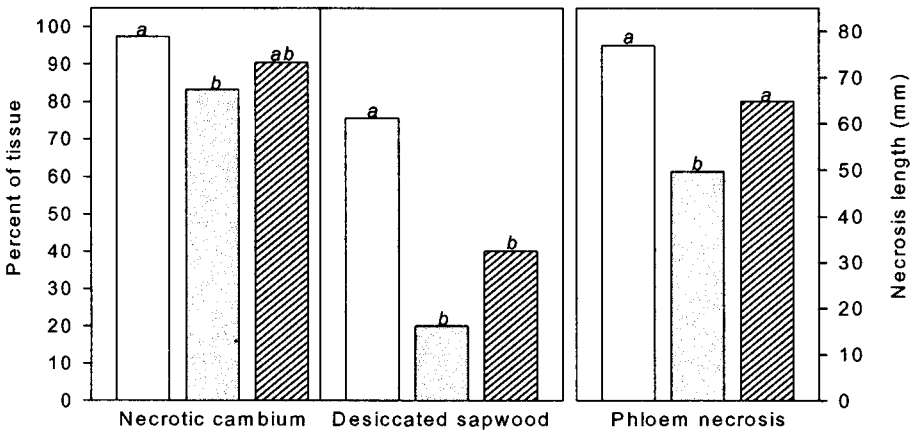

Figure 1. Symptoms of fungal mass-inoculation in 1998 in Scots pine trees pretreated with a low (50 inoculations/m^2, grey bars) or high dosage (100 inoculations/m^2, hatched bars) of *L. wingfieldii* inoculations 4 weeks before they were mass-inoculated with *L. wingfieldii* at 800 inoculations/m^2. Control trees (white bars) did not receive any pretreatment before mass-inoculation. Bars with different letters were significantly different by the LSD test ($p = 0.05$) following ANOVA.

different symptoms of fungal colonisation after challenge inoculation (% necrotic cambium: $p = 0.04$, $F = 3.91$; % desiccated sapwood: $p = 0.002$, $F = 9.73$; phloem necrosis length: $p = 0.0001$, $F = 5.45$) (Figure 1). The effect of pretreatment was most pronounced in the sapwood, where the low dosage of *L. wingfieldii* reduced host symptoms by 74% relative to the control. In the phloem, the reduction was 35% and in the cambium it was only 15%. However, for all tissues, symptoms were significantly less severe in trees pretreated with the low dosage of *L. wingfieldii* than in control trees. The high pretreatment dosage resulted in significantly less severe symptoms than the control in the sapwood, but not in the cambium or phloem (Figure 1).

In 1999, there were also significant differences in host symptoms between control trees and trees assigned to different pretreatments (% necrotic cambium: $p = 0.002$, $F = 6.15$; % desiccated sapwood: $p = 0.002$, $F = 6.67$; phloem necrosis length: $p = 0.0001$, $F = 9.78$) (Figure 2). As in 1998, the differences were most pronounced in the sapwood and phloem. Trees that were pretreated with *L. wingfieldii* or *O. canum* had significantly less severe symptoms than untreated control trees in both phloem, cambium and sapwood. Trees pretreated with sterile wounds had significantly less severe symptoms than control trees in the sapwood ($p = 0.025$) and phloem ($p = 0.0003$), whereas in the cambium the difference was marginally significant ($p = 0.056$).

In 1998, *L. wingfieldii* was re-isolated from all 18 trees and from 98% of the mass-inoculation points. In 1999, the fungus was re-isolated from all 32 trees and from all inoculation points in each tree.

Discussion

Pretreatment with fungus and mechanical wounding enhanced the resistance of Scots pine trees to subsequent massive inoculation with *L. wingfieldii*. Thus, induced resistance similar to that observed in Norway spruce (Christiansen et al., 1999; Krokene et al., 1999) appears to be present in Scots pine. Such induced resistance was apparently not activated in loblolly pine (*Pinus taeda* L.) or shortleaf pine (*P. echinata* Mill.) pretreated with a few fungal inoculations or sub-lethal bark beetle attacks (Paine and Stephen, 1987; Cook and Hain, 1988). However, in those studies the resistance of pretreated trees was evaluated by single fungal inoculations, and this method may not be sensitive enough to distinguish between resistant and susceptible trees. Massive fungal inoculation, which overwhelms the total defensive capacity of susceptible trees, probably gives more reliable estimates of tree resistance (Krokene and Solheim, 1999).

