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Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway spruce

Patogenitet på gran til noen fargeskadesopper som følger granbarkbillen

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Abstract

SOLHEIM, H. 1988. Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway spruce. (Patogenitet på gran til noen fargeskadesopper som følger granbarkbillen.) Medd. Nor. inst. skogforsk. *40*(14): 1–11.

To test the pathogenicity of *Ips typographus*-associated fungi to Norway spruce, trees were inoculated with four species, *Ophiostoma polonicum, O. bicolor, O. penicillatum* and *Graphium* sp. Trees inoculated with the three *Ophiostoma* species produced strong hypersensitive responses in the phloem and exuded more resin than did the control trees. Only *O. polonicum* was able to invade the sapwood and consequently able to kill trees. Inoculation with *Graphium* sp. gave the same response as inoculation with the control.

Key words: *Picea abies, Ophiostoma* spp., blue-stain, *Ips typographus,* artificial inoculation, pathogenicity, wound reaction, resinosis.

Utdrag

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For å teste patogeniteten til *Ophiostoma polonicum, O. bicolor, O. penicillatum* og *Graphium* sp., som er assosiert med granbarkbillen, *Ips typographus,* ble grantrær inokulert med soppene. Etter inokulering med de tre *Ophiostoma*-artene produserte trærne sterke reaksjonssoner i barken og de skilte ut mer kvae enn kontrolltrærne. Bare *O. polonicum* klarte å trenge inn i veden og således i stand til å drepe grantrær. Inokulering med *Graphium* sp. ga det samme resultat som kontrollen.

Nøkkelord: *Picea abies, Ophiostoma* spp., fargeskadesopp, *Ips typographus,* kunstig inokulering, patogenitet, sårreaksjon, kvaeutflod.

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Preface

This work is a part of the project «Fungal detriment in beetle-killed spruce» financed by The Agricultural Research Council of Norway and carried out at the Norwegian Forest Research Institute, Division of forest ecology, Department of forest pathology. Skilful technical assistance was given by Liisa Lassen, Thomas Midttun and Pamela Paulsboe. Linda Hjeljord has corrected the English text. Erik Christiansen, Richard Horntvedt and Kåre Venn, of the Norwegian Forest Research Institute, have critically read the manuscript and given valuable suggestions. The above-mentioned persons and institutions are gratefully acknowledged.

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Content

Introduction

Rapid desiccation of living trees attacked by certain bark beetles is caused by blue-stain fungi transferred by these beetles. This is well known from studies of different bark beetle-fungus associations, and supported by several inoculation studies, the first by NELSON & BEAL (1929).

Conifers have generally two different resistance mechanisms that may prevent or reduce colonization by bark beetles and associated fungi: (1) Resin ducts holding preformed or primary resin and (2) an induced hypersensitive tissue response involving production of secondary resin (REID, WHITNEY & WATSON 1967; BERRYMAN 1972; WONG & BERRYMAN 1977; SHRIMPTON 1978; RAFFA & BERRYMAN, 1982B; STEPHEN, PAINE & LIH 1983; CHRISTIANSEN, WARING & BERRYMAN 1987). The induced hypersensitive response produces resin-soaked lesions surrounding the infection points. The stronger and more rapid the response, the more resistant are the trees.

Inoculation studies on Norway spruce (*Picea abies* (L.) Karst.) with blue-stain fungi associated with *Ips typographus* L. were initiated by HORNTVEDT et al. (1983). Trees inoculated with *Ophiostoma polonium* Siem. were infested, while trees inoculated with *O. penicillatum* (Gros.) Siem. were not. Later *O. polonicum* has been used to study the resistance of Norway spruce (CHRISTIANSEN & HORNTVEDT 1983; CHRISTIANSEN 1985A, B; CHRISTIANSEN & ERICSSON 1986; HORNTVEDT 1988).

Many blue-stain fungi have been found in association with *I. typographus*. *O. penicillatum* was described first (GROSMANN 1931, 1932). Later other species have been mentioned, but *O. penicillatum* has been considered to be the most important one (MATHIESEN 1950; RENNERFELT 1950; MATHIESEN–KÄÄRIK 1953; KÄÄRIK 1975).

Studies by SOLHEIM (1989a) during an *I. typographus* epidemic in Norway, indicated that *O. polonicum* was the first species to invade the sapwood of Norway spruce. The species following closely behind *O. polonicum* were *O. bicolor* Davids. & Wells, *O. penicillatum* and *Graphium* sp. All these species are frequently isolated from *I. typographus* (FURNISS, SOLHEIM & CHRISTIANSEN 1989) and are probably introduced together by the beetles.

