Status in 2002 of Experimental Fungal Inoculations to Promote Establishment of Cavity Nests and Wildlife Habitat in Selected Trees in Managed Forests

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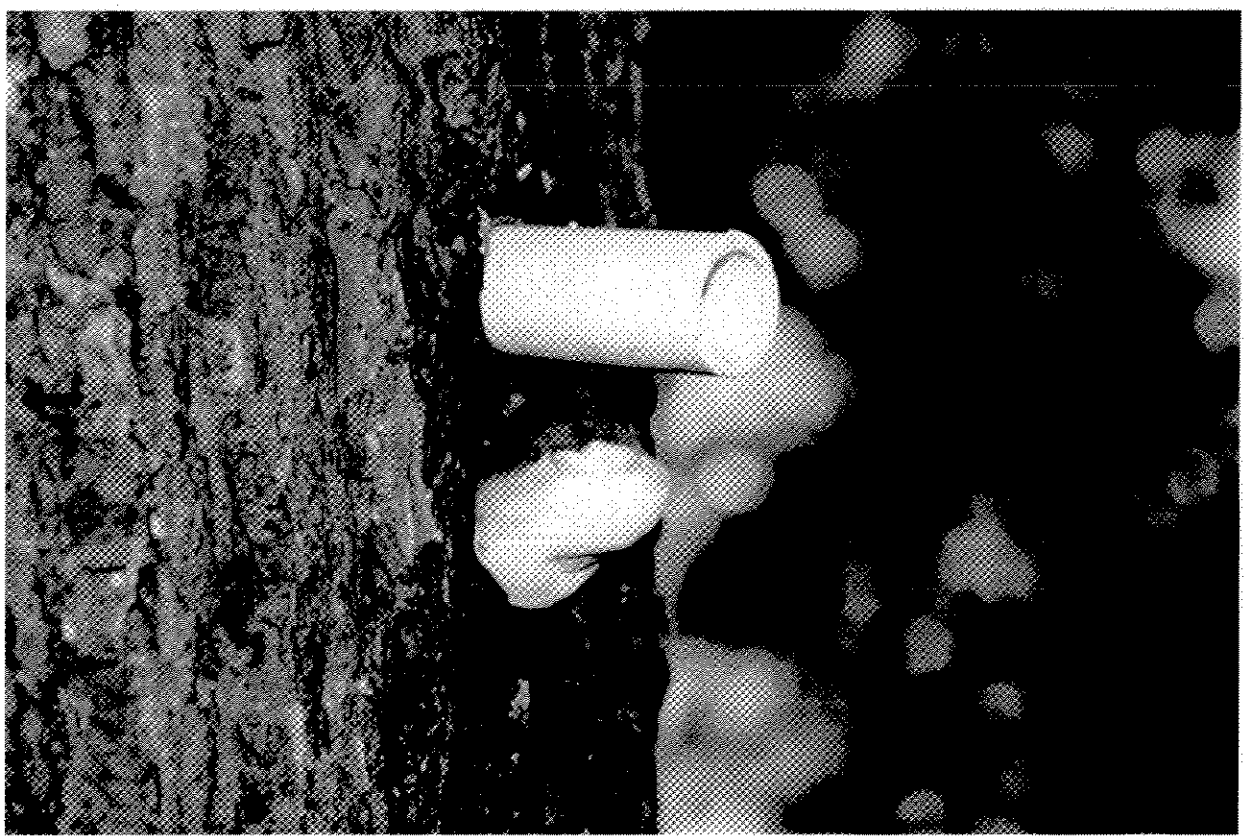

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ABSTRACT

Managed forests contain fewer trees suitable for use by cavity-using wildlife than are generally found in old-growth forest. Because of short timber-harvest rotations, relatively few trees in industrial forests are infected with the heart-wood decaying fungi that soften wood, enabling primary cavity-nesters (e.g., *Piciodes* woodpecker spp.) to excavate cavities. A lack of woodpecker activity and resulting deficiency of available nest and roost cavities limits the diversity and abundance of many wildlife species. We have implemented and are presently evaluating an innovative management approach wherein a wood-decaying fungus, the red-belted conk (*Fomitopsis pinicola*), is introduced into selected trees to enhance the suitability of managed forests for woodpeckers. In 2002, we revisited 650 trees that were inoculated experimentally in 1997 and 1998, and inspected each tree for the presence of fungal growth and signs of woodpecker activity. We also took wood samples from 20 control and 21 treated trees to test for fungal growth.

Of the 650 trees originally inoculated with fungus ($N = 330$) or a sterile control ($N = 320$), 50 trees at three different sites were harvested. We were able to locate and visually inspect all but one of the remaining 600 trees. We re-marked the remaining inoculated and control trees, and made arrangements with all landowners to protect these from future harvest. Of the remaining 300 treatment trees examined, three (1%) had been blown down by wind, one (0.3%) was standing dead, and one (0.3%) had its top broken off above the inoculation point. Of the 299 remaining control trees inspected, six (2%) were broken above the inoculation point, two (6%) had been topped above the inoculation point, two (0.6%) were standing dead, and 10 (3%) had been blown down.

A significantly higher proportion of treatment trees displayed *F. pinicola* conks (0.173) and mycelia (0.063) than did control trees (0 conks, 0 mycelia, $p < 0.001$). We also found that western hemlock (*Tsuga heterophylla*) trees had a higher incidence of fungal growth as compared to Douglas-fir trees (*Pseudotsuga menziesii*). Western hemlocks had a higher proportion of conks (0.312) and mycelia (0.106, $N = 160$) than was observed on Douglas-fir trees (0.014, $p < 0.001$ and 0.014, $p < 0.001$, respectively; $N = 140$). We did not observe any significant difference in the proportion of trees with fungal growth in comparisons between larger-diameter trees (>37 cm DBH; 0.256) and smaller-diameter trees (<37 cm DBH, 0.193). During this inspection, we did not observe any evidence of woodpecker excavations associated with the fungal inoculations. This finding was expected, as woodpecker use may begin between 6-10 years after inoculation.

Thus far, the fungal inoculations completed in 1997 and 1998 appear to be successful. Our findings indicate that the red-belted conk has been introduced successfully into at least 23% of trees in the treatment clusters. Also it is clearly evident that in addition to *Fomitopsis pinicola*, that an ensemble of wood-decay organisms has worked their way into the wounds created by the inoculation of trees in the Control and Treatment Groups of this extensive field experiment. Decay columns have been established, although the quality of the two methods (i.e., with or without introduction of *F. pinicola*) does not appear to be the same based on our current evaluation.

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INTRODUCTION

Intensive forest management often includes the removal of dead and dying trees that provide nesting and foraging substrate for a number of different wildlife species (Brandeis et al. 2002). Trees of the highest value for wildlife are those with existing cavities or those that are suitable for cavity excavation. A deficiency of suitable cavity trees in the managed forest landscape can result in a reduction of the number of cavity-nesting and cavity-roosting birds and mammals (Rosenburg and Anthony 1993, Conner et al. 1994, Henjum et al. 1994). This potential loss of cavity-using species represents a deterioration of a functioning forest ecosystem and could result in declining production of timber products over the long term. For example, tree stands may be more susceptible to insect damage if numbers of insectivorous birds are reduced due to a lack of nest and roost cavities (Otvos 1979).

Most woodpeckers and secondary cavity-nesters have generally been associated with old-growth forest (e.g., Mannan et al. 1980, Lundquist 1988). Many researchers have suggested that the resident woodpeckers of the Pacific Northwest require old-growth forest for their nesting and foraging activities (Jackman 1975, Nelson 1988). Over the course of previous research on the Olympic Peninsula, however, we have determined that hairy woodpeckers (*Picoides villosus*) and northern flickers (*Colaptes auratus*) seem to have adapted well to the changing landscape of industrial timberlands. Moreover, our research has documented that the hairy woodpecker is the most common primary cavity-nester in western Washington forests, and that this bird requires dead and dying trees for both nesting and foraging (Huss et al. 1999, Ripper et al. 2002). The nest trees of this species tend to be associated with wood-decaying fungi (Huss et al. 1999). Therefore, providing decaying substrate within the managed forest habitat may be necessary to maintain woodpecker populations and the wildlife communities that depend on woodpeckers.

