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The efficacy of fungal inoculation of live trees to create wood decay and wildlife-use trees in managed forests of western Washington, USA

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ABSTRACT

Decaying wood plays a vital role in maintaining biological diversity and ecological processes within forest ecosystems. One enhancement that may help to maintain wood-decay processes in managed forests involves the inoculation of healthy trees with fungus to create potential habitat trees to enable excavation for foraging and nesting by primary cavity nesters (i.e., woodpeckers); however, this approach has only received limited evaluation. We evaluated the inoculation of *Fomitopsis pinicola* into live trees in managed forests in western Washington in 1997 and 1998. In 2006, we revisited trees that were inoculated with live fungus or sterile controls, and inspected each tree for the presence of fungal growth and woodpecker activity. Of 650 trees inoculated with fungus ($n = 330$) or a sterile control ($n = 320$), 528 (81.2%) were alive and standing in 2006 ($n = 276$ with fungus, 83.6%; $n = 252$ control trees, 78.8%). Trees had been lost to harvest (11.1%), blowdown (3.8%), breakage (2.9%), and had died of undetermined causes (0.9%). A higher proportion of treatment trees displayed *F. pinicola* conks (0.200) and mycelia (0.073; inferred to be *F. pinicola*) than did control trees (0.004 conks [unknown species], 0.012 mycelia), although the difference for mycelia was marginally significant. We also found that western hemlock (*Tsuga heterophylla*) had a higher proportion of conks (0.313) and evidence of any fungal growth (conks or mycelia; 0.393) than Douglas-firs (*Pseudotsuga menziesii*; 0.064 and 0.112, respectively). Further, we observed evidence of significantly ($P = 0.010$) more woodpecker excavations and sapsucker (*Sphyrapicus* spp.) foraging holes associated with the fungal inoculations (6.2%) than at control trees (1.2%). Although use by woodpeckers was limited, we suggest that this finding is ecologically significant as we observed no woodpecker use, except for limited sapsucker foraging, when we inspected trees in 2002. The fungal inoculations completed 1997–1998, to some extent, were successful as *F. pinicola* was documented in at least 36.8% of the treated trees. In addition to *F. pinicola*, an ensemble of fungi and other microorganisms was established into the inoculation wounds of both control and treatment trees, suggesting that wounding of a healthy tree under the right circumstances may be sufficient to initiate this natural process in younger-aged forests as it occurs in old-growth forests.

1. Introduction

The maintenance of deadwood, including the presence of snags, and natural wood-decay processes is critical to functioning forest ecosystems worldwide. Dead and decaying wood play vital roles in maintaining biodiversity, including associated complex biological interactions fundamental to the support of a variety of ecological processes; soil development and porosity; water infiltration; nutrient and gas dynamics; natural resistance to a variety of pests; slope stability; control of sediment loss; site productivity; and long-term sustainable harvests (e.g., Brockerhoff et al., 2008; Chambers et al., 1997; Rose et al., 2001). Moreover, the amount of dead wood and associated decay dynamics in forest stands has been documented to decline with successive management actions (e.g., Bunnell et al., 2002a; Ohmann and Waddell, 2002; Franklin et al., 1997; Hanson et al., 1991; Kroll et al., 2012b; Rose et al., 2001). Thus, to maintain healthy functioning forests and sustainable harvests, it is integral to foster natural decay dynamics in intensively managed forests (Arnett et al., 2010; Brockerhoff et al., 2008; Bunnell et al., 2002b; Cline et al., 1980; Kroll et al., 2012a; Vuidot et al., 2011). Unfortunately, the effective management of these processes is limited by the lack of understanding of the dynamics and associated biological interactions of cavity formation and loss both in managed and natural forests (Bednarz et al., 2004; Jackson and Jackson, 2004; Kroll et al., 2012b; Remm et al., 2006).

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Several techniques have been developed to provide structural enhancements to managed forests to foster communities of primary and secondary cavity nesting birds, which are interdependent with a multitude of natural ecological processes that occur within forests (e.g., natural control of insect pests). These include topping of trees with chain saws, feller-bunchers, or explosives; girdling; injection of herbicides; pheromone applications to attract wood-boring beetles; and fungal inoculations (Arnett et al., 2010; Brandeis et al., 2002; Bull and Partridge, 1986; Huss et al., 2002; Rose et al., 2001). Of these techniques, fungal inoculation of live trees has received limited study and results have been confounded because of varied approaches used to inoculate trees (e.g., use of different species of fungi, differences in target tree species, use of single versus multiple inoculations of single trees, and varying heights of inoculation; Brandeis et al., 2002; Bull and Partridge, 1986; Filip et al., 2004, 2011; Huss et al., 2002). Also, monitoring of fungal inoculations has produced mixed results, and tree species and species of inocula seem to be important related to the efficacy of establishing wood decay (Brandeis et al., 2002; Bull and Partridge, 1986; Filip et al., 2004, 2011; Huss et al., 2002; Parks et al., 1996). One of the most encouraging reports to date was by Parks et al. (1996), who reported woodpecker nesting cavities in 14% of 60 live western larch (*Larix occidentalis*) within 5 years of inoculation in northeastern Oregon. Other studies showed limited success or reported that more time may be required before woodpecker or other wildlife use can be demonstrated (Bull and Partridge, 1986; Brandeis et al., 2002; Filip et al., 2004, 2011; Huss et al., 2002).

However, use of fungal inoculations to establish decay in live trees to potentially supplement the provision of suitable snags and wildlife trees has several potential advantages over topping trees below the live crown to create snags (e.g., Arnett et al., 2010; Bull and Partridge, 1986; Walter and Maguire, 2005). (1) Live, degraded trees with rot have been shown to be preferred by a number of cavity-dependent species of birds and other wildlife over snags (Remm et al., 2006; Rose et al., 2001). (2) Some cavity-dependent birds and deadwood-dependent organisms use habitat on relatively tall snags or dead wood near the tops of live trees; habitat higher than can be provided by topping trees below the lowest live whorl of branches (e.g., Bull and Holthausen, 1993; Kroll et al., 2012b; Lacki and Baker, 2007; Ormsbee and McComb, 1998). (3) By establishing fungal decay in live trees, such trees may provide relatively longer-term usable foraging and nesting substrates for birds and other organisms (Brandeis et al., 2002; Jackson and Jackson, 2004; Rose et al., 2001; Welsh and Capen, 1992). (4) Fungal inoculations may facilitate long-term recruitment of additional snags within harvest units without multiple entries to top trees at different times during the harvest rotation interval (Bunnell et al., 2002b). Green tree retention is currently the primary method employed to provide for the recruitment of future snags, but the efficacy of this approach in providing future snags is extremely variable as many retained green trees fall victim to blow down and the timing of tree death is unpredictable (Keeton and Franklin, 2004; Rosenvald and Lõhmus, 2008; Zenner, 2000). (5) Fungal inoculation may simulate more natural processes and accelerate wildlife-beneficial decay compared to creating simulated snags by tree topping (Huss et al., 2002; Jackson and Jackson, 2004). Therefore, further investigation of the various approaches and potential benefits of establishing wood decay and the delayed creation of snags through fungal inoculation of live trees should remain a priority for the potential enhancement of forest management techniques (Filip et al., 2011).

