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Pathogenicity of *Ophiostoma polonicum* to Norway spruce: The effect of isolate age and inoculum dose

Patogenitet av Ophiostoma polonicum *på gran: Virkning av soppisolatets alder og av inokuleringsdosen*

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Abstract

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Inoculation experiments in order to test the pathogenicity of *Ophiostoma polonicum* to Norway spruce have given variable results. An experiment was designed to test if the age of the fungus isolate and the inoculum dose could explain some of this variation.

Thirty spruce trees were inoculated with 0-, 1-, and 3-yr-old isolates of *O. polonicum* in two or four rings of inoculation points 100-145 cm above ground. The trees were harvested 40 days after inoculation, and resin flow, bark necroses and blue-stained sapwood were measured.

There was no effect of isolate age. The cross-sectional proportion of blue-stained sapwood was about doubled with a dose of four rings of inoculation points as compared to two rings. Individual variation in resistance between trees and inoculation points was strongly correlated to resin flow.

Utdrag

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I infeksjonsforsøk for å teste patogeniteten av *Ophiostoma polonicum* på gran har resultatene vært variable. Et forsøk ble utført for å se om alder på soppisolatet og inokuleringsdosen kunne forklare noe av denne variasjonen.

I alt 30 grantrær ble inokulert med 0, 1, og 3 år gamle isolat av *O. polonicum* i 2 og 4 ringer med inokuleringspunkter rundt stammen i 100-145 cm høyde. Trærne ble høstet etter 40 dager, og kvaeutflod, barknekroser og blå yteved ble målt.

Det var ingen virkning av isolatets alder. Tverrsnittarealet av blåved var omtrent dobbelt så stort ved en dose på 4 ringer som ved en dose på 2 ringer. Individuelle variasjoner i resistens mellom trær og inokuleringspunkter viste sterk sammenheng med kvaeutflod.

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Preface

The present study was done within the projects «Resistance of conifers to bark beetles, drought and fungi» and «Fungal detriment in beetle-killed spruce». We thank Olaug Olsen and Thomas Midttun for technical assistance, Erik Christiansen and Kåre Venn for valuable criticism of the manuscript, and Linda G. Hjeljord for linguistic corrections. The project was partly financed by The Agricultural Research Council of Norway.

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Introduction

Following a severe autumn gale in 1969 and extreme summer drought in the years 1974-1976, populations of the spruce bark beetle in southern Norway rose to epidemic levels. Some one million cubic meters of spruce trees were killed yearly during 1978-1980 (CHRISTIANSEN & BAKKE 1988).

When attacking trees, the spruce bark beetle *Ips typographus* L. infects the phloem with different species of blue-stain fungi (FURNISS, SOLHEIM & CHRISTIANSEN 1990). We started infection experiments with some of these fungi in 1981. *Ophiostoma polonicum* Siem. proved to be highly pathogenic. Extensive blue-staining of the sapwood developed in all trees inoculated with *O. polonicum* in contrast to no blue-stain in trees inoculated with *O. penicillatum* or with sterile agar (HORNTVEDT et al. 1983)

In 1982 another experiment was carried out to compare the pathogenicity of four species of fungi, including *O. polonicum*. To our surprise, only one of 20 inoculated trees was infected, indeed with *O. polonicum*

The following experimental differences, among others, could have contributed to the diverging results:

- The same isolate of *O. polonicum* was used both years. Isolation was done in 1980, and the isolate was thus one year old in 1981 and two years old in 1982. Its virulence could have been reduced when kept in culture.

- A higher dose of inoculum was used in the 1981 experiment than in 1982. We were at that time not aware of the importance of the inoculum dose. In 1982, two rings of inoculum points were used. This could have been too low a dose for successful infection.

To test these two hypotheses another experiment was set up in 1983, using three isolates of different age, and two doses of inoculum.

Material and methods

A 25-year-old plantation of Norway spruce (*Picea abies* (L.) Karst.) at Ås, 30 km south of Oslo, was used for the experiment. The trees were 8-13 m high and had relative crown lengths of 60-80 percent.

Six trees were selected in each of five diameter classes:
<=10, 11, 12, 13, and =>14 cm at breast height. Three isolates of *Ophiostoma polonicum* and two inoculum doses were randomly assigned to each diameter class.

The three isolates of *O. polonicum* were taken from spruce trees in Ås newly attacked by *Ips typographus*.