Our ongoing research study implements and evaluates an innovative technique of introducing a wood-decaying fungus (i.e., *Fomitopsis pinicola*) into selected trees to foster occupancy by woodpeckers (e.g., *Picoides* spp.) and secondary cavity nesters (Huss et al. 1999). This technique could promote the colonization and use of relatively young, managed forests by woodpeckers and other wildlife typically associated with older forest stands. We propose that this proactive management approach could provide for the conservation of a diverse wildlife community in concert with the commercial production of timber.

In 1996 and 1997, we established that the fungus, known as the red-belted conk (*Fomitopsis pinicola*), was present in at least at 55.1% of 78 occupied woodpecker nest trees sampled for fungi (Huss et al. 2002). We isolated and cultured fungi taken directly from nest trees and used it to impregnate wooden dowels for experimental fungal inoculations. In 1997 and 1998, we inoculated 650 trees with either *F. pinicola* (treatment, $N = 330$ trees) or sterile wooden dowels (control, $N = 320$).

Experimental inoculations consisted of inserting the wooden dowel into a drilled hole approximately 8 m above the ground into the north side of trees (Huss et al. 1999). For the inoculation experiment, we developed a sampling design that would allow us to test the effects of: (1) tree species, (2) the abundance of available snags, and (3) size/age classes of trees on the resulting use of inoculated trees by woodpeckers (Huss et al. 1999). As this experiment was organized in a hierarchical fashion, we hope to examine these effects independently and the interactions of these factors. Evaluating the efficacy of this management technique to create

trees that will be used by woodpeckers is the long-term objective of this study; this assessment may take 10 to 20 years.

This report provides the results of a comprehensive inspection conducted in June 2002 of all sites inoculated in 1997 and 1998. Wood cores retrieved from near the initial point of inoculation of a sample of "Control" and "Treatment" trees were returned to Arkansas State University at Jonesboro for primary isolation of fungi (during 2002 and 2003) that could be grown on culture medium and identification of the fungi found. Specifically, our objectives for the 2002 field and laboratory research were as follows:

Objectives

1) Revisit all inoculation sites and visually-inspect each individual tree. Record the condition of the tree and the presence of any fungal growth in the form of conks (i.e., reproductive structures, also called fruiting bodies or basidiocarps) or filamentous vegetative (i.e., hyphae or mycelium) growth.
2) Determine whether woodpeckers are using inoculation trees as evidenced by nesting and foraging excavations or presence of birds.
3) Obtain wood samples of two control trees and two treatment trees at each of 10 randomly-selected sites to determine through laboratory analyses if *Fomitopsis pinicola* is present.
4) Increase the awareness of involved timber producers and landowners regarding the location, status, and protection of inoculated sites.

STUDY AREA AND METHODS

Inoculation Sites

In 1997 and 1998, we completed inoculation of 650 trees with the fungus known as the red-belted conk, fulfilling our original proposed experimental design (Bednarz et al. 1997). A total of 24 stands were inoculated in 1997. Fifteen of these stands were on Rayonier lands and nine were on the Washington Department of Natural Resources (DNR) lands. The Rayonier sites included 12 western hemlock and 3 Douglas-fir stands; DNR sites were comprised of 6 western hemlock and 3 Douglas-fir stands. Fifteen stands (10 Rayonier, 5 DNR) were located in Clallam County, seven (3 Rayonier, 4 DNR) were in Jefferson County, and two were in Grays Harbor County (2 Rayonier).

In 1998, we inoculated trees in 10 Douglas-fir forest stands. The Campbell Group now owns four of these stands, in Pierce County, and four are located on Weyerhaeuser Company land in Lewis County. Two Grays Harbor sites formerly owned by the John Hancock Timber Resources Group were sold after treatment. One of these was purchased by The Fruit Growers (98-09) and the other (98-08) was purchased by Weyerhaeuser Company (Appendices A and B). For comprehensive maps of inoculation sites refer to Figures 1 and 5 in Huss et al. 1999, Figure 1 in Huss et al. 2002. Locations of inoculation and control sites are included in Appendix B.

Also, a pilot study was conducted on three stands owned by Rayonier in Grays Harbor County, Washington approximately 3 mi (4.8 km) west of the town of Humptulips. Trees inoculated in these stands were substantially younger (16-23 yr) than those in the main study (most inoculated stands were at least 50 yr; Huss et al. 1999) and were inoculated at about 1.5 m above the ground (instead of at about 8 m). There were no control treatments at the ground-based sites.

All study areas were comprised of state or private lands managed primarily for timber production. Most inoculation sites ($N = 23$, 67.8%) were located in Riparian Management Zones (RMZ) to protect experimental trees against loss from harvest. Forest habitats surrounding experimental stands consisted of a mosaic of different even-aged forest stands ranging from recently clear cut to more than 100 yr old. Habitats adjacent to the inoculation sites generally consisted of small forest stands (5-100 acres [2-40 ha]), often bordered by areas which had been clear cut within the last 5 yr.

2002 Inspection of Inoculation Trees

We inspected all trees in the 34 stands inoculated in 1997 and 1998, and verified location records and written directions to all sites. Inspections were made from the ground using binoculars. We carefully inspected the entire tree, with special attention given to the area within 1 m of the PVC tube. We recorded the presence of any fungal conks growing within the PVC tube or within 1 m of the inoculation site. We also inspected the inside of the PVC tube for the presence of fungal mycelia from the ground using binoculars. Any fungal growth on the tree was noted; when a conk was present and clearly associated with the inoculation (i.e., was located within 1 m of the PVC tube), we estimated the conk's length, width, and depth. Any woodpecker excavations on the tree were noted; only excavations that were associated with the fungal inoculation were considered to be a response to the inoculation.

We recorded whether the metal identification tag was still present on each tree and replaced any missing tags. The presence of the PVC tube was also noted; when a PVC tube was missing from a tree, in almost all cases we were able to locate the wound on the tree where the inoculation took place. All of the aforementioned observations were recorded for standing trees, trees broken above the inoculation point, and those trees that had blown down. Trees that had been broken or topped below the inoculation point were excluded from further analysis. Each tree was re-marked with tree-marking paint; we used orange for treatment trees and yellow for controls.

We randomly selected 10 treatment and 10 control clusters. From these clusters, we collected wood samples using an increment borer (one sample per tree) from two randomly-selected trees to determine if the desired fungus was established. Core samples were taken at a point 2 – 5 cm below the PVC tube (or associated wound if the PVC tube was no longer present).