Induced disease resistance in Scots pine was not specific to the pretreatment, since pretreatment with *O. canum* and mechanical wounding also protected trees against massive *L. wingfieldii*-infection. This

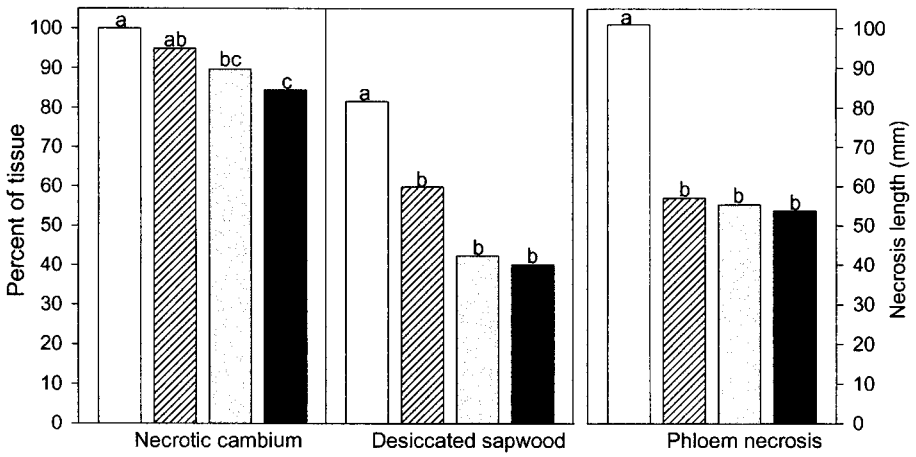

Figure 2. Symptoms of fungal mass-inoculation in 1999 in Scots pine trees pretreated with 50 inoculations/m^2 of sterile agar (hatched bars), *L. wingfieldii* (grey bars), or *O. canum* (black bars) 4 weeks before they were mass-inoculated with *L. wingfieldii* at 800 inoculations/m^2. Control trees (white bars) did not receive any pretreatment before mass-inoculation. Bars with different letters were significantly different by the LSD test ($p = 0.05$) following ANOVA.

agrees with results from Norway spruce, where pretreatment with mechanical wounding and different fungi was found to protect trees against *C. polonica*-infection (Krokene et al., 1999; 2000). *L. wingfieldii* and *O. canum* seemed to be equally effective inducers of resistance in Scots pine. The virulent *L. wingfieldii* produces long phloem necroses in Scots pine (Lieutier et al., 1989; Långström et al., 1993; Solheim et al., 1993; 2000), whereas *O. canum* produces only negligible necrosis (Solheim et al., 2000). Mechanical wounding, which produces very little necrosis, also induced disease resistance in Scots pine. Thus, the amount of necrotic phloem produced by the pretreatment does not seem to determine its effectiveness as an inducer of disease resistance in Scots pine. This differs from Norway spruce, where the strength of the induced resistance response seems to depend on the amount of host tissues that is destroyed by the pretreatment (Krokene et al., 2000).

Because trees were pretreated and mass-inoculated in the same area, it is not known whether the enhanced resistance effect in Scots pine is systemic or local. However, in Norway spruce the resistance response does not appear to extend beyond the pretreated area when trees are mass-inoculated 3 weeks after pretreatment (Krokene et al., 1999). It is possible, however, that the effect will spread further if the interval between pretreatment and mass-inoculation is extended. The mechanisms responsible for enhanced disease resistance in Norway spruce and Scots pine are unknown, but may involve spread of inducible defence reactions, such as traumatic resin duct formation or activation of phenol-rich parenchyma cells, beyond the local lesion (Franceschi et al., 1998; 2000; Nagy et al., 2000). Similar inducible defence reactions, such as rapid induction of phenolic metabolism and lignification, have been observed in cell suspension cultures of different pines (Campbell and Ellis, 1992; Hotter, 1997), and in Monterey pine seedlings (Reglinski et al., 1998).

This study demonstrated that induced resistance against bark beetle-associated blue-stain fungi can be activated in Scots pine, and thus this type of resistance occurs in Scots pine, Norway spruce, and Monterey pine (Reglinski et al., 1998; Christiansen et al., 1999; Krokene et al., 1999). Inducible disease resistance could be widespread in conifers, and this defence mechanism deserves further attention.

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