Our research implements and evaluates a proposed technique of introducing wood-decaying fungi (*Fomitopsis pinicola*) into selected live trees to encourage habitat use by woodpeckers (e.g., *Picoides* spp.) and secondary cavity users in northwestern coniferous forests (Huss et al., 2002). Specifically, we isolated and cultured fungi taken directly from woodpecker nesting 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, $n = 320$).

Here, we report the results of revisiting all inoculation sites and specifically (1) summarize the condition of treatment and control trees; (2) quantify the presence of visible fungal growth in the form of conks or mycelia present in 2006, 8–9 years after treatment; (3) assess if there was any woodpecker use of inoculation trees as evidenced by nesting or foraging excavations; and (4) report our results from random sampling of two trees at each of 10 randomly selected sites to determine if *F. pinicola* and other fungal species were present.

2. Methods

2.1. Study area

Inoculation stands were identified by working with timberland managers of cooperating landowners in 1997 and 1998, which included Rayonier, Washington Department of Natural Resources, Weyerhaeuser, Port Blakely Tree Farms L.P., I.P. Pacific timberland, Inc., and Hancock Timber Resource Group. All inoculation clusters (each including 10 inoculated trees) are in western Washington in Clallam, Grays Habor, Jefferson, Lewis, and Pierce counties (Fig. 1). All study stands were dominated by western hemlock (*Tsuga heterophylla*), Douglas-fir, or both species were co-dominant. Sitka spruce (*Picea sitchensis*) was also present in many of the forest stands inoculated.

All study areas were comprised of state or private lands managed primarily for timber production (Fig. 1). Most inoculation clusters (67.8%; $n = 33$) 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 clearcut to >100 year old. Habitats adjacent to the inoculation sites generally consisted of small forest stands (2–40 ha), often bordered by areas which had been clearcut within the past 5 year.

2.2. Experimental design

We developed an experimental design to test the effects of tree species (Douglas-fir or western hemlock), age/size classes of trees (approximately 50 years old, 30–45 cm dbh versus approximately 70 years old, 40–60 cm dbh), and the density of available snags (>7 or <7 snags ha^{-1}), and on the resulting use of inoculated trees by woodpeckers. Stand age and mean tree diameter data were derived from forest stand data provided by the landowner. If the site inspection of the candidate revealed that many trees were of ages or diameter sizes not consistent with forest stand data, we rejected the site for further consideration as an inoculation site. Density of snags was initially assessed by visual inspection and placed in one of two classes (i.e., large snags conspicuously present or large snags mostly absent). To confirm initial classifications, we counted all snags (>50 cm dbh) in a 0.202 ha circular plot centered on the candidate inoculation site. In most cases, inoculated and control clusters (each including 10 trees) were located in the same forest stand. In some cases, stands were either not large enough to accommodate two clusters of experimental trees or the structure varied within the stand. In these cases, we located the control cluster in a nearby stand. When experimental and control clusters were placed in separate stands, we made an effort to use stands

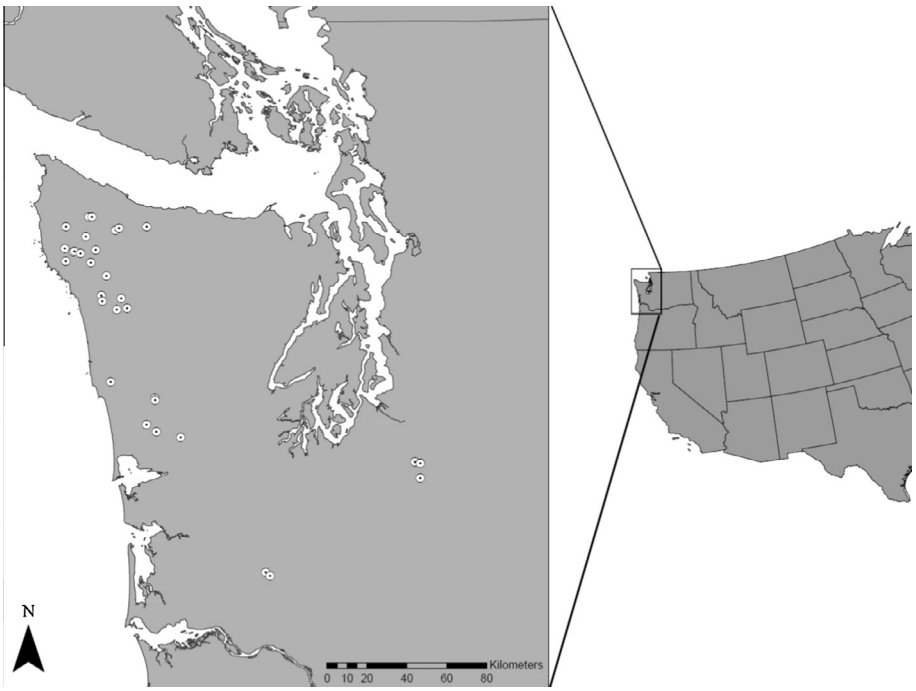

Fig. 1. Locations inoculation tree clusters (each with 10 fungal inoculated and/or control inoculated trees) on private and state managed forest stands in western Washington, USA.

of similar characteristics (i.e., same dominant tree species, age, snag density). This experiment was set up in a hierarchical fashion to enable us to examine these effects independently and the interaction of these factors during long-term monitoring (see Huss et al., 2002) and required the inoculation of 320 experimental trees and 320 control trees.

In 1997 and 1998, we completed inoculation of 650 trees with the fungus *F. pinicola*, fulfilling our original proposed experimental design, including the inadvertent inoculation of one additional treatment site of 10 trees; resulting in a total of 330 experimental trees and 320 control trees. A total of 24 stands, involving all living trees, were inoculated in 1997. Fifteen of these stands were on Rayonier lands and 9 were on 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 (Rayonier). One of the Rayonier sites, a hemlock stand in Jefferson County, is now owned by the Hoh River Trust.

