USDA Forest Service
Forest Health Protection
Pacific Northwest Region
Portland, Oregon
August 2011

Seven- to 14-Year Effects of Artificially Inoculating Living Conifers to Promote Stem Decay and Subsequent Wildlife Use in Oregon and Washington Forests

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Summary

We destructively sampled 75 inoculated trees on 19 sites in 8 different geographical areas in Oregon and Washington. Tree species sampled included Douglas-fir, western larch, grand fir, and ponderosa pine. Inoculated fungal species sampled included *Fomitopsis cajanderi*, *F. pinicola*, *F. officinalis*, *Porodaedalia pini*, *Neolentinus lepideus*, *Echinodontium tinctorium*, *Stereum sanguinolentum*, and *Wolfiporia cocos*. After 7 to 14 years, there was relatively little decay associated with inoculated trees, and no conks or nesting cavities were found on any sampled trees associated with the inoculations. Despite the low level of internal tree decay and subsequent wildlife use, we believe that live-tree inoculations may be continued in Oregon and Washington but only after substantial modification. We provide discussion, recommendations, and future research needs regarding live-tree inoculations.

Introduction

Cavity-nesting birds require decayed wood in standing trees to excavate cavities for nesting. Some other bird species and mammals are dependent on these excavated cavities (Bull et al. 1997). Although dead or partially dead trees eventually decay and provide suitable material for cavity creation, living decayed trees with intact crowns remain standing much longer, thus providing suitable habitat for many more years than dead or partially dead trees. In some forest ecosystems there is a lack of large-diameter living trees with sufficient decay for some cavity-nesting birds to utilize. Artificial inoculation with decay fungi has been reported to be successful in causing decay in living trees with subsequent use by wildlife in some geographical areas and on some sites in the US.

Artificial inoculation of living loblolly pines in Texas and subsequent wood decay were first reported by Conner and Locke (1983). Nesting cavities in 14% of 60 artificially inoculated live western larch were reported by Parks et al. (1996, 1998) in NE Oregon. Fungal inoculation with rifle or shotgun also has been successful in causing decay after 5 years in living Douglas-fir in western Oregon (Filip et al. 2004) and British Columbia (Manning 2008). Although inoculation of living larch in NE Oregon resulted in stem decay and subsequent use by woodpeckers 6 years after treatment, similar successful inoculation and wildlife use in other living tree species in other geographical areas in the Pacific Northwest have not been adequately determined.

Fig.1 – A tree climber is drilling a hole to insert a colonized dowel into a tree bole about 30 ft. above ground.

Objectives of our project were to evaluate the amount of internal decay in four species of living conifers that were artificially inoculated with stem-decay fungi 7 to 14 years ago. Treated trees were Douglas-fir, western larch, ponderosa pine, and grand fir that were inoculated on four National Forests and lands managed by the Washington Department of Natural Resources**.** Specifically, we sampled a set of previously inoculated conifers by destructively sampling each tree and determining the following:

1) Presence and extent of internal decay associated with inoculated trunk sections
2) Species of fungi associated with the decayed wood
3) Type and extent of wildlife use such as foraging or nesting holes associated with inoculated trunks
4) Site, stand, and tree characteristics that may have contributed to the success or failure of wildlife use of inoculated trees

Methods

Tree Inoculations

Between fall 1996 and 2003, live trees, mostly Douglas-fir, western larch, ponderosa pine, or grand fir were artificially inoculated with one of eight species of decay fungi. Inoculated trees were located in National and State forests in Oregon and Washington. Methods included drilling 1 to 6 inoculation holes spaced at 2- to 10-ft intervals on one side of each tree bole (Parks and Hildebrand 2002). Selected decay fungi (primarily *Porodaedalia [Phellinus] pini, Stereum sanguinolentum, Neolentinus lepideus, Echinodontium tinctorium, Wolfiporia cocos, Fomitopsis cajanderi, F. officinalis,* and *F. pinicola*) were collected locally and grown on ¾-in. or 11/16-in. diam. by 4-in. long, hollow, hemlock dowels. Dowels were soaked overnight in distilled water and placed in autoclavable bags with

vermiculite, malt-dextrose broth, and distilled water. Bags and dowels were autoclaved for 35 min., cooled, and inoculated with a malt-sawdust-peptone broth culture of a single test-fungus species. Bags and inoculated dowels were stored at room temperature in the dark for a minimum of 6 months before use (Parks and Hildebrand 2002).

In the field, a fungal-colonized dowel was placed into a hole drilled at least 5 in. deep and 7/8-in. diameter about 15-80 ft above ground to optimize decay at the optimal height for most primary cavity-nesting birds (Fig. 1). In most trees, a 13/16-in. outside diameter, 4-in.-long plastic pipe was then inserted half way into each inoculation hole against the end of the dowel (Fig. 2). In some cases, larger diameter pipes or shorter pipes were used.

From 2002 to 2008 about 50 of the inoculated trees were sampled by climbing and increment boring to determine any decay development (Hildebrand 2009). Although decay was present in most of the inoculated trees sampled 5 to 8 years after treatment, there appeared to be little or no use by wildlife, especially no cavity creation (Woolley et al. 2007; Hildebrand 2009).

Tree Sampling

From August to November 2010, we destructively sampled 75 inoculated trees on 19 sites for presence of internal decay and subsequent wildlife use (Table 1). Wildlife use included foraging and nesting holes. We also examined trees for fungal conks near the inoculation holes. In addition, the following stand and site data were collected in the vicinity of the inoculated trees: 1) stand location, 2) aspect (N, NE, E, SE, S, SW, W, NW, N), 3) elevation (ft.), 4) topographical position (upper slope, mid-slope, valley-bottom), and 5) percent slope (nearest 10%).

Fig. 2 – An inoculated trunk section of a sampled Douglas-fir. Note the white mycelium on the colonized dowel (right) and the plastic pipe inserted partway into the drilled hole (left). Only a small amount of decay is shown here that is associated with the colonized dowel after 10 years.

For each inoculated tree, we recorded the following data before felling: 1) tag number, 2) tree species, 3) dbh (nearest 0.1 in.), 5) live-crown ratio (nearest 10%), 6) number of plastic pipes (inoculations), 7) pipe aspect(s), 8) number and species of bole conks, and 9) number of any nest cavities or foraging holes.

Inoculated trees were selected to avoid felling more than 2 trees/acre and felled as close to the ground as possible. Tree felling and bucking were done by a USFS-certified faller, and all safety procedures were strictly followed. After felling, we marked the side of the tree with a chainsaw line from about 5 ft. above the top pipe down along the side of all pipes to 5 feet below the last pipe. This facilitated dowel location in relation to decay after the wood disks were cut. Total tree height (nearest ft.) was recorded with a logger's tape. We recorded tree age and last 10-year radial growth (2 sides, nearest 1/20 in.) at the stump.