After shipment of wood samples to Arkansas State University, these samples were packaged in sterile bags and stored in the refrigerator until processed. Samples were processed using standard lab techniques and vegetative compatibility analyses to confirm whether *Fomitopsis pinicola* used in the original inoculation was present and viable in experimental trees and what other fungi might be present (see Huss et al. 1999 and Huss et al. 2002 for description of laboratory procedures). Isolation of fungi from wood samples ($N = 21$ from Control Trees; N

= 20 from Treatment Trees) on the primary isolation medium (Huss et al. 2002) was attempted on three separate occasions (18 October 2002 with 12 isolation attempts per sample; 10 December 2002 with 9 isolation attempts per sample; 21 June 2003 with 12 isolation attempts per wood sample). Isolation attempts were attempted multiple times to verify repeatability of results and increase the probability of isolating fungi of interest to this study; in all, 33 attempts were made for each of the 41 wood samples). Visual observations were made of any colonies that grew and some of this material transferred to Malt Extract Agar (MEA) to grow and maintain the separate fungal colonies. Tease mount slides with fungal material was suspended in lactophenol with cotton blue (Larone 1995), these examined under the microscope, and voucher slides numbered and stored for future reference at Arkansas State University (Fungal Culture and Specimen Curator: Martin Huss). All laboratory procedures were conducted as in previous studies (Huss et al. 1999, 2002). However, yeast were only identified, when possible to genus, based on morphological characteristics (e.g., texture and color) of colonies growing on Malt Extract Agar or microscopic characteristics (e.g., presence or absence of cellular capsule when cells suspended in India ink, cell shape, size) as seen in preparations under magnification with a light microscope. No physiological assays were run on yeast cultures to aid in identification (i.e., Analytical Profile Index system for diagnosis of yeasts; diagnostic kits available from bioMérieux Viteck, Inc., Hazelwood, MO), as have been done at times in previous culture identification attempts.

Statistical Analysis

We conducted a series of comparisons using two-proportion tests to determine whether there were any significant differences between the frequency of fungal growth in treatment and control trees. We compared the number of trees bearing fungal conks, the number of trees with mycelia observed in PVC tubes, and the number of trees showing either type of growth between control and treatment sites. We also compared these data between western hemlock and Douglas-fir trees, as well as between large-diameter trees (>37 cm mean DBH) and small-diameter trees (<37 cm mean DBH) stands to determine if tree species or tree diameter contributed to a difference in the growth of the red-belted conk. For trees where conks were present, we determined the mean width, height, and outward growth of conks.

RESULTS AND DISCUSSION

Status of Inoculated Trees

All 34 inoculation sites, as well as the three ground-based inoculation sites, were inspected in 2002. All treatment trees at ground-based inoculation sites were intact. We found that three of the 34 inoculation sites had been partially or entirely cut during harvest operations. Trees at one inoculation site owned by Rayonier (T97-02) adjacent to Highway 101 near Forks were all removed or topped at 8 m as required by the Clallam County Pacific Utility District (PUD) to eliminate any possibility that these trees might fall and disrupt power lines and/or block the road. This site was in an RMZ. At two other sites, 98-01 and 98-07, we found that all treated and control trees had been cut. Champion and Weyerhaeuser, the landowners responsible for these two sites, originally agreed to retain the experimental trees. However, because these sites were

not in permanently protected RMZs, and the forestry personnel involved in the original study design were no longer present, the inoculation sites were inadvertently harvested.

At the remaining sites, we found that most trees (98% of treated trees and 94% of control trees) were still present and alive. Of the 300 remaining treatment trees, three (1%) had blown down, one (0.3%) was dead, and one (0.3%) had its top broken off above the inoculation point. Of the 300 remaining control trees inspected, six (2%) were broken above the inoculation point, two (0.6%) had been topped above the inoculation point, two (0.6%) were standing dead, and 10 (3%) had been blown down. We were unable to locate only one tree, a control tree at site 97-08. All trees that were uncut and for which we were able to identify the point of inoculation ($N = 599$) were included in further analysis.

Visual Evidence of Fungal Growth on Control and Treatment Trees

Significantly more treatment trees (0.173) had conks than control trees (0.0, two-proportion test, $p < 0.001$; Table 1). This demonstrates that the inoculation technique of impregnated wooden dowels was vital to introducing the red-belted conk to treated trees, and that it was the presence of a small open wound that facilitated this fungal colonization. We also observed significantly more mycelia within the PVC tubes of treated trees (0.063) than in control tubes (0.0, $p < 0.001$, Table 1). Overall presence of fungal growth (including both conks emerging from the bark and mycelia observed within the PVC tube and surrounding area) was higher in treated trees (0.227), as we observed no fungal growth associated with the blank inoculations in control trees (0, $p < 0.001$).

Table 1. Comparison of the frequency of fungal growth observed in 2002 on trees inoculated with *Fomitopsis pinicola* ($N = 300$) and control trees ($N = 299$) in western Washington during 1997-1998. Data analyzed using two-proportion tests.

Type of fungal growth	Proportion of treated trees	Proportion of control trees	p –value
conks on tree	0.173	0	<0.001
mycelia observed within PVC tube	0.063	0	<0.001
conks and mycelia	0.227	0	<0.001

Evidence of Fungal Growth by Tree Species based on Visual Inspections

Comparisons between the presence of fungal growth in western hemlock and Douglas-fir trees suggests that western hemlocks are more susceptible to red-belted conk infection or that the fungus grows more quickly in this species than in Douglas-fir trees. Of the 300 treated trees, fungal conks were observed in a significantly higher proportion (0.400) in western hemlocks than in Douglas-firs (0.028, $p < 0.001$, Table 2). Treated western hemlock trees showed a higher incidence of both fungal conks (0.312) and mycelia (0.106) as compared to Douglas-fir (0.014 and 0.014, respectively, $p < 0.001$; Table 2).

Table 2. Comparison of the frequency of fungal growth observed on western hemlock (N = 160) Douglas-fir trees (N = 140) inoculated with *Fomitopsis pinicola* in western Washington during 1997-1998. Data analyzed using two-proportion tests.

Type of fungal growth	Proportion of western hemlocks	Proportion of Douglas-firs	p –value
conks on tree	0.400	0.028	<0.001
mycelia observed within PVC tube	0.312	0.014	<0.001
conks and mycelia	0.106	0.014	<0.001

These findings are supported by patterns of woodpecker use of nest trees. Most woodpecker nests located in 1996-1998 were located in western hemlock trees (73%, N = 57, Huss et al. 1999), while a similar frequency was observed in 2000-2001 (80%, N = 45, Ripper et al. 2002). In both studies, however, areas where nest searching was conducted were comprised mostly of western hemlock-dominant stands. The selection of hemlocks as nest trees by resident woodpeckers may be a result of their disproportionate availability; alternatively, the woodpeckers may be using western hemlocks due to the higher incidence of fungal heart-rot.

None of the treatment trees at the three ground-based inoculation sites (i.e., pilot-study sites) showed evidence of conks or mycelia at or near the point of inoculation. There were no control trees at these sites.

Presence of Fungal Growth by Tree Diameter

We found no significant difference in comparing the incidence of fungal growth (conks and/or mycelia) between treated trees in inoculation sites with large-diameter trees (0.256, N = 140) and small diameter trees (0.193, N = 160, p = 0.187; Table 3). The frequency of conks was not significantly different in large trees (0.157) than in small trees (0.187, p = 0.486). We also did not observe any difference in the presence of mycelia in large trees (0.075) as compared to small trees (0.050, p = 0.369; Table 3). Future inspections will provide more information about the effects of stand age on fungal growth. If we continue to observe that tree age/size has no effect on the efficacy of red-belted conk introduction, this technique may prove to be even more valuable in the managed forest context, where many stands are maintained as younger, smaller trees.

Table 3. Comparison of the frequency of fungal growth observed in large trees (>37 cm DBH, N = 140) and small trees (<37 cm DBH, N = 160) inoculated with *Fomitopsis pinicola* in western Washington during 1997-1998. Data analyzed using two-proportion tests.

Type of fungal growth	Proportion of large trees	Proportion of small trees	p –value
conks on tree	0.187	0.157	0.486
mycelia observed within PVC tube	0.075	0.050	0.193
conks and mycelia	0.256	0.193	0.187

Woodpecker Use of Experimental Trees

We detected no signs of woodpecker use that we considered to be associated with the introduced fungal growth in any of the inoculated trees. Several trees ($N = 13$) in both the treated and control sites showed evidence of sapsucker foraging, while one control tree had pileated woodpecker (*Dryocopus pileatus*) foraging excavations near the base. We note that 4-5 yr post-inoculation is probably too early to expect woodpecker excavations associated with the inoculation, especially considering that extensive heart-rot is necessary for cavity creation (Conner et al. 1976). A similar study involving snag creation by various methods, including topping, girdling, silvicide treatment, and fungal inoculation of Douglas-fir trees, supports our findings that woodpecker activity is scarce or absent in the 5 yr after treatment (Brandeis et al. 2002). During future inspections of inoculated sites (e.g., in 2005 or 2008), woodpecker occupancy of treated trees may be more likely.