In 1998, we inoculated living trees in 10 Douglas-fir forest stands. Four of these stands are now owned by the Hancock Timber Resources Group in Pierce County, and 5 are located on Weyerhaeuser land in Lewis County. The remaining stand is located in Grays Harbor County and is currently owned by Fruit Growers.

2.3. Preparation of wooden dowel spawn

Dowel plugs (12.8 cm long) were cut from 2.24 cm diameter hardwood. A 0.96 cm diameter hole was drilled down the center of each dowel using an auger drill bit to create an axial channel. Preparation of wooden dowel spawn is detailed in Huss et al. (2002). In brief, after sterilization the dowels were inoculated with mycelium of *F. pinicola* (a brown-rot fungus originally isolated from basidiocarp tissue and grown as pure laboratory culture) collected off of dead wood from 2 trees (designated 36A and 64A, respectively) that contained hairy woodpecker (*Picoides villosus*) nests in 1996 located in Clallam County; isolates that had been grown previously on 2% malt extract agar. Material was incubated at room temperature for 2–3 mo and growth was monitored.

Inoculations of trees were made by climbing approximately 8 m (to the height of approximately 2 standard errors lower than the mean height of woodpecker nests; mean height = 10.9 ± 2.2 m [SE × 2], $n = 57$ hairy woodpecker nests; JCB unpubl. data) up a Douglas-fir or western hemlock tree and a 2.24–2.56 cm diameter hole was drilled, 5–8 cm deep using an battery-operated drill. A manually-operated brace was then used to drill out the remaining hole to a depth of 13–15 cm. A single blank or inoculated dowel (for further description on methodology for preparation of blank and inoculated dowels see Huss et al., 2002) was inserted into the hole and a piece of PVC tubing (7.70 cm long × 2.56 cm in diameter) was inserted in the hole with the dowel. About 5 cm of the tubing remained outside the entrance of the hole to prevent the tree from healing over the wound. Holes were made on the north sides of trees, which was the orientation where woodpeckers most often excavated their nests (61.5%, $n = 57$; JCB unpubl. data) in western Washington. Fungal inoculations were made using dowels impregnated with cultures 36A and 64A of *F. pinicola*.

2.4. Inspection of inoculation trees

Field work was conducted in July and August 2002 and 2006. We attempted to find and visit all inoculated trees in 2002 and all trees in 32 of 34 inoculated clusters in 2006 (excluding those completely cut prior to 2002). We carefully inspected the entire tree, with special attention given to the area within 1 m of the PVC tube where trees were treated with either inoculated or control dowels. We recorded the presence of any fungal conks growing in the PVC tube or within 1 m of the inoculation site and identified species based on external characteristics. Using binoculars, we also inspected the interior of each PVC tube for the presence of fungal mycelia. The presence of mycelia appeared as a dirty white mass in PCV tubes and was easily discernable with 8× or 10× binoculars

from the ground. For several selected tubes with observed fungal growth from the ground, we climbed the tree and inspected the tubes to confirm that fungal mycelia were growing within the tube. 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 relative to the dimensions of the PVC tube. 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 analyses. We re-marked each tree with tree-marking paint; we used orange for treatment trees and yellow for controls.

2.5. Collection and processing of wood samples

We randomly selected 10 treatment and 10 control clusters originally inoculated in 1997 or 1998 for collection of wood samples in 2006. From these clusters, we collected samples using an increment borer (2 samples per tree) from 2 randomly-selected trees to determine if the desired fungus was established. Core samples were taken at the point of inoculation (i.e., point 2–5 cm below the PVC tube or associated wound if the PVC tube was no longer present) and 30 cm below the point of inoculation. We acknowledge that sampling with an increment borer in 2 locations on a tree did not allow us to measure the volume of decay and limits any conclusions related to the extent of the decay column. In total, we collected 80 wood samples from 40 trees. Two samples from a single tree (i.e., tree No. 14 from cluster T97-16) were not processed as they were misplaced during transportation to the lab for processing, reducing the sample size from $n = 80$ to $n = 78$.

We processed wood samples using standard lab and fungal culturing techniques (Huss et al., 2002). To increase the probability of isolating fungi, we made 6 attempts to isolate fungi from each wood sample ($n = 40$ from 20 control trees; $n = 38$ from 19 Treatment Trees) on the primary isolation medium (Huss et al., 2002). We then incubated cultures at room temperature and periodically inspected the colonies that grew. We prepared tease mount slides with fungal material grown suspended in lactophenol with cotton blue (Larone, 1995) and examined these slides under a microscope. We then identified fungal isolates to the lowest taxonomic level possible (i.e., genus) or category based on microscopic characteristics (Huss et al., 2002). Cultures that we preliminarily identified as *F. pinicola*, the fungus used in the original inoculation, were processed using vegetative-compatibility analysis to confirm whether the specific culture originally inoculated into a tree was still present and viable in experimental trees (Huss et al., 2002; Filip et al., 2011).

2.6. Data analysis

We conducted a series of comparisons using Generalized Linear Mixed Models (GLMM) with a logit link function and assuming a binomial distribution (Littell et al., 2006) based on data collected during 2006 inspection of inoculated trees. This allowed us to assess the efficacy of the inoculations and the response by woodpeckers 9 and 8 year after the trees were originally inoculated in 1997 and 1998, respectively. We treated forest stand and the treatment by forest stand combinations as random effects to account for the potentially non-independent responses arising from spatial proximity. Our response variables were the presence or absence of fungal growth (fungal conks, mycelia in PVC tubes, or either type of growth) and woodpecker excavations on each tree. We included treatment (i.e., control versus inoculation), tree species (western hemlock or Douglas-fir), and the treatment by tree species interaction as fixed effects. For all analyses, comparisons with $P < 0.05$ were considered statistically significant and results with $0.05 \leqslant P < 0.10$ were considered marginally significant.

3. Results

Over the years, trees in the study were lost to harvest and natural causes. Of the 650 trees originally inoculated with fungus ($n = 330$) or a sterile control ($n = 320$), 528 (81.2%) were alive and without damage during the 2006 field check ($n = 276$ with fungus, 83.6%; $n = 252$ control, 78.8%). Most, 52 trees at 3 different sites, were lost to harvest prior to 2002, and 20 more were lost between surveys in 2002 and 2006. We found 8 (2.4%) treatment trees blown down by wind and 6 (1.8%) had their tops broken off and were dead. Among control trees, 17 (6.7%) were blown down, 13 (5.0%) were broken off and had died, and 6 (2.4%) had died, but the cause was not determined. Four control trees had broken tops, but were still alive. We were unable to locate one control tree, which was probably lost when the bank of the Hoh River collapsed since our 2002 check.