At each plastic pipe, we recorded the following data: 1) mean height(s) above ground (nearest ft.) and 2) tree diameter outside bark (nearest 0.1 in.).

To determine the amount of internal tree stain and decay, each tree was dissected into about 1-ft. sections from about 5 ft. above the top pipe to about 5 ft. below the last pipe or until stain/decay disappeared. The following decay estimates were recorded: 1) decay/stain column maximum diameter (nearest in.), 2) decay/stain length up the bole from hole (nearest in.), 3) decay/stain length down bole,

4) log diameter outside bark at upper end of decay/stain (nearest 0.1 in.), and 5) log diameter outside bark at lower end of decay. Multiple decay columns that merged were measured as one decay column. Because stain (incipient decay) and decay (advanced decay) were often difficult to separate in the field, there was no attempt to differentiate them. Where the maximum diameter of the decay was not measured from a circle but a square, rectangle, or asymmetrical, the shortest and longest measure across the decay was averaged for a mean maximum diameter.

Total volume of the bole (log) segment containing decay was calculated using Smalian's formula: V (vol.) = (A+a)/2*L where A = cross-sectional area (πr^2) of large end of the bole, a = cross-sectional area of small end of the bole, and L = length of the bole (nearest in.).

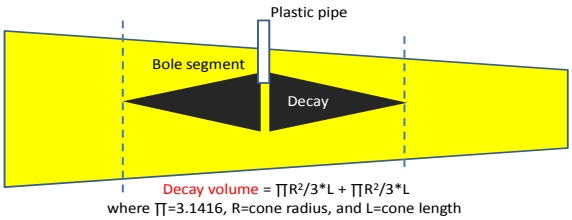

Fig. 3 – Diagram of a bole (log) section showing the typical shape of internal decay from one inoculation hole and plastic pipe per tree. Decay volume was calculated by summing the volumes of the two cones.

Decay columns resulting from inoculated dowels usually were widest at the point of inoculation and then tapered to a point above and below the inoculation hole, thus resembling two cones (Fig. 3). The volume of stained and/or decayed wood was calculated by summing the volumes of the cones above and below the pipe (inoculation point) using the equation for the volume of a cone: V = A/3*L where A = cross-sectional area (πr^2) of the decay column at its widest point, and L is the length of the decay column from the inoculation point to its end. For trees with more than one pipe or dowel, decay from each dowel often merged into one column or cylinder, and the volume of stain/decay was calculated as a cylinder and two end cones that contained all of the decay from the multiple inoculation points (Fig. 4). The cylinder represented the volume of decay between two or more pipes: V = A*L: where A = cross-sectional area (πr^2) of the decay column at the ends of the cylinder and L is the distance between the top and bottom pipes.

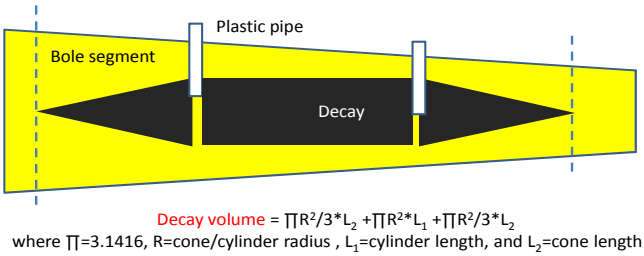

Fig. 4 – Diagram of a bole section showing the typical shape of internal decay from two or more inoculation holes and plastic pipes per tree. Decay volume was calculated by summing the volumes of the two cones and the cylinder.

Decay amounts for each tree were expressed as decay widths, decay volume per dowel, and percentage of bole-segment volume. Decay widths are easy to visualize relative to the size of decay needed for bird cavities. Decay volume per dowel is the total volume of decay associated with each infected dowel. If there was more than one dowel per log, then the entire decay volume was divided by the number of dowels to obtain the decay volume per dowel. Decay percentage is the proportion of the bole segment that was decayed. Larger-diameter logs would have a lower percentage of decay than smaller-diameter logs that would be associated with a colonized dowel. The decay width and volume values in Table 2 represent

the mean of all the trees in that class including trees with no decay.

At most field sites, at least three samples per tree (approx. 4x4x4 in.) of representative decay and stain were collected. A sample was taken at each decay column end and at the maximum diameter of decay, usually at the inoculation site, or at each pipe if more than one pipe was present. Samples were placed in plastic bags with a tag of stand location, tree number, and sample number. Samples were kept cool (40-50^{o}F) and returned to the lab for culturing and fungal identification.

Lab Procedures

To verify the species of decay fungi from sampled trees, wood chips were removed from decay areas of the sample trees and cultured on agar media in petri plates to isolate the causal fungi. To collect the wood chips for culturing, each wood sample was split with a sterile hatchet (Fig. 5). From the freshly split surface, a sterile 7/16 in. "plug cutter" mounted in an electric drill was used to extract wood samples for relatively stained but sound wood (Fig. 5). Each wood plug was then split into 5 or 6 pieces of about 1/8 in. The end pieces were discarded, and the three center pieces were placed into a petri plate containing one of two types of agar media.

Fig. 5 – In the lab, wood samples were split with a sterilized hatchet before the plugs were removed (below left). Plugs were drilled from samples of stained or sound wood and then sectioned into pieces for plating onto agar media (above).

For incipient decay, 1/8 to 3/16 in.-wide strips of wood with a triangular cross-section about 1 in. long were made with a sterile knife. Each strip was then cut into 3 pieces and placed into a petri plate containing the agar media. For advanced or crumbly decay, small chips of wood were removed with a sterile forceps, and 3 chips placed into each agar plate. None of the plated wood pieces were surface sterilized.

Wood chips from sampled wood sections were placed on two types of agar media: (1) benomyl-dichloran-streptomycin (BDS) malt-extract agar and (2) malt-extract streptomycin (MS) agar. Both types of agar contained 15g/l malt extract, 15g/l bacteriological agar, and 1 liter purified water autoclaved for 20 min. After cooling for 10 min. at 50^{o}C, 100 mg streptomycin powder was added to both media. Ten ml of stock solution in 50% ethanol containing 40 mg Benlate and 20 mg dichloran per 10 ml was added to the BDS medium. The MS medium was used for decay fungi with slow or no growth on BDS such as *Porodaedalia pini*. Two agar plates were used for each decay sample.

Initial fungal growth was characterized one to three weeks after plating and additionally as

needed as follows: Relative growth was rated on a scale of 0 to 3 where 0 = no decay fungus, 1 = fungus on wood, 2 = fungus on medium, and 3 = fungus ¼ in. into medium. The rare presence of other micro-organisms was noted as follows: Bac = bacteria present and NDecay = non-decay fungus present.

