

Bark beetles and fungal associates colonizing white spruce in the Great Lakes region

Kirsten E. Haberkern, Barbara L. Illman, and Kenneth F. Raffa

Abstract: We examined the major bark beetles and associated fungi colonizing subcortical tissues of white spruce (*Picea glauca* (Moench) Voss) in the Great Lakes region. Trees were felled at one northwestern Wisconsin site in a preliminary study in 1997 and at 10 sites throughout northern Wisconsin, Minnesota, and Michigan in 1998. Fungal isolations were made from beetles colonizing felled trees, beetles that emerged from felled trees, tissue of colonized trees, and tissue of uncolonized trees. *Dryocoetes affaber* (Mannerheim) and *Polygraphus rufipennis* (Kirby) accounted for over 90% of the insects that emerged from logs. Time of colonization had a significant effect on abundance and composition of emerging insects. New records include *Dendroctonus rufipennis* (Kirby) in Wisconsin and two Michigan counties and *Crypturgus borealis* (Swaine) in Wisconsin and Minnesota and one Michigan county. Five fungal species from two genera were isolated both from beetles and colonized tree tissue. None were isolated from uncolonized trees. Ten new beetle–fungal associations were identified. The association of specific fungi with specific bark beetles, both as they colonize and emerge from hosts and the isolation of these fungi from subcortical tissues of colonized but not uncolonized trees, is consistent with vector relationships. We compare our results with bark beetle–fungal associations reported elsewhere in spruce and suggest possible mechanisms constraining population growth by *Dendroctonus rufipennis* in the Great Lakes region.

Résumé : Nous avons étudié les principaux scolytes et les champignons associés qui colonisent les tissus sous-corticaux de l'épinette blanche (*Picea glauca* (Moench) Voss) dans la région des Grands-Lacs. Des arbres ont été abattus dans un site du Nord-Ouest du Wisconsin lors d'une étude préliminaire en 1997 et à 10 endroits dans le Nord du Wisconsin, du Minnesota et du Michigan en 1998. Les champignons ont été isolés à partir des insectes qui colonisaient les arbres abattus, de ceux qui émergeaient des arbres abattus, des tissus des arbres colonisés et des tissus des arbres non colonisés. *Dycoetes affaber* (Mannerheim) et *Polygraphus rufipennis* (Kirby) représentaient plus de 90% des insectes qui ont émergé des billes. Le moment de la colonisation avait un effet significatif sur l'abondance et la composition des insectes qui émergeaient. C'est la première mention de *Dendroctonus rufipennis* (Kirby) au Wisconsin et dans deux comtés du Michigan et de *Crypturgus borealis* (Swaine) au Wisconsin et au Minnesota et dans un comté du Michigan. Cinq espèces de champignons appartenant à deux genres ont été isolées à partir des insectes et des tissus des arbres colonisés. Aucun champignon n'a été isolé chez les arbres non colonisés. Dix nouvelles associations insectes–champignons ont été identifiées. L'association de certains champignons avec certains dendroctones, lorsqu'ils colonisent et lorsqu'ils émergent des hôtes, et l'isolation de ces champignons dans les tissus sous-corticaux des arbres colonisés, mais non des arbres non colonisés, sont consistantes avec le type de relations rencontrées dans le cas d'un vecteur. Nous comparons nos résultats avec les associations entre dendroctones et champignons rapportées ailleurs chez l'épinette et nous suggérons des mécanismes qui pourraient limiter la croissance de la population de *Dendroctonus rufipennis* dans la région des Grands-Lacs.

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Introduction

White spruce (*Picea glauca* (Moench) Voss) is an important economic resource and ecological component of the boreal forests of North America (Lindgren and Lewis 1997; Uzunovic et al. 1999). Its natural range extends into the central Great Lakes region, which includes northern Wisconsin,

northern Minnesota, and the Upper Peninsula of Michigan. Natural stands often occur as spruce–pine–fir forests (Schmid and Hinds 1974; Uzunovic et al. 1999),

Several species of insects and microorganisms exert significant impacts on *Picea glauca* throughout its range, in both commercial and unmanaged stands (Parish et al. 1999). Bark beetles (Coleoptera: Scolytidae; alternative: Curculionidae: Scolytinae) and their associated fungi are among the most important of these groups (Harrington and Cobb 1988; Reynolds 1992; Lindgren and Lewis 1997). For example, the spruce beetle, *Dendroctonus rufipennis* (Kirby), is the leading cause of mortality to white spruce in Alaska (Werner and Holsten 1985; Gara et al. 1995) and western Canada (Safranyik and Linton 1988) and an important mortality agent in New England (Werner and Holsten 1985; Harrington and Cobb 1988) and eastern Canada (Safranyik and Linton 1988). It is also the leading cause of

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mortality to Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) in the Rocky Mountains (Schmid 1981). Mortality of healthy spruce due to bark beetles is less common in the Great Lakes region, but attacks on stressed, windthrown, or recently cut trees are often observed. Both primary bark beetles, such as *Dendroctonus rufipennis*, and secondary bark beetles introduce fungi that stain host tree tissues (Whitney 1982; Klepzig et al. 1991; Poland and Borden 1998a; Oshsawa et al. 2000).

Bark beetles and their associated microorganisms play important roles in forest nutrient cycling, succession, and gap regeneration by initiating the process of wood degradation and the release of organic compounds (Schowalter and Filip 1993; Castello et al. 1995). They also contribute to biodiversity by providing an important food base for insectivorous birds, such as woodpeckers, beginning the process of tree cavity formation that provides habitat for various bird and mammal species and initiating the process of within-tree succession that supports a diverse guild of invertebrates and microorganisms (Coulson et al. 1986; Ross et al. 1997).

Scolytid life cycles include three phases: dispersal, host colonization, and development (Safranyik et al. 1983). Most of their lives are spent within the phloem tissue beneath the bark, which provides protection and nutrients (Wood 1982). The time required to complete development varies with temperature (Werner and Holsten 1985; Bentz et al. 1991; Bentz and Mullins 1999; Logan and Bentz 1999).

Microorganisms show close associations with bark beetles (Whitney 1982; Six and Paine 1999a, 1999b; Six et al. 1999). Beetles provide transportation to host trees (Barras and Perry 1972; Harrington 1993; Furniss 1990, 1993, and microorganisms often enhance beetle fitness by facilitating nutrient uptake (Barras 1973; Bridges 1981; Coppedge et al. 1995; Six and Paine 1998; Ayres et al. 2000), reducing the toxicity of host defense compounds (Leufven 1991; Paine et al. 1997), or obstructing water-transporting conduits (Reynolds 1992; Solheim and Safranyik 1997; Krokene and Solheim 1996, 1998). Some fungal associates can reduce the developmental success of bark beetles by competing for nutrients or inhibiting mutualistic fungi (Barras 1970; Bridges et al. 1985; Paine et al. 1997). Scolytids may vector fungal spores casually in cuticular pits on their elytra (Furniss et al. 1990, 1995; Lewinsohn et al. 1994) or in specialized pouches in their exoskeleton, called mycangia (Barras and Perry 1971, 1972; Six and Paine 1996). Several species of ascomyceteous fungi, such as *Leptographium abietinum* (Peck), cause a blue or black discoloration that decreases the value of the wood (Gibbs 1993; Seifert 1993; Croan and Highley 1995; Uzunovic and Webber 1998). Ascomyceteous fungi associated with bark beetles include the genera *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis*, and the anamorphic genera *Graphium* and *Leptographium* (Harrington 1993; Upadhyay 1993; Wingfield et al. 1997).