The purpose of this study was to test the pathogenicity of the blue-stain fungi found in association with *I. typographus* using artificial mass inoculations and to evaluate the host tree response.

Material and methods

The inoculation experiment was performed in a 25-year-old plantation of Norway spruce at Ås, in south-eastern Norway.

The following species and isolates were used: *Ophiostoma bicolor* (NFRI 80–48/36 and NFRI 80–93/30), *O. penicillatum* (NFRI 60–21 and NFRI 80–91/54), *O. polonicum* (NFRI 80–53/7 and NFRI 80–65/19) and *Graphium* sp. (NFRI 80–52/24 and NFRI 80–93/46).

The species of *Ophiostoma* have been treated earlier by SOLHEIM (1986). *Graphium* sp. will be described in a separate paper.

Twenty healthy trees were selected and grouped into four classes, according to diameter at breast height. The smallest diameter was 6 cm and the largest 15 cm. One tree in each diameter class was randomly assigned to each treatment: inoculation with young mycelium of the four blue-stain fungi or with sterile malt agar (control). The inoculations were done during 16–21 May 1984. Bark plugs were removed with a 5 mm cork borer and small pieces of inoculum were inserted into the holes before the bark plugs were replaced. The inocula were placed with their edges 2 cm apart in six rings around the stems between 120 and 170 cm above the ground. The two isolates of each species were inoculated on half of the circumference of each tree.

Five weeks after inoculation the length of the resin stripes under each inoculation point was measured. This was repeated after ten weeks when the trees were harvested. At this time the outer dead bark was removed and the vertical length of the lesion surrounding each inoculation point measured. After felling the trees, thin discs were cut at the inoculation rings for delineation and area calculation of sapwood and desiccation zone. The blue-stained area of the desiccation zone was also measured. Reisolations were done from random discs.

Results

There was no difference between the two isolates of each species with respect to pathogenicity, and they have consequently been treated together. Neither was there any difference between trees of different diameters as to the response to inoculation.

After inoculation with *O. polonicum*, about one fourth of the sapwood was desiccated (Table 1) and most of this zone was blue-stained. *O. polonicum* was always reisolated from the blue-stained area.

The inoculations with the other tree species, *O. bicolor, O. penicillatum,* and *Graphium* sp., produced only small desiccation zones, not significantly different from that following inoculation with the control (Table 1). These

Table 1. The desiccation zones in per cent of the sapwood area 10 weeks after inoculation with *Ips typographus*-associated fungi into Norway spruce

	Desiccation zone/sapwood (%)
Ophiostoma polonicum	27.5 a
O. penicillatum	8.7 b
O. bicolor	7.1 b
Graphium sp.	5.6 b
Control	4.7 b

Values followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

desiccation zones were generally impregnated with resin, and no blue-stain was observed. The three species were occasionally reisolated from the current annual ring.

Great differences in wound reaction were detected, depending on treatment. After inoculation with *Graphium* sp. and sterile malt agar (control), only a few cells around each inoculation point were dead (Fig. 1). Inoculation with the *Ophiostoma* species, however, gave a strong hypersensitive reaction in the tissues around the inoculation points (Fig. 1). Lesions after inoculation with *O. penicillatum* were significantly longer than those caused by *O. polonicum* and *O. bicolor* (Table 2). Lesions associated with *O. polonicum* were, however, broader than the others.