Representative examples of fungi that had grown >3/4 in. onto the media were examined with a compound microscope for identifying morphological characteristics such as the type of clamp connections. Representative fungal cultures from each sampled tree were then transferred to malt-extract agar without any antibiotics and also onto BDS media for comparisons.

For identification purposes, each transferred isolate (test culture) was paired to observe reactions between fungi. Test cultures were paired with 1) other test cultures from the same location, 2) the presumed original culture used for dowel inoculation (reference culture), and 3) a culture of the most similar related species (i.e. a *Fomitopsis* with a different species of *Fomitopsis*). Multiple pairings for a test culture were spaced evenly around the petri plate. Each pairing was between 3x3 mm blocks of colonized medium of test and reference cultures separated by 1mm.

After several weeks of growth, paired cultures were examined for fusion, barrage reactions, or zone lines. Fusion indicates that the pairings were the same species and maybe the same genet. Barrage reaction indicated genetic difference, usually within the same species. Zone lines indicated different species.

A selected number of cultures were sent to the USDA Forest Service, Northern Research Station, Madison, WI for identification of decay fungi.

Results

In 2010, we destructively sampled 75 inoculated trees on 19 sites in 8 different geographical areas in Oregon and Washington (Table 1). Tree species sampled included inoculated Douglas-fir, western larch, grand fir, and ponderosa pine. Sampled trees had been inoculated with fungal species that included *Fomitopsis cajanderi*, *F. pinicola*, *F. officinalis*, *Porodaedalia pini*, *Neolentinus lepideus*, *Echinodontium tinctorium*, *Stereum sanguinolentum*, and *Wolfiporia cocos*. After 7 to 14 years, inoculated trees had relatively little decay associated with inoculation holes (Table 2 and Fig. 6), and no conks or nesting cavities were found on any sampled trees associated with the inoculations. Because of the relatively low amount of decay and no nest-cavity formation, it was difficult to evaluate the possible effects that site or stand characteristics may have had on nest-cavity formation. The results in this report are presented for each geographical area.

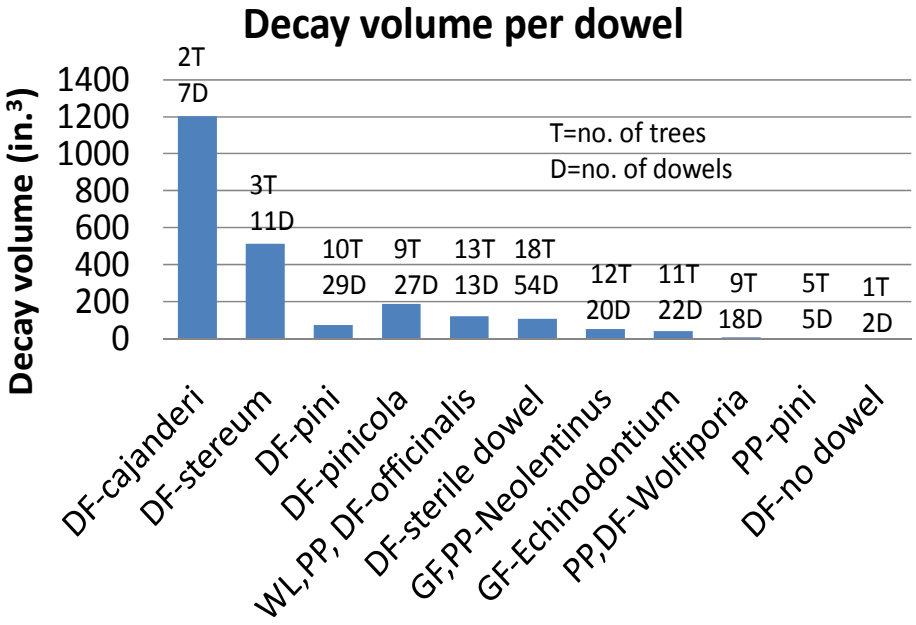

Fig. 6 – Bole-decay volume per dowel for 75 trees sampled on 19 sites in Oregon and Washington by tree and fungal species. WL=western larch, DF=Douglas-fir, PP=ponderosa pine, and GF=grand fir

Table 1. Site and stand characteristics for 19 sites that were sampled for internal decay in trees that were artificially inoculated with decay fungi. All sites are on USDA Forest Service-administered lands except for Littlerock and Ashford, WA which are on Washington Department of Natural Resources lands.

Site location	Elev.	Slope	Aspect	Slope position	Tree species[1]	No. of trees	Mean tree age	Mean tree dbh	Mean tree total height	Mean tree live crown	Mean current radial growth
	(ft.)	(%)					(yrs.)	(in.)	(ft.)	(%)	(in./yr.)
Conconully, WA	4600	20	S	Upper	WL	2	105	24.8	113	60	0.07
	3950	15	S	Mid	WL	2	130	20.6	114	55	0.04
Republic, WA	3300	10	S	Mid	PP	5	148	22.2	92	62	0.04
	4150	10	SE	Mid	DF	4	133	20.9	93	38	0.04
Littlerock, WA	2000	5	N	Mid	DF	3	61	22.4	115	50	0.13
	1900	15	SE	Mid	DF	7	71	24.6	126	37	0.13
Ashford, WA	2090	10	S	Bottom	DF	8	79	32.0	166	33	0.17
Halfway, OR	4800	5	SE	Mid	GF	4	107	21.8	106	64	0.13
	5000	25	E	Mid	GF	3	155	20.2	89	83	0.09
	4100	20	SE	Bottom	GF	9	98	17.9	87	88	0.10
Baker City, OR	4700	10	E	Upper	PP	3	109	20.6	84	50	0.07
	4900	15	NE	Mid	PP-DF	6	150	21.8	94	44	0.08
	5100	20	N	Upper	PP-DF	3	185	21.7	84	63	0.04
Crescent, OR	5200	5	W	Upper	PP	4	144	22.1	95	44	0.04
	4700	5	N	Upper	PP	3	145	18.9	79	52	0.07
	4500	5	W	Upper	PP	2	84	22.6	88	60	0.10
Blue River, OR	1500	20	SE	Mid	DF	3	81	21.9	126	33	0.10
	1800	35	NE	Upper	DF	1	-	21.4	108	35	-
	1400	20	N	Mid	DF	3	57	24.4	127	32	0.10

[1]WL=western larch, DF=Douglas-fir, PP=ponderosa pine, and GF=grand fir

Table 2. Amount of bole decay in four artificially inoculated conifer species 7-14 years after treatment in Oregon and Washington. All sites are on USDA Forest Service-administered lands except for Littlerock and Ashford, WA which are on Washington Department of Natural Resources lands.