The guild of bark beetle species and associated fungi colonizing spruce trees in the Great Lakes region is not well characterized. Moreover, the distribution of *Dendroctonus rufipennis*, and the potential for future outbreaks in this region is unknown (Bright 1976; Wood 1982). The goals of this project were to identify the bark beetles colonizing white spruce in the Great Lakes region, identify the

ascomyceteous fungi associated with these beetles, and evaluate potential vector relationships.

Materials and methods

General approach

Ten research sites in northern Wisconsin, northeastern Minnesota, and the Upper Peninsula of Michigan were sampled to characterize the predominant bark beetles and fungal symbionts associated with white spruce in the Great Lakes region. The effects of time within the season, height along the bole, and tree diameter on the number and species composition of emerging beetles were evaluated. Trees were felled at each site and baited. After colonization, trees were sectioned and transported to the laboratory. Fungal isolations were made from beetles colonizing felled trees, beetles that emerged from felled trees, tissue of colonized trees, and tissue of uncolonized trees.

Site selection and description

One site was established for a preliminary trial in Washburn County, Wisconsin, in 1997 and was sampled as described below. In April of 1998, ten 0.13- to 0.20-ha sites were established. Each site was located in a monoculture stand that contained at least 80% *Picea glauca* and ranged in size from 0.81 to 56.68 ha (Fig. 1). Mean stand age was 44 years, and the diameter at breast height (DBH) of the trees ranged from 15.2 to 25.4 cm (Table 1). The surrounding landscape was predominantly forested with a 50:50 mixture of conifer and deciduous trees. Detailed site descriptions are in Haherkern (2001).

Sampling and rearing of bark beetles

Three 15- to 18-cm DBH trees were felled at each site in early May, and again in early July, of 1998. (Trees were not felled at Gull Lake in July because of insufficient trees remaining in the designated diameter class.) Felled trees were baited with membrane release lures containing the synthetic pheromone of *Dendroctonus rufipennis* (frontalin and α -pinene, emission rates 2.6 and 1.5 mg/day, respectively, at 23°C; Phero Tech Inc., Delta, B.C.). In a parallel study this lure had no effect on the bark beetle composition in this region, as there was no cross attraction or inhibition (Haherkern 2001). Similarly, Greenwood and Borden (2000) concluded that cobaiting for *Dendroctonus rufipennis* and *Dryocoetes confusus* Swaine did not cause interference. Felled trees were laid flat on the forest floor in the shade and left for 7 weeks to allow for colonization. Hereafter, we use the terms lower, middle, and upper to refer to the position along the height of the vertical stem and top and bottom to refer to the surface of the felled stem.

After 7 weeks, three 30 cm long sections were cut from each tree and transported to the University of Wisconsin-Madison. The lower stem section was cut from the base of the stump, the middle section was cut 2.13 m from the stump, and the upper section was cut 3.96 m from the stump. The tree sections were placed individually in metal rearing cans, 31 cm diameter $\times$ 41 cm high. The sides of the cans had two 6 cm holes through which adult progeny could pass into 250-mL glass collecting jars containing a

Fig. 1. Site locations for sampling bark beetles and fungal associates of white spruce in the Great Lakes region. The shaded portion represents the natural range of white spruce in this region (Little 1971), and the circles are the study plots.

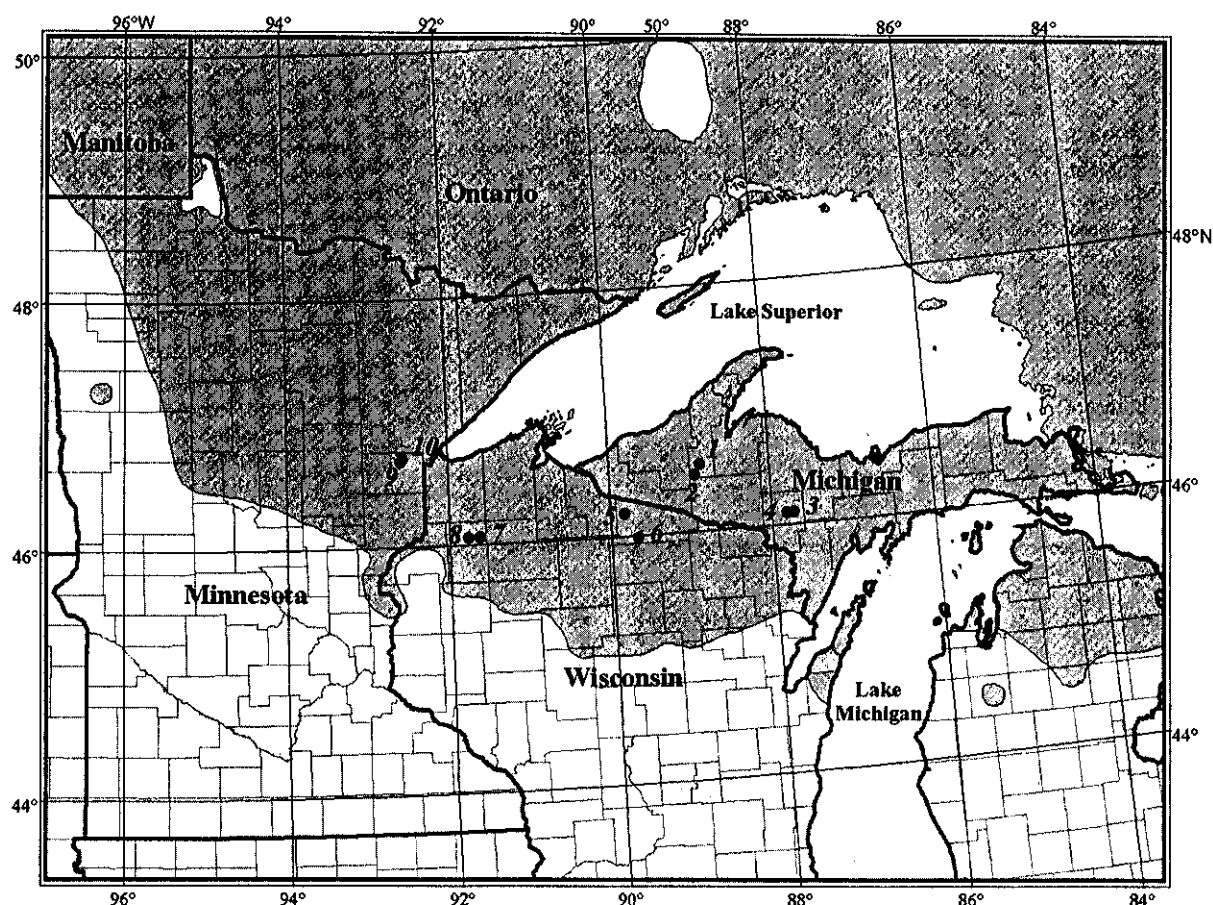


Table 1. Locations of white spruce research sites for characterization of bark beetles and fungal associates.