Incidence of Fungal Growth in Trees based on Visual Inspections

Our findings regarding the efficacy of fungal inoculations on the basis of tree species and age may be integrated with recent documentation of hairy woodpecker ranging patterns within this study area. Ripper et al. (2002) recently determined that hairy woodpeckers tend to use 61-80 yr forest stands disproportionately within their home ranges, and use 41-60 yr stands in proportion with their availability. This study also documented that this woodpecker tends to under use younger succession stages (6-40 yr; Ripper et al. 2002). This information, coupled with our recent findings regarding the susceptibility of western hemlock trees to colonization by the red-belted conk and the similarity in the presence of fungi between old and young trees, suggests that fungal inoculations should be targeted towards mid-aged stands (≥40 yr). Furthermore, previous research on the Olympic Peninsula by Huss et al. (1999) in 1996-1998 and Ripper et al. (2002) during 2000-2001 suggests that Hairy Woodpeckers tend to nest on or near the edges of clearcuts and in >40 yr stands. Based on this information, we suggest placing future inoculation sites in proximity to >40 yr stand edges, preferably clustered with other retained trees in order to reduce blowdown.

Incidence of Fungal Growth in Wood Samples

A summary of isolation attempts from the sample of trees in the Control and Treatment Groups is found in Appendix C. Regardless of wood sample source, a plethora of fungi were found occupying the wood samples. The wounding of the tree during the control procedure and prevention of healing through the insertion of a piece of PVC tubing into the drilled out hole seems sufficient to allow a host of organisms to exploit this particular niche. A summary of the fungi found in trees based on tree type and experimental subgroup are given in Tables 4 and 5.

Among the Control Group that were inoculated in 1997 and 1998 with "blank" wooden dowels, fungi were isolated from all samples of wood collected, although the types of fungi isolated varied from tree to tree. Overall, we found 13 different types of fungi. The mean number of fungal types isolated per wood sample regardless of tree species (western hemlock and Douglas-fir) was 2.8 taxa per wood sample. Likewise, fungi were isolated from all samples of wood collected in 2002 from trees within the Treatment Group that were inoculated in 1997 and 1998 with wooden dowels impregnated with the mycelium of *Fomitopsis pinicola*. In

treatment trees, we found 19 different types of fungi. The mean number of fungal types isolated per wood sample regardless of tree species (western hemlock and Douglas-fir) was 2.9 taxa per wood sample. These data suggest that although the mean number of fungi retrieved per wood sample did not vary much among the trees between the Control and Treatment Groups, the diversity of fungi found was greater in those trees inoculated with *F. pinicola*. We did find that the two fields of data for control and treatment trees did on occasion overlap in fungal taxa present (e.g., *Aspergillus*, *Cryptococcus*, *Oedocephalum*).

We note that the incidence of basidiomycetes isolated from trees within the Control Group was higher 38.1% overall when compared the 15.0% recovered from wood samples from trees in the Treatment Group. Some members of the phylum Basidomycota are known to be actively involved in brown rot (such as *F. pinicola*), degrading cellulose and hemicellulose but leaving behind lignin, whereas other species can cause white rot and attack cellulose, hemicellulose, lignin, and other components of wood. Conks or the fruit bodies of basidiomycetes (presumed to be *F. pinicola*) from the visual inspection in the field of trees were found only on trees inoculated with this fungus (17.3% of all treated trees; 0.00% on all control trees). Recovery of *F. pinicola* in 1997 and 1998 indicates that *F. pinicola* colonies are able to form decay columns in inoculated trees (Huss et al. 2002). The lower incidence of *F. pinicola* or other fungal invasion by basidiomycetes of treatment trees vs. control trees we found in 2002 (4-5 yr after inoculation) suggests the possibility that the fungal mycelium may have advanced deeper into the wood out of reach of the point from where wood samples were collected (i.e., just below the initial wound and inoculate site). We suspect that movement of *F. pinicola* mycelium deeper into the wood is then followed by an ensemble of other decay organism in a succession of decomposers advancing into the localized region of decay in the wood. In controls, the initial wounding made to simulate the effects of inoculation, but with "blank" wooden dowels containing no *F. pinicola*, may have become a site available for the recruitment of fungi from airborne spores and the animal activity (e.g., bark beetles, woodpeckers; Kerry et al. 2004). The process of inoculating with live fungus may "jump start" a natural process of fungal succession necessary for the formation of decay columns in a tree, which may be exploited by woodpeckers for the excavation of nest cavities.

Establishment of *Fomitopsis pinicola* in Trees from the Control and Treatment Groups

When separate colonies of *F. pinicola* grow toward and encounter one another on the same culture medium (i.e., malt extract agar) one of two interactions occur. If a zone of demarcation develops between the two colonies, this is a good indication that the two colonies represent different strains or genetically distinct individuals. The zone of demarcation, represented by a line of dead hyphae, is caused as a result of an antagonistic interaction instigated by a self/non-self recognition system. If both colonies are identical or genetically similar, the two colonies will not form a zone of antagonism but grow toward and into one another. No boundary is formed and the two colonies are denoted as belonging to the same vegetative compatibility group (VCG). This intra-species interaction is well documented (Mounce 1929, Worrall 1997) for this fungus, serving in our previous work to determine whether the fungus retrieved from inoculated trees is the same as that originally introduced (Huss et al. 1999, Huss et al. 2002). One of three genetically distinct strains of *F. pinicola* (43A, 36A, and 64A; originally isolated from conks obtained from woodpecker nest trees in western Washington in 1996) was used in the inoculation of trees in the Treatment Group. These are also currently used in the experiments for VCG analysis.

Table 4. Fungi recovered from wood samples obtained from a sample of trees within the Control Group for experimental inoculation in western WA.

CONTROL GROUP	Category or Species	Incidence	Percent Occurrence
Western Hemlock (*N* = 6)	*Acremonium* or *Fusarium* sp. (only microconidia present)	3	50.00%
Douglas-Fir (*N* =15)		4	26.67%
Total (*N*=21)		7	33.33%
Western Hemlock (*N*=6)	*Aspergillus* sp.	1	16.67%
Douglas-Fir (*N*=15)		5	33.33%
Total (*N*=21)		6	28.57%
Western Hemlock (*N* = 6)	Basidiomycetes (hyphae with clamp connections and for some presence of chlamydospores; some these cultures may be *Fomitopsis pinicola*)	2	33.33%
Douglas-Fir (*N* =15)		6	40.00%
Total (*N*=21)		8	38.10%
Western Hemlock (*N* = 6)	*Cryptococcus* sp. (slimy yeast with polysaccharide capsules)	2	33.33%
Douglas-Fir (*N* =15)		3	20.00%
Total (*N*=21)		5	23.81%
Western Hemlock (*N* = 6)	*Gliocladium* sp.	0	0.00%
Douglas-Fir (*N* =15)		1	6.67%
Total (*N*=21)		1	4.76%
Western Hemlock (*N* = 6)	*Oedocephalum* sp. (possible asexual form or anamorph of *Heterobasidion annosum*, cause of butt & root rot in confers; Barnett and Hunter 1972; Agrios 1997)	2	33.33%
Douglas-Fir (*N* =15)		1	6.67%
Total (*N*=21)		3	14.29%
Western Hemlock (*N* = 6)	*Penicillium* sp.	0	0.00%
Douglas-Fir (*N* =15)		5	33.33%
Total (*N*=21)		5	23.81%

