Pathogenicity of Four Blue-Stain Fungi Associated with Aggressive and Nonaggressive Bark Beetles

Paal Krokene and Halvor Solheim

Norwegian Forest Research Institute, Høgskoleveien 12, N-1432 Ås, Norway.

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ABSTRACT

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The pathogenicity of two isolates of each of four bark beetle-associated blue-stain fungi was evaluated after mass inoculation of about 40-year-old Norway spruce trees (*Picea abies*). Trees were inoculated with a different isolate of each fungus in 1995 and 1996 at a density of 400 inoculations per m² in a 1.2-m-wide band on the lower bole (about 270 inoculations per tree). Trees were felled 15 weeks after inoculation. In 1995, *Ceratocystis polonica* was the only fungus that had stained the sapwood (56.3% of cross-sectional sapwood area). It induced five times longer phloem necroses, 21 times more dead cambium, and 11 times more dead phloem than any other fungus. In 1996, *C. polonica* induced less extensive host symptoms and an unidentified *Ambrosiella* sp. induced comparable

symptoms to *C. polonica* in the phloem and cambium. No trees showed any foliar symptoms 15 weeks after inoculation, but six out of eight trees inoculated with *C. polonica* in 1995 had only 0 to 25% functional sapwood and probably would have died if felling had been delayed. This study confirms that *C. polonica*, an associate of the aggressive bark beetle *Ips typographus*, is pathogenic to Norway spruce. The pathogenicity of the *Ambrosiella* sp., which is associated with a nonaggressive bark beetle, seems moderate and varies between isolates. The two remaining fungi included in this study (*Ophiostoma piceae* and a dark fungus with sterile mycelium), which are associated with nonaggressive bark beetles, were nonpathogenic in both experiments. These results are consistent with the hypothesis that aggressive bark beetle species vector virulent fungi that may help them kill trees, but the results also show that some nonaggressive bark beetles may vector phytopathogenic fungi.

Microbes often play important roles as mediators in plant-insect interactions (1). Bark beetles feeding in the phloem of conifers have a wide variety of microbial associates that are transported in mycangia, on the exoskeleton, or in the gut (3,33,52). Bacteria, yeasts, and other fungi may condition the host substrate for egg laying and feeding (52), serve as an additional food source for the developing brood (13,15), and produce population-aggregating pheromones (4); in the case of aggressive bark beetles that are able to kill healthy trees, phytopathogenic fungi may help the beetles to overwhelm host defenses (20,49,52). Mediation by phytopathogenic fungi has been proposed as one of the main hypotheses to explain how aggressive bark beetles can overwhelm and kill healthy trees (10,11,37).

The fungi suspected to be involved in overwhelming host defenses are blue-stain fungi in the genera *Ceratocystis* and *Ophiostoma* and related anamorph genera such as *Graphium* and *Leptographium* (17, 18,52). Most blue-stain fungi are associated with bark beetles, and most conifer bark beetles vector some species of these fungi (16, 35). However, the phytopathogenicity of the fungal associates varies considerably, even between the associates of aggressive bark beetles (17,33). Some blue-stain fungi are relatively pathogenic, and inoculation studies have demonstrated that they can kill mature trees in the absence of their bark beetle vectors (7,8,29,48). For most aggressive bark beetles, the symbiosis between the beetle and their pathogenic blue-stain fungi appears to be mutualistic, since each partner can reduce the difficulties encountered by the other in host colonization (39). The beetles may benefit, since the phytopathogenic fungi can increase the aggressiveness of each beetle and, thus, help them exhaust tree defenses. Benefits to the fungi include dispersal and inoculation into suitable host trees (28).

Although bark beetles are generally regarded as forest pests, only a very small proportion of the approximately 5,800 species in the world (54) can kill healthy trees. The few aggressive species play important ecological and economic roles, particularly in temperate conifer forests. In North America, they have been estimated to be responsible for more tree mortality than all other natural factors combined (53). The majority of the bark beetles are nonaggressive and colonize only dead trees (i.e., saprophagous species) or severely stressed or dying trees (i.e., facultatively parasitic species [40]).