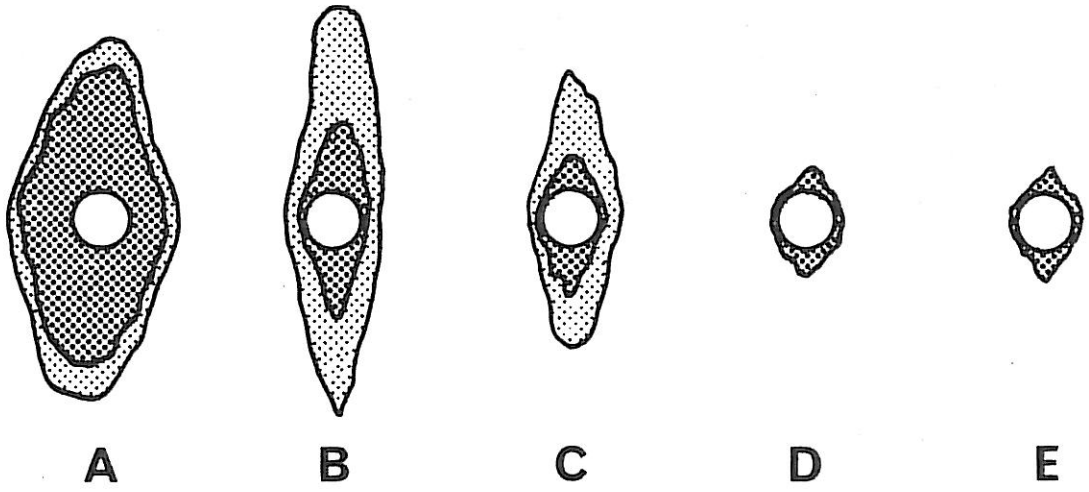

Fig. 1. Typical wound reaction in the phloem of Norway spruce 10 weeks after inoculation with some *Ips typographus*-associated fungi,
a) *Ophiostoma polonicum*, b) *O. penicillatum*, c) *O. bicolor*, d) *Graphium* sp., e) control.

The wound reaction in the phloem characteristically consists of two different zones: an inner dark-coloured area enclosing the fungi, and an outer, lighter area with no fungal growth. The ratio between the inner and outer areas of the lesions, however, was not the same for the *Ophiostoma* species. Inoculation with *O. bicolor* and *O. penicillatum* caused small, dark brown inner zones (Fig. 1), as opposed to the long, broad and greyish-brown inner zones produced by *O. polonicum*.

Inoculation with the three *Ophiostoma* species gave much longer resin stripes on the bark surface (i.e. larger resin yield) than inoculation with *Graphium* sp. or the control (Table 2). There was no significant difference between *Graphium* sp. and the control. At the first inspection, *O. penicillatum* inoculations had produced the longest resin stripes, but after 10 weeks there was no significant difference between *O. penicillatum* and *O. polonicum*. The length of the resin stripes increased from week 5 to 10 for all treatments.

Table 2. The mean length of resin stripes on the bark surface and phloem lesions produced in response to inoculation of *Ips typographus*-associated fungi into Norway spruce

	Resin stripes after 5 weeks (cm)	Resin stripes after 10 weeks (cm)	Lesion length (cm)
Ophiostoma polonicum	4.7 a	7.9 a	4.2 a
O. penicillatum	6.7 b	8.4 a	4.8 b
O. bicolor	4.1 c	7.2 b	3.1 c
Graphium sp.	1.2 d	2.6 c	1.4 d
Control	0.8 d	2.4 c	1.4 d

Values within columns followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

Discussion

O. polonicum was, in this experiment, able to infect the sapwood of Norway spruce. There was a considerable difference between *O. polonicum* on the one hand and *O. bicolor, O. penicillatum* and *Graphium* sp. on the other hand. The latter resulted in no more desiccation or visible blue-stain than in the control trees. None of these three species were able to invade the sapwood of healthy Norway spruce alone, as *O. polonicum* does, even though they are introduced together with *O. polonicum* by the beetles (FURNISS et al. 1989) and follow close behind *O. polonicum* into the sapwood after *I. typographus* attacks (SOLHEIM 1989a). *O. penicillatum,* which has been considered to be the most important blue-stain fungus associated with *I. typographus,* was neither in this experiment nor in an earlier one (HORNTVEDT et al. 1983) able to invade the sapwood.

Although *O. polonicum* proved able to infect the sapwood, the infection was not as successful as could be expected after an earlier inoculation experiment with this species (HORNTVEDT et al. 1983). According to HORNTVEDT (1988), the tree resistance may vary throughout the growth season, being higher in May, when the present experiment was initiated, than in June, when the other experiment was started. Climatic influences may also be important (CHRISTIANSEN et al. 1987).

Development of large lesions and exudation of long resin stripes after inoculation with *Ophiostoma* species show the hypersensitive response of Norway spruce to invasion by bark beetle-associated blue-stain fungi. In contrast to *Graphium* sp., inoculations with *O. bicolor* and *O. penicillatum* gave rather large wound reactions in the inner bark. *O. penicillatum* produced even longer lesions and *O. bicolor* only slightly shorter lesions than the highly pathogenic *O. polonicum.*

The induced hypersensitive response of lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelmann) and grand fir (*Abies grandis* (Douglas) Lindley) has been thoroughly studied by REID et al. (1967) and BERRYMAN (1969). The response of Norway spruce has also been investigated (CHRIS-