Table 4. Fungi recovered from wood samples obtained from a sample of trees within the Control Group for experimental inoculation in western WA (continued from previous page)

CONTROL GROUP	Category or Species	Incidence	Percent Occurrence
Western Hemlock (*N* = 6)	*Rhodotorula* sp. (pink or red yeast)	1	16.67%
Douglas-Fir (*N* =15)		1	6.67%
Total (*N*=21)		2	9.52%
Western Hemlock (*N* = 6)	*Scopulariopsis* sp.	0	0.00%
Douglas-Fir (*N* =15)		1	6.67%
Total (*N*=21)		1	4.76%
Western Hemlock (*N* = 6)	*Trichoderma* sp.	1	16.67%
Douglas-Fir (*N* =15)		0	0.00%
Total (*N*=21)		1	4.76%
Western Hemlock (*N* = 6)	Unidentified dematiaceous (darkly pigmented) filamentous fungi - lack of distinguishing morphological characteristics for conclusive diagnosis	1	16.67%
Douglas-Fir (*N* =15)		2	13.33%
Total (*N*=21)		3	14.29%
Western Hemlock (*N* = 6)	Unidentified hyaline (colorless or lightly colored) filamentous fungi - lack of distinguishing morphological characteristics for conclusive diagnosis	3	50.00%
Douglas-Fir (*N* =15)		9	60.00%
Total (*N*=21)		12	57.14%
Western Hemlock (*N* = 6)	Unidentified yeast or yeast-like fungi; some with pseudohyphae	1	16.67%
Douglas-Fir (*N* =15)		1	6.67%
Total (*N*=21)		2	9.52%

Table 5. Fungi recovered from wood samples obtained from a sample of trees within the Treatment Group for experimental inoculation in western WA.

TREATMENT GROUP (Inoculated with *Fomitopsis pinicola*)	Category or Species	Incidence	Percent Occurrence
Western Hemlock (*N* = 12)	*Acremonium* or *Fusarium* sp. (only microconidia present)	9	75.00%
Douglas-Fir (*N* =8)		3	37.50%
Total (*N*=20)		12	60.00%
Western Hemlock (*N*=12)	*Aspergillus* sp.	0	0.00%
Douglas-Fir (*N*=8)		2	25.00%
Total (*N*=20)		2	10.00%
Western Hemlock (*N*=12)	*Aureobasidium* sp.	1	8.33%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		1	5.00%
Western Hemlock (*N*=12)	Basidiomycetes (hyphae with clamp connections and for some presence of chlamydospores; some these cultures may be *Fomitopsis pinicola*)	1	8.33%
Douglas-Fir (*N*=8)		2	25.00%
Total (*N*=20)		3	15.00%
Western Hemlock (*N*=12)	*Botrytis* sp. (with sclerotia)	0	0.00%
Douglas-Fir (*N*=8)		1	12.5%
Total (*N*=20)		1	5.00%
Western Hemlock (*N*=12)	*Cladosporium* sp.	0	0.00%
Douglas-Fir (*N*=8)		1	12.5%
Total (*N*=20)		1	5.00%

Table 5. Fungi recovered from wood samples obtained from a sample of trees within the Treatment Group for experimental inoculation in western WA (continued from previous page).

TREATMENT GROUP (Inoculated with *Fomitopsis pinicola*)	Category or Species	Incidence	Percent Occurrence
Western Hemlock (*N*=12)	*Cryptococcus* sp. (slimy yeast with polysaccharide capsules)	3	25.00%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		3	15.00%
Western Hemlock (*N*=12)	*Leptographium* sp. or *Verticicladiella* sp.	0	0.00%
Douglas-Fir (*N*=8)		1	12.50%
Total (*N*=20)		1	5.00%
Western Hemlock (*N*=12)	*Monilia* sp.	1	8.33%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		1	5.00%
Western Hemlock (*N*=12)	*Oedocephalum* sp. (possible asexual form or anamorph of *Heterobasidion annosum*, cause of butt & root rot in confers; Barnett and Hunter 1972; Agrios 1997)	3	25.00%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		3	15.00%
Western Hemlock (*N*=12)	*Penicillium* sp.	1	8.33%
Douglas-Fir (*N*=8)		3	37.50%
Total (*N*=20)		4	20.00%
Western Hemlock (*N*=12)	*Phoma* sp.	0	0.00%
Douglas-Fir (*N*=8)		1	12.50%
Total (*N*=20)		1	5.00%
Western Hemlock (*N*=12)	*Rhodotorula* sp. (pink or red yeast)	0	0.00%
Douglas-Fir (*N*=8)		1	12.50%
Total (*N*=20)		1	5.00%

Table 5. Fungi recovered from wood samples obtained from a sample of trees within the Treatment Group for experimental inoculation in western WA (continued from previous page).			
TREATMENT GROUP (Inoculated with *Fomitopsis pinicola*)	**Category or Species**	**Incidence**	**Percent Occurrence**
Western Hemlock (*N*=12)	*Torula* sp.	2	16.67%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		2	10.00%
Western Hemlock (*N*=12)	*Trichoderma* sp.	5	41.67%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		5	25.00%
Western Hemlock (*N*=12)	Unidentified dematiaceous (darkly pigmented) filamentous fungi - lack of distinguishing morphological characteristics for conclusive diagnosis	2	16.67%
Douglas-Fir (*N*=8)		2	25.00%
Total (*N*=20)		4	20.00%
Western Hemlock (*N*=12)	Unidentified hyaline (colorless or lightly colored) filamentous fungi - lack of distinguishing morphological characteristics for conclusive diagnosis	3	25.00%
Douglas-Fir (*N*=8)		6	75.00%
Total (*N*=20)		9	45.00%
Western Hemlock (*N*=12)	Unidentified yeast or yeast-like fungi; some with pseudohyphae	2	16.67%
Douglas-Fir (*N*=8)		0	0.00%
Total (*N*=20)		2	10.00%
Western Hemlock (*N*=12)	*Verticillium* sp.	0	0.00%
Douglas-Fir (*N*=8)		1	12.5%
Total (*N*=20)		1	5.00%

Cultures retrieved from wood samples from both Control and Treatment Groups that shared characteristics in common with the vegetative or mycelial form of *Fomitopsis pinicola* were subjected to a pairing experiment with three known tester strains of this fungal species (15 October 2003). After pairing the "unknown" culture with the three tester strains on a sterilized culture medium (i.e., malt extract agar) in a Petri dish, these were incubated at room temperature for several weeks to allow colonies to grow and the interactions between colonies to unfold. Observations were taken of the interactions that took place.

Four types of intra- and interspecific interactions of paired growing cultures were observed. In addition to intraspecific responses, we encountered responses that are likely due to interspecific interactions. Interspecific interactions between colonies of different fungal species that encounter one another while growing in close proximity to one another can also occur, but the "behavior" of the interaction could vary from mutualistic to neutral to antagonistic, depending on the two species involved. This point is mentioned as it was not known with certainty whether some of the pairing cultures (i.e., the "unknowns") represented members of *F. pinicola* or another type of basidiomycete of similar cultural morphology.

- Interaction 1: Strong Lines of Demarcation between known tester strains of *F. pinicola* but not when paired with self. None of the cultures tested demonstrated any cultures compatible with *F. pinicola*. Three cultures, numbers 123, 124, and 154 (refer to Appendix C) demonstrated strong zones of demarcation when paired with all three-tester strains, suggesting that these three cultures represented a genetically-unique strain of *F. pinicola*. These cultures were obtained from two separate western hemlock trees from within the Treatment Group (i.e., T97-07-08 and T97-24-09).