In this study, we compare the phytopathogenicity of four blue-stain fungi associated with different bark beetles that range from aggressive to saprophagous. All beetle species colonize a common host, Norway spruce (*Picea abies* (L.) H. Karst.). Our objective was to determine if fungi vectored by the less aggressive beetles could invade healthy trees and to test the hypothesis that aggressive bark beetles are associated with more pathogenic fungi than nonaggressive species.

MATERIALS AND METHODS

Fungal species. Norway spruce trees were mass-inoculated with the following four fungi: *Ceratocystis polonica* (Siem.) C. Moreau, *Ophiostoma piceae* (Münch) H. Sydow & P. Sydow, an apparent *Ambrosiella* sp., and a sterile fungus with dark mycelium (Dark sterile sp. A). A different isolate of each fungus was used in 1995 and 1996 (Table 1). The fungi were originally isolated in a survey of the fungal associates of five species of bark beetles colonizing Norway spruce (25). The four fungi were chosen from all the different fungi that are associated with these bark beetles because they are consistent associates of one or more of the beetles and because their pathogenicity to Norway spruce has not been tested before. The pathogenicity of the other major fungal associates of these bark beetles has been tested previously (45; H. Solheim, unpublished data).

Corresponding author: P. Krokene; E-mail address: paal.krokene@nisk.no

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C. polonica is the most important fungal associate of *Ips typographus* L. In Norway, 35 to 94% of the beetles carry this fungus (14, 25,44,46). *C. polonica* has also been isolated consistently from *I. duplicatus* (25), and its likely synonym *C. laricicola* (19,50) has been isolated in low frequencies from *I. cembrae* in Scotland and Japan (41). *O. piceae* is a common sapstain fungus of lumber in both Europe and North America (43). It has been isolated from *I. typographus*, *Pityogenes chalcographus*, *Hylurgops palliatus*, and several other species of Scolytidae, Curculionidae, and Nitidulidae, and is, thus, nonspecific with respect to vectors (25,31,44). In a recent study in Norway, *O. piceae* was isolated from 31 and 17% of *I. typographus* and *H. palliatus* individuals, respectively (25). Dark sterile sp. A and the *Ambrosiella* sp. have been isolated from a high percentage of individuals of the nonaggressive bark beetles *H. palliatus* (69%) and *Polygraphus poligraphus* (28%), respectively (25).

Inoculation and sampling. Sixty Norway spruce trees of similar size and age were selected from a stand in Ås, Norway (Tables 2 and 3). Eight trees in 1995 and four trees in 1996 were randomly assigned to each of the four fungi and to the control. Trees were mass-inoculated at a density of 400 inoculations per m² in a 1.2-m-wide band from about 0.8 to 2 m above ground on 5 to 7 June 1995 and 15 to 16 July 1996. Trees were inoculated by removing a bark plug

with a 5-mm cork borer, inserting inoculum in the wound, and replacing the bark plug. Inoculum consisted of actively growing mycelium of the four fungi on malt agar (2% malt and 1.5% agar) and sterile malt agar as a control. Fourteen weeks after inoculation, we measured resin flow from inoculation wounds by determining the proportion of a random sample of 90 to 100 inoculation points per tree with resin flow longer than 3 mm. At the same time, we removed the outer bark over six of the uppermost and six of the lowermost inoculation sites on each tree and measured maximum width and length of the necrosis surrounding each inoculation point. Necrosis length was measured from the center of the inoculation wound and in one direction away from the inoculated band. This was done because necroses often coalesce in the inoculation band on trees inoculated with *C. polonica*.