The landowners responsible for inoculation stands originally agreed to retain the experimental trees. Ten treatment and 3 control trees at one RMZ site owned by Rayonier (T97-02) adjacent to Highway 101 near Forks were removed or topped at 8 m as required by the Clallam County Pacific Utility District to eliminate a potential road hazard. All treated and control trees were cut at sites 98-01 (Campbell Group, now Hancock Timber Resource Group), 98-07 (Weyerhaeuser), and 97-09 (Rayonier) because of forestry management personnel turnover or site location data were inadvertently omitted from company computer-based lists of protected sites. The remaining 528 inoculated trees (81.2%) that were alive and undamaged in 2006 constitute the sample in the analyses that follow.

Significantly more treatment trees (0.200) had *F. pinicola* conks than control trees (0.004; Table 1). Conks near inoculation sites on treatment trees were all identified as *F. pinicola* mostly growing directly under the PVC tube, but several were immediately to the right or left of the tube and some grew directly out of the tube. There were no significant interactions between inoculation treatment and tree species for proportion of trees with mycelia, or either conks or mycelia ($F_{1.48} = 0.06$, $P = 0.80$; $F_{1.54}$ 2.82, $P = 0.10$,

Table 1
Proportion of trees with fungal growth in 2006 on trees inoculated with *Fomitopsis pinicola* ($n = 275$) and control trees ($n = 251$) in western Washington during 1997–1998.

Type of growth	Observed prop. of treated[a]	Observed prop. of control[a]	*F*	*P*	Odds ratio	95% CI
Conks	0.200	0.004	15.22	<0.001	61.900	7.434–515.399
Mycelia	0.073	0.012	3.79	0.058	4.498	0.947–21.364
Conks or mycelia	0.265	0.016	27.90	<0.001	22.895	6.980–75.098

[a] Observed proportion reported; statistical analysis was based on the Generalized Linear Mixed Models least-squares means (Littell et al., 2006).

Table 2
Proportion of trees with fungal growth in 2006 on western hemlock (n = 150) and Douglas fir trees (n = 125) inoculated with *Fomitopsis pinicola* in western Washington during 1997–1998. Data analyzed using Generalized Linear Mixed Models (Littell et al., 2006).

Type of growth	Observed prop. of hemlock[a]	Observed prop. of fir[a]	F	P	Odds ratio	95% CI
Conks	0.313	0.064	12.34	0.002	6.929	2.236–21.466
Mycelia	0.093	0.048	1.02	0.322	2.554	0.380–17.161
Conks or mycelia	0.393	0.112	12.75	0.001	5.894	2.127–16.333

[a] Observed proportion reported; statistical analysis was based on the Generalized Linear Mixed Models least-squares means (Littell et al., 2006).

Table 3
Comparison of the frequency of fungal growth observed in inoculated (treatments and controls) stands with large trees (>37 cm DBH at the time of inoculation, n = 278) and stands with small trees (<37 cm DBH at the time of inoculation, n = 248) in western Washington during 1997–1998.

Type of fungal growth	Prop. of large trees	Prop. of small trees	F	P
Conks	0.094	0.121	0.40	0.535
Mycelia	0.065	0.022	1.65	0.205
Conks or mycelia	0.151	0.141	0.04	0.845

respectively). This demonstrates that the inoculation technique of impregnated wooden dowels was vital to the successful introduction of *F. pinicola* to treated trees, and the presence of a small open wound alone in control trees did not facilitate infection of this fungal species. We also observed more mycelia within the PVC tubes of treated trees (0.073) than in control tubes (0.012; Table 1), although this difference was marginally significant. Overall, the presence of obvious fungal growth (including either conks or mycelia) was greater in treated trees (0.265) than in control trees (0.016; Table 1). We did note subjectively that some *F. pinicola* conks at inoculation sites first observed during the 2002 check and that appeared vigorous and brightly colored, seemed to be degraded and the colors considerably faded during our 2006 visit.

Comparisons between the presence of fungal growth in western hemlock and Douglas-fir trees indicated that western hemlocks were more susceptible to *F. pincola* infection or that the fungus grew more quickly in this species than in Douglas-fir trees. Of the 275 treated trees surveyed, fungal growth (conks or mycelia) was observed in a significantly higher proportion (0.393; F = 12.75, P = 0.001) in western hemlocks than in Douglas-firs (0.112; Table 2). Western hemlock trees also showed a higher incidence of *F. pincola* conks (0.313; F = 12.34, P = 0.002) compared to Douglas-fir (0.064; Table 2). There was no difference between the proportion of western hemlock trees with mycelia only (0.093) and Douglas-firs with mycelia (0.048; F = 1.02, P = 0.322; Table 2).

We found no trends in the incidence of fungal growth (conks or mycelia) between treated and control trees at inoculation stands with large-diameter trees (0.151, n = 278) and those with small-diameter trees (0.141, n = 248; Table 3). Likewise, the frequency of conks was not significantly different in stands of large trees (0.094) compared to stands with relatively small trees (0.121, Table 3). We also did not observe any clear difference in the presence of mycelia in large-tree stands (0.065) as compared to small-tree stands (0.022; Table 3). Thus far, these data indicated no difference in the efficacy of inoculating stands of relatively large (40–60 cm dbh) or small trees (30–45 cm dbh).

Seven live trees (2.6%) in treated and no live trees in control sites had woodpecker excavations associated with inoculation areas on trees, 8–9 years after inoculations. These excavations appeared to be foraging holes (>3 cm in diameter), but some could have been nesting cavity starts, and based on the large fracturing of wood were likely made by pileated woodpeckers (*Dryocopus pileatus*). Further, we recorded no excavations in these specific trees when previously surveyed in 2002, 4–5 years after initial inoculation. There were no differences in frequency of sapsucker (*Sphyrapicus* spp.) foraging holes on live and dead trees in control versus treatment groups, but there were more live western hemlock (0.029) than Douglas-fir trees with sapsucker foraging (0.012), although this difference was marginally significant (F = 3.68, P = 0.060; Table 4). For woodpecker excavations and sapsucker foraging combined, there was a significantly greater proportion of foraging present (0.062) at inoculation relative to control trees (0.012; F = 7.07, P = 0.01; Table 4, Fig. 2). However, there was no difference (F = 0.40, P = 0.533) between proportion of western hemlock (0.042) and Douglas-fir trees (0.034) with woodpecker excavations or sapsucker foraging (0.035 and 0.004, respectively; F = 3.68, P = 0.60; Fig. 2) and no inoculation treatment by tree species interaction ($F_{1,54}$ = 0.05, P = 0.819). Although woodpecker excavations were limited to relatively few trees 8–9 years after inoculation, none of these trees had excavations when last surveyed in 2002 (4–5 years after inoculation). Because of the relatively low incidence of woodpecker excavation and foraging sign, it was not informative to statistically examine the potential influence of snag density related to woodpecker use.