- Interaction 2: Weak interactions. With exceptions of pairings of known tester strains with the other known tester strains (the controls in this pairing experiment), none of the following cultures (nos. 2, 3, 4, 6, 7, 10, 21, 22, 41, 51, 52, 56, 58, 80, 84, 86, 87) interacted in a pattern consistent with vegetative compatibility or incompatibility. The interactions consisted of slightly more than raised regions of mycelium along the points where two colonies encountered one another. These results indicate some initiation of the self/non-self recognition system but with no substantial change occurring over time. These observations further suggest that colonies encountering one another do not possess a strong affinity for or against the tester strains of *F. pinicola* as the interactions did not vary between the colony being tested and the three separate tester strains. If these cultures do represent *F. pinicola*, then none of these are likely compatible with the tester strains. If so, then this suggests multiple invasions of wound sites by airborne spores or another vector. Some of the interaction observed could be a result of pairings of congeneric species or another type of closely related fungal species.

- Interaction 3: Crowding. The tester strains grew and overtook the colony of the fungal culture paired with it. All three tester strains seemed to inhibit the growth of the culture paired with which included cultures nos. 19, 29, 31, 35, 38, 39, 48, 49, 50, 116, 117, 118, 119, 121, 125, 147, 148, 158, 159, 189, 193, and 197. Of these, cultures nos. 29, 31, 48, 49, 121, 158, and 159 represented *Oedocephalum* sp., which had been identified to genus prior to the experiment. None of the other cultures, which based on their microscopic

characteristics (e.g., presence of clamp connections) represented basidiomycetes, appear to represent members of *F. pinicola*.

- Interaction 4: Growth inhibition. A few pairing cultures produced colonies that were able to grow and inhibit the growth of the three tester strains. The observations suggest the diffusion of an inhibitory substance into the culture medium that prevent active growth by the tester strains of *F. pinicola*. Cultures capable of this included 8, 9, 138, 139, and 156. These cultures are unlikely to represent members of *F. pinicola*.

The results of these pairings demonstrated that retrieval of *F. pinicola* from trees previously inoculated with this fungus was not possible 4-5 years after initial inoculation. However, as field evidence demonstrated these fungi were present in the form of reproductive structures protruding from many of these previously inoculated trees (i.e., basidiocarps or conks). This supports the notion that either the fungus dies off after a time or that it has grown deep enough into the wood away from the point of inoculation origin not to be detected through our current retrieval methods. Indigenous basidiomycetes and other fungi clearly have made their way to the trees wounded in the manner used to treat the trees in both the Control and Treatment Groups. The quality and diversity profile of the fungal population likely varies though between the two forms of treatment. More time is needed to determine whether conditions have been created conducive toward the end result; that is, the occupation of these "wildlife trees" by nest excavators and their successors.

CONCLUSIONS

- As evidenced by the presence of fungal conks or mycelia, we have successfully introduced the red-belted conk to at least 23% of treated trees.

- Western hemlock appears to be more susceptible to localized infection by the red-belted conk fungus, as 42% of treated hemlock trees showed evidence of fungal growth (conks and/or mycelia). Only 3% of treated Douglas-fir trees displayed evidence of conks and/or mycelia.

- Data from this inspection suggest that the age/size of treated trees does not have an effect on the success of fungal inoculation. There was no significant difference in the incidence of fungal growth in small trees (19%) as compared to large trees (26%).

- We found that trees at three inoculation sites (9%) had been cut since the inoculations took place. We request that landowners be aware of the location of the remaining inoculation sites and make a concerted effort to preserve these sites for future evaluation and monitoring.

- Fungi were recovered from wood samples retrieved from trees both in the Control and Treatment Groups inoculated in 1997 and 1998. The mean number of fungal taxa from the Control Groups was 2.8 taxa per wood sample ($N = 21$) and for the Treatment Group, 2.9 taxa per wood sample.

- From the wood samples recovered from trees in the Control Group, a minimum of 13 different fungal taxa were recovered, while 19 were recovered from wood samples from trees in the Treatment Group. Although both test groups share similar fungi, the diversity of fungi overall is greater within the Treatment Group. Notable though is that those fungi most likely to be associated with primary wood decay were more frequently recovered from wood samples in the Control Group.

- A succession of wood decay organisms are working their way in and around the initial wound site created by the inoculation procedure initiated in western hemlock and Douglas-fir trees in 1997 and 1998.

- Within the Treatment Group of trees, data suggest that the *Fomitopsis pinicola* (red belted conk) has moved beyond the initial point of entry. This would explain our inability to retrieve this fungus from wood samples collected near the wound entrance. The fungus is likely present deep in the wood of many of the inoculated trees as evidenced by the presence of conks protruding from their surfaces.

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Appendix A. Characteristics of stands and results of 2002 inspection for fungal growth of inoculation sites in western Washington. Except as indicated below, there were 10 treatment trees and 10 control trees at each stand. None of the control trees had conks or mycelia upon inspection.

SITE	LANDOWNER	Riparian Management Zone (RMZ)	Tree Species	Percent treatment trees with conks	Percent trees with mycelia
97-01	Rayonier	RMZ	w. hemlock	20	30
97-02	Rayonier	RMZ	w. hemlock	TREES CUT	
97-03	Rayonier	RMZ	w. hemlock	30	10
97-04	Rayonier	RMZ	w. hemlock	10	0
97-05	Rayonier	RMZ	w. hemlock	20	10
97-06	Rayonier	not RMZ	w. hemlock	0	0
97-07	Rayonier	RMZ	w. hemlock	50	0
97-08	Rayonier	RMZ	w. hemlock	50	10
97-09	Fruit Growers	not RMZ	w. hemlock	40	0
97-10	Rayonier	RMZ	w. hemlock	40	10
97-11	Rayonier	RMZ	w. hemlock	30	10
97-12	Rayonier	RMZ	w. hemlock	60	10
97-13	Rayonier	not RMZ	Douglas-fir	10	0
97-18	Rayonier	not RMZ	Douglas-fir	0	0
97-19	Rayonier	not RMZ	Douglas-fir	0	0
98-09	Rayonier	RMZ	Douglas-fir	0	0
97-14	DNR[a]	RMZ	Douglas-fir	0	0
97-15[b]	DNR	not RMZ	w. hemlock	50	0
97-16	DNR	RMZ	Douglas-fir	0	0
97-17	DNR	RMZ	Douglas-fir	0	0
97-20	DNR	not RMZ	w. hemlock	0	10
97-21	DNR	not RMZ	w. hemlock	0	30
97-22[c]	DNR	RMZ	w. hemlock	0	0
97-23	DNR	RMZ	w. hemlock	40	0
97-24	DNR	not RMZ	w. hemlock	50	40
98-01	Campbell Group	not RMZ	Douglas-fir	TREES CUT	

Appendix A continued. Characteristics of stands and results of 2002 inspection for fungal growth of inoculation sites in western Washington. Except as indicated below, there were 10 treatment trees and 10 control trees at each stand. None of the control trees had conks or mycelia upon inspection.

SITE	LANDOWNER	Riparian Management Zone (RMZ)	Tree Species	Percent treatment tree with conks	Percent trees with mycelia
98-02	Campbell Group	RMZ	Douglas-fir	10	0
98-03	Campbell Group	RMZ	Douglas-fir	0	0
98-10	Campbell Group	RMZ	Douglas-fir	0	0
98-04	Weyerhaeuser	RMZ	Douglas-fir	0	0
98-05	Weyerhaeuser	RMZ	Douglas-fir	0	10
98-06	Weyerhaeuser	RMZ	Douglas-fir	0	0
98-07	Weyerhaeuser	not RMZ	Douglas-fir	TREES CUT	
98-08	Weyerhaeuser	RMZ	Douglas-fir	0	10

[a] DNR = Washington Department of Natural Resources.
[b] Site with treatment trees only.
[c] Site with control trees only.