The trees were felled 15 weeks after inoculation. We measured tree height growth for the previous three growth seasons, length of green crown, and total tree height. The central 0.6 m of each inoculated stem section was removed and taken to the laboratory, where it was processed within 24 h. From the 0.6-m bolts, we cut two 5-mm-thick stem disks about 0.1 m from the upper and lower end. Based on translucency, we outlined the sapwood-heartwood border, the border between desiccated and apparently healthy sap-

TABLE 1. Isolate number and collection information for the four blue-stain fungi inoculated in Norway spruce in 1995 and 1996

Species	Isolate no. ^z	Inoculated	Collected	Substrate
<i>Ceratocystis polonica</i>	93-208/115	1995	1993, Akershus, Norway	<i>Picea abies</i> , <i>Polygraphus poligraphus</i>
	94-169/13	1996	1994, Akershus, Norway	<i>Picea abies</i> , <i>Ips typographus</i>
<i>Ophiostoma piceae</i>	94-169/56	1995	1994, Akershus, Norway	<i>Picea abies</i> , <i>Ips typographus</i>
	96-65/27	1996	1996, Akershus, Norway	<i>Hylurgops palliatus</i>
<i>Ambrosiella</i> sp.	93-208/44	1995	1993, Akershus, Norway	<i>Picea abies</i> , <i>Polygraphus poligraphus</i>
	94-176B/4	1996	1994, Akershus, Norway	<i>Polygraphus poligraphus</i>
Dark sterile sp. A	94-166/39	1995	1994, Akershus, Norway	<i>Picea abies</i> , <i>Hylurgops palliatus</i>
	94-166/25	1996	1994, Akershus, Norway	<i>Picea abies</i> , <i>Hylurgops palliatus</i>

^z Numbers refer to the reference collection at Norwegian Forest Research Institute.

TABLE 2. Characteristics of Norway spruce trees mass-inoculated with four different fungi or sterile agar control in 1995

Treatment	DBH ^w (cm)	Tree age	Tree height (m)	Crown length (m)	Height growth (cm)			Annual ring width (mm)	
					1995 ^x	1994 ^x	1993 ^x	1995 ^y	(1992–1994)/3
<i>Ceratocystis polonica</i>	18.3	39.8	18.2	11.2	22.2	17.3	15.6	0.53 a	0.95
<i>Ophiostoma piceae</i>	17.6	41.9	18.1	10.4	28.2	17.4	15.6	1.11 c	0.69
<i>Ambrosiella</i> sp.	17.5	43.1	19.0	10.9	21.6	13.6	18.4	1.01 c	0.70
Dark sterile sp. A	17.7	41.5	17.7	12.0	18.9	12.4	14.1	0.88 bc	0.82
Control	17.7	40.5	18.9	11.6	25.1	18.0	12.3	0.70 ab	0.63
<i>F</i> value ^z	0.89	1.02	1.00	0.50	1.13	2.13	1.04	4.82	1.51
<i>R</i> ²	0.09	0.10	0.10	0.05	0.12	0.20	0.11	0.36	0.15
<i>P</i>	0.48	0.41	0.42	0.74	0.36	0.10	0.40	0.004	0.22

^w Diameter at breast height.

^x Data were transformed ($\log_{10} y + 1$) before analysis; nontransformed values are reported.

^y Values followed by the same letter were not significantly different according to the least significant difference test ($P < 0.05$). The test was employed only if the analysis of variance was significant at $P < 0.05$.

^z Degrees of freedom (df) = 4, 35 for all tests, except for height growth in which df = 4, 34.