Site No.	Location (city, state, county)	Name	Longitude, latitude	Age (years)	Size (ha)
1	Kenton, Michigan, Houghton	Ottawa National Forest	46°32.2'N, 88°50.9'W	58	2.83
2	Kenton, Michigan, Houghton	Ottawa National Forest	46°26.9'N, 88°55.4'W	59	56.68
3	Felch, Michigan, Dickinson	Copper County State Forest	46°04.36'N, 87°43.83'W	36	9.72
4	Felch, Michigan, Dickinson	Copper County State Forest	46°04.02'N, 87°48.34'W	38	5.67
5	Boulder Junction, Wisconsin, Vilas	Northern Highland State Forest	46°09.3'N, 89°48.8'W	34	21.46
6	Arbor Vitae, Wisconsin, Vilas	American Legion State Forest	45°56.9'N, 89°39.6'W	33	5.67
7	Stinnett, Wisconsin, Washburn	Washburn County Forest	46°01.4'N, 91°37.0'W	48	10.53
8	Gull Lake, Wisconsin, Washburn	Washburn County Forest	46°46.11'N, 91°45.4'W	45	6.48
9	Cloquet, Minnesota, Carlton	University of Minnesota, Cloquet Forestry Center	46°41.08'N, 92°34.85'W	47	1.30
10	Cloquet, Minnesota, Carlton	University of Minnesota, Cloquet Forestry Center	46°42.69'N, 92°33.14'W	36	0.81

Kimwipe® (Kimberly-Clark Inc., Roswell, Ga.) tissue paper that minimized contact among beetles. A 21.5 cm diameter screen mesh in the lid allowed for air circulation. A black cloth (37.5 cm²) underneath the lid blocked light from all directions except the jars. Rearing cans were kept under a constant light source at 20–25°C and 50–70% RH. Live bark beetles were collected from the rearing cans twice weekly, placed in glass vials containing a Kimwipe® tissue, and stored in a refrigerator at 4°C. Prior to fungal isolations,

bark beetles were identified to species using keys (Bright 1976; Wood 1982) and by comparison with specimens from the University of Wisconsin-Madison Insect Research Collection (IRC). Insect identifications were confirmed by Steven Krauth, IRC curator, and voucher specimens were deposited in the IRC.

Additional insect collections were made from the 30 trees felled in May, prior to their sectioning and placement in containers. We systematically examined four sections of each

Table 2. Summary of emerged insects from white spruce trees felled in the central Great Lakes region.

Coleoptera: Scolytidae							
Site NO.	Location	No. of trees	<i>Dryocoetes affaber</i>	<i>Polygraphus rufipennis</i>	<i>Ips pini</i>	<i>Dryocoetes autographus</i>	<i>Crypturgus borealis</i>
1	Michigan	6	3 505	438	17	15	7
2	Michigan	6	1004	102	210	15	0
3	Michigan	6	5162	771	141	85	0
4	Michigan	6	792	694	16	15	14
5	Wisconsin	6	940	425	98	11	2
6	Wisconsin	6	356	904	55	24	0
7	Wisconsin	6	882	21	15	127	0
8	Wisconsin	3	51	14	20	14	0
9	Minnesota	6	229	193	24	33	77
10	Minnesota	6	5 763	257	99	339	14
Total		57	18 684	3819	695	678	114
Mean			312.25	63.88	11.92	11.53	1.90
SE			169.54	34.66	8.82	6.74	1.60

Note: Values for each insect are the totals for each site.

tree: the stump, a 1.52-m portion between the lower and middle stem sections, a 1.52-m portion between the middle and upper sections, and a 0.33-m section above the upper stem sections. The bark and phloem tissue were removed with a knife. Colonizing adult beetles were collected from their galleries and placed either in vials containing 70% ethanol for storage or in sterile vials for fungal isolations.

Fungal isolations from bark beetles

A modified dilution plating method was used to isolate fungi from adult bark beetles. One to five live beetles of each species were ground in a sterile glass tissue homogenizer with 9 mL of sterile water (Juzwik and French 1983; Klepzig et al. 1991). The liquid portion of the homogenate was decanted and set aside. The process was repeated twice for a total of three liquid homogenates. Each homogenate was purified through four 10^{-1} dilutions, resulting in three rows each with a spore concentration range of 10^{-1} to 10^{-4} . The tubes containing 10^{-2} to 10^{-4} concentration were shaken, and 0.5 mL was removed and spread evenly across the surface of a 100×15 mm Petri dish (Falcon; Becton Dickinson Co., Sparks, Md.) that contained potato dextrose agar (PDA; Becton Dickinson Co., Sparks, Md.) amended with gentamicin (ICN Biomedicals, Costa Mesa, Calif.). A pipetter (Ranin Inc., Woodburn, Mass.) was used to extract the liquid and a sterile glass "L" rod on a turntable (Fisher Scientific, Pittsburgh, Pa.) was used to spread the spore suspension on the surface of the media. The Petri dishes were cultured in a growth chamber at 22°C on an 8 h light : 16 h dark schedule.

Ascomyceteous fungi were distinguished from other fungi based on hyphal morphology (de Hoog 1993; Kendrick et al. 1993). Fungi were classified into putative types according to hyphal morphology and characteristics of the fruiting structures produced during the anamorph stage (Seifert and Okada 1993; Upadhyay 1993; Wingfield 1993). Each morphological type was treated as a potentially different fungal species.

A 3-mm² area of actively growing hyphae from each morphological type was transferred to a Petri dish containing

PDA using a sterilized wire dissection needle. These dishes were cultured in a growth chamber at 22°C with an 8 h light : 16 h dark schedule for 7–10 days. Dishes containing monocultures were individually sealed with parafilm and stored in plastic bags in a refrigerator (4°C) until identification. Petri dishes from dilution plating were screened for additional ascomyceteous fungi weekly for a month. Transfers for monocultures were performed within 24 h of each screening. Monocultures stored in the refrigerator were visually inspected every 28–30 days for the presence of contaminant fungi, i.e., any genera not within the ascomycete group. When contaminant fungi were present, hyphae from ascomycete fungi were transferred to new PDA dishes.

Fungal isolations from colonized trees

An increment core borer was used to extract 0.5 cm diameter $\times$ 6 cm long tissue samples containing bark, phloem, and xylem. The core borer was sterilized with ethanol and a flame prior to each sample extraction. Core samples were extracted from random points around the circumference of the felled colonized trees at the following heights: three from the stump (0–0.1 m), five from the lower stem (0.6 m), three from the middle stem (2.4 m), and three from the upper stem (4.3 m). All core samples were placed in sterile glass tubes in the field. The core samples were examined in the laboratory for the presence of blue or black stain, and fungal isolations were performed on each core sample.

