Dissemination of Hymenomycetes by *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae)

T. C. Harrington, M. M. Furniss, and Charles Gardner Shaw

Former graduate research assistant, Department of Plant Pathology, Washington State University; principal entomologist, U.S. Forest Service, Intermountain Forest and Range Experiment Station, Moscow, ID; and professor, Department of Plant Pathology, Washington State University, Pullman 99164.
Present address of senior author: Department of Plant Pathology, University of California, Berkeley 94720.
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ABSTRACT

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Isolations of Hymenomycetes on semiselective media were attempted from preflight, in-flight, and postflight Douglas-fir beetles (DFB), *Dendroctonus pseudotsugae*. Eighty adult DFB either removed or allowed to emerge from Douglas-fir bolts (preflight) yielded no Hymenomycete. In contrast, 178 of 222 DFB collected in flight yielded at least one Hymenomycete: *Fomitopsis pinicola* from 169; *Cryptoporus volvatus* (often obscured by the faster growing *F. pinicola*) from five; and other Hymenomycetes (less important in sapwood decay) from 31. Basidiospores acquired during the flight period, rather than dikaryotic mycelial fragments, are hypothesized to have been the propagules disseminated, since most of these isolates were without clamps (monokaryotic). Of 122 postflight adults removed from egg-laying galleries, *F. pinicola* was isolated from five and *C. volvatus* from one. We suggest that many of the propagules were detached in moist phloem during tunneling. These DFB-vectored propagules may be significant in the enhanced sapwood decay noted in trees attacked by DFB.

Additional key words: bark beetles, dead tree deterioration, *Aleurodiscus lividocoeruleus*, *Coriolus versicolor*, *Phlebia subserialis*, *Oedocephalum* sp.

Catastrophic events such as fires and insect outbreaks often result in large quantities of dead timber. Dead trees are as desirable as green timber for many uses, including lumber, if salvaged quickly. However, sapwood of freshly killed Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and other conifers decays rapidly, especially if the trees have been attacked by bark beetles (5,22,25) or ambrosia beetles (20). Hymenomycetes destroy most of the sapwood beyond use within 3 yr after tree death (15,16,25), which is often before salvage operations can be completed. Portions of the bole heavily attacked by bark beetles may have twice the decay of lightly attacked portions (22).

The Douglas-fir beetle (DFB), *Dendroctonus pseudotsugae* Hopk., infests trees in dense mature stands and trees damaged by wind, fire, or defoliators (9). Adults fly in spring and bore into fresh inner bark (phloem) of Douglas fir where they excavate galleries and lay eggs. Larvae feed on phloem tissue until summer, transform to pupae, then overwinter as adults that emerge the following spring to infest new trees. Adults are believed to promote sapwood staining by vectoring blue stain fungi (Ascomycetes and Fungi Imperfecti) (16,25), but the role of DFB in decay acceleration remains uncertain. Holes bored by bark beetles may provide infection courts for decay fungi (15,16) otherwise unable to gain entry. Alternatively, adult DFB may hasten sapwood decay by carrying these Hymenomycetes into the egg galleries.

Castello et al (4) isolated *Fomitopsis* (*Fomes*) *pinicola* (Schwartz ex Fr.) Karst. and *Cryptoporus* (*Polyporus*) *volvatus* (Pk.) Shear from DFB trapped in flight. They proposed that the propagules disseminated were fragments of mycelium lodged in exoskeletal crevices. Introduction of fungal propagules into fresh trees by tunneling beetles could be an important means of dissemination of these sapwood rotters, especially *C. volvatus*, sporophores of which are associated with bark beetle galleries (3,4,12,13,15,16,23). We attempted to isolate fungi from DFB collected before, during, and after flight to examine further their role as vectors of wood decay fungi.

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MATERIALS AND METHODS

DFB were collected at three sites in northern Idaho (Latah and Clearwater counties). The Meadow Ridge site was described by Castello et al (4). The Jackson Mountain site is 10 km and the Slate Creek site is 37 km northwest of Meadow Ridge. They are located at ca 1,100 m elevation; the forest type is mixed with *P. menziesii* and *Abies grandis* (Dougl.) Lindl. predominating.