TABLE 3. Characteristics of Norway spruce trees mass-inoculated with four different fungi or sterile agar control in 1996

Treatment	DBH ^x (cm)	Tree age	Tree height (m)	Crown length (m)	Height growth (cm)			Annual ring width (mm)	
					1996 ^y	1995 ^y	1994 ^y	1996	(1993–1995)/3
<i>Ceratocystis polonica</i>	16.60	42.3	17.9	9.8	19.3	19.0	15.8	0.58	0.50
<i>Ophiostoma piceae</i>	17.01	40.5	17.6	10.0	26.8	24.0	18.8	1.03	0.84
<i>Ambrosiella</i> sp.	16.40	42.5	17.6	9.0	27.0	16.5	15.3	0.54	0.66
Dark sterile sp. A	17.49	43.0	18.6	10.0	21.0	16.0	13.0	0.50	0.62
Control	16.31	41.8	17.2	11.7	34.8	25.3	22.0	0.71	0.86
<i>F</i> value ^z	1.38	0.38	0.25	0.82	0.70	1.48	1.70	1.34	1.35
<i>R</i> ²	0.27	0.09	0.06	0.18	0.17	0.30	0.33	0.28	0.28
<i>P</i>	0.29	0.82	0.91	0.53	0.61	0.26	0.21	0.30	0.30

^x Diameter at breast height.

^y Data were transformed ($\log_{10} y + 1$) before analysis; nontransformed values are reported.

^z Degrees of freedom = 4, 14 for all tests.

wood, and the border between blue-stained and desiccated sapwood on the upper surface of each disk (10). The surface area of heartwood, total sapwood, and blue-stained, desiccated, and normal sapwood was later determined with a computer-connected planimeter (Ultima: GTCO Corporation, Columbia, MD; "Blåveden" software: G. Fjone, Nisk, Ås, Norway). Along the disk circumference, we measured the proportion of dead and live cambium. On the lower disk, taken about 1.2 m above ground, we estimated tree age and measured annual ring width for the last 4 years of growth along four radii. On each 0.6-m bolt, we removed the outer bark and traced all phloem necroses in a 0.062-m² area onto clear acetate. Total area of dead phloem was later determined from the acetate sheets with a planimeter. Fungi were reisolated from phloem and sapwood around three inoculation points on each tree.

Data analysis. For each year, length and width of individual necrotic zones, percent dead phloem, percent dead cambium, and percent blue-stained and desiccated sapwood area were subjected to one-factor analysis of variance (ANOVA). The ANOVA was unbalanced for the 1996 data, since one tree inoculated with the *Ambrosiella* sp. was attacked by bark beetles and had to be discarded. Tree characteristics such as age, height growth, and annual ring width were also subjected to one-factor ANOVA. For height growth in 1995, the ANOVA was unbalanced, since one tree with a broken top (inoculated with *O. piceae*) had to be discarded. Mean values for each tree were used in all analyses.

Some data were transformed before ANOVA (Tables 2 to 5) to correct for unequal variances and departures from normality as indicated by graphical analysis of residuals (30). If treatments were significantly different ($P < 0.05$), means were separated using the least significant difference test at $P < 0.05$ (30). The nontrans-

formed means are reported. All statistical analyses were performed with the general linear models procedure of SAS (SAS Institute Inc., Cary, NC).

RESULTS

In 1995, *C. polonica* was much more pathogenic than the other fungi based on all criteria of fungal pathogenicity (Table 4). It was the only fungus that caused blue-stain, and it induced more sapwood desiccation, 21 times more dead cambium area, 11 times more dead phloem area, and twice as wide and five times longer phloem necrotic zones than any other fungus (Table 4). Two trees inoculated with *C. polonica* in 1995 had less than 2% functioning sapwood, and four others had 14 to 25% functioning sapwood. Except for resin exudation and shallow depressions on the bark around inoculations with *C. polonica* in 1995 and 1996 and the *Ambrosiella* sp. in 1996, the trees showed no visible external symptoms of infection. In 1995, *O. piceae*, the *Ambrosiella* sp., and Dark sterile sp. A did not differ significantly in any criterion of fungal pathogenicity (Table 4). They were all significantly different from the control, except for percent dead cambium and width of necrosis (Table 4). All fungi induced much more sapwood desiccation than the control (Table 4). There were no significant differences in resin exudation among trees inoculated with different fungi, but control trees exuded significantly less resin than inoculated trees (Table 4).