Regardless of wood sample source, a plethora of fungi and other microorganisms were found occupying the wood samples (Appendices A and B). 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 was sufficient to allow a host of organisms to exploit this particular niche. Among the control group that were inoculated in 1997 and 1998 with sterilized "blank" wooden dowels, fungi were isolated from all 40 samples of wood collected, although the types of fungi isolated varied from tree to tree. The most common genus cultured from control group was

Table 4
Proportion of live trees with sapsucker foraging holes and woodpecker excavations in 2006 on trees inoculated with *Fomitopsis pinicola* (n = 273) and control trees (n = 251) in western Washington during 1997–1998. Data analyzed using Generalized Linear Mixed Models (Littell et al., 2006).

Woodpecker use	Observed prop. of treated[a]	Observed prop. of control[a]	F	P	Odds ratio	95% CI
Sapsucker foraging	0.029	0.012	1.72	0.195	2.481	0.619–9.937
Woodpecker excavations	0.040	0.000	0.00	0.996	–[b]	–[b]
Excavations or sapsucker foraging	0.062	0.012	7.07	0.010	5.489	1.522–19.802

[a] Observed proportion reported; statistical analysis was based on the Generalized Linear Mixed Models least-squares means (Littell et al., 2006).
[b] The absence of excavations on control trees precludes calculation of an odds ratio.

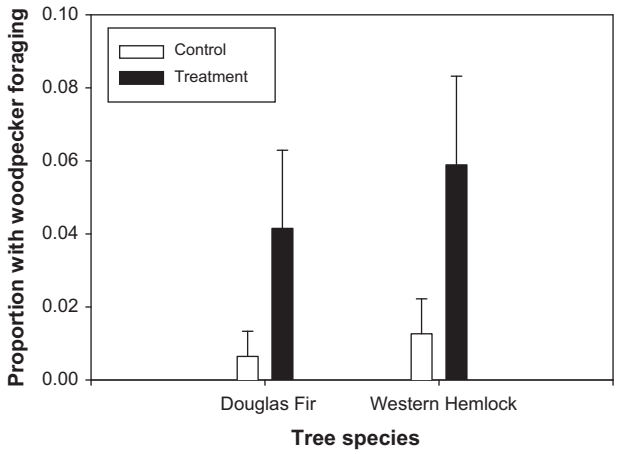

Fig. 2. Proportion of live control and treatment Douglas-fir and western hemlock trees with sapsucker foraging holes or woodpecker excavations during surveys in western Washington during 2006. The error bars are 1 standard error around the least-squares estimated proportion.

Table 5
Mean number of fungal and related taxa isolated and identified from wood samples from trees in control and treatment stands of trees.

Treatment group	Western hemlock (n = 6)[a]	Douglas-fir (n = 13)	Tree species pooled (n = 19)
Mean no. of taxa recovered at point of original inoculation	1.67	1.69	1.68
Mean no. of taxa recovered at 30 cm below point of inoculation	2.17	1.85	1.95
Mean no. of taxa recovered per tree	3.00	3.23	3.16
Control group	**Western hemlock (n = 10)**	**Douglas-fir (n = 10)**	**Tree species pooled (n = 20)**
Mean no. of taxa recovered at point of original inoculation	1.40	1.80	1.60
Mean no. of taxa recovered at 30 cm below point of inoculation	1.50	1.80	1.65
Mean no. of taxa recovered per tree	2.30	3.00	2.65

Trichoderma spp. that occurred in at least 50.00% of the trees sampled (70.00% of western hemlock, n = 10; 30.00% of Douglas-fir, n = 10). Overall, we found 16 different types of fungi and related microorganisms in wood samples from the control trees (Appendix A). The mean number of fungi isolated per wood sample regardless of tree species (western hemlock and Douglas-fir) was 2.65 taxa per wood sample (Table 5).

Likewise, fungi were isolated from all 38 samples of wood collected in 2006 from trees within the treatment group that were inoculated in 1997 and 1998. The most common genus cultured from treatment group was *Penicillium* spp. that occurred in at least 42.11% of the trees sampled (16.67% of western hemlock, n = 6; 53.85% of Douglas-fir, n = 13). In treatment trees, we found 18 different types of fungi and microorganisms in wood samples (Appendix B). The mean number of fungal types isolated per wood sample regardless of tree species (western hemlock and Douglas-fir) was 3.16 taxa per wood sample (Table 5).

We found 11 taxa (e.g., *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium*, and *Trichoderma*) in wood samples from both control and treatment trees. Five unique taxa were detected in the control group trees, while 7 unique taxa were identified in Treatment Trees (Appendices A and B).

Cultures retrieved from wood samples suspected to be *F. pinicola* found in wood samples were tested using vegetative compatibility group (VCG) analysis to determine if the fungi originally introduced in treatment trees was the same fungus inoculated in the tree 8 or 9 years previously. With this test, if a zone of demarcation develops between two colonies, this indicates the two colonies represent different strains or genetically distinct individuals. Conversely, if other colonies are genetically similar, the two colonies may be classified as belonging to the same VCG (Mounce, 1929; Worrall, 1997). Collected strains from wood samples were tested with three distinct, but indigenous strains of *F. pinicola* (43A, 36A, and 64A), only one of which was inoculated into each treatment tree. Seven cultures demonstrated the organism retrieved in 2006 was the same inoculation strain originally introduced in those 7 trees 8–9 years previously (36.8%, n = 19 treatment trees).

Eight cultures from 7 different trees demonstrated strong zones of demarcation when paired with all three tester strains suggesting that these represented a genetically-unique strain of *F. pinicola*. These samples were retrieved from 4 control trees (20.00%, n = 20) and 3 treatment trees (15.79, n = 19). *F. pinicola* or a closely-related fungal species found natural avenues for invasion into the wood of live experimental trees (both treatment and control trees), most likely via airborne spores entering the wounds of created by the inoculation process (Filip et al., 2011).

Other VCG pairings revealed the presence of fungal taxa other than *F. pinicola*. In 10 cultures, tester strains grew and overtook the fungal culture retrieved from the wood samples, indicating the organism was not related to *F. pinicola*. The fungi were identified as *Mucor* spp. in 2 of these cases; other cultures could not be identified to genus. In 7 cultures, the organisms from the wood samples were able to inhibit the growth of all 3 tester strains. Three of these cultures were identified as *Trichoderma* spp. based on diagnostic characteristics; the remaining cultures could not be identified to genus.