Site location	Number of trees	Species[1]	Dowels per tree	Year treated	Fungal species inoc.[2]	Trees with decay	Mean max decay width[3]	Mean decay volume[4]		Total fungal recovery[5]		Fungal recovery per dowel[6]	
						%	in.	in.^{3}	%	no.	%	no.	%
Conconully, WA	4	WL	1	1996	*Fomitopsis officinalis*	100	5.1	679.7	3.0	66	45	4	50
Republic, WA	5	PP	1	1996	*F. officinalis*	100	3.0	117.7	1.0	90	80	5	100
	4	DF	1	1996	*F. officinalis*	100	3.1	94.4	1.3	72	17	4	75
Littlerock, WA	5	DF	6	2000	*P. pini*	100	1.9	90.4	0.8	45	0	15	0
					sterile dowel	40	0.8	33.9	0.5	45	11	15	13
	5	DF	6	2000	*F. pinicola*	100	3.8	367.8	3.6	45	16	15	53
					sterile dowel	100	2.4	193.5	2.2	45	18	15	13
Ashford, WA	4	DF	6	2000	*P. pini*	75	1.6	106.3	0.5	36	3	12	8
					sterile dowel	50	1.5	100.1	0.3	36	17	12	17
	4	DF	6	2000	*F. pinicola*	50	0.8	4.6	0.2	36	0	12	0
					sterile dowel	75	2.0	97.5	0.6	36	19	12	17
Halfway, OR	11	GF	2	1997	*E. tinctorium*	36	0.8	37.9	0.4	-	-	-	-
	5	GF	2	1997	*Neolentinus lepideus*	20	0.7	134.6	0.6	-	-	-	-
Baker City, OR	3	PP	2	1997	*N. lepideus*	33	0.3	1.0	0.1	-	-	-	-
	5	PP	2	1997	*Wolfiporia cocos*	0	0	0	0	-	-	-	-
	4	DF	2	1997	*W. cocos*	75	0.6	12.8	0.2	-	-	-	-
Crescent, OR	4	PP	1	1997	*N. lepideus*	75	1.4	17.8	0.4	58	55	3	100
	5	PP	1	1997	*P. pini*	0	0	0	0	-	-	-	-
Blue River, OR	1	DF	2	1998	no dowel	0	0	0	0	12	0	2	0
	3	DF	2,5, 5	1998 2003	*Stereum sanguino-lentum*	100	2.9	511.6	2.2	102	44	12	50
	2	DF	2,5	1996 2003	*F. cajanderi*	100	5.3	1202.7	9.4	66	31	7	29
	1	DF	2	1998	*P. pini*	100	2.7	18.7	1.5	12	0	2	0

[1]Tree species: WL=western larch, PP=ponderosa pine, DF=Douglas-fir, GF=grand fir
[2]At all sites each tree was inoculated with one fungal species. At WADNR sites, each tree was treated with 6 dowels; 3 colonized with a fungus and 3 that were sterile when initially inserted. At Blue River, "no dowel" indicates that pipes were inserted but no dowels.
[3]Mean maximum decay width is the average maximum diameter of stain/decay associated with each dowel for all trees sampled including those without decay. Stain and decay were not differentiated.
[4]Mean decay volume is the average volume of stain/decay associated with each dowel for all trees sampled including those without decay. Percentage decay volume is decay volume divided by the volume of the bole (log) segment containing the decay multiplied by 100 for all trees sampled.
[5]Total fungal recovery is the total number of pieces of wood used in isolation attempts and percentage that were positive for the inoculated fungal species. Samples were not collected at Halfway or Baker City, OR. The

brown-cubical-rot fungi, *Antrodia carbonica* and *Postia placenta*, were isolated from decayed wood associated with sterile dowels.
[6]Total number of occurrences where wood adjacent to dowels was sampled and the percentage of samples with positive isolations

Okanogan-Wenatchee National Forest

Conconully, WA

On August 17, 2010, we sampled four western larch trees on two sites NE of Conconully, WA (Cazadero 19 – N48°38'31.46", W119°47'40.38" and Cazadero 17 – N48°38'40.99", W119°49'40.35"). The larch averaged 105 and 130-years old, 25 and 21 in. dbh, and 113 and 114 ft. in height for the two sites (Table 1). Each tree was inoculated with one dowel colonized by *Fomitopsis officinalis* in 1996 (Table 2). All of the inoculated trees had decay associated with the dowels. Decay amount was relatively high with a mean maximum decay width of 5.1 in. and volume of 679.7 in.^{3} per dowel or 3% of the bole volume (Table 2). In the lab, 45% of the attempted isolations yielded *Fomitopsis officinalis*, and the fungus was recovered from decayed wood associated with 2 of the 4 sampled dowels.

Republic, WA

On August 18, five ponderosa pines were sampled SW of Republic, WA (Lyman Lake 17 – N48°29'32.14", W119°0'59.95"). The pines averaged 148-years old, 22 in. dbh, and 92 ft. tall (Table 1). Each tree was inoculated with one dowel colonized by *F. officinalis* in 1996 (Table 2). All of the inoculated trees had decay associated with the dowels. Decay amount was relatively high with a mean maximum decay width of 3.0 in. and volume of 117.7 in.^{3} per dowel or 1% of the bole volume (Table 2). In the lab, 80% of the attempted isolations yielded *Fomitopsis officinalis*, and the fungus was recovered from decayed wood associated with all of the 5 sampled dowels.

On August 19, four Douglas-firs were sampled SW of Republic, WA (Dragon – N48°38'50.63", W118°54'54.82"). The firs averaged 133-years old, 21 in. dbh, and 93 ft. tall (Table 1). Each tree was inoculated with one dowel colonized by *F. officinalis* in 1996 (Table 2). All of the inoculated trees had decay associated with the dowels. Decay amount was relatively high with a mean maximum decay width of 3.1 in. and volume of 94.4 in.^{3} per dowel or 1.3% of the bole volume (Table 2). In the lab, only 17% of the attempted isolations yielded *Fomitopsis officinalis*, but the fungus was recovered from decayed wood associated with 3 of the 4 sampled dowels.

Washington Department of Natural Resources Forestland

Littlerock, WA

On November 1-2, 2010, two sites W of Littlerock, WA (Capitol Forest - N46°58'16.198", W123°7'14.027" and N46°57'46.742, W123°7'46.157) were sampled for inoculated Douglas-fir with five trees sampled at each site. For the two sites, the Douglas-firs averaged 61 and 71–years old, 22 and 25 in. dbh, and 115 and 126 ft. in height (Table 1). Each tree was treated with 6 dowels separated by 3 to 4 ft. with the upper three dowels on one side of the tree and the lower three dowels on the opposite side of the tree. Three dowels were colonized by either *Fomitopsis pinicola* or *Porodaedalia pini* (Table 2). The other three dowels were autoclaved (sterile). All trees treated in 2000 with *F. pinicola* or *P. pini* had some decay but at relatively moderate amounts.