0-yr-old: Isolated in June 1983
1-yr-old: Isolated in October 1982
3-yr-old: Isolated in September 1980

The isolates were stored on malt agar at 3°C according to ROLL-HANSEN & ROLL-HANSEN (1982) and were transferred to fresh agar one week before inoculation.

The inoculum doses were either two rings of inoculum points 100 and 115 cm above the ground, or four rings of inoculum points 100, 115, 130 and 145 cm above the ground. The centers of the inoculum points were spaced 2 cm apart. At each point a 5 mm ø bark plug was removed with a cork borer, a piece of mycelium and agar was inoculated, and the bark plug was replaced (HORNTVEDT et al. 1983).

Due to a mistake during inoculation, one tree in diameter class <=10 cm got four instead of two rings of the 1-year-old isolate.

The trees were inoculated on June 29, 1983. The experiment was terminated on August 8, 1983.

Length of resin flow on the bark surface, size of bark necroses, and cross-sectional area of blue-stained sapwood were measured as described by HORNTVEDT (1988). «Proportion of blue-stained sapwood» refers to cross-sectional area measurements on stem discs cut at the inoculation rings and at 10 cm intervals above and below these.

Results

The main results are presented in Table 1. There were no effects of tree diameter, hence mean values for all diameter classes are given.

The proportion of blue-stained sapwood was always largest at the lower inoculation ring(s). The values for blue-stain given in Table 1 are the means of the maximum values per tree. There was a significant effect of inoculation dose. Four rings of inoculation points gave about the double amount of blue-stain, as compared to two rings. There was no effect of isolate age.

Table 1. Proportion of sapwood blue-stained, length of bark necroses and length of resin flow, resulting from inoculations of Norway spruce with *O. polonicum* at two inoculation doses and three isolate ages.

Inoc. dose	Isolate age	Blue-stain (%)	Bark necroses up/down (mm)	Resin flow (mm)
2 rings	0-yr-old	24	17/21	37
2 rings	1-yr-old	24	13/15	81
2 rings	3-yr-old	39	13/17	51
4 rings	0-yr-old	66	20/28	12
4 rings	1-yr-old	68	18/33	10
4 rings	3-yr-old	66	18/31	24

The proportions of blue-stained sapwood at various heights in the trees are shown in Fig. 1. Maximum extension along the stem was about 60 cm in both directions from the inoculation rings. However, the proportion of blue-stained sapwood decreased more rapidly upward from the upper ring than downward from the lower. This difference was highly significant and was evident in almost all trees.

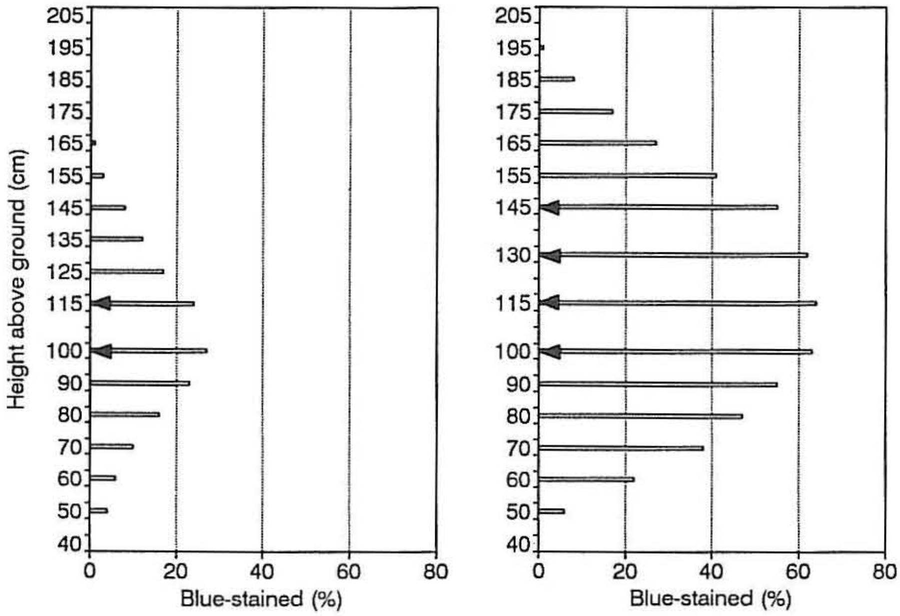

Fig. 1. Proportion of sapwood blue-stained from inoculations with *O. polonicum* at four rings around the stem (right), or at two rings (left). The arrows indicate the position of the inoculation rings. Each curve represents mean values of 14-16 trees.