Appendix B. Global Positioning System (GPS) coordinates (NAD-27, U.S. ft.) of sites with trees inoculated experimentally with *Fomitopsis pinicola* or with control trees treated in 1997 and 1998. All sites are located in western Washington.

Site	Landowner	*X*	*Y*
97-01	Rayonier	5319887	396886
97-02	Rayonier	5312155	390619
97-03	Rayonier	5312111	390601
97-04	Rayonier	5307797	386856
97-05	Rayonier	5306911	399395
97-06	Rayonier	5312637	391111
97-07	Rayonier	5329753	398429
97-08	Rayonier	5311631	394219
97-09	Rayonier	5290296	404602
97-10	Rayonier	5247054	409262
97-11	Rayonier	5287653	405146
97-12	Rayonier	5324864	386877
97-13	Rayonier	5329808	400014
97-18	Rayonier	5237905	431664
97-19	Rayonier	5238947	431456
98-09	Rayonier	5222089	432215
97-14	DNR[a]	5324152	413623
97-15	DNR	5313456	401789
97-16	DNR	5322978	411818
97-17	DNR	5325110	427438
97-20	DNR	5283530	412420

Appendix B continued. Global Positioning System (GPS) coordinates (NAD-27, U.S. ft.) of sites with trees inoculated experimentally with *Fomitopsis pinicola* or with control trees treated in 1997 and 1998. All sites are located in western Washington.

Site	Landowner	*X*	*Y*
97-21	DNR	5284034	417516
97-22	DNR	5288835	414582
97-23	DNR	5300388	407236
97-24	DNR	5313892	386317
98-01	Campbell Group	5206225	564529
98-02	Campbell Group	5206198	564512
98-03	Campbell Group	5206966	561849
98-10	Campbell Group	5198974	564525
98-04	Weyerhaeuser	5150103	489222
98-05	Weyerhaeuser	5151712	487030
98-06	Weyerhaeuser	5225498	427301
98-07	Weyerhaeuser	Unavailable	Unavailable
98-08	Weyerhaeuser	5219433	444376
GB1	Rayonier	5228803	418106
GB2	Rayonier	5231068	421394
GB3	Rayonier	5225502	427299

[a]DNR = Washington Department of Natural Resources.

APPENDIX C

Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola*.

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
C97-04	4	W. Hemlock	Hyaline colonies; white and slow growing colonies; greenish gray and fast growing colonies.	69 - Unidentified fungus: septate, hyaline hyphae; 70, 71 - *Aspergillus*.
C97-04	8	W. Hemlock	Hyaline to brown fungal colonies.	65 -*Acremonium* or *Fusarium* (microconidia present); 66, 67, 68 - Unidentified fungus - green, small to large globose spores.
C97-06	4	W. Hemlock	All hyaline colonies; some yeast-like; colonies white to orange in color.	45, 46, 47 - *Acremonium* or *Fusarium* (microconidia present); 48, 49 - *Oedocephalum*.
C97-06	6	W. Hemlock	Yeast (cream white or red); white hyaline and black dematiaceous filamentous colonies.	11 - Pink yeast (*Rhodotorula*); 12 - Slimy yeast with capsule (*Cryptococcus*); 13 - *Trichoderma* (atypical); 14, 15 - *Acremonium* or *Fusarium* (microconidia present); 16 - Unidentified fungus: septate hyphae with terminal chlamydospores.
C97-16	5	Douglas-fir	All hyaline colonies; light green to white in color.	23, 24, 25 - *Aspergillus*.
C97-16	8	Douglas-fir	Cream-colored yeast; hyaline white and gray to black dematiaceous filamentous colonies.	32, 33 - Slimy yeast with capsule (*Cryptococcus*); 34 - *Aspergillus*; 35 - Unidentified fungus: mostly non-septate hyaline filamentous hyphae; 36 - Unidentified fungus: non-sporulating dematiaceous septate hyphae.
C97-17	4	Douglas-fir	No growth seen on plates or if so, hyaline white to dematiaceous slow-growing colonies.	37 - Unidentified fungus: septate, hyaline hyphae; 38, 39, 40, and 41 - Unidentified fungus: mostly non-septate filamentous hyaline hyphae.
C97-17	10	Douglas-fir	All hyaline colonies; white to green in color. Yeast colony present.	96, 97, 100 - *Penicillium*; 98, 99 - *Aspergillus*; 101 - Slimy yeast with capsule (*Cryptococcus*).

Table continued on next page

Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola*. (continued from previous page).

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
C97-18	1	Douglas-fir	Red yeast; hyaline white to cream-colored filamentous colonies.	6, 7 - *Oedocephalum*; 8, 9 - Unidentified fungus: non-sporulating septate hyaline hyphae; 10 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?).
C97-18	5	Douglas-fir	Hyaline white to black dematiaceous colonies; one green colony.	26 - *Scopulariopsis*; 50 - Mostly non-septate non-sporulating hyaline hyphae; 51, 52 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?); 53, 54 - Unidentified fungus - non-sporulating septate dematiaceous hyphae (No. 53 contaminated with *Aureobasidium*?).
C97-18	9	Douglas-fir	Fast- and slow-growing colonies of hyaline orange-colored mycelia.	42, 43, 44 - *Acremonium* or *Fusarium* (microconidia present).
C97-19	4	Douglas-fir	All hyaline colonies; white, yellowish, orange to dusty brown in color. One yeast or bacterial colony observed.	60, 61 - *Aspergillus*; 62 - Unidentified fungus: mostly non-septate filamentous hyphae; 63 - *Acremonium* or *Fusarium* (microconidia present); 64 – Unidentified bacterium.
C97-19	10	Douglas-fir	Hyaline (white, cream, green) and black dematiaceous filamentous fungal colonies; yeast.	77 - Slimy yeast with capsule (*Cryptococcus*); 78, 79 - Unidentified fungus: yeast or conidia floating among hyphae; 80 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?); 81, 82, 83 - *Aspergillus*.
C97-22	3	W. Hemlock	Hyaline filamentous colonies and white cream-colored yeast (slimy).	27 - Slimy yeast with capsule (*Cryptococcus*); 28 - Unidentified fungus: round to oblong yeast cells and "pseudohyphae"; 29, 30, 31 - *Oedocephalum*.
C97-22	4	W. Hemlock	Hyaline filamentous colonies; light brown to white in color.	58 - Unidentified fungus: non-sporulating septate hyaline hyphae; 59 - Basidiomycete: non-sporulating septate hyaline hyphae with clamp connections.

Table continued on next page

Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola* (continued from previous page).

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
C98-03	1	Douglas-fir	Hyaline and dematiaceous isolated colonies; white, emerald green, gray green, or brownish pink in color.	84, 85, 86, 87, 88 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?); 89 - *Penicillium*; 90 - Unidentified fungus: non-sporulating dematiaceous septate hyphae.
C98-03	10	Douglas-fir	All hyaline colonies; white, orange or green in color.	91, 94, 95 - Unidentified fungus: mostly non-septate filamentous hyaline hyphae; 92 - *Penicillium*; 93 - *Acremonium* or *Fusarium* (microconidia present).
C98-05	1	Douglas-fir	Hyaline and white fast-growing filamentous colonies.	1 - *Penicillium*; 2, 3, and 4 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?); 5 - Pink yeast (*Rhodotorula*).
C98-05	9	Douglas-fir	Hyaline colonies: orange to white in color.	17 - *Gliocladium*; 18, 19 - Unidentified fungus: non-sporulating septate hyaline hyphae; 20 - *Acremonium*; 21, 22 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?).
C98-06	3	Douglas-fir	Slow-growing brown hyaline fungal colonies; and several fast-growing white hyaline filamentous colonies.	55 - Unidentified fungus: non-sporulating hyaline hyphae (gray colony); 56, 57 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?).
C98-06	8	Douglas-fir	Hyaline filamentous colonies; green, light brown, and white in color.	72, 73 - Unidentified fungus; 74, 75, 76 - *Penicillium*.

