

Isolation of *Cryptoporus volvatus* and *Fomes pinicola* from *Dendroctonus pseudotsugae*

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ABSTRACT

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Cryptoporus volvatus and *Fomes pinicola* were isolated from Douglas-fir beetles (*Dendroctonus pseudotsugae*) trapped in flight (24% and 37%, respectively). *Dendroctonus pseudotsugae* is not an inhabitant of sporophores; consequently, mycelial fragments are considered to be the propagules disseminated. *Fomes pinicola* also was isolated from beetles removed from galleries in *Pseudotsuga menziesii*. Upon entering the tree after flight, the beetles

probably dislodge mycelial fragments during their tunneling activities. Difco malt agar (4.5%) containing benomyl at 30 µg/ml, streptomycin-sulfate at 100 µg/ml, and 85% lactic acid at 4,000 µg/ml provided a selective medium for isolation of *C. volvatus* and *F. pinicola*. Results indicate that *D. pseudotsugae* is a major vector of *C. volvatus* on *P. menziesii* in northern Idaho, and also that it is an effective vector of *F. pinicola*.

Additional key words: sap rot, heart rot, wood decay, bark beetles, fungal dissemination, *Polyporus volvatus*, *Fomitopsis pinicola*.

Sporophores of *Cryptoporus volvatus* (Pk.) Hubbard (6) (= *Polyporus volvatus* Pk.) consistently are associated with sites of attack by subcortical insects; e.g., *Dendroctonus pseudotsugae* Hopk. on Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], *Scolytus ventralis* Le Conte on Grand fir [*Abies grandis* (Dougl.) Lindl.], and *Ips pini* (Say) on ponderosa pine [*Pinus ponderosa* Laws. (3, 10)]. Sporophores occur over much of the bole of infected trees only 1-3 years after their death, which supports the hypothesis of simultaneous entry of the fungus at many points. Although the fungus and decay that it causes have been known for about 100 years, no conclusive information regarding its dissemination heretofore has been available. Occurrence of sporophores of *C. volvatus* at holes created by bark beetles suggested that the fungus might be disseminated by them. Consequently, we investigated the possible role of one beetle, *D. pseudotsugae*, as a vector of *C. volvatus*.

MATERIALS AND METHODS

On 4 June 1974, we set up a trap (Fig. 1) 9.6 km southwest of Elk River, Clearwater County, Idaho. Two open vials containing 300 mg of the Douglas-fir beetle aggregating pheromone, frontalin, and alphapinene (5), respectively, were suspended within the trap.

On 13 June 200 Douglas-fir beetles were removed from the trap; and 200 adult beetles that had flown for the first time that spring were removed from a nearby, recently felled Douglas-fir. The beetles were placed in sterile glass vials, which then were placed in a chest filled with ice to minimize their activity and prevent dislodging of any fungal propagules present.

On 18 June 1974, whole beetles were plated on a medium containing 45 g Difco malt agar, 60 mg Benlate benomyl fungicide (50% WP), 10 mg streptomycin-sulfate, and 2 ml of 85% lactic acid per liter. Lactic acid and streptomycin-sulfate were added after the other ingredients had been autoclaved and cooled to 45-50 C.

On 5 May 1975, three pheromone-baited traps were set up approximately 100 m apart in the same study area. The traps were located in an uncut, mature stand adjacent to a clear-cut area. The tree species near the traps were mainly Douglas-fir and Grand fir. Some recently killed Grand fir bore sporophores of *C. volvatus*.

Before assembly, traps were washed and scrubbed with ethanol to reduce contamination. The traps were suspended between trees and 3 m above the ground. Crumpled tissue paper was placed in the lower compartment of each trap to reduce contact between beetles.

Beetles were removed from traps every 7 to 10 days from 15 May to 23 July 1975. The number of beetles trapped on 23 July was negligible; therefore, beetle flights were considered to have terminated by that date. The

beetles were placed in sterile vials and brought to the laboratory where they were sexed, fragmented, plated, and incubated for 10 days.

The medium described above was used for beetles trapped through 30 May 1975. Beetles trapped subsequently were plated on media to which 4 ml (instead of 2 ml) of 85% lactic acid had been added.

RESULTS AND DISCUSSION

A fungus with white, floccose mycelium consisting of hyphae bearing clamps was isolated from 35.5% of the beetles collected in the trap in 1974. The cultures reacted negatively to Noble's gum guaiac solution and produced no stain when plated on tannic acid agar (4, 7). This fungus was identified as *Fomes pinicola* (Schwartz ex Fr.) Cke. [\equiv *Fomitopsis pinicola* (Schwartz ex Fr.) Karst.]. All plates also contained white bacterial colonies, yeast colonies, and many other fungi. *Alternaria* sp. was isolated from 86% of the beetles. Cultures of some 500 nonbasidiomycetous isolates were retained for future study and identification. Despite the addition of benomyl, streptomycin, and lactic acid, the growth of

other fungi and bacteria was profuse; thus, isolation of basidiomycetes was difficult.