Length of resin flow from the inoculation points is summarized in Table 1. Each figure is a mean value of all individual inoculation points. The effect of inoculation dose was highly significant. On the average, inoculations in four rings resulted in less than a third of the resin flow resulting from two rings. There were no effects of isolate age.

With four rings of inoculation points the mean length of resin flow increased significantly from the lower to the upper ring (Table 2). With two rings the difference between the lower and upper ring was not significant (53 vs. 55 mm).

Table 2. Mean length of resin flow (mm) from the inoculation points, by inoculation height (cm) and tree no. Three trees with no resin flow at all have been omitted.

Inoc.	Tree no.													
height	1	2	3	4	5	6	7	8	9	10	11	12	13	All
100	9	37	5	11	4	1	11	24	51	1	0	0	0	10
115	1	34	3	8	2	0	10	43	93	0	5	0	0	12
130	1	51	0	16	4	0	5	26	136	3	13	2	2	16
145	16	51	5	7	5	0	29	70	128	0	14	19	5	22

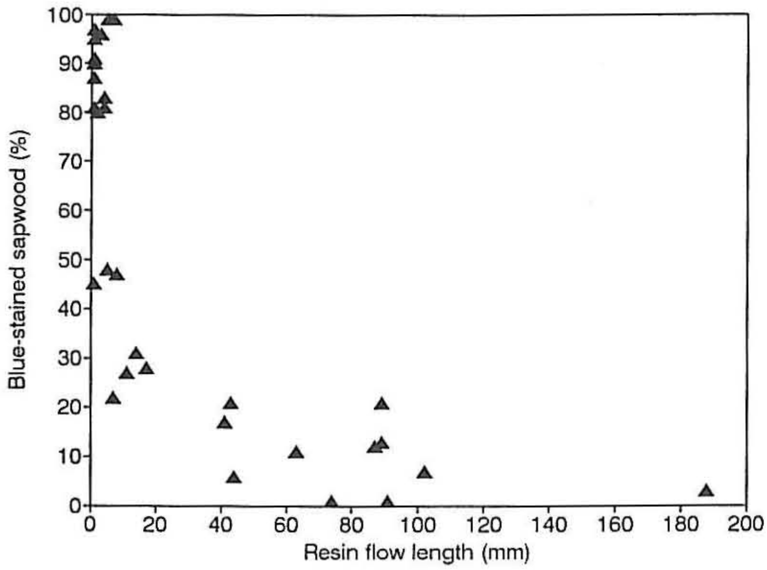

Fig. 2. Proportion of sapwood blue-stained from inoculations with *O. polonicum* related to length of resin flow. The points represent the maximum proportion of blue-stained sapwood and the mean length of resin flow from all inoculation points per tree.

The amount of blue-stained sapwood was related to resin flow (Fig. 2). With close to zero resin flow the proportion of blue-stained sapwood was usually over 80 percent. With more than 20 mm of mean resin flow per tree the proportion of blue- stained sapwood was less than 20 percent.

In most trees bark necroses from individual inoculation points or even rings had coalesced and were indistinguishable. The criterion of bark necroses presented in Table 1 is the maximum extension upward from the upper inoculation ring and downward from the lower. These figures were significantly higher with four rings than with two rings. There was no effect of isolate age.

With few exceptions the extension of bark necroses upward from the upper ring was less than downward from the lower. This was both an effect of the ring position and, to a lesser degree, of the fact that bark necroses at individual inoculation points tend to extend more downward than upwards.

When established the fungus apparently expanded more rapidly in the sapwood than in the bark. Whenever significant amounts of blue-stain were present, the vertical extension of sapwood blue-stain was longer than that of bark necroses.

Discussion

The inoculated trees showed defense reactions and symptoms of infection in accordance with other experiments of this type (HORNTVEDT et al. 1983, CHRISTIANSEN & HORNTVEDT 1983, CHRISTIANSEN 1985a, CHRISTIANSEN, WARING & BERRYMAN 1987, HORNTVEDT 1988, SOLHEIM 1988).

The experiment could not demonstrate any difference in pathogenicity between the isolates of *O. polonicum*. An isolate which had been kept in culture for three years was as pathogenic as one isolated one month before inoculation.

In general, the storage method will influence vitality and virulence of fungi (ONIONS 1983). At our institute, cold storage at 3°C and transferring every 1.5-2 years is used (ROLL-HANSEN & ROLL-HANSEN 1982). With this storage method a 24-year-old isolate of *O. penicillatum* was as virulent as a 4- year-old isolate when inoculated in Norway spruce trees (SOLHEIM 1988).