Beetle collection. Preflight DFB were removed aseptically from infested Douglas-fir stem sections (bolts) brought into the laboratory 1 yr after DFB attack. Other DFB emerged through the bark of bolts or slabs (bark and approximately 5 cm of sapwood) enclosed in a dark box containing sterile glass vials inserted into one side. Emerging adults were attracted by the room light and flew into the vials, which were subsequently removed and capped.

In-flight DFB were collected with the traps and procedures described by Castello et al (4). Other in-flight DFB were captured individually in sterile vials as they landed on screening around Douglas-fir trees baited with the aggregating pheromone, frontalin (10).

Postflight adults were removed in the field from freshly initiated egg galleries in Douglas-fir trees. Three trees were felled and cut into logs the previous fall. A fourth was a recently windthrown green tree. Beetles from all collections were either plated immediately or stored individually in sterile vials at 4 C until plated (within 24 hr).

Hymenomycete isolation. Two media were used. BSLM was a semiselective medium for Hymenomycetes, slightly modified from Castello et al (4). A mixture of 15 g malt extract, 2 g yeast extract, 15 g agar, 50 mg of 50% wettable powder (WP) benomyl, and 965 ml of water was autoclaved. After cooling, 100 mg of streptomycin-sulfate and 4 ml of 85% lactic acid were added. PBSLM consisted of the above formulation with 200 mg of 75% WP pentachloronitrobenzene (PCNB) added before autoclaving.

Each DFB was quartered (head, two pieces of thorax, and abdomen) after sex determination (14), and the four sections were placed in a 90-mm-diameter petri dish of solidified medium. Plates were incubated at 20–26 C for 6 wk and all suspected

Hymenomycetes were subcultured. Some DFB trapped in flight at Slate Creek were rinsed for 2 min in a solution containing 1% $HgCl_2$, 0.85% NaCl, and 0.1% Tween-80. These beetles were then rinsed for 1 min in sterile water prior to plating. Equal numbers were plated without rinsing.

Tentative identifications were based on cultural characters and physiological tests (18,19,21). Tentatively identified cultures were paired with known isolates from sporophores or previously identified isolates. Dikaryotization, as indicated by clamp formation by the previously clampless isolates in the presence of known cultures, confirmed their identity. Isolates with clamps were confirmed by dikaryotization with known tester isolates originating from single basidiospores.

RESULTS

Preflight beetles. No Hymenomycete was isolated from the 60 DFB emerging from the bolts or slabs enclosed in dark boxes. Twenty-five of these beetles emerged from naturally infested bolts collected in June 1978 at Meadow Ridge. The other 35 emerged from slabs cut in June 1979 at Slate Creek from two trees infested in 1978. One tree was baited with frontalin to induce infestation; the other was a green tree attacked after felling. Twenty additional DFB removed before emergence from bolts taken from the latter tree also failed to yield a Hymenomycete. *Sistotrema hirschii* (Donk) Donk was the only Hymenomycete found sporulating in 1-yr-old DFB galleries examined at ×20. Isolates from the hymenium of *S. hirschii* grew very slowly on malt extract agar.

In-flight beetles. In contrast to preflight beetles, many Hymenomycetes were isolated from in-flight DFB (Table 1). At Slate Creek, where beetles were collected every 3–6 days in 1978, 90% of the DFB plated yielded one or more cultures of *F. pinicola*. Other Hymenomycetes were isolated in fewer numbers. Of the 80 DFB that yielded at least one Hymenomycete, only 19 (24%) yielded cultures (all *F. pinicola*) that had hyphae with clamps. All the species isolated have clamp connections in the dikaryotic state (17,18,21). Male beetles predominated over females in all collections of in-flight DFB because they are more strongly attracted to frontalin (10). However, as in the previous study (4), no significant difference in frequency of Hymenomycete isolation was noted between sexes.

The 1979 trappings at Jackson Mountain, where DFB were collected every 1–10 days, gave results similar to the foregoing, but frequencies of isolation were lower for *F. pinicola*, *Oedocephalum* sp., *Phlebia* (*Corticium*) *subserialis* (Bourd, et Galz.) Donk and *Aleurodiscus lividocoeruleus* (Karst.) Lemke (Table 1). *Cryptoporus volvatus* was the only fungus whose frequency did not drop. Seven (23%) of the DFB with Hymenomycetes yielded *F. pinicola* cultures with clamps; the other 23 yielded clampless hymenomycetous isolates.