A basidiomycete with the same characteristics as that isolated from the trapped beetles also was isolated from 76% of the beetles removed from galleries. *Alternaria* sp. developed from 28.5% of these beetles; colonies of bacteria, yeast(s), and other fungi developed from all beetles.

A second fungus with whitish, appressed mycelium bearing clamps developed sparsely among the contaminants and *F. pinicola*. Initial attempts to isolate this basidiomycete were not consistently successful; often it was overgrown by other fungi. Isolation was facilitated by use of the modified medium. White yeasts were still present, but their rates of growth were so reduced that both basidiomycetes could outgrow them. The second basidiomycete produced white- to cream-colored mycelium which was water-soaked in appearance and appressed to the agar surface. The hyphae were much branched and bore abundant, intermittent swellings. Fresh cultures gave a positive reaction for phenyl oxidase with gum guaiac and a slight stain on the reverse side of the culture when plated on tannic acid (4). These are the cultural characters of *C. volvatus* (8).

In 1974, *F. pinicola* was isolated more than twice as frequently (76%) from beetles excavated from galleries as from beetles trapped in flight (35.5%). Conversely, nonbasidiomycetes were obtained less frequently from beetles removed from logs than from those trapped in flight. Upon emergence, beetles would be subjected to surface contamination prior to being trapped. Tunneling through and underneath the bark apparently removes microorganisms present on the exoskeleton which interfere with isolation of basidiomycetes. However, the propagules of *F. pinicola* and *C. volvatus*, if worked into intersegmental areas prior to emergence, presumably would not be dislodged easily. Thus, we surmise that the increased frequency of isolation of *F. pinicola* from beetles removed from logs versus those trapped in flight is inversely correlated with the total number of other organisms isolated on the 2% lactic acid medium.

The seasonal history of the Douglas-fir beetle has been determined in the northern Rocky Mountains by the junior author. One generation with two broods occurs per year; the more abundant brood overwinters as new adults whereas the less abundant one overwinters as larvae.

Flight and attack by overwintered adult beetles begin in early May at elevations comparable to the study area. Attacks reach peak intensity during the latter part of May or early June, depending upon seasonal weather. Broods produced from these spring attacks transform to the adult stage by late August but do not emerge and fly until the following spring, at which time they infest new hosts.

Broods that overwinter as larvae usually constitute a small fraction (generally less than 5%) of the beetle population. They mature and fly to attack new hosts in late June and early July. Also attacking new hosts at that time are some reemerged adults that had attacked in the spring. The combined summer attacks generally are less intense than the spring attacks, and they involve different trees. Eggs that are laid late in the season overwinter as larvae, thus perpetuating the one-generation and two-brood cycle.

During the spring and summer of 1975, 3,148 Douglas-

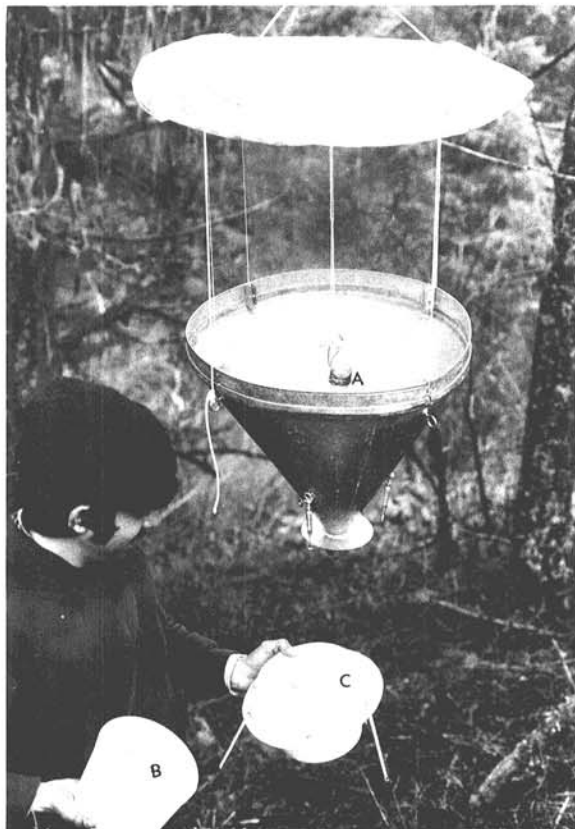


Fig. 1-(A to C). Trap used to collect Douglas-fir beetles in flight. A) Plastic deflectors, container with pheromone, and funnel. B-C) Compartment components. Component B is inverted from its assembled position. Beetles fall through B into C through a screen (visible as a square on top of C). Larger insects, including most predators, remain in B.

fir beetles were trapped in flight and plated (Table 1). About 2,300 were first-flight beetles. The first flight apparently terminated between 26 June and 3 July when only 36 beetles were trapped. The peak of the second flight (602 beetles) occurred between 3 July and 7 July (Table 1). *Cryptoporus volvatus* and *F. pinicola* were isolated from both first- and second-flight beetles (Table 1).