The dose of inocula was of vital importance. Doubling the dose from two to four rings of inoculation points resulted on the average in twice as much blue-stained sapwood, even if the density of inocula along the stem periphery was the same. With two rings, two out of 14 trees were not infected at all, and only two trees got more than 50 percent blue-stained sapwood. With four rings, all trees were infected and 11 of 16 trees had more than 50 percent blue-stained sapwood. Thus it is evident that total inoculum dose more than density determines the tree's response.

CHRISTIANSEN (1985b) has compiled dose response results from inoculation experiments and pheromone-induced *Ips typographus* attacks. Considering a tree of 20 cm DBH, a dose of about 100 inocula or beetle attacks apparently is the critical dose. At lower doses no or little infection occurs; at higher doses blue-stain and tree mortality increase rapidly. The trees were inoculated or attacks induced by late May.

In the present experiment, two and four rings of inocula are equivalent to doses of about 60 and 120 inocula, respectively, in a tree of 20 cm DBH. Our results thus indicate a somewhat lower threshold dose than that reported by CHRISTIANSEN (1985b). In his experiments the inocula were not placed in rings around the stem, but were spaced evenly within a stem section.

The trees' resistance may vary considerably during the growing season (HORNTVEDT 1988). Timing of the inoculation or induction of attack is therefore an important factor. The present experiment was started on 29 June, when the trees' resistance probably is lower than earlier in the season. This may also explain why significant infection occurred even at the lower dose.

The experiment in 1982 started about one month earlier than the present. This may partly explain the difference between the results.

Like in other experiments of this type, the trees' resistance against *O. polonicum* was related to resinosis at the inoculation points (CHRISTIANSEN 1985a, CHRISTIANSEN & ERICSSON 1986, HORNTVEDT 1988). The effect of inoculation height was interesting in this connection. By all criteria the defense reaction was stronger and the infection weaker at the higher than at the lower ring of inoculation points. An assumed desiccation from the lower to the upper ring appeared to be of minor importance. Flow of resin or resin precursors downward in phloem and sapwood seems to be the main defense mechanism.

There was no effect of tree dimension in this experiment. Although the largest trees had a DBH fifty percent larger than the smallest and thus had grown much faster (all trees were of the same age), none of them seemed to suffer from strong competition stress. Relative crown length and sapwood area were not significantly different between the DBH classes. Thus, this experiment neither supports nor refutes the hypothesis that tree vigor is important for defense against bark beetle-transmitted fungi, as suggested by WARING & PITMAN (1983), MULOCK & CHRISTIANSEN (1986), CHRISTIANSEN, WARING & BERRYMAN (1987).

Patogenitet av *Ophiostoma polonicum* på gran: Virkning av soppisolatets alder og av inokuleringsdosen

Ophiostoma polonicum er en av de blåvedsoppene som granbarkbillen fører med seg, og som hjelper billen å drepe trærne. I infeksjonsforsøk for å teste patogeniteten av *O. polonicum* på gran har resultatene vært variable.

Et forsøk ble utført for å se om soppisolatets alder eller inokuleringsdosen kunne forklare noe av denne variasjonen. I alt 30 grantrær ble inokulert (smittet) med 0, 1, og 3 år gamle isolat av *O. polonicum* i 2 og 4 ringer med inokuleringspunkter rundt stammen. Trærne ble høstet etter 40 dager, og kvaeutflod, barknekroser og blå yteved ble målt.

Det kunne ikke påvises noen forskjeller mellom soppisolatene. Et isolat som var holdt i kultur i tre år var like patogent som ett som var isolert en måned før inokulering (Tabell 1).

Tverrsnittarealet av blåved var omtrent dobbelt så stort ved en dose på 4 ringer som ved en dose på 2 ringer, selv om avstanden mellom inokuleringspunktene langs omkretsen av stammen var den samme (Tabell 1).

Individuelle variasjoner i resistens mellom trær og inokuleringspunkter viste sterk sammenheng med kvaeutflod (Fig. 2). Kvaeutfloden var i gjennomsnitt større ved øverste enn ved nederste inokuleringsring (Tabell 2). I samsvar med dette avtok blåvedandelen raskere oppover fra øvre inokuleringsring enn nedover fra nedre (Fig. 1).

Det kunne ikke påvises noen virkning av trærnes diameter på forsvarsreaksjoner eller infeksjon. Trærne var plantet i jevnt forband, og konkurransen var trolig ennå for liten til å påvirke trærnes resistens.

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