4. Discussion

The ultimate goal of providing structural enhancements in managed forests is to foster the full range of natural ecological processes, to the extent possible, found in a healthy forest and to support sustainable timber production. Current research has provided credible evidence that some of the ecological functions related to wood decay processes may be provided in managed forests through topping trees (generally by mechanical means) in western coniferous forests (Bull and Partridge, 1986; Kroll et al., 2012a). However, this approach really only simulates one form of natural tree death, top rot, and does not encompass the full range of ecological interactions related to the multitude of organisms dependent on decaying wood, cavity-excavating and secondary cavity-using species, and their associated ecological interactions. Another important process related to wood decay is fungal infection of a living bole typically due to some sort of wound that leads to fungal rot of sapwood and heartwood, creating a pocket of decay suitable for cavity excavation and surrounded by sound wood (Jackson and Jackson, 2004; Rose et al., 2001). This type of compartmentalization of fungal rot on living trees provides both foraging habitat for woodpeckers and a variety of other forest organisms, and importantly, very durable and long-lasting cavities for many species of wildlife (Jackson and Jackson, 2004; Remm et al., 2006). Such live, decadent trees in the forest are also likely important in providing long-lasting wood decay habitat and the future provision of snags (Bunnell et al., 2002b; Rose et al., 2001).

The technique of fungal inoculation to initiate wood decay has often been compared to tree topping as a means to create rapid tree death and cavity habitat for primary cavity excavating birds in managed forests (e.g., Brandeis et al., 2002). In this context, and based on limited assessments, tree topping performs much better than artificial fungal inoculation in the short-term as the former approach results in rapid tree death and use by primary cavity excavating species typically in less than 5 years (Arnett et al., 2010; Bull and Partridge, 1986; Chambers et al., 1997; Hallett et al., 2001; Walter and Maguire, 2005). However, we suggest this may not be the most appropriate context in which to evaluate the efficacy of fungal inoculation techniques in terms of forest wildlife and ecosystem function enhancement. Rather, we propose that the establishment of natural wood decay processes and the facilitation of long-term deadwood biota usage on live trees with compartmentalized fungal rot would be a more suitable assessment of the success of this proposed forest management approach. Specifically, do fungal inoculations provide long-term wildlife habitat benefits compared to the relatively rapid tree death and the provision of suitable snag habitat of topped trees?

Our data showed that trees treated with fungal inoculations had significantly higher incidence of observable sign of wood decay (26.5%; conks or mycelia) compared to trees receiving blank inoculations (1.6%; Table 1). Importantly, these results demonstrated the inoculation technique that we employed enhanced the rate of fungal infection over just the process of creating a minor wound in the tree (i.e., blank inoculation with insertion of the PVC tube). Our results were somewhat different than observed by Brandeis et al. (2002), who found that artificial inoculation did not significantly affect the numbers of visible fruiting bodies on newly created snags compared to Douglas-fir snags created by injection, girdling, and topping that were not inoculated. There may not have been enough time elapsed (4 year) for conk formation since inoculation in the Brandeis et al. (2002) study. These workers did not report the incidence of conks or mycelia on the 9 live control trees inoculated simultaneously with 4 species of fungi, results that may have been more relevant to our study. Further, Bull and Partridge (1986) found that trees topped by chainsaw had greater use by woodpeckers after 5 years than both (1) trees topped with dynamite and inoculated with *Dichomitus squalens* and (2) girdled trees inoculated with *Dichomitus squalens* and *F. pinicola*. Collectively, these results suggest that fungal inoculation is probably not useful to employ in conjunction with some other snag-creating techniques (i.e., tree topping, herbicide injection). The relatively rapid death caused by these techniques and the natural exposure to attacking bark beetles and the ambient presence of spores and hyphae are probably satisfactory to quickly and naturally infect snags with local fungi. However, these collective results also suggest that artificial inoculation with the appropriate fungal taxa (i.e., native and non-pathogenic wood-decay fungi) and target tree species may potentially create a compartment of fungal decay in live trees.

The efficacy of our fungal inoculation experiment also was far from ideal as about 60% of treated trees never showed any visible signs of fungal growth. Also, we observed some apparent decline in the vigor of selected *F. pinicola* conks between our 2002 and 2006 evaluation visits. Furthermore, based on a partial survey of 62 of our inoculated trees in 2011, the frequency of trees with visible conks declined from 31.8% to 4.8% for that subsample (D. Varland, unpubl. data). We speculate that about 9 years after inoculation, the trees' defenses may have overwhelmed the introduced fungus and stabilized the extent of the compartmentalized wood decay; however, we did not cut and dissect trees to confirm this possibility.

Importantly, our data showed that western hemlock trees had a significantly higher incidence of both fungal conks (0.313) and mycelia (0.093) compared to Douglas-fir (0.064 and 0.048, respectively; Table 3). These results suggested that Douglas-fir, likely related to the hardness of wood or its ability to defend against fungal infections, were more resistant to inoculations of *F. pinicola* than western hemlock. Filip et al. (2004) did have some success inoculating 7 Douglas-fir trees with rifles and shotguns resulting in a mean decay area of 68.7 cm^2 with *Phellinus pini* or *Fomitopsis cajanderi* 5 years after treatment. However, they observed there was no apparent difference in internal decay area between sterile and viable inocula, but their sample size was limited to 11 treated trees of each fungus and 12 control trees. Based destructively sampling 75 trees involving 4 tree species and 8 fungi species in various treatment situations, Filip et al. (2011) recommended that *F. cajanderi* may cause the most decay in Douglas-firs relative to other species they used for inoculation, including *F. pinicola*. However, this recommendation was based on a sample of 2 trees that showed a mean maximum decay width of 13.5 cm and volume of 19,709 cm^3 or 9.4% of the bole volume. Based on our results, we concluded that *F. pinicola* inoculations were more effective on western hemlock ($\geqslant$39% displaying evidence of fungal growth) than Douglas-fir ($\geqslant$11% with visible fungal growth).

These findings may be related to patterns in woodpecker use of nest trees in our study area. Specifically, our initial surveys of woodpecker nests in the same area of Washington in 1996–1998 found the majority of active nests in western hemlock trees (73%, n = 57 nests; JCB, unpubl. data) rather than Douglas-fir, a co-dominant species in most of the surveyed stands. A companion survey of hairy woodpeckers in 2000–2001, also in the same study area, showed the same trend, with 80% (n = 45) of the nests were in western hemlock (Ripper, 2002). It is unknown at this time whether woodpeckers select western hemlock for cavity excavation because it is more susceptible to fungal rot in general than Douglas-fir or for other reasons.