DFB individually captured in 1979 yielded more Hymenomycetes than did DFB trapped at the same site (Table 1). Of the 68 DFB that yielded one or more Hymenomycetes, only 12 (18%) yielded dikaryotic isolates; these were all *F. pinicola*.

Isolation frequencies from in-flight DFB rinsed with $HgCl_2$ were lower than from nonrinsed DFB collected in the same traps (Table

TABLE 1. Isolation frequencies of Hymenomycetes from in-flight Douglas-fir beetles (DFB) collected in northern Idaho at Slate Creek in 1978 and at Jackson Mountain in 1979

Collection method and year	Date	Number of beetles sampled	Number of beetles yielding Hymenomycetes	*Fomitopsis pinicola*	*Cryptoporus volvatus*	*Oedocephalum* sp.	*Phlebia subserialis*	*Aleurodiscus lividocoeruleus*	Unidentified Hymenomycetes
Trapped 1978	8 June	34	32	30	1	1	3	0	2
	14 June	23	21	21	0	2	1	0	1
	24 June	17	17	16	0	2	1	1	1
	28 June	10	10	9	1	1	2	1	0
	Total	84	80**[a]	76**	2	6	7*	2	4
Captured 1979	2 June	11	9	9	0	0	0	0	1
	3 June	39	38	37	0	2	0	0	2
	27 June	25	21	19	1	0	1	0	1
	Total	75	68**	65**	1	2	1	0	4
Trapped 1979	3 June	2	2	2	0	0	0	0	0
	13 June	20	14	13	1	0	0	0	2
	22 June	13	1	0	1	0	0	0	0
	27 June	28	13	13	0	0	0	1	2
	Total	63	30	28	2	0	0	1	4

[a] Totals for fungus species significantly different by chi-square analysis from totals for the same species from DFB trapped in 1979 are followed by: *, $P = 0.05$; or **, $P = 0.01$.

TABLE 2. Isolation frequencies of Hymenomycetes from $HgCl_2$-rinsed and nonrinsed in-flight Douglas-fir beetles (DFB) trapped at Slate Creek, ID, in 1978

Trapping date	Treatment	Number of beetles sampled	Number of beetles yielding Hymenomycetes	*Fomitopsis pinicola*	*Cryptoporus volvatus*	*Oedocephalum* sp.	*Phlebia subserialis*	*Aleurodiscus lividocoeruleus*	*Coriolus versicolor*	Unidentified Hymenomycetes
June 8	Nonrinsed	34	32	30	1	1	3	0	0	2
	Rinsed	34	16	12	3	1	0	0	3	0
June 24	Nonrinsed	17	17	16	0	2	1	1	0	1
	Rinsed	17	10	10	1	0	0	0	1	0
June 28	Nonrinsed	10	10	9	1	1	2	1	0	0
	Rinsed	10	3	2	0	0	0	0	1	0
Total	Nonrinsed	61	59	55	2	4	6	2	0	3
	Rinsed	61	29**[a]	24**	4	1	0*	1	5	0

[a] Totals for fungus species from $HgCl_2$-rinsed DFB significantly different by chi-square analysis from totals for the same species from nonrinsed beetles are followed by: *, $P = 0.05$; or **, $P = 0.01$.

2). Isolations of the faster-growing (on BSLM and PBSLM) Hymenomycetes were reduced. Two slower-growing fungi, *C. volvatus* and *Coriolus versicolor* (L. ex Fr.) Quél., were less obscured by *F. pinicola* colonies when isolated from rinsed beetles and were isolated more frequently (Table 2).

Postflight beetles. Adults removed in June from newly initiated egg galleries (avg length 17 cm) at Jackson Mt. (Table 3) yielded fewer Hymenomycetes (six clampless isolates) than did in-flight DFB. Whereas 87% of the DFB captured in flight yielded *F. pinicola* (Table 1), only 4% of the postflight DFB, collected just 100 m away during the same month, yielded that fungus (Table 3).