Trees inoculated with *P. pini* had a mean maximum decay width of 1.9 in. and volume of 90.4 in^3 per dowel at 0.8% of the bole volume (Table 2). Trees inoculated with *F. pinicola* had a mean maximum decay width of 3.8 in. and volume of 367.8 in^3 at 3.6%. In the lab, none of the 45 attempted isolations yielded the inoculated fungus, *P. pini* at the Littlerock site. Of 45 attempted isolations, only 16% yielded *F. pinicola*, but the fungus was recovered from decayed wood associated with 53% of the 15 sampled dowels.

Forty and 100% of the trees inoculated with sterile dowels surprisingly had decay but at relatively low amounts with mean maximum decay widths of 0.8 and 2.4 in. and volumes of 33.9 and 193.5 in^3 per dowel at 0.5 and 2.2% of the bole volume (Table 2). Fungi apparently naturally infected the drill holes and the inserted sterile dowels, thus causing some decay. From 45 attempted isolations from decay associated with sterile dowels in the *P. pini*-inoculated trees, 11% of the isolation attempts yielded decay fungi, and the fungus was recovered from decayed wood associated with 13% of the 15 sampled dowels.

From 45 attempted isolations from decay associated with sterile dowels in the *F. pinicola*-inoculated trees, 18% yielded decay fungi, and the fungus was recovered from decayed wood associated with 13% of the 15 sampled dowels. Both *Postia placenta* and *Antroidia carbonica*, causes of brown-cubical rot, were isolated from decayed wood associated with sterile dowels.

Ashford, WA

On November 3-4, one site S of Ashford, WA (Trap Cat – N46°43'21.496", W122°0'30.153") was sampled for inoculated Douglas-fir with 10 trees sampled. The Douglas-firs averaged 79–years old, 32 in. dbh, and 166 ft. in height, the largest trees sampled in the entire project (Table 1). As at the Littlerock sites, each tree was treated with 6 dowels separated by 3 to 4 ft. with the upper 3 dowels on one side of the tree and the lower three dowels on the opposite side of the tree. Three dowels were colonized by either *F. pinicola* or *P. pini* (Table 2). The other three dowels were autoclaved (sterile). All trees were treated in 2000.

Two of four firs inoculated with *F. pinicola* had decay, and three of four firs inoculated with *P. pini* had decay but at relatively low amounts. Trees inoculated with *P. pini* had a mean maximum decay width of 1.6 in. and volume of 106.3 in^3 per dowel at 0.5% of the bole volume. Trees inoculated with *F. pinicola* had a mean maximum decay width of 0.8 in. and volume of 4.6 in^3 at 0.2%. In the lab, only 3% of the 36 attempted isolations yielded the inoculated fungus, *P. pini,* and the fungus was recovered from decayed wood associated with 8% of the 12 sampled dowels. Of 36 attempted isolations, none yielded *F. pinicola*.

Fifty and 75% of the trees inoculated with sterile dowels surprisingly had decay but at relatively low amounts with mean maximum decay widths of 1.5 and 2.0 in. and volumes of 100.1 and 97.5 in^3 per dowel at 0.3 and 0.6% of the bole volume. As at the Littlerock site, fungi apparently naturally infected the drill holes and the inserted sterile dowels, thus causing some decay. From 36 attempted isolations from decay associated with sterile dowels in the *P. pini*-inoculated trees, 17% of the isolation attempts yielded decay fungi, and the fungus was recovered from decayed wood associated with 17% of the 12 sampled dowels (Table 2).

From 36 attempted isolations from decay associated with sterile dowels in the *F. pinicola*-inoculated trees, 19% yielded decay fungi, and the fungus was recovered from decayed wood associated with 17% of the 12 sampled dowels. Both *Postia placenta* and *Antroidia carbonica*, causes of brown-cubical rot, were isolated from decayed wood associated with sterile dowels (Table 2).

Wallowa-Whitman National Forest

Halfway, OR

On September 28-29, 2010, 11 grand firs on three sites were sampled NW of Halfway, OR (Summit 1 – N44°55'13.01", W117°12'48.31"; Summit 4 – N44°56'44.91", W117°13'43.28"; and Westwall – N44°53'48.07", W117°10'55.71"). The grand firs averaged 107, 155, and 98-years old; 22, 20, and 18 in. dbh; and 106, 89, and 87 ft. in height for the three sites (Table 1). Each tree was inoculated with two dowels 4 to 7 ft. apart. At the Summit 1 site, 2 sampled trees were inoculated with *Echinodontium tinctorium* and 2 trees with *Neolentinus lepideus* in 1997 (Table 2). At the Summit 4 site, all sampled firs were inoculated with *N. lepideus* in 1997. At the Westwall site, all sampled firs were inoculated with *E. tinctorium* in 1997.

Only four of 11 firs inoculated with *E. tinctorium* had decay associated with the dowels; decay amount was relatively low with a mean maximum decay width of 0.8 in. and volume of 37.9 in.^{3} per dowel or 0.4% of the bole volume (Table 2, figure at right). No samples of decay were collected in the field for culturing. Only one of five firs inoculated with *N. lepideus* had decay associated with the dowels; decay amount was relatively low with a mean maximum decay width of 0.7 in. and volume of 134.6 in.^{3} or 0.6% of the bole volume (Table 2). No samples of decay were collected in the field for culturing.

Baker City, OR

On September 30, eight ponderosa pines and four Douglas-firs on three sites were sampled S of Baker City, OR (Rancheria 3 – N44°36'36.84", W117°53'51.56"; Rancheria 4 – N44°35'01.78", W117°52'32.45", and Rancheria 6 – N44°34'22.00", W117°52'16.84"). The pines averaged 109-years old; 21in. dbh; and 84 ft. in height on the Rancheria 3 site (Table 1). The pines and Douglas-fir averaged 150 and 185-years old, 21 and 22 in. dbh, and 94 and 84 ft. in height and the other two sites. Each tree was inoculated with two dowels 4 to 5 ft. apart. At the Rancheria 3 site, all sampled pines were inoculated with *N. lepideus* in 1997 (Table 2). At the Rancheria 4 and 6 sites, all sampled trees were inoculated with *Wolfiporia cocos* in 1997.