Multiple isolation methods were conducted to account for the fact that stain fungi vary among species in their requirements to produce the sexual and asexual fruiting structures necessary for identification (Seifert et al. 1993). Some species, such as *Ophiostoma nigrocarpum* (Davidson), produce sexual fruiting structures on PDA, most species, such as *Ophiostoma piceaperdum* (Rumbold) Arx, require low nutrient conditions and conifer sapwood tissue (Seifert et al. 1993), and some species, such as *Ceratocystis rufipenni* (Wingfield), require extended incubation at a low temperature for sporulation to occur (Wingfield et al. 1997). There-

<i>Dendroctonus rufipennis</i>	<i>Trypodendron lineatum</i>	<i>Orthotomicus caelatus</i>	<i>Ips grandicollis</i>	Coleoptera: Curculionidae <i>Pissodes nemorensis</i>	Coleoptera: Cleridae <i>Thanasimus dubius</i>	Hymenoptera: Pteromalidae <i>Roptrocercus xylophagorum</i>
4	0	1	0	0	2	242
3	7	0	0	0	5	28
1	0	0	1	14	2	20
0	0	0	1	1	2	33
0	0	3	0	1	2	15
4	0	1	3	11	5	64
0	0	0	0	0	1	9
0	0	0	0	0	0	1
0	0	0	0	0	0	37
0	0	1	0	2	4	13
12	7	6	5	29	23	462
0.20	0.12	0.10	0.08	0.48	0.38	7.72
0.15	0.07	0.17	0.07	0.31	0.22	4.71

fore, three complementary methods were used: plating on PDA, plating on water agar, and incubating at 4–15°C.

The PDA method was used on six trees. Core samples were cut into three pieces (1, 1, and 4 cm in length). Each piece was placed in the center of a Petri dish containing PDA, cultured at 22°C, and checked weekly. Hyphae of putative ascomyceteous fungi were transferred into monocultures as described previously, and monocultures were identified to species as described below (see fungal identification). The water agar method was used on 15 trees. Core samples were cut into three pieces and placed individually on Petri dishes containing water agar (Bacto-Agar®, Beckton Dickinson Co., Sparks, Md.). These samples were cultured in a growth chamber at 15°C, checked weekly for 6 weeks, and transferred to new dishes when necessary. Fruiting structures growing from core samples were slide mounted for identification (Seifert et al. 1993). Monocultures were processed for identification from hyphae growing from core samples that did not produce fruiting structures. The cool temperature incubation method was used on 10 trees, including one on which the water agar method was also used. Core samples were left in the sterile tubes and incubated at 15°C for 6 weeks. Fruiting structures growing from the cores were slide mounted for identification. Core samples without fruiting structures were stored at 4°C for 4 months and cultured as described above in the PDA method.

Fungal isolations from uncolonized trees

Tissue samples were collected from 30 uncolonized standing trees, including 15 each at sites 5 and 6. These trees showed normal growth and crown structure and had no visible signs or symptoms of attack by insects or pathogens. A sterilized increment core borer was used to collect two tissue samples from each tree, 0.30 and 1.52 m above the ground. All 60 samples were examined for the presence of stain fungi, and the water agar method was used for fungal isolations. Transfers of hyphae for monocultures and culturing fruiting structures were performed as described above.

Fungal identification

Monocultures of potential ascomyceteous fungi were identified to species based on hyphal morphology, hyphal physiology, and the production of fruiting structures during the anamorph and teleomorph stages (Olchowecki and Reid 1974; Nag Raj and Kendrick 1975; Upadhyay 1981; Grylls and Seifert 1993).

The sexual and asexual fruiting structures were mounted on glass slides, stained with cotton methyl blue, and coverslips were sealed with nail polish. Fruiting structures and spores were measured using the ocular scale of a compound microscope. To ensure homogeneity of species, spores from individual fruiting structures were smeared on PDA, cultured at 22°C for 7–10 days, and then anamorph and teleomorph stages were reevaluated (Seifert et al. 1993). Identification of ascomyceteous species were confirmed by Thomas Harrington (Department of Plant Pathology, Iowa State University, Ames) and specimens were deposited in his collection. Identification of other fungi were confirmed by Eugene Smalley (Department of Plant Pathology, University of Wisconsin, Madison).

Statistical analysis

Overall sources of variation in the abundance of beetle emergence from felled trees were analyzed with an analysis of variance (ANOVA). Because the interaction term between site and time was significant we proceeded to a split-plot analysis (see Results). The split-plot analysis (PROC MIXED; Littrell et al. 1996) included tree within time period as the whole plot, height as the split plot, and site as a random effect. For *Dryocoetes affaber* (Mannerheim), a one-fourth root transformation was required for both analysis to normalize the variance. Other species did not require a transformation. For the two most abundant species, which together accounted for 91.74% of all emerged insects, we also evaluated an analysis of covariance (ANCOVA). This ANCOVA model was identical to the split-plot model but included hole diameter as a continuous variable.

Table 3. Analysis of variance for bark beetles emerging from felled white spruce trees using (A) all variables for all trees, (B) separate analyses based on all felled trees and colonized trees only, and (C) separate analyses by time of colonization.

(A) All variables for all trees.										
Source of variation	<i>Dryocoetes affaber</i>		<i>Polygraphus rufipennis</i>		<i>Ips pini</i>		<i>Dryocoetes autographus</i>		<i>Crypturgus borealis</i>	
	F	P	F	P	F	P	F	P	F	P
Site (S)	3.67	0.0005	2.13	0.33	1.49	0.16	1.66	0.106	2.42	0.015
Time (T)	16.31	<0.0001	20.58	<0.0001	8.42	0.005	1.59	0.21	4.66	0.033
S × T	4.49	<0.0001	2.33	0.023	1.56	0.145	1.9	0.066	2.76	0.008
Height (H)	0.30	0.739	2.68	0.073	0.13	0.881	0.18	0.833	1.1	0.336
S × H	0.53	0.936	0.61	0.886	0.28	0.999	0.73	0.771	1.64	0.061
T × H	0.42	0.658	0.87	0.421	0.05	0.954	0.12	0.891	0.79	0.456
S × T × H	0.47	0.958	0.79	0.692	0.46	0.964	0.74	0.751	1.9	0.027
(B) Separate analyses based on all felled trees and colonized trees only .										
All trees										
Site (S)	2.06	0.058	1.77	0.106	0.64	0.757	2.18	0.045	1.65	0.136
Time (T)	9.17	0.004	14.49	0.001	3.61	0.065	2.08	0.157	3.19	0.082
S × T	2.52	0.026	1.64	0.146	0.67	0.715	2.50	0.028	1.89	0.091
Colonized trees only										
S	1.67	0.134	1.67	0.132	0.64	0.756	1.12	0.384	0.39	0.794
T	7.50	0.010	13.72	0.001	3.50	0.070	1.27	0.271	—*	—
S × T	1.99	0.079	1.56	0.170	0.75	0.651	1.46	0.227	—	—
(C) Separate analyses by time of colonization										
May–June										
Site (S)	1.35	0.232	2.45	0.019	1.34	0.236	5.52	<0.001	2.6	0.013
Height (H)	0.69	0.507	1.68	0.195	3.22	0.047	0.47	0.625	2.03	0.363
S × H	0.64	0.853	0.68	0.818	0.87	0.613	0.65	0.843	1.77	0.052
July–August										
S	4.15	<0.001	1.81	0.096	1.54	0.167	1.69	0.122	1.00	0.447
H	0.33	0.721	3.02	0.057	0.03	0.972	0.14	0.861	1.00	0.375
S × H	0.50	0.935	1.16	0.329	0.35	0.988	0.74	0.742	1.00	0.471

*Insufficient data.