In 1996, *C. polonica* generally caused less extensive symptoms than in 1995 and the differences in pathogenicity between the four inoculated fungi were smaller (Table 5). In particular, inoculation with the *Ambrosiella* sp. and *C. polonica* induced comparable

TABLE 4. Indices of fungal success 15 weeks after mass inoculation of four blue-stain fungi and sterile agar in Norway spruce, 1995 experiment¹

Treatment	Phloem necrosis (mm)		% Sapwood area ¹		% Dead phloem ^x	% Dead cambium ^x	Resin flow ^y
	Length ^{v,w}	Width ^w	Blue-stained	Blue-stained and desiccated ^x			
<i>Ceratocystis polonica</i>	65.4 a	17.7 a	56.3	77.9 a	80.3 a	71.9 a	54.0 a
<i>Ophiostoma piceae</i>	10.1 b	8.8 b	0.0	16.0 b	5.9 b	3.1 b	40.0 a
<i>Ambrosiella</i> sp.	13.1 b	8.9 b	0.0	12.5 b	7.1 b	3.4 b	53.0 a
Dark sterile sp. A	12.2 b	10.2 b	0.0	15.2 b	7.3 b	3.2 b	49.0 a
Control	6.8 c	9.5 b	0.0	1.6 c	4.0 c	1.8 b	22.0 b
<i>F</i> ratio ^z	75.29	21.06	...	35.35	152.37	119.62	5.54
<i>R</i> ²	0.90	0.71	...	0.80	0.95	0.93	0.39
<i>P</i>	0.0001	0.0001	...	0.0001	0.0001	0.0001	0.002

¹ For each column, values followed by the same letter were not significantly different according to the least significant difference test ($P < 0.05$). The test was employed only if analysis of variance was significant at $P < 0.05$.

² Percentage of cross-sectional sapwood area on a stem disk taken inside the mass-inoculated part of the bole.

^v Necroses were measured in only one direction from the point of inoculation.

^w Data were transformed ($\log_{10} y + 1$) before analysis; nontransformed values are reported.

^x Data were transformed ($\arcsin \sqrt{y}$) before analysis; nontransformed values are reported.

^y Percentage of 90 to 100 inoculation points per tree with external resin flow longer than 3 mm.

^z Degrees of freedom = 4, 35 for all tests.

TABLE 5. Indices of fungal success 15 weeks after mass inoculation of four blue-stain fungi and sterile agar in Norway spruce, 1996 experiment^w

Treatment	Phloem necrosis (mm)		% Desiccated sapwood area ^y	% Dead phloem ^y	% Dead cambium ^y	Resin flow
	Length ^x	Width ^x				
<i>Ceratocystis polonica</i>	42.52 a	22.31 a	53.2 a	47.2 a	42.8 a	87.2 a
<i>Ophiostoma piceae</i>	13.48 b	11.19 b	16.1 bc	7.4 b	8.6 b	65.5 b
<i>Ambrosiella</i> sp.	34.08 a	19.39 a	27.5 ab	53.2 a	39.3 a	74.2 b
Dark sterile sp. A	8.23 bc	9.44 b	3.5 c	5.0 b	7.3 b	43.8 c
Control	7.33 c	9.79 b	8.3 bc	4.2 b	3.4 b	40.4 c
<i>F</i> ratio ^z	16.62	9.28	6.02	15.11	9.34	20.91
<i>R</i> ²	0.83	0.73	0.67	0.81	0.73	0.86
<i>P</i>	0.0001	0.0007	0.007	0.0001	0.0007	0.0001

^w For each column, values followed by the same letter were not significantly different according to the least significant difference test ($P < 0.05$). The test was employed only if analysis of variance was significant at $P < 0.05$.

^x Data were transformed ($\log_{10} y + 1$) before analysis; nontransformed values are reported.

^y Data were transformed ($\arcsin \sqrt{y}$) before analysis; nontransformed values are reported.