Fomes pinicola and *C. volvatus* both were isolated in higher percentages from beetles plated after the amount of lactic acid (85%) was increased from 2% to 4% (Table 1). After this change, both fungi were often isolated from the same beetle and sometimes from the same beetle segment. *Fomes pinicola* still grew faster, and overran colonies of *C. volvatus* growing from the same or a nearby segment. To some extent this accounts for the higher isolation frequencies of *F. pinicola* from most collections of beetles even after the change in the medium (Table 1).

The frequencies of isolation of these fungi were not correlated with sex; both were isolated with similar frequencies from male and female beetles. Both fungi were isolated with greater frequency from wing covers and legs than from head, thorax, or abdominal segments.

The results of this study demonstrate the role of *D. pseudotsugae* in dissemination of *C. volvatus* and *F. pinicola* on Douglas-fir in northern Idaho. The demonstration that two important wood decay fungi, one that causes a sap rot and the other a heart rot (8), are carried by *D. pseudotsugae* raises the possibility that other scolytids, buprestids, cerambycids, and other subcortical insects also may be vectors of wood-decay fungi.

What remains to be clarified is the nature of the propagules and the mechanism of their dissemination. Examinations of hundreds of fresh sporophores of *C. volvatus* over many years has not revealed a single specimen of *D. pseudotsugae* therein. The pores of *C. volvatus* are covered by a volva. Most of its basidiospores are retained within the volva as a caked layer; thus, wind apparently is not a major means of dissemination. We hypothesize that, during tunneling in tissue colonized by

C. volvatus and *F. pinicola*, fragments of mycelium are forced into the intersegmental areas of the beetles. These fragments are retained during flight (Table 1); and some are released during postflight tunneling in trees and logs not previously attacked by beetles. Even after beetles had tunneled for several inches, however, *F. pinicola* could still be isolated from them. Thus, those that survive to participate in the second flight still carry these fungi (Table 1, data for 9-23 July). With the improved medium now in use, we believe the presence of *C. volvatus* on beetles removed from logs also could be demonstrated.

Some of the bark beetles attacking other conifers may also be vectors of *C. volvatus* and *F. pinicola*. Waldron's (10) investigations of *C. volvatus* implicated the pine engraver (*I. pini*) in the dissemination of this fungus on ponderosa pine (*Pinus ponderosa*).

Cryptoporus volvatus is found in association with the galleries and larval mines of *D. pseudotsugae* on *P. menziesii*. Sporophores emerge from holes bored through the bark by beetles. Borden and McClaren (1, 2) point to *Temnochila chlorodia* (Mannerheim), a predator of *D. pseudotsugae* and other bark beetles, as a possible insect vector of *C. volvatus* in British Columbia and suggest that basidiospores are the propagules disseminated by it. Populations of *T. chlorodia* are much smaller than those of *D. pseudotsugae*; no *T. chlorodia* were caught in our traps. In examining some 200 sporophores of *C. volvatus* in the past 4 years, we have found three adult *T. chlorodia* therein. Furthermore, the predator does not enter the tunnels made by *D. pseudotsugae*; females merely oviposit their eggs within the entry hole. On the basis of the evidence presented here, *D. pseudotsugae* very likely is a more common vector on Douglas-fir.

Bark beetles probably are not the principal vectors of *F. pinicola*, which produces wind-disseminated basidiospores. Wounds and dead branch stubs are considered the usual portals of entry for this fungus (9). Although bark beetles may play a lesser role in its dissemination, spread by bark beetles provides immediate and direct introduction of *F. pinicola* into infection courts inside the main bole of dead and dying trees.

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TABLE 1. Isolations of *Fomes pinicola* and *Cryptoporus volvatus* from flown *Dendroctonus pseudotsugae*

Date (1975)	Beetles plated (no.)	Percentage of beetles yielding	
		<i>F. pinicola</i>	<i>C. volvatus</i>
15-26 May ^a	139	12.9	5.6
30 May	207	1.0	7.2
6 June ^b	969 ^c	30.3	19.8
13 June	640	47.0	43.3
17 June	153	40.5	62.7
26 June	173	26.6	21.4
3 July	36	41.7	44.4
9 July	602	56.3	13.3
16 July	189	41.3	19.6
23 July	40	50.0	20.0
Totals	3148	37.2	24.3

^aCollections on three dates combined.

^bBeetles collected on this date and subsequently plated on selective medium (described in text) containing 4 ml of 85% lactic acid per liter instead of 2 ml/liter.

^cIncluding 787 males.

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