Results

Bark beetles colonizing white spruce

A total of 1745 insects emerged from the three trees felled in 1997. All were bark beetles, and six species were collected: *Crypturgus borealis* (Swaine), 56%; *Polygraphus rufipennis* (Kirby), 24%; *Dryocoetes affaber*, 20%; *Ips pini* (Say), 0.5%; *Dryocoetes autographus* (Ratzeburg), 0.2%; and *Dendroctonus rufipennis* (Kirby), 0.1%. A total of 24 530 insects emerged from felled white spruce in 1998. Bark beetles represented 97.91%; root-colonizing weevils, 0.12%; predatory beetles, 0.09%; and parasitoids, 1.88% of all emerging insects (Table 2). Nine species of bark beetles, including five of the six species obtained in 1997, emerged from these logs. The most abundant species were *Dryocoetes affaber* (76.17%) and *Polygraphus rufipennis* (15.57%). Fifteen of an unidentified species (Coleoptera: Cerambycidae) also emerged.

In the overall analysis that included all variable for all trees, *Dryocoetes affaber* and *C. borealis* showed significant site effects (Table 3A). We repeated the analysis separately for all trees and colonized trees only, with the insignificant terms from the first analysis removed (Table 3B). The site

effect was significant for *Dryocoetes autographus* and weakly for *Dryocoetes affaber*, but only when all trees were considered. Therefore, variation is due more to the number of trees colonized per site than the emergence per colonized tree. The time at which trees were felled was a significant source of variation for all species except *Dryocoetes autographus* and had a stronger effect than site for *Dryocoetes affaber*, *Polygraphus rufipennis*, and *I. pini* (Table 3A). Moreover, there were strong site by time effects. Therefore, we conducted separate analyses based on time of colonization (Table 3C). Site remained significant during those periods when each insect was most prevalent, i.e., May–June for *Polygraphus rufipennis*, *Dryocoetes autographus*, and *C. borealis* and July–August for *Dryocoetes affaber*, but not when each insect was less prevalent. Height was not significant in the overall analysis for any species but was significant for *I. pini* in May–June. No interactions with height were significant except for the three-way interaction involving *C. borealis*.

The split-plot analyses that included all trees, and those that included only colonized trees always yielded the same results (Haberkern 2001). Therefore, the former are used for all subsequent discussion. *Dryocoetes affaber*, *Polygraphus*

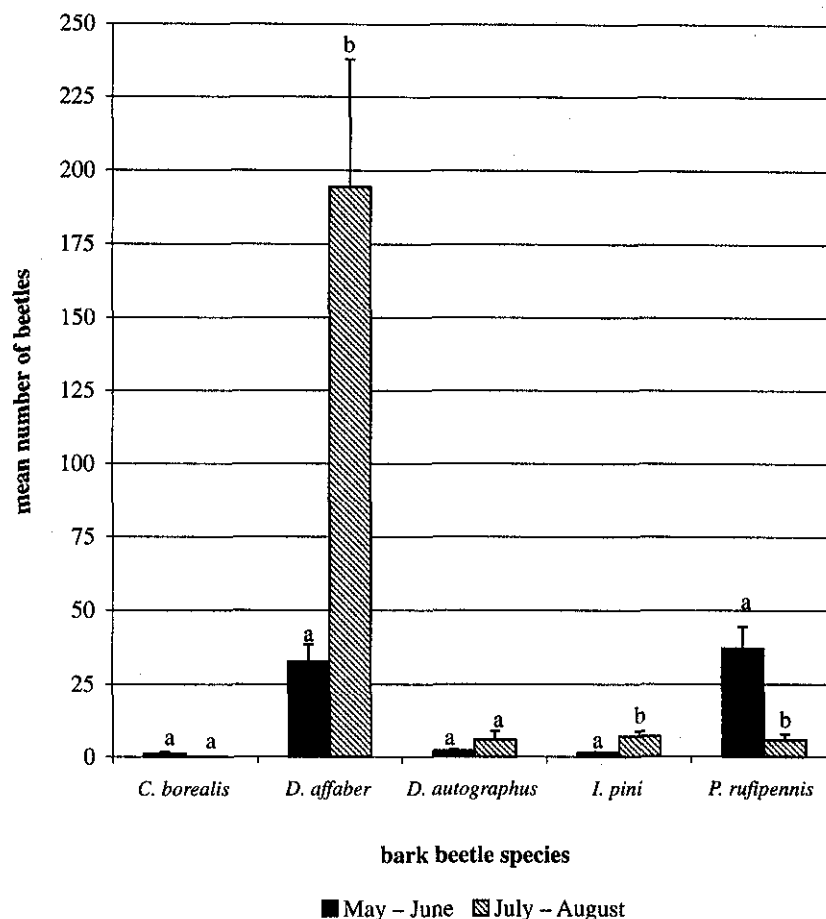
Table 4. Split-plot analysis of the effects of time of colonization and height along bole on emergence abundance of bark beetles from felled white spruce in Michigan, Minnesota, and Wisconsin in 1998.

Source of variation	<i>Dryocoetes affaber</i>		<i>Polygraphus rufipennis</i>		<i>Ips pini</i>		<i>Dryocoetes autographus</i>		<i>Crypturgus borealis</i>	
	F	P	F	P	F	P	F	P	F	P
Time (T) ^a	4.50	0.039	11.14	0.002	4.13	0.048	1.72	0.196	2.41	0.127
Height (H) ^b	1.63	0.200	3.33	0.039	0.33	0.717	0.20	0.816	1.13	0.327
T × H	0.52	0.593	0.89	0.415	0.19	0.827	0.15	0.863	0.61	0.547

^adf (numerator, denominator) = 1, 46.

^bdf (numerator, denominator) = 2, 110.

Fig. 2. Mean number of bark beetles emerged from felled white spruce trees colonized during two periods in 1998 in Michigan, Minnesota, and Wisconsin. Different letters within a species indicate significant differences between times at $p < 0.05$ ($n = 30$ trees for May–June, $n = 27$ trees for time July–August). Error bars are SEs.



rufipennis, and *I. pini* showed significant variation due to time (Table 4). *Polygraphus rufipennis* also showed significant within-tree effects due to height. The interaction between time and height of colonization was not significant for any species. Emergence of *Dryocoetes affaber* was six times greater in trees colonized during July–August than during May–June (Fig. 2). Emergence of *Polygraphus rufipennis* was seven times greater in trees colonized during May–June than during July–August. *Ips pini* was five times more abundant in trees colonized during July–August than May–June. *Polygraphus rufipennis* was twice as abundant in the lower stem than in the middle or upper portions of the stem (Fig. 3).