^z Degrees of freedom = 4, 14 for all tests.

symptoms, and these two fungi generally induced significantly more extensive symptoms than the other two fungi. In 1996, Dark sterile sp. A. and *O. piceae* did not differ significantly from the control for most criteria of fungal pathogenicity (Table 5). None of the fungi inoculated in 1996 caused any sapwood blue-stain. Trees inoculated with *C. polonica* in 1996 exuded significantly more resin than the other trees (Table 5). For all treatments except Dark sterile sp. A, resin exudation was stronger in 1996 compared with 1995. Trees inoculated with *O. piceae* and the *Ambrosiella* sp. in 1996 generally had more extensive symptoms in the phloem and cambium than the trees inoculated in 1995.

The reisolations yielded high frequencies of most of the inoculated fungi both years (42 to 100% of inoculations positive). Experimental fungi were normally not isolated from sites other than where they had been inoculated, except for *O. piceae*, which was reisolated occasionally from control trees and trees inoculated with Dark sterile sp. A in 1996. *O. piceae* was the only fungus that was reisolated from all inoculations, but most of the inoculations with the other fungi probably resulted in successful colonization, as indicated by the presence of phloem necroses around most inoculation sites. In 1995, *C. polonica* was generally reisolated further into the sapwood than the other fungi.

There were no significant differences among trees allocated to the different treatments with respect to any tree characteristics in either 1995 or 1996 (Tables 2 and 3). The only exception was that trees inoculated with *C. polonica* in 1995 had very narrow growth rings that year (Table 2). When annual ring width for 1995 was divided by the average for the years 1992 to 1994, *C. polonica* had a value of only 55%, compared with 106 to 160% for the other treatments ($P < 0.0008$, $F = 6.17$, $R^2 = 0.42$). In 1996, the corresponding figures were 112% versus 75 to 129% ($P = 0.21$, $F = 1.35$, $R^2 = 0.32$). Trees inoculated in 1995 and 1996 were generally very similar in size and growth, although the trees inoculated in 1996 were slightly smaller (Tables 2 and 3).

DISCUSSION

The pathogenicity of *C. polonica* that was demonstrated in the current study is consistent with results from previous mass inoculations of Norway spruce with this fungus (7,8,23,45). In the 1996 test, the *Ambrosiella* sp. was moderately pathogenic to Norway spruce. This fungus is associated with the facultatively parasitic *Polygraphus poligraphus* (25), which can colonize stressed and weakened Norway spruce when its population level is high. *O. piceae* and Dark sterile sp. A are associated with various nonaggressive bark beetles colonizing Norway spruce (25) and are not particularly pathogenic. Other fungi that have been isolated from these and other bark beetles colonizing Norway spruce are either saprophagous (25) or have been shown to be relatively nonpathogenic in previous mass-inoculation experiments (45; H. Solheim, unpublished data). One exception is *O. penicillatum*, associated with the aggressive bark beetle *I. typographus* and the facultatively parasitic *I. duplicatus*, which is moderately pathogenic to Norway spruce (45). These results are consistent with the hypothesis that aggressive bark beetle species vector virulent fungi that may aid them in killing trees, but it appears that some nonaggressive bark beetles also may vector pathogenic fungi.

Nonaggressive bark beetles that attack the roots or base of dead, weakened, or healthy trees vector the pathogenic blue-stain fungus *Leptographium terebrantis* (e.g., *D. valens*, *D. terebrans*, and *Hylastes porculus* [17,24]). These beetles do not engage in mass attacks, but colonize parts of the tree with high oleoresin pressure and water content and correspondingly low oxygen levels. Pathogenic blue-stain fungi are probably well adapted to colonize such environments (47), and the beetles may be dependent on a fast-growing pathogen to colonize such tissue (17). The facultatively parasitic *I. duplicatus* is consistently associated with *C. polonica* in Norway (25). However, this beetle may be more aggressive than previously

recognized, as it has recently killed Norway spruce over large areas in Central Europe (36).