Although woodpecker use (including sapsucker use) was limited to relatively a few trees 8–9 years after inoculation, there was significantly more use of trees treated with viable inocula (6.2%) compared to control trees that received sterile dowels (1.2%; Fig. 2). In fact, all 7 trees documented with woodpecker excavations occurred in treatment trees and none in control trees. Similar to results reported by Filip et al. (2011), our data that decay formation is especially slow in live trees and may require many years to achieve sufficient decay to be suitable for woodpecker excavation. We suggest that the 4–5 year interval between inoculations and our first monitoring check of treated trees was probably too early for woodpeckers to initiate substantial excavations, given that extensive heart-rot is necessary for cavity creation (Conner et al., 1976; Filip et al., 2011). Importantly, the finding of a significant difference of woodpecker and sapsucker use of treated trees versus control trees indicates the inoculation approach can provide useable woodpecker habitat and this response is not simply due to wounding of the tree by the inoculation process. This result is somewhat contrary to some previous evaluations of the inoculation technique that showed no trends in woodpecker responses between inoculated and control trees (Brandeis et al., 2002; Filip et al., 2004, 2011). However, both of these studies only monitored inoculation sites 4–5 years after treatment. Even though we showed a significant response by woodpeckers to inoculation treatments, our response rate was still relatively low 8–9 years after introduction of the fungus, suggesting further adjustments in the inoculation approach are probably necessary to better address desired management outcomes.

Our findings regarding the efficacy of fungal inoculations and the biased use of western hemlock may be integrated with findings on hairy woodpecker habitat use in the same study areas. Ripper et al. (2007) determined that hairy woodpeckers used 61–80 year forest stands disproportionately within their home ranges, and used 41–60 year stands in proportion with their availability. These

workers also documented that hairy woodpeckers underuse younger successional stages (6–40 year; Ripper et al., 2007). This information, coupled with our findings regarding the susceptibility of western hemlock trees to infection by *F. pinicola* and the similarity in the presence of fungi between old and young trees, suggests that fungal inoculations should be targeted towards mid-aged or older stands (>40 year). Furthermore, previous research in western Washington by Ripper (2002) suggested that hairy woodpeckers nest on or near the edges of clearcuts and >40 year stands.

Our results of the VCG tests demonstrated that fungal inoculations successfully introduced *F. pinicola* colonies in at least 36.8% of the trees in treatment clusters. We emphasize that culturing wood samples collected with an increment borer has limited success in isolating all organisms actually present in treatment trees at or near the inoculation site. Therefore, unquestionably, our rate of successful inoculation and survival of the original inocula after 8–9 years in treated tree is much higher than 37%. However, we did not destructively sample and dissect trees and could not measure the volume of decayed associated with inoculations.

We have also documented that an ensemble of endophythic organisms have worked their way into the wounds created by the inoculation process of trees both in the control and treated groups (Appendices A and B). The process of drilling a hole and the placement of a PVC tube to prevent the bark from sealing over the wound allowed indigenous basidiomycetes (including *F. pinicola*) and other fungi to infect the experimental trees. The trees treated with viable inoculums not only were infected with the selected *F. pinicola* strain, but also supported slightly more taxa per treated tree (mean = 3.16) and a slightly greater diversity of decay organisms (18 taxa identified) than the control trees (mean = 2.65 taxa; 16 total taxa). However, these trends were not statistically significant. The diversity of taxa we documented were similar to organisms isolated by Kiser et al. (2010) both in damaged Douglas-firs 14 years after commercial thinning and control trees. Interestingly, Kiser et al. (2010) found that *Penicillium* spp. was the most common fungi isolated (49.6% of the trees sampled) in Oregon and we isolated this taxa in 33.0% of all trees we sampled in Washington, and it was particularly frequent in treated Douglas-firs (47.8%). The most common taxa identified in our Washington study was *Trichoderma* spp., which was isolated from 35.9% of the 39 trees we sampled.

We estimated the cost of preparing a limited number of inoculated and uninocolated dowels based on materials (e.g., culture media, chemicals, autoclavable spawn bags, aluminum foil pans, hardwood dowels) and labor in our lab at approximately $8–9 per wooden dowel spawn for use in the field (Huss et al., 2002). If dowels were prepared in greater volume, this cost would be substantially reduced per dowel. Using our inoculation procedure, we estimate the cost for field inoculations (based on $200/day labor and 80 km [$0.353/km] for travel/day of work) of one dowel would cost $14–15 per live tree. If multiple infected dowels were used per tree as suggested by Filip et al. (2011) the cost would probably be in the range of $20–25 per inoculated tree. These costs for inoculation compare quite favorably to topping trees by chainsaw at an average of $30 per tree (1990s cost estimate; Chambers et al., 1997). Further, the long-term wildlife use benefits (foraging and cavity nesting use) of inoculated trees may exceed those of topped snags (Jackson and Jackson, 2004; Rose et al., 2001; Welsh and Capen, 1992), but more long-term monitoring of both management enhancements are needed.

5. Research and management implications

Based on the results of our work, we recommend that experimental fungal inoculation should be continued, adjusted, and evaluated further in managed forest landscapes. Importantly, inoculation approaches should be assessed on how they contribute to the replacement of natural wood decay processes and associated ecological functions beyond those benefits provided by the structural enhancements of topping trees. Specifically, what are the benefits of creating live decadent wildlife trees with compartmentalized fungal rot? Will such trees facilitate the excavation of long-lasting durable cavities and be used by secondary cavity-dependent species including bats? What is the chronology of the wildlife benefits of inoculated trees compared to snags created by topping or girdling and retained green trees? How often and how long are such trees used by foraging birds and wildlife?

Although we showed some significant success in introducing decay fungus to western hemlock and Douglas-fir trees and a positive woodpecker response compared to control trees 8–9 years after inoculation, our results were far from ideal. Thus, the inoculation approach needs to be further refined. We recommend that trees be inoculated with live fresh cultures obtained by non-destructive sampling and isolation from trees within the same landscape in which any inoculation program is planned (Filip et al., 2011). Further, selection of the species used on the trees species of interest should be based on evidence that the fungus causes a reasonable degree of decay and is associated with primary-cavity excavator nests (Huss et al., 2002). We recommend *F. pinicola* for inoculation into western hemlock and for other trees species with similar wood hardness and characteristics in the Pacific Northwest, but not for the inoculation of Douglas-fir. Based on limited results reported by Filip et al. (2011), *F. cajanderi* or *Fumaria officinalis* might be a useful species to evaluate further for inoculation into Douglas-fir. Importantly, inoculations should be made at least at the height of the lower live crown of trees, but higher would probably be more effective as decay columns grow downward more rapidly with the force of gravity (Jackson and Jackson, 2004). Probably, heights of 8–15 m would be satisfactory.