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Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola* (continued from previous page).

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
T97-03	1	W. Hemlock	Isolated hyaline white to gray colonies; greenish black dematiaceous colonies present; slimy cream-colored yeast present.	178 - *Torula* (black sheen on colony); 179 - Slimy yeast with capsule (*Cryptococcus*); 180 - *Oedocephalum*; 181-183 - *Acremonium* or *Fusarium* (microconidia present, dark diffusible pigment present, but hyphae are hyaline).
T97-03	7	W. Hemlock	Multiple isolations of brown dematiaceous and hyaline white fungal colonies.	222, 223 - *Trichoderma* - globose microconidia and chlamydospores present; 224, 225, 226 - *Acremonium* or *Fusarium* (microconidia present).
T97-05	3	W. Hemlock	Isolated mostly green to black dematiaceous fungi; yeast.	169-74 - *Acremonium* or *Fusarium* (microconidia present, dark diffusible pigment present, but hyphae are hyaline); 175 - Unidentified yeast (not slimy, no capsule present, colony creamy white in color); 176, 177 - *Torula* (black sheen on colony).
T97-05	7	W. Hemlock	Isolated mostly dematiaceous and gray to green colored hyaline fungi.	217 - *Acremonium* or *Fusarium* (microconidia present); 218-221 - *Trichoderma* (variable microcondia [globose to elliptical] and globose chlamydospores; phialides *Trichoderma*-like to nondescript appearance).
T97-07	5	W. Hemlock	All isolation attempts yielded white fungus with a bull's eye pattern of growth.	136, 137 - *Acremonium* or *Fusarium* (microconidia present); 138, 139 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?).
T97-07	8	W. Hemlock	Hyaline white filamentous colonies; dematiaceous (green, brown, or black) and yeast-like fungal colonies also present.	123, 124 - Unknown fungus: septate hyaline hyphae with extracellular debris present; 125, 128 - Unidentified fungus: septate, hyaline hyphae (no debris present (125 is a white colony; 128 is yellow brown in color); 126 - *Penicillium*; 127, 129 - *Aureobasidium*.

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Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola* (continued from previous page).

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
T97-11	5	W. Hemlock	Isolated dematiaceous (brownish- and greenish-colored) and hyaline white filamentous colonies.	199, 200, 201, 203, 204, 205 - *Acremonium* or *Fusarium* (microconidia present); 202, 206, 207, 208 - *Trichoderma* (variable microcondia [globose to elliptical] and globose chlamydospores).
T97-11	6	W. Hemlock	All isolated colonies appear to be hyaline with green conidia - *Trichoderma*?	227, 228, 229, 230 - *Trichoderma* (variable microcondia [globose to elliptical] and globose chlamydospores).
T97-14	2	Douglas-fir	Most isolation attempts yielded hyaline filamentous colonies that were white to green in color.	140-146 - *Penicillium*; 147 - Unidentified fungus: mostly non-septate filamentous hyaline hyphae; 148 - Basidiomycete: hyaline septate hyphae with clamp connections and chlamydospores (*Fomitopsis pinicola*?).
T97-14	4	Douglas-fir	Isolated small to sparsely-growing colonies; hyaline filamentous colonies: gray white to brownish red in appearance.	130, 131 - *Phoma* with pynidia; 131, 133, 134 - *Penicillium*; 135 - *Verticillium*.
T97-17	3	Douglas-fir	Isolation attempts yielded a few slow-growing white to brown filamentous and several dematiaceous colonies.	190 - *Acremonium* or *Fusarium* (microconidia present); 191 - *Aspergillus*; 192-198 - Unknown Basidiomycete: Hyaline non-pigmented septate non-sporulating hyphae (Nos. 195-196 with a few clamp connections).
T97-17	7	Douglas-fir	Colonies for the most part absent except for several dematiaceous and some slow-growing hyaline white colonies.	109, 110, 111 - Slow growing colonies, nonsporulating nonseptate to septate hyaline hyphae; 112 - *Verticicladiella* or *Leptographium*; 113 - Non-sporulating dematiaceous septate hyphae; 114, 115 - *Cladosporium*.
T97-20	7	W. Hemlock	Some colonies present; hyaline (white, cream, and brown in color) to dematiaceous (gray green, black in color); yeast.	209 - *Monilia*; 210 - *Acremonium* or *Fusarium* (microconidia present); 211, 212 - *Trichoderma* (variable microcondia [globose to elliptical] and globose chlamydospores); 213, 214, 215, and 216 - Unknown fungus: white-colored yeast consisting of elliptically-shaped cells and pseudohyphae.

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Results of isolation and culturing of fungi from wood samples collected in 2002 from trees inoculated in 1997 and 1998 with and without the red-belted conk fungus, *Fomitopsis pinicola* (continued from previous page).

Tree Cluster[1]	Tree No.	Tree Species	Visual descriptions of growth on primary isolation plates containing Isolation Medium (IM)	Culture/Slide No. & Taxon
T97-20	10	W. Hemlock	Flat cream to gray white colored to white hyaline filamentous colonies. Cream white yeast also observed.	157b, 158, 159 - *Oedocephalum*; 160 - Slimy yeast with capsule (*Cryptococcus*); 161, 162, 163 - *Acremonium* or *Fusarium* (microconidia present).
T97-24	5	W. Hemlock	Small colonies that are white to brown; most isolation attempts failed to yield fungal colonies; some yeast present.	120 - Slimy yeast with capsule (*Cryptococcus*); 121, 122 - *Oedocephalum.*
T97-24	9	W. Hemlock	Isolated hyaline fungal colonies; white, brown and pink in color.	149-153 - Dematiaceous colonies and oval-shaped spores, probably a species of *Cladosporium*; 154-156 - Non-sporulating hyaline septate fungus; 157a - *Acremonium* or *Fusarium* (microconidia present).
T98-03	2	Douglas-fir	Isolated several white to dark brown fast-growing colonies (dematiaceous fungi); bacterium or yeast present.	164 – Non-sporulating hyaline septate wide hyphae, *Rhizoctonia*?; 165 - Non-sporulating dematiaceous septate hyphae; 166, 167,168 - *Botrytis* (with sclerotia).
T98-03	6	Douglas-fir	A few slow-growing colonies; hyaline filamentous (cream to orange-yellow) to dematiaceous and brownish-white colonies.	102 - Unknown fungus: mostly non-septate hyaline slow-growing hyphae (in) with no spores; 103, 104, 105 - *Acremonium* or *Fusarium* (microconidia present); 106, 107, 108 - Non-sporulating septate dematiaceous (brown) hyphae.
T98-10	8	Douglas-fir	Isolated a red-colored yeast, some filamentous colonies - some dematiaceous, others hyaline white and brownish-red.	184, 185 - *Acremonium* or *Fusarium* (microconidia present); 186 - Red yeast (*Rhodotorula*?); 187 - *Penicillium*; 188 - *Aspergillus*; 189 - Unknown fungus: hyaline nonpigmented, mostly nonseptate and nonsporulating hyphae.
T98-10	10	Douglas-fir	Colonies mostly absent; hyaline, white and slow growing filamentous colonies.	116-119 - Unknown fungus: slow-growing white colonies, mostly non-septate hyaline hyphae with cysts or spherical crystals present.

[1]Tree Cluster: C=Control, T=Treatment; 97=Trees inoculated in 1997, 98=trees inoculated in 1998; number indicates stand designation from experiment. In the Control set, 21 trees were sampled; in the Treatment Set, 20 trees were sampled.