C. polonica killed a large proportion of the phloem, cambium, and sapwood of the trees inoculated in 1995, and most of the trees inoculated with this fungus would probably have developed foliar symptoms if we had delayed harvesting. We did not delay harvesting because foliar symptoms may not occur until the growing season after inoculation and, before then, the trees are likely to be attacked by secondary bark beetles (23). In some previous mass inoculations of Norway spruce with *C. polonica*, the foliage turned brown and started to drop 10 to 14 weeks after inoculation (7,8). The higher inoculation densities used in those studies may have been responsible for the earlier appearance of symptoms. Tree age and timing of inoculation may also affect the pathogenicity of *C. polonica* (7,9,22).

C. polonica was the only fungus that reduced radial growth of the trees during the season of inoculation relative to three previous seasons. This occurred in 1995, the only season when trees were seriously affected by any treatment. Reduced radial growth after mass inoculation has been observed previously in Norway spruce inoculated with *C. polonica* (7,9) and in Sitka spruce (*P. sitchensis*) inoculated with *C. rufipenni* (48). The reduced growth suggests that trees inoculated with *C. polonica* in 1995 may have allocated more resources to defense and less to growth than trees inoculated with other fungi or the control (9). Growth may also have been reduced as a result of water stress (9) following fungal colonization of the sapwood. Inoculation with *C. polonica* did not affect height growth in either year (Tables 2 and 3), contrary to the finding of Christiansen (7). This discrepancy may be a result of the timing of the inoculations. Our inoculations were done 2 and 8 weeks later than Christiansen's, and it is possible that the trees had completed most of their height growth before they were seriously stressed by *C. polonica*. When Sitka spruce was mass-inoculated with *C. rufipenni* partway through the growing season, there was no reduction in height growth (48). Height growth is generally less affected by stress than radial growth (27).

C. polonica produced more and deeper stain/desiccation and was reisolated further into the sapwood than the other fungi. This is consistent with previous studies that have shown *C. polonica* to be at the leading edge of fungal penetration all the way to the heartwood in Norway spruce attacked by *I. typographus* (46). The ability to invade fresh sapwood may be the most important characteristic of the pathogenic associates of aggressive bark beetles (34).

O. piceae is regarded as a weak bark pathogen (5). Its pathogenicity to Norway spruce has not been tested previously, but, based on phloem necrosis length, it was considered to be relatively pathogenic when inoculated into mature white spruce (*P. glauca*) (42) and moderately pathogenic to seedlings of eastern white pine (*Pinus strobus*) (31). *O. piceae* seemed to be less pathogenic to Norway spruce in the current study. Inoculation with *O. piceae*, the *Ambrosiella* sp., and Dark sterile sp. A resulted in 4.0 to 28.0% desiccated sapwood (Tables 4 and 5). The pathological significance of this sapwood desiccation is uncertain. It did not seem to affect stem growth, since there were no significant differences in annual ring width in the season of inoculation between the three fungi and the control. In fact, trees inoculated with *O. piceae* and the *Ambrosiella* sp., the fungi that caused the largest proportion of desiccated sapwood, had the largest basal area increment of all trees in 1995 (175 and 166%, respectively, of the average during 1992 to 1994). *O. piceae*, the *Ambrosiella* sp., and Dark sterile sp. A induced much more sapwood desiccation in Norway spruce than several nonpathogenic blue-stain fungi associated with *I. typographus* (45).

The higher pathogenicity observed for the *Ambrosiella* sp. and, to some extent, for *O. piceae* in 1996 compared with 1995 may have been caused by year-to-year variation in experimental conditions, different timing of inoculation relative to host phenology, or different virulence of different fungal isolates. Inoculations were made 6 weeks later in 1996 than in 1995, and mean temperature

the first month after inoculation was almost 2°C higher. The higher temperature may have had a positive effect on the fungi and increased their ability to colonize host tissues. This agrees with an earlier study in Norway spruce (22) that found trees to be more resistant to infection by *C. polonica* in spring/early summer (May to June) than later in the growth season. Thus, the higher pathogenicity observed for the *Ambrosiella* sp. and *O. piceae* in 1996 may have been a combined effect of using more virulent fungal isolates in 1996 and inoculating the trees later in the growth season.