Diameters of logs ranged from 9 to 21 cm. Diameter had no effect on the abundance of emerging *Dryocoetes affaber* for either colonization time period or when times were pooled ($df = 1$, $F = 0.06$, $P = 0.803$). Diameter affected the overall emergence of *Polygraphus rufipennis* ($df = 1$, $F = 6.22$, $P = 0.014$), but this can be attributed solely to the second time period ($df = 1$, $F = 10.30$, $P = 0.002$): emerged *Polygraphus rufipennis* = $130.95 + 2.51 \times \text{diameter (cm)}$. Although statistically significant, the relationship between emergence and diameter is weak ($R^2 = 0.118$).

During field dissections of colonized trees *Dendroctonus rufipennis* was only found in the bottom of the lower and

Fig. 3. Mean number of bark beetles that emerged from three heights along the stem of felled spruce trees. Different letters within a species indicated significant differences at $p < .005$ ($n = 57$ for each height). Error bars are SEs.

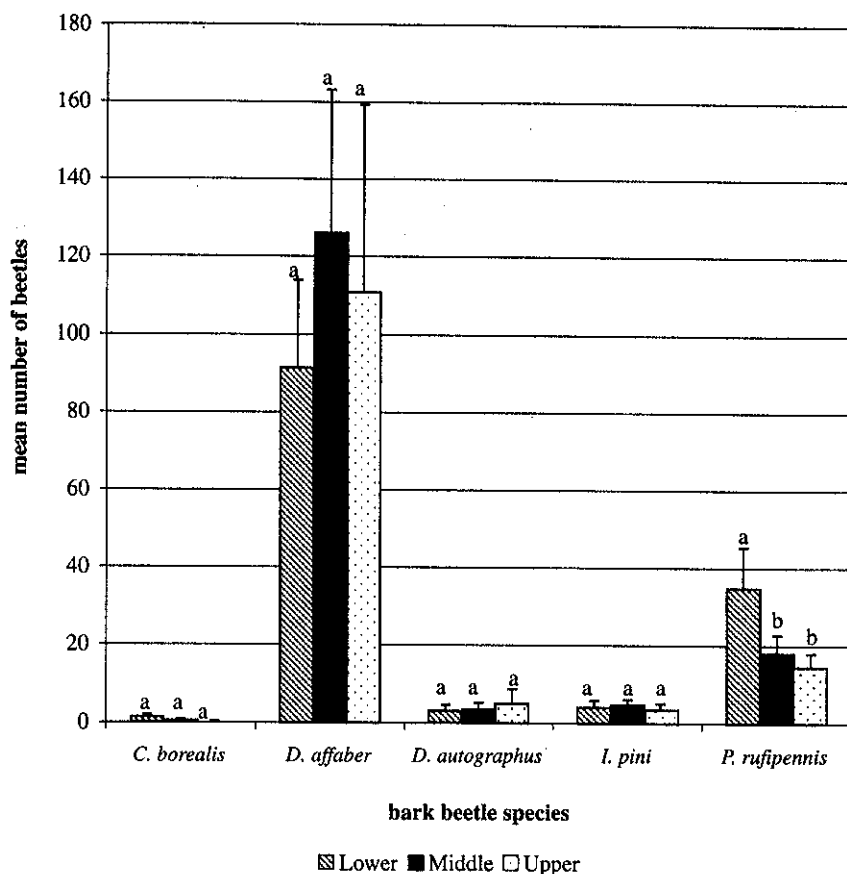


Table 5. (A) Percentage of trees in which sections contained galleries of various bark beetle species in white spruce ($N = 30$ trees) and (B) contingency analyses of the percentages.

(A) Percentage of trees in which sections contained galleries of various species.

Height	<i>Dendroctonus rufipennis</i>			<i>Dryocoetes autographus</i>			<i>Polygraphus rufipennis</i>			<i>Dryocoetes affaber</i>		
	Bottom	Top	Total ^a	Bottom	Top	Total	Bottom	Top	Total	Bottom	Top	Total
Stump	na ^b	na	0.0	na	na	30.0	na	na	26.1	na	na	0.0
Lower	30.0	0.0	30.0	60.0	0.0	60.0	83.3	83.3	83.3	50.0	50.0	50.0
Middle	16.7	0.0	16.7	36.7	0.0	36.7	83.3	83.3	83.3	53.3	53.3	53.3
Upper	0.0	0.0	0.0	6.7	0.0	6.7	83.3	83.3	83.3	56.7	56.7	56.7
Total	30.0	0.0	30.0	60.0	0.0	60.0	83.3	83.3	83.3	60.0	60.0	60.0

(B) Contingency analysis.

Source of variation	<i>Dendroctonus rufipennis</i>		<i>Dryocoetes autographus</i>		<i>Polygraphus rufipennis</i>		<i>Dryocoetes affaber</i>	
	c ²	P	c ²	P	c ²	P	c ²	P
Height (df = 3)	16.3	0.001	13.0	0.005	10.5	0.02	16.2	0.001
Surface (df = 1)	9.0	0.003	18.0	0.00002	0.0	1.00	0.0	1.000

Note: Lower, middle, and upper refer to height along the bole: bottom and top refer to the surface of the felled tree. The contingency analyses are based on raw counts.

^aThe total percent colonization is sometimes less than the sum of columns or rows because some trees had multiple sections colonized.

^bNot available.

Table 6. Percentage of various bark beetle species from which fungi were isolated after they emerged from white spruce.

Fungus	<i>Dryocoetes affaber</i> (n = 185)	<i>Polygraphus rufipennis</i> (n = 125)	<i>Ips pini</i> (n = 8)	<i>Dryocoetes autographus</i> (n = 5)	Total % presence ^a
<i>Leptographium abietinum</i>	2.1	8.8	0.0	0.0	4.9
<i>Ophiostoma bicolor</i>	3.2	4.0	12.5	0.0	3.7
<i>Ophiostoma piceaperdum</i>	36.8	5.6	12.5	80.0	24.8
<i>Ophiostoma ips</i>	1.6	2.4	75.0	0.0	3.7
<i>Ophiostoma piceae</i>	3.2	0.0	0.0	0.0	1.9
<i>Ophiostoma nigrocarpum</i>	0.0	0.0	0.0	0.0	0.0
Total % beetles with fungi ^b	46.5	19.2	75.0	80.0	31.2

^aWeighted by total number of all beetles.