Only one of three pines inoculated with *N. lepideus* had decay associated with the dowels; decay amount was relatively low with a mean maximum decay width of 0.3 in. and volume of 1.0 in.^{3} per dowel or 0.1% of the bole volume (Table 2). None of the five pines inoculated with *W. cocos* had decay associated with the dowels. Three of the four Douglas-firs inoculated with *W. cocos* had decay associated with the dowels; decay amount was relatively low with a mean maximum decay width of 0.6 in. and volume of 12.8 in.^{3} per dowel or 0.2% of the bole volume. No samples of decay were collected in the field for culturing.

Deschutes National Forest

Crescent, OR

On October 27-28, 2010, nine ponderosa pine on three sites were sampled S of Crescent, OR (East Slope 8 – N43°16'50.78", W121°49'13.47"; Railroad Rimrose 9 – N43°19'58.11", W121°47'38.85"; and Railroad Rimrose 14A – N43°24'18.85", W121°44'6.58"). The pines averaged 144, 145, and 84-years old; 22, 19, and 23 in. dbh; and 95, 79, and 88 ft. in height for the three sites (Table 1). Each tree was inoculated with one dowel. At the East Slope 8 site, all sampled pines were inoculated with *Neolentinus lepideus* in 1997 (Table 2). At both of the Railroad Rimrose sites, all sampled pines were inoculated with *Porodaedalia pini* in 1997.

Three of the four trees inoculated with *N. lepideus* had decay associated with the dowels; decay amount was relatively low with a mean maximum decay width of 1.4 in. and volume of 17.8 $in.^3$ per dowel or 0.4% of the bole volume (Table 2). In the lab, 55% of 58 attempted isolations yielded *N. lepideus*, and the fungus was recovered from decayed wood associated with all 3 sampled dowels. None of the five pines inoculated with *P. pini* had decay associated with the dowels; therefore, no samples of decay were collected in the field.

Willamette National Forest

Blue River, OR

On August 4-5, 2010, seven Douglas-firs on three sites were sampled near Blue River, OR (Blue River Thin 11 – N4°10'30.59", W122°17'32.55"; Blue River Thin 6 - N44°10'11.15", W122°17'34.91"; and Delta Thin 1 – N44°9'9.17", W122°16'11.16"). The firs averaged 81 and 57-years old; 22, 21, and 24 in. dbh; and 126, 108, and 127 ft. in height for the three sites (Table 1). Each tree was inoculated with two dowels at 5 ft. apart at the two Blue River sites and five dowels at 4-5 ft. apart at the Delta Thin site. At the three sites treated in 1998, 1996, or 2003, one sampled tree was inoculated with *Porodaedalia pini*, two trees with *Fomitopsis cajanderi*, three trees with *Stereum sanguinolentum*, and one tree was drilled twice and pipes inserted but no dowels were used.

The one *P. pini*-inoculated fir had a relatively small amount of decay with a mean maximum decay width of 2.7 in. and volume of 18.7 in.^{3} per dowel or 1.5% of the bole volume (Table 2). In the lab, none of the 12 attempted isolations yielded the inoculated fungus. The two *F. cajanderi*-inoculated trees had the highest amount of decay sampled with a mean maximum decay width of 5.3 in. and volume of 1202.7 in.^{3} or 9.4% of the bole volume (Table 2). In the lab, 31% of the 66 attempted isolations yielded the inoculated fungus, and the fungus was recovered from decayed wood associated with 29% of the 7 sampled dowels.

The three *S. sanguinolentum*-inoculated trees had relatively high amounts of decay with a mean maximum decay width of 2.9 in. and volume of 511.6 in.^{3} per dowel or 2.2% of the bole volume (Table 2). In the lab, 44% of the 102 attempted isolations yielded the inoculated fungus, and the fungus was recovered from decayed wood associated with half of the 12 sampled dowels.

The one drilled tree with pipes but no dowels had no stain or decay associated with the two drill-holes.

Discussion

The most critical finding was that the amount of decay was relatively low or absent in conifers that had been drilled and inoculated 7-14 years previously. Why was the amount of decay relatively low or absent in inoculated conifers 7-14 years after treatment? Successful inoculations and subsequent nest-cavity formation occurred in some western larch artificially inoculated 6-years earlier in 1988 (Parks et al. 1990). In that study, each tree received six infected dowels with three placed equidistantly around the bole at 21 ft. above ground and three placed only 2 ft. away at 23 ft. above ground. None of the trees that we sampled in 2010 were inoculated at multiple points at the same height. Apparently, there were concerns that if too much decay developed early, stems might break at the multiple-inoculation sites.

As wood is colonized by decay fungi, fungal growth and decay are initially slow but become very rapid over time. Decay rates are based on the amount of enzymes or peroxides released in the wood, and these are based on the amount of fungus present. Colonization and wood decay can greatly accelerate once a decay fungus reaches a higher density in the wood. Using multiple inoculation sites at the same or nearly the same height may magnify the effect of inoculations in causing decay with an additive or synergistic effect. Even in our sample, where trees were not inoculated at multiple points at the same height, some decay columns from some inoculation holes extended to the next inoculation hole 5 ft. away, but decay columns were thin (1-2 in. diam.).

We speculate, however, that if there had been more than one inoculation site at the same tree height, then perhaps enough decay would have occurred after 7-14 years to allow for nest-cavity formation as had occurred from multiple larch inoculations in 1988. Even with observed decay widths as narrow as 2 in., three inoculations at the same height may have resulted in at least three times that amount for a minimum 6 in. decay width assuming that all three dowels would cause equal decay and coalesce (Fig. 7). At a 5 in. decay width, as we found for *F. cajanderi* in two inoculated Douglas-firs, three inoculations might have resulted in a 15 in. decay width, which probably is sufficient for nest-cavity formation. This may even be an underestimation of decay width if there is synergism of fungal growth among the coalescing decay columns.

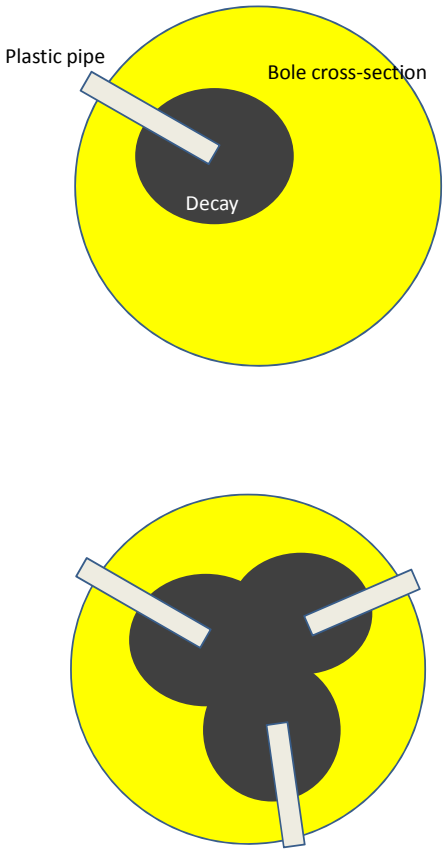

Fig. 7 – Simulated tree inoculation with one colonized dowel (upper) and three colonized dowels (lower) at the same tree height. Three dowels at the same height would create more usable decay for nest-cavity creation.