For *C. polonica*, different virulence between isolates seems to be the most likely explanation for the lower pathogenicity observed in 1996, since the late inoculation date should have led to increased virulence in this season (22). Lower virulence of the isolate used in 1996 was also apparent when the two isolates were inoculated in seedlings and logs of Norway spruce (H. Solheim and P. Krokene, unpublished data). Although the isolate used in 1996 was less virulent than the isolate used in 1995, it induced equally extensive host symptoms as two isolates used in a previous mass-inoculation experiment (45). Lower virulence of the isolate used in 1996 may explain the lack of any blue-staining in trees inoculated in 1996. Staining occurs when the mycelium has reached a certain density in the sapwood (52), and it is likely that the *C. polonica* isolate used in 1996 was unable to colonize the sapwood sufficiently to cause blue-stain.

The inoculation density (400 inoculations per m²) that was used in this study is equivalent to a high natural attack density for *I. typographus*, which normally colonizes trees at 100 to 400 attacks per m² (7,51). However, attack densities of up to 1,800 per m² have been recorded (51). Because one beetle gallery is probably a more severe challenge to the tree's defenses than one inoculation (23,38) and most individuals of *I. typographus* carry *C. polonica* (14,25), attack by beetles is probably more detrimental to the trees than our inoculations. Furthermore, a natural mass attack extends over a large part of the stem, while our inoculations were restricted to a 1.2-m-long segment. Total number of inoculations is probably more important than the inoculation pattern (7).

The relative importance of the fungus and the beetles in the killing of the host has been the subject of a long and continuing debate (6,11,17,21). *I. typographus* and most other aggressive bark beetles seem to be associated with at least one pathogenic fungus, although there is significant variation in the pathogenicity of the fungi associated with different beetles (18,35,52). For example, the associates of *I. typographus*, *Dendroctonus ponderosae*, and *D. rufipennis* seem to be more pathogenic than those of *D. frontalis* and *D. brevicornis* (18,48). *D. frontalis* and *D. brevicornis* may also have a looser association with their pathogenic fungus, as instances of successful mass attacks with little or no visible sign of blue-stain fungi have been recorded (6). Thus, the question of whether association with pathogenic fungi is essential to overwhelm host defenses for all aggressive bark beetles remains unresolved (33). In some bark beetle-blue-stain fungi-host tree interactions, the fungi seem to play an important role in tree killing, while, in other systems, the role of the fungi is more uncertain. However, since all aggressive bark beetles are associated with more or less pathogenic fungi, the tree will always face an attacking beetle-fungus complex that probably acts synergistically to overwhelm host defenses (39,52).

The most important advantage aggressive bark beetles gain from a pathogenic fungus may be that the fungus prevents the host from expressing its potential resistance against attack. For example, multiple inoculations with *C. polonica* reduces the quantity of monoterpenes that accumulates at each wound site (7). Fungi may also prevent the host from recovering after the initial attack and killing the developing brood with secondary resinosis (2,12). The fungus probably interferes with the water relations of the tree, either directly through hyphal growth into the water conducting vessels, indirectly through the production of toxins (20), or through induction of tracheid cavitation (26).

The conditions under which association with pathogenic fungi are favorable to the beetle may be limited, however, and may be restricted to periods with high beetle activity (32). When the beetles colonize dead and dying trees during nonepidemic periods, the fungus may act as a competitor for the limited nutrients in the phloem (2). To speculate further, the benefits accrued to the beetles by an association with pathogenic fungi may be less during nonepidemic periods, when it is less likely that a mass attack can be initiated. This could conceivably select against association with pathogenic fungi in aggressive species during nonepidemic periods and against association at any time for nonaggressive species.

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