^bThe total % beetles with fungi is sometimes less than the sum of the columns, because 1.86% of the beetles carried multiple species of fungi.

middle stem sections (Table 5). *Dryocoetes autographus* was predominantly found in the bottom of the lower stem, but also in the bottom of the middle, and occasionally in the bottom of the upper. *Dryocoetes autographus* and *Polygraphus rufipennis* were the only bark beetles found in the stump. *Dryocoetes affaber* and *Polygraphus rufipennis* were found equally in the bottoms and tops of all stem sections.

Fungal isolations from bark beetles

Approximately 37% of the 323 beetles carried ascomyceteous fungi (Table 6). *Ophiostoma piceaperdum* (synonymous with *Ophiostoma europaoides* (Wright & Cain); Jacobs et al. 2000) was the most abundant fungus (25%), the only fungus found on all of the four beetle species that carried fungi, and the only species isolated from *Dryocoetes autographus*. *Leptographium abietinum* (Peck) was the second most abundant fungus (5%) and was isolated from *Polygraphus rufipennis*, and *Dryocoetes affaber*. *Ophiostoma bicolor* (David & Wells) and *Ophiostoma ips* (Rumbold) Nannf. were each isolated from 3.7% of the beetles and were associated with *Dryocoetes affaber*, *I. pini*, and *Polygraphus rufipennis*. *Ophiostoma piceae* (Munch) was the least abundant species (2%) and was only associated with *Dryocoetes affaber* (Table 6).

Two of three *Dryocoetes autographus* and two of three *Polygraphus rufipennis* excavated from their entrance galleries carried *O. bicolor*, one each carried both *O. bicolor* and *O. piceae*, and one each carried no fungi. One-third of these beetles also carried *O. nigrocarpum* or *O. piceaperdum*.

Fungal isolations from host tissue

Overall, 20% of the 420 tissue samples taken from colonized trees contained fungi (Table 7). Some species (10.5%) failed to produce spores and could only be classified as *Ophiostoma* spp. *Ophiostoma piceae* was the most prevalent species isolated from tissue samples. This fungus was isolated from all four heights along the tree using each of the three methods. *Ophiostoma piceaperdum* was isolated from all heights of the tree using the PDA and cool temperature incubation methods. *Leptographium abietinum* was also isolated from all heights of the tree using each of the three methods. *Ophiostoma bicolor* was also isolated from all heights of the tree using the PDA and cool incubation methods. *Ophiostoma ips* was isolated from the lower and upper

stem tissue samples, using the PDA method. *Ophiostoma nigrocarpum* was isolated from the stump, lower, and upper stem samples, using the water agar method.

No ascomyceteous fungi were isolated from the 60 tissue samples of the 30 trees not colonized by bark beetles.

Sapstain on host tissue

The percentage of core samples with stain were 30% from the stump, 29% from the lower stem, 28% from the middle stem, and 28% from the upper stem. The presence of stain was consistently higher than the presence of fungi. For example, the percentages of fungi from the stump, lower, middle and upper stem were 21, 24, 14, and 17%, respectively (Table 7). No stain was observed in samples from trees not colonized by bark beetles (n = 60).

Discussion

The overwhelming majority of insects emerging from white spruce logs were scolytids, and most of these were *Dryocoetes affaber* and *Polygraphus rufipennis*. *Ips pini*, the third most abundant insect, is usually found in pine but has been reported in spruce (Thomas 1961; Wood 1982). The composition of phloeophagous insect fauna associated with spruce in the Great Lakes region is generally similar to that reported in western North America. *Dryocoetes affaber* and *Polygraphus rufipennis*, as well as *Ips tridentis* (Mannerheim) in Canada, *Ips perturbatus* (Eichoff) in Alaska, and *Ips* spp. in Colorado, are the most common associates of spruce trees infested with *Dendroctonus rufipennis*. Eleven of the 12 insect species collected in this study have been recorded previously in spruce in Canada, Alaska, and Colorado (McCambridge and Knight 1972; Werner and Holsten 1984; Gara et al. 1995; Bowers et al. 1996a, 1996b; Poland and Borden 1998a). To the best of our knowledge *Ips grandicollis* (Eichoff) (Bright 1976; Wood 1982; Drooz 1985) has not been recorded in *Picea* previous to this study.

Several new geographical records for bark beetles and new associations of fungi with bark beetles were obtained (Table 8). *Dendroctonus rufipennis* was collected in Wisconsin and in two counties in Michigan for the first time (Bright 1976; Wood 1982; Drooz 1985). *Crypturgus borealis* was collected in Minnesota, Wisconsin, and one county in Michigan for the first time (Bright 1976; Wood 1982; Drooz 1985). Ten new beetle–fungus associations were observed

Table 7. Percentage of tissue samples extracted from multiple heights of colonized spruce trees that yielded fungi, using three isolation

Fungus	Stump				Lower stem			
	PDA (n = 45)	Water agar (n = 18)	Cool incubation (n = 27)	Total (n = 90)	PDA (n = 70)	Water agar (n = 30)	Cool incubation (n = 50)	Total (n = 150)
<i>Leptographium abietinum</i>	0.00	0.00	3.70	1.11	1.43	3.33	0.00	1.33
<i>Ophiostoma bicolor</i>	0.00	0.00	7.41	2.22	1.43	0.00	0.00	0.67
<i>Ophiostoma piceaperdum</i>	4.44	0.00	3.70	3.33	2.85	0.00	0.00	1.33
<i>Ophiostoma ips</i>	0.00	0.00	0.00	0.00	1.43	0.00	0.00	0.67
<i>Ophiostoma piceae</i>	8.89	5.56	0.00	5.56	7.14	3.33	2.00	4.67
<i>Ophiostoma nigrocarpum</i>	0.00	5.56	0.00	1.11	0.00	3.33	0.00	0.67
<i>Ophiostoma</i> spp.	6.67	0.00	22.22	10.00	11.43	0.00	30.00	15.33
Total % samples with fungi	20.00	5.56	33.33	21.11	24.29	10.00	32.00	24.00

Table 8. (A) New geographical records for *Dendroctonus rufipennis* and *Crypturgus borealis* and (B) new associations of fungi with bark beetles.

(A) Bark beetle records.				
Species	Record	County, State	No. collected	Year
<i>Dendroctonus rufipennis</i>	State	Vilas, Wisconsin	10	1998
		Washburn, Wisconsin	2	1997
	County	Dickinson, Michigan	1	1998
		Houghton, Michigan	22	1998
<i>Crypturgus borealis</i>	State	Vilas, Wisconsin	2	1998
		Washburn, Wisconsin	972	1997
	State	Carlton, Minnesota	91	1998
	County	Houghton, Michigan	7	1998
		Dickinson, Michigan	14	1998
(B) Fungi isolated from bark beetles				
Fungal species		Beetle species		
<i>Leptographium abietinum</i> , <i>O. bicolor</i> , <i>O. ips</i> , <i>O. piceaperdum</i>		<i>Dryocoetes affaber</i>		
<i>Ophiostoma piceaperdum</i>		<i>Dryocoetes autographus</i>		
<i>Ophiostoma bicolor</i> , <i>O. piceaperdum</i>		<i>Ips pini</i>		
<i>Leptographium abietinum</i> , <i>O. bicolor</i> , <i>O. ips</i>		<i>Polygraphus rufipennis</i>		

among four species of fungi and four species of bark beetles. Although there were no previous records of fungi associated with *Dryocoetes autographus*, we isolated *Ophiostoma piceaperdum* from 80% of the adults assayed.