In some cases the best fungal species or clones to cause decay in a particular tree species or area may not have been used. The best fungal species for artificial inoculation may not be species that normally cause the most decay. For example, there was little or no decay in grand firs inoculated 14 years previously with dowels colonized by the Indian paint fungus (*E. tinctorium*). The Indian paint fungus is the principal cause of heart rot in grand fir. Also, because they are highly susceptible to decay after wounding, wounds on grand fir almost always result in some decay in 10 years. We speculate that because the Indian paint fungus does not enter trees through large or deep wounds but instead infects through shade-killed branchlets on

suppressed trees, inoculation through a deep wound was not successful. Also, the presence of the *E. tinctorium*-colonized dowel in the drill hole actually may have prevented infection from windborne spores of other decay fungi in such a deep wound.

There also may be more effective alternatives to climbing, drilling, and inoculating living trees with colonized dowels. Inoculation trials using rifles and shotguns appeared to be successful in initiating decay after 5 years in Douglas-fir near Corvallis, OR (Filip et al. 2004). Based on sampling seven inoculated trees, decay-column widths averaged 3.7 in. after 5 years. Decay widths did not differ depending on whether the bullets contained wood inoculated with *F. cajanderi*, *P. pini*, or were sterile. In 10 years, decay widths would average 7.4 in., assuming constant decay rates, which is larger than any decay widths that we sampled associated with holes drilled after 7-14 years. Bole wounds created by rifle or shotgun are relatively large and open (Fig. 8) compared to the standard drilled holes, and this may be more favorable for decay development by creating more area for initial infection and increasing oxygen levels in the wood that stimulated subsequent fungal colonization.

Fig. 8 – Stain and decay caused by a fungal-inoculated rifle bullet (white spot below stain) in a live Douglas-fir inoculated 5 years previously. Note the large, open wound at the top of the disk made by the bullet.

We offer some additional thoughts and questions on the use of fungal inoculations of live trees to create wildlife habitat. Future usefulness of decay depends on its accessibility to cavity nesters. Rate of invasion of healthy wood and the radial-growth rate of trees at the time of inoculation may indicate potential for use by cavity nesters, and also predict time until breakage or loss of the nesting resource. Slow radial growth keeps a rind of sound wood thin while decay develops. For example, a tree with a radial-growth rate of 0.07 in. per year would add only 0.7 in. of sound wood in 10 years. Trees with very slow radial growth may develop useful decay even without a fungus needing to breach the resistant barrier of the growth ring from the year the tree was wounded during inoculation.

Decay in trees with currently fast radial growth might become useful to wildlife if the decay fungus is also a pathogen that can breach the growth ring (i.e., a canker rot fungus), or if radial growth slows. Trees growing at 0.2 in./year would add 3 in. in 15 years which may be too thick for bird penetration. Trees sampled in our project were all within 15 years of inoculation, which may be too early to detect significant decay from one inoculation point or multiple points that are several feet apart.

Some tree-species and site mixes might be better than others for providing habitat. Short-lived species such as true firs have recurrent mortality from fir engravers and disease and may be needed in the mix. Stand structure should match what is known about the cavity-utilizing species if these efforts are to be used as effective treatments to promote wildlife.

Are tree and fungus species chosen for inoculation correct? Have inoculations taken into account any cavity-nesters' preferences for tree species? Were fungi chosen for their known field utility or for other reasons, such as ease or past experience isolating and growing fungal species. From previous sampling (Hildebrand 2009) and our sampling in 2010, *Porodaedalia pini* had poor decay results and recovery from inoculated wood and stored cultures.

Topped or girdled trees that die from treatment rapidly develop saprot but may be of shorter duration for standing habitat (Fig. 9, Bull et al. 1997). Do methods with less input, such as non-inoculated stands with topping or side-wounding of trees have potential to provide more useful nesting and feeding resources than stands with artificial inoculation? Multiple entries of topping could

provide a more certain, consistent supply of decayed but short-lived trees, but there may be temperature and light differences in the bole associated with changes in stand structure that affect decay development and wildlife use.

Should an inoculation program choose a different optimum number of inoculation sites on the bole or arrangement/closeness of inoculation sites for different combinations of tree and fungus species? Success rate of tree decay depends on the success rate of the inoculum sites or holes and tree/fungus species combinations. One can choose a success rate desired for getting at least one colonization. Simplistically, you can calculate success $S_{n\ inoculation\ sites} = 1 - F^n$, where n is the number of inoculation sites per tree and F is the rate of failure of a single inoculation. For example, if one desires 90% success in getting one or more successful decay sites per tree, and if inoculation rate of success is 70%, then at 2 dowels per tree, S=91%. If inoculation rate of success is 50%, then at 3 dowels per tree, S=93.75%. More sophisticated statistical methods may be needed to get probability levels. Interactions of multiple decay columns may speed colonization.

Were cultures of the fungi used in this project really representative of the species' effectiveness? Dowels may have been inoculated with whatever isolates were easy to obtain from labs or conks. Wood-block decay tests might be used to choose isolates for superior decaying ability. Those from a forest area where they will be eventually used should be climatically and ecologically adapted. Fresh cultures can be obtained by non-destructive sampling and isolation from trees with cavity nests. Sampling can be done at times of the year to avoid nesting periods, but would require a climber and collaboration with wildlife biologists.

In conclusion, based on the results of our survey of 19 sites, we believe that live-tree inoculations may continue in Oregon and Washington but only after substantial modification. The USDA Forest Service, Forest Health Protection unit in Portland will no longer provide inoculum for infecting trees for wildlife purposes; there are private groups that can supply inoculum for infecting trees. In addition, we provide the following recommendations to modify and improve inoculation techniques as well as a list of future trials and research concerning tree decay and subsequent wildlife use.

Fig. 9 – Topped or girdled trees often die and are short-lived cavity-nesting habitat.

Recommendations

1. Inoculate trees with three dowels of the same fungal species at the same height on the tree bole equally spaced around the bole on smaller trees (<25 in. dbh, Fig. 8). Four or more inoculations at the same height, however, may sufficiently weaken the tree bole to cause breakage. Fungi are slow to cause decay, but several adjacent cultures of the same species may coalesce into a larger decay column that may be sufficient for nest-cavity excavation in <10 years. Inoculations at one bole height are more effective than inoculations on one side of the tree bole separated by several feet.
2. If very large trees (≥25 in. dbh) are treated, consider placing the three drill holes in the same quadrant of the tree at 10-12 in. apart at the same height (Fig. 10). This may allow the individual decay columns from each dowel to grow together in less time.