We used a single inoculation and demonstrated effective establishment of a decay column and a positive response by foraging woodpeckers. Filip et al. (2011) recommends using 3 drill inoculation sites at the same height within 30 cm on each tree, which would facilitate the coalescence of individual decay columns to form a larger decay compartment in shorter time (5–10 years). Placing multiple drill holes with the same orientation, but spaced out vertically ($\leqslant$30 cm apart) would also be worth experimentation. The efficacy of the orientation of inoculation sites is still unclear; however, we would initially recommend the placing the inoculation sites with the same orientation trends seen in local populations of primary-cavity excavators. Based on our data, we recommend that inoculation sites be oriented northeast to northwest in the Pacific Northwest (see Jackson and Jackson, 2004 for review of patterns in other parts of the US).

Other approaches that should be evaluated include drilling holes and placing at least a 7.7 cm plastic tube inserted hole with or without a blank dowel (see Filip et al., 2011). Further, as the vigor of some of our visible fungal growths seemed to decline 4 or more years after inoculation, we recommend assessment of some combined approaches including inoculation of girdled and half-girdled trees (Hallett et al., 2001). In addition, we recommend that experimental and management inoculations of fungi be implemented in groups or clusters of trees that also could include retained snags and green trees. This approach would be operationally effective (Arnett et al., 2010; Kroll et al., 2012b) and enable efficient long-term monitoring. Further, there is evidence that snags and decadent wildlife trees often exhibit clumped patterns in natural old-growth forests (Marcot, 2002; Ohmann and Waddell, 2002; Rosenvald and Lõhmus, 2008). Moreover, experimental evaluations of fungal inoculation approaches as advocated above should be monitored periodically over long-term periods

Table A1
Fungi recovered from wood samples obtained from trees within the control group for experimental inoculation in western Washington, USA.

Category or taxon recovered from wood samples	Western hemlock $n = 10$				Douglas-fir $n = 10$			
	Point of inoculation		30 cm below point of inoculation		Point of inoculation		30 cm below point of inoculation	
	Incidence	Percent	Incidence	Percent	Incidence	Percent	Incidence	Percent
Acremonium or *Fusarium* sp. (only microconidia present)	1	10.00	1	10.00	1	10.00	0	0
Amoeba (free-living cells and cysts)	0	0.00	0	0.00	1	10.00	0	0.00
Aspergillus sp.	1	10.00	0	0.00	0	0.00	0	0.00
Basidiomycetes (hyphae with clamp connections, chlamydospores, and basidia present)	1	10.00	0	0.00	3	30.00	3	30.00
Basidiomycetes (hyphae with clamp connections; chlamydospores sometimes observed)	0	0.00	2	20.00	2	20.00	3	30.00
Bipolaris sp.	0	0.00	0	0.00	1	10.00	0	0.00
Chrysoporium sp.	0	0.00	0	0.00	0	0.00	2	20.00
Cladosporium sp.	0	0.00	0	0.00	0	0.00	1	10.00
Dematiaceous (darkly pigmented) septate filamentous fungus – lack of distinguishing morphological characteristics for conclusive diagnosis (with and without asexual spores in the form of condidia)	1	10.00	0	0.00	2	20.00	0	0.00
Eppicoccum sp.	0	0.00	1	10.00	0	0.00	0	0.00
Hyaline septate (colorless or lightly colored; sometimes light gray blue, brown, to red diffusible pigment present) filamentous fungus – lack of distinguishing morphological characteristics for diagnosis (with and without asexual spores in the form of conidia and chlamydospores)	5	50.00	3	30.00	4	40.00	4	40.00
Mucor sp.	0	0.00	0	0.00	0	0.00	1	10.00
Penicillium sp.	0	0.00	1	10.00	3	30.00	1	10.00
Trichoderma sp.	6	60.00	5	50.00	0	0.00	3	30.00
Ulocladium sp.	0	0.00	2	20.00	0	0.00	0	0.00
Unidentified yeast	0	0.00	0	0.00	1	10.00	0	0.00

Table B1
Fungi recovered from wood samples obtained from trees within the treatment group for experimental inoculation in western Washington, USA.

Category or taxon recovered from wood samples	Western hemlock $n = 6$				Douglas-fir $n = 13$			
	Point of inoculation		30 cm below point of inoculation		Point of inoculation		30 cm below point of inoculation	
	Incidence	Percent	Incidence	Percent	Incidence	Percent	Incidence	Percent
Acremonium or *Fusarium* sp. (only microconidia present)	1	16.67	1	16.67	1	7.69	2	15.38
Altermaria sp.	0	0.00	0	0.00	1	7.69	0	0.00
Aspergillus sp.	1	16.67	0	0.00	0	0.00	0	0.00
Aureobasidium sp.	0	0.00	0	0.00	0	0.00	1	7.69
Basidiomycetes (hyphae with clamp connections; chlamydospores sometimes observed)	2	33.33	2	33.33	5	38.46	0	0.00
Cladosporium sp.	0	0.00	1	16.67	0	0.00	3	23.08
Dematiaceous (darkly pigmented) septate filamentous fungus – lack of distinguishing morphological characteristics for diagnosis	1	16.67	0	0.00	0	0.00	0	0.00
Eppicoccum sp.	1	7.69	0	0.00	0	0.00	1	7.69
Fusarium sp. (asexual lunate macroconidia and ascocarps in the form of perthecia present)	0	0.00	1	16.67	0	0.00	0	0.00
Hyaline septate (colorless or lightly colored; sometimes light gray blue, brown, to red diffusible pigment present) filamentous fungus – lack of distinguishing morphological characteristics for diagnosis (with and without asexual spores in the form of conidia and chlamydospores)	0	0.00	4	66.67	6	46.15	6	46.15
Monilia sp.	0	0.00	0	0.00	1	7.69	0	0.00
Mucor sp.	0	0.00	0	0.00	0	0.00	2	15.38
Penicillium sp.	1	16.67	1	16.67	3	23.08	7	53.85
Phialophora sp.	0	0.00	0	0.00	2	15.85	0	0.00
Trichoderma sp.	1	16.67	1	16.67	3	23.08	1	7.69
Unidentified hyaline non-septate hyphae – lack of distinguishing morphological characteristics for diagnosis	0	0.00	1	16.67	0	0.00	0	0.00
Unidentified yeast	1	16.67	0	0.00	0	0.00	0	0.00
Verticillium sp.	2	33.00	1	16.67	0	0.00	1	7.69

(e.g., >20 years) and results should be adaptively integrated into future inoculation programs implemented in managed forests.

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Appendix A

Table A1.

Appendix B

Table B1.

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