Overall, 37% of all bark beetles carried fungi, with the combined frequencies of associations ranging from 20 to 80% (Table 6). *Ophiostoma piceaperdum*, a common associate of *Dendroctonus rufipennis* (Whitney 1982; Safranyik et al. 1983), was the fungus most frequently isolated from bark beetles. Four of the five fungi isolated from beetles in this study have been isolated from *Dendroctonus rufipennis* in other regions (Whitney 1982; Safranyik et al. 1983). *Ophiostoma ips* was predominantly isolated from *I. pini* and was the only fungus that has not been reported previously on phloeophagous insects in spruce (Whitney 1982; Safranyik et al. 1983; Ohsawa et al. 2000). Each beetle species carried predominantly one fungal species, and the frequency of association ranged from 9 to 80% (Table 6). Using similar isolation techniques Klepzig et al. (1991) found that *O. ips* was the predominant fungus of *I. pini* and *I. grandicollis* (100% each) colonizing red pine in central Wisconsin, and Krokene and Solheim (1996) found that *Ceratocystis polonicum*

(Siemaszko) was the predominant fungus of *Ips typographus* (L.) and *Ips duplicatus* (Sahlberg) (94% each) colonizing spruce in Europe.

The fungal associations we observed were consistent with vector relationships. First, no fungi were recovered from trees that were not colonized by bark beetles. Second, four of the six fungal species were isolated from beetles as they colonized trees, beetles after they emerged from trees, and from tissue of the colonized trees. Third, there was a high degree of correspondence between isolation of various fungi from tissue of specific host sections and beetles that emerged from those sections (Table 9). Fungal species found at particular heights along the tree stem were frequently isolated from *Dryocoetes affaber* and *Polygraphus rufipennis* that emerged from the same section of the tree. Similar relationships have been reported between fungi associated with *Polygraphus rufipennis* and spruce tissue in Newfoundland (Bowers et al. 19966) and Alberta (Ohsawa et al. 2000), Canada.

Multiple methods of isolation and evaluation appear necessary to obtain the full diversity of fungi occurring in white spruce phloem. The PDA method yielded a high rate of fun-

techniques.

Middle stem				Upper stem				Total % presence (n = 420)
PDA (n = 45)	Water agar (n = 18)	Cool incubation (n = 27)	Total (n = 90)	PDA (n = 45)	Water agar (n = 18)	Cool incubation (n = 27)	Total (n = 90)	
4.44	0.00	0.00	2.22	4.44	0.00	0.00	2.22	1.67
2.22	0.00	0.00	1.11	2.22	0.00	0.00	1.11	1.19
6.67	0.00	0.00	3.33	11.11	0.00	0.00	5.56	3.10
0.00	0.00	0.00	0.00	2.22	0.00	0.00	1.11	0.24
6.67	0.00	0.00	3.33	0.00	5.56	0.00	1.11	3.81
0.00	0.00	0.00	0.00	0.00	5.56	0.00	1.11	0.71
2.22	0.00	18.52	6.67	2.22	0.00	18.52	6.67	10.48
17.78	0.00	18.52	14.44	17.78	11.11	18.52	17.77	19.76

Table 9. Association of fungi among bark beetle and host tissue in white spruce in the Great Lakes region.

Height along bole	<i>Ophiostoma europioides</i>		<i>Leptographium abietinum</i>		<i>Ophiostoma piceae</i>		<i>Ophiostoma bicolor</i>		<i>Ophiostoma ips</i>		<i>Ophiostoma nigrocarpum</i>	
	Beetle ^a	Tissue ^b	Beetle	Tissue	Beetle	Tissue	Beetle	Tissue	Beetle	Tissue	Beetle	Tissue
<i>Dryocoetes affaber</i>												
Stump	na	+	na	+	na	+	na	–	na	–	na	+
Lower stem	+	+	+	+	+	+	+	+	–	+	–	+
Middle stem	+	+	+	+	+	+	+	+	+	–	–	–
Upper stem	+	+	+	+	+	–	+	+	+	+	–	+
<i>Polygraphus rufipennis</i>												
Stump	na	+	na	+	na	+	na	–	na	–	na	+
Lower stem	+	+	+	+	–	+	+	+	+	+	–	+
Middle stem	+	+	–	+	–	+	–	+	–	–	–	–
Upper stem	–	+	+	+	–	–	–	+	–	+	–	+

Note: No fungi or beetles were found in standing trees without symptoms of bark beetle attack.

^aBeetles were reared from tree sections and fungal isolations were performed.

^bFungal isolations were performed on phloem and sapwood tissue.

gal recovery, ranging from 17 to 24%, and a high percentage of these samples were identifiable to species (89–98%). For example, *O. ips* was isolated only when the PDA method was used (Table 7). The water agar method yielded a low rate of recovery, 5–11%, but all samples were identifiable to species. The cool temperature incubation method yielded a high recovery rate of fungi, 18–33%; however, 18–30% of those samples were identifiable to genus only.

The colonization periods of bark beetles associated with white spruce in the Great Lakes region have not been previously recorded. Most colonization by *Dryocoetes affaber* occurred in July–August (Fig. 2), in contrast to June in Newfoundland (Bowers et al. 1996a), and July in Alaska and Colorado (Beckwith 1972; McCambridge and Knight 1972). Most colonization by *P. rufipennis* occurred in May–June, compared with June in Newfoundland and Maine (Hoskings and Knight 1975; Bowers et al. 1996a), and July in Alaska and Colorado (Beckwith 1972; McCambridge and Knight 1972). The peak colonization period of *I. pini*, July–August, was similar to that reported in red pine plantations in southern Wisconsin (Aukema et al. 2000) and pine and spruce stands in Ontario, Canada (Thomas 1961).

The absent, or weak, relationships between the number of emerging *Dryocoetes affaber* and *Polygraphus rufipennis* with stem diameter probably reflects their small size, in ad-

We currently lack sufficient information to explain why outbreaks of *Dendroctonus rufipennis* have historically been less pronounced in the Great Lakes region than in coastal Alaska, British Columbia, and the Rocky Mountains. A likely explanation is the more diverse forest composition, in contrast with the contiguous spruce forests of western North America. Other factors may include higher abundance of natural enemies (Haherkern 2001) than in western populations (Gara et al. 1995), behavioral differences associated with population density (Wallin 2001), differences in phloem moisture (Werner et al. 2002), and temperature (Logan and Powell 2001). Also, the three bark beetle species that accounted for over 90% of our total collection have been shown to be important competitors of *Dendroctonus rufipennis* in British Columbia (Poland and Borden 1998b), Colorado (McCambridge and Knight 1972), and Alaska (Gara et al. 1995). Additional research is needed to determine factors that prevent outbreaks of *Dendroctonus rufipennis* in the Great Lakes region.

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