TIANSEN & HORNTVEDT 1983; HORNTVEDT et al. 1983). To distinguish between resistant or less resistant trees, measurement of the concentration of monoterpenes and other extractives in the lesions has been attempted (CHRISTIANSEN & HORNTVEDT 1983; RAFFA & BERRYMAN 1983; CHRISTIANSEN 1985a). RAFFA & BERRYMAN (1982a, b) have also correlated resistance to the concentration of monoterpenes. Lesion weights (STEPHEN et al. 1983; PAIN, 1984) and lengths (REID & SHRIMPTON 1971; STEPHEN & PAINE 1985) have been attempted used as a measure of host-tree resistance. On some pine species longer lesions have been considered characteristic of the more resistant trees. In contrast, relatively small, heavily resin-soaked lesions are produced by Norway spruce trees resistant to *O. polonicum*. Resistant trees quickly react to the fungus and will not use more energy than necessary to overcome the intrusion. Less resistant trees apparently fight for a longer time against the pathogen and consequently the lesions become longer. Occasionally the fungus may break out of the lesions first produced and additional zones outside the first one may be created (CHRISTIANSEN et al. 1987). A negative correlation between lesion lengths and resistance of Norway spruce was demonstrated by HORNTVEDT (1988).

Lesion length has also been used in comparison of pathogenicity of different species (MOLNAR 1965; SAFRANYIK, SHRIMPTON & WHITNEY 1983; PAINE 1984). This seems to be of limited value in Norway spruce, since *O. penicillatum,* which could not invade sapwood, produced longer lesions than *O. polonicum.*

In this study the length of the resin stripes showed great variation, as did lesion lengths. Length of resin stripes, however, is a very crude measure depending of site and time. More elaborate quantifications, e.g. gravimetrical measurements (CHRISTIANSEN 1985a), however, seem to correlate well with stripe length (HORNTVEDT 1988).

Under normal conditions, *I. typographus* populations survive in weak and wind-felled trees, unbarked timber and logging debris, but may occasionally become epidemic, killing healthy spruce. When a complex of species is introduced together by the bark beetles, the species have different preferences. *O. polonicum* seems to tolerate low oxygen concentration (SOLHEIM 1989b) and is consequently well-adapted to colonize wet sapwood of living trees, thus opening up the way for other species. These species, such as *O. bicolor* and *O. penicillatum,* may be better adapted to live in and colonize the inner bark and wood of unbarked timber and logging debris. In such material, *O. penicillatum* may be the dominating species, as indicated in earlier studies of *I. typographus* (MATHIESEN 1950; RENNERFELT 1950; MATHIESEN–KÄÄRIK 1953; KÄÄRIK 1975).

Patogenitet på gran til noen fargeskadesopper som følger granbarkbillen

Granbarkbillen, *Ips typographus*, fører med seg en variabel sammensetning av soppsporer som den inokulerer i barken på angrepne grantrær. Enkelte av disse soppene infiserer den friske yteveden og fører til at vanntransporten stopper opp og trærne tørker. Hensikten med dette arbeidet var å se hvilke sopparter som klarte å trenge inn i frisk yteved og å studere granas reaksjon i barken mot soppinntrengingen.

Tjue trær ble inokulert med fargeskadesoppene *Ophiostoma polonicum, O. bicolor, O. penicillatum, Graphium* sp. eller med sterilt inokulum (kontroll). Inokula ble plassert i små hull i barken laget med korkbor, med 2 cm avstand i 6 ringer rundt stammen, 120–170 cm over bakken.

Ti uker etter inokulering med *O. polonicum* hadde omtrent en fjerdedel av yteveden tørket ut (Tabell 1) og var for det meste infisert av soppen. Svært lite av yteveden tørket ut ved de andre behandlingene og uttørkingssonene var vanligvis impregnert med kvae.

Trær som var inokulert med *Ophiostoma*-artene produserte store reaksjonssoner i barken rundt inokuleringsstedet (Fig. 1). Inokulering med *O. polonicum* ga lange, brede soner som for det meste var fylt opp med en mørk gråbrun innersone. Inokulering med *O. bicolor* og *O. penicillatum* ga lange, smale soner med små mørkebrune innersoner. Trær som var inokulert med *Ophiostoma*-artene skilte også ut lange kvaestriper på barkoverflaten (Tabell 2). *Graphium* sp. ga ingen av disse reaksjonene i barken hos trærne.

De forskjellige soppartene som føres inn i gangsystemene med *I. typographus* har nok forskjellige fortrinn. *O. polonicum* synes å være godt tilpasset etablering i yteveden hos friske grantrær og er følgelig i stand til å drepe trærne raskt. Etter etablering i yteveden kan andre arter følge etter, bl.a. *O. bicolor* og *O. penicillatum* som begge gir en viss reaksjon i barken hos gran. De sistnevnte soppene kan være bedre tilpasset barken og veden i svekkede trær eller tømmer som substrat.

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