BSLM and PBSLM were equally effective for isolating Hymenomycetes from DFB. In paired isolations of 61 in-flight DFB on each medium, no significant difference in isolation frequency of any Hymenomycete was noted; therefore, data obtained from both media (used in about the same frequency) were combined.

TABLE 3. Isolation frequencies of Hymenomycetes from postflight Douglas-fir beetles removed from fresh egg galleries within Douglas-fir logs at Jackson Mountain, ID, in 1979

Tree	Removal date	Number of beetles sampled	Number of beetles yielding Hymenomycetes	*Fomitopsis pinicola*	*Cryptoporus volvatus*
A	2 June	50	1	1	0
B	3 June	17	2	1	1
C	3 June	9	0	0	0
D	13 June	46	3	3	0
	Males	35	2	2	0
	Females	87	4	3	1
	Total	122	6	5	1

DISCUSSION

Hymenomycetes, particularly *Fomitopsis pinicola*, were frequently isolated from in-flight, but not from preflight, DFB. These isolates were predominately monokaryotic as indicated by absence of clamps. After a period of tunneling in fresh trees, the beetles yielded fewer Hymenomycetes than did preattack beetles. Rinsing in-flight beetles in $HgCl_2$ also reduced isolation frequencies. Several interpretations of these data are possible.

Perhaps these fungi are present on or in the beetles at emergence. The hypothesis of acquisition of mycelial fragments (4) would require mechanisms that prevent isolation of Hymenomycetes from preflight DFB, and induce monokaryotization of dikaryotic propagules (17,21). This hypothesis also must account for the isolation of fungi uncommon on Douglas fir (eg, *Phlebia subserialis*) and for failure to isolate the common sapwood rotter, *Hirschioporus* (*Polyporus*) *abietinus* (Dicks. ex Fr.) Donk (15,16,20,22,25). Basidiomycetes have been found in the mycangia of some bark beetles (11,24) and can reproduce in these structures (1). However, mycangia have not been found in DFB (8), and no mechanism is known that would prevent isolation of Hymenomycetes from emerging beetles. Furthermore, fungal reproduction within a mycangium does not explain the reduction in successful isolations from tunneling or rinsed beetles.

Our data are more consistent with the simpler hypothesis that bark beetles acquire monokaryotic basidiospores at some time after emergence. Castello (3), using SEM, noted the presence of basidiospores on the undersurfaces of wing covers of DFB trapped in flight. Forest atmospheres contain high concentrations of such spores (6). *Cryptoporus volvatus* has been shown to release large quantities of airborne basidiospores during DFB flight periods (12), and *F. pinicola*, like other fungi with large basidiocarps (13), would also be expected to do so. Interestingly, fresh basidiocarps of *H. abietinus* were not seen in the study area until late August 1979, after appreciable rainfall and after DFB flights had terminated.

The hypothesis of postemergence acquisition of basidiospores accounts for the failure to isolate Hymenomycetes from preflight beetles, the inability to isolate *H. abietinus* from in-flight beetles, and the monokaryotic condition of most isolates. Those few isolates with clamps could have resulted from fusions of compatible mycelia developing from two or more basidiospores. Because the inoculum would be superficial, dislodgment during tunneling would be expected. Rinsing with $HgCl_2$ would also reduce the number of viable propagules.

How DFB surfaces become contaminated is not known. Bark beetles are not known to visit basidiocarps. They may acquire spores from bark or other surfaces. Alternatively, they may passively acquire airborne spores while in flight or at rest.

The enhanced decay of freshly killed Douglas fir heavily attacked by DFB has been documented (5,22,25). Similar decay acceleration has been correlated with attacks by ambrosia beetles on Douglas fir (20) and with other bark beetles on other conifers (2,7). Increased aeration around beetle holes may help explain decay enhancement (5,20), but it does not explain the increased foci on infection (7,22). Von Schrenk (23) was first to consider bark beetle holes as major infection courts for *F. pinicola* and *C. volvatus*. Others have reiturated that beetle holes are important avenues of infection (2,7,15,16). Our evidence indicates that most DFB acquire Hymenomycete propagules during the postemergence period, and are likely to be vectors of these wood-decay fungi. However, the relative importance of these beetle-vectored propagules in initiating sapwood decay remains to be determined.

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