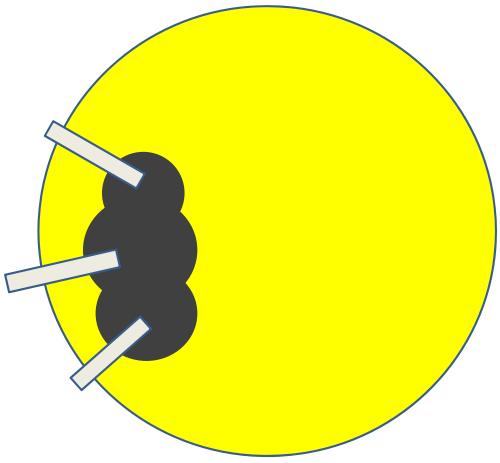

Fig. 10 - Inoculation holes should be drilled in one quadrant of large-diameter trees to allow for timely fusion of decay columns resulting from each colonized dowel.

3. Use only dowels colonized with locally collected cultures and only from cultures that are less than 10-years old. Local isolates are more adapted to cause decay. Older cultures lose viability and ability to cause wood decay.
4. For westside Douglas-fir, *Fomitopsis cajanderi* appears to cause the most decay followed by *Stereum sanguinolentum, F. pinicola, and Porodaedalia pini*.
5. For eastside ponderosa pine, we recommend inoculating with *F. officinalis*. We do not recommend inoculating with *Wolfiporia cocos, Neolentinus lepideus.*, or *P. pini*.
6. *Fomitopsis officinalis* appears to be effective in causing decay in eastside western larch, ponderosa pine, and Douglas-fir in eastern Washington. We recommend that *F. officinalis* also be used in eastern and southern Oregon with these tree species.
7. We do not recommend using *Neolentinus lepideus* or *Wolfiporia cocos* for inoculations of any tree species since decay amounts were none or relatively low.
8. We do not recommend that grand fir or other true firs be artificially inoculated with decay fungi, especially *Echinodontium tinctorium* or *N. lepideus*. If there is a need to create decay in true firs, drilling or wounding the tree should be sufficient to result in decay.
9. Use at least a 6-in.-long plastic pipe inserted halfway into each hole to prevent sealing of the inoculated hole by resin and subsequent tree growth. Holes without pipes or shorter pipes will seal over more quickly and retard or prevent

decay formation. Short pipes also may be expelled by the tree and the hole sealed before decay forms.

10. Inoculate only relatively slow-growing trees. Faster growing trees will form a larger annual rind of healthy wood around the decay that may be too difficult for cavity-nesting birds to penetrate. Consider topping or girdling the upper-third or less of the live crown to slow tree growth. Removing too much live crown, however, may overly stress the tree and result in premature tree death.
11. Drilling holes and using plastic pipes without inserting dowels does not appear to be as effective in causing decay, especially in resinous species such as Douglas-fir, ponderosa pine, and larch. Fungal-colonized dowels in drilled holes are more effective in causing decay than uncolonized dowels, which are better than no dowels.

Future Research and Trials

1. Given good methodology, will inoculations to promote decay be significantly more effective over the long-term than trees left alone or treated by other methods?
2. Do either topped trees or suppressed trees have the same useful structural characteristics as hollow, slow-growing, old-growth trees?
3. Do inoculated trees develop usable decay when cavity nesters' other needs can be met? Can cavity-nester needs for feeding and appropriate structure be met in stands that are typically being chosen for treatment?
4. Do cavity nesters select decay in living trees caused by specific fungi?
5. Can the potential period of wildlife utilization be predicted for either inoculated or non-inoculated trees? This would involve comparing the time until potential usage and potential length of usage. The statistical distribution of decay creation and tree survival after decay formation will also be important; a right-tailed distribution with a few long-remaining trees might be preferable to a peak event with a more rapid decline. True firs that died of natural causes may be utilized for food and nesting at some sites but may not be standing for very long.
6. Would the drilling of four or more holes and colonized-dowel placement at the same tree height result in unacceptable live-tree breakage due to too many drill holes and subsequent decay (figure below)?

7. Can *F. officinalis* be artificially inoculated into westside Douglas-fir and cause decay in a relatively short time for cavity nesters? This fungus caused decay

in 14 years in all trees that we sampled in eastern Washington (Table 2).

8. Can *Phaeolus schweinitzii* be used to inoculate conifers in OR/WA. Although *P. schweinitzii* causes mainly a root and butt rot in several conifer species, it has been found associated with nesting cavities in larch (Parks et al. 1990) and observed on the upper boles of coastal Douglas-fir.
9. Can other fungal species be used to inoculate living trees to produce decay? Decay fungi such as *Hericium abietis* and *Phellinus hartigii* were used to artificially inoculate trees in Oregon and Washington but were not sampled in 2010.
10. Should inoculation trials be repeated with rifles and shotguns throughout OR/WA, with and without inoculated bullets (Filip et al. 2004)?
11. Would other methods of live-tree wounding and inoculation including the creation of larger diameter wounds result in more usable decay in less time?
12. Is there an optimum time of year to make wounds and inoculate trees to optimize decay development?
13. How well do cultures of wood-decay fungi maintain effectiveness to decay under long-term storage on artificial media? What is the maximum age in culture that can be accepted for effective cultures under current protocols? What is better: fewer vs. more frequent culture transfers? Active vs. dormant condition? High vs. low media nutrients? Are current or other preservation methods for the cultures better suited to preserve fungal activity? Are there alternatives to culture plates of medium, such as storage of colonized agar plugs under sterile water or using the slow desiccation/-80^{o}C method. Early tests show it is effective even for difficult haploid Basidiomycetes, Chytridiomycetes, and some Oomycetes (Zambino 2004).
14. If additional trials are made to determine potentially effective fungi, inoculations should be made at multiple sites at the same tree level, and only with fungi that have shown to cause significant decay.

Acknowledgements

We thank the following people for their help on this project: Penny Harris (McKenzie RD); Blue River fallers Todd and Lyle Lowry; Jeff Henshaw (Crescent RD); Joe Sciarrino (Pine RD); Jim Gilsdorf (Baker City RD), Wallowa-Whitman fallers Sam Vaughan, Kevin May, and Erik Lisle; Matt Marsh (Tonasket RD), and Jessie Glaeser and John Haight (Northern Research Station). All photos were taken by the authors.

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