112 Extension Note

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Results of Fungal Inoculation Treatments as a Habitat Enhancement Tool in the East Kootenay Region of British Columbia: 2007–2013

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Executive Summary

Wildlife trees provide critical nesting, denning, roosting, and feeding habitat for more than 70 species of birds, mammals, and amphibians in British Columbia, including some species that are considered at risk provincially and federally. Depending on the age, condition, and disturbance type and history of the forested landscape, wildlife trees can be in short supply in some areas. This is the case in parts of the Columbia River valley in the East Kootenay region of British Columbia, where wildlife tree enhancement treatments using inoculation with native heart-rot decay fungi and mechanical stem modifications have been conducted since 2007 to increase local habitat supply. Thirteen properties in this region that are managed by several partners for ecosystem restoration, biological diversity conservation, and wildlife habitat maintenance or enhancement were selected for wildlife tree treatments using fungal inoculation between 2007 and 2013.

The overall project goals were to enhance wildlife tree habitat in areas that currently lack wildlife trees, and to increase the abundance of wildlife trees in areas that have high habitat capability for Lewis's Woodpecker (*Melanerpes lewis*), Flammulated Owl (*Psiloscops flammeolus*), and other cavity-dependent wildlife.

In total, 369 trees (217 Douglas-fir, 139 ponderosa pine, and 13 western larch) were inoculated and mechanically modified to enhance or create wildlife tree habitat in the treatment areas. All trees were live and appeared healthy prior to treatment.

To determine if the inoculation and mechanical modification treatments were producing internal decay, a limited destructive sampling program was conducted in 2010 at the Hoodoo-Hofert property using trees that had been inoculated in 2007; the program was repeated in 2013 at the Dutch Findlay property using trees that had been inoculated in 2010. Heart-rot decay in its early to moderately advanced stages occurred within 3 years post-treatment in some of the sampled trees. This timeframe is very rapid compared to natural heart-rot decay dynamics, which often require more than 100 years from initial tree wounding for significant decay to become established, and suggests that the revised drilling and girdling techniques employed during the latter part of the project period improved the efficacy of the inoculation treat-

British Columbia

ments. The results of this study shed new light on ways to improve the efficacy and application of the inoculation techniques, and are consistent with other assessments of fungal inoculation trials in British Columbia, Washington State, and Oregon.

Recommendations for improving the efficacy and application of fungal inoculation and mechanical stem modifications as a useful wildlife habitat enhancement tool are also discussed. Fungal inoculation treatments can provide accelerated creation, enhancement, and recruitment of wildlife trees in areas where there is a shortage of such habitat, and where management goals include increasing wildlife habitat supply and restoring old forest-like structural characteristics. We recommend continued effectiveness monitoring of inoculated trees to verify the presence and extent of internal decay and subsequent use by cavity-dependent wildlife over time.

Background

In general, in Pacific Northwest forests, 25–30% of bird and mammal species are dependent on dead and decaying trees as habitat (Bunnell et al. 1999). In British Columbia, wildlife trees provide critical nesting, denning, roosting, and feeding habitat for more than 70 species of birds, mammals, and amphibians. There is an extensive body of literature from North America and elsewhere on the ecology of cavity-dependent wildlife and the importance of standing dead and dying trees in forest ecosystems (e.g., Davis et al. 1983; Wright and Wales 1993; Samuelsson et al. 1994; Machmer and Steeger 1995; WTC 1995; Steeger et al. 1996; Manning et al. 2001; Laudenslayer et al. 2002; Lofroth et al. 2011). Fenger et al. (2006) provide a thorough summary of the biology and habitat ecology of wildlife tree obligate species in British Columbia.

Depending on the age, condition, and disturbance type and history of the forested landscape, wildlife trees can be in short supply in some areas, particularly where forest harvesting or agriculture have been practiced for many years and second-growth stands are predominant, or where ecosystem restoration may be required; the result can be a lack of suitable habitat for cavity-dependent wildlife, which can in turn affect the relative abundance and distribution of such species. The establishment of heart-rot fungi and the formation of internal decay relies on natural processes of stem and branch wounding and subsequent infection by resident decay fungi; therefore, it can often take 100+ years for trees of sufficient size and decay condition to function as useful wildlife trees. Even healthy, second-growth stands containing trees that are 50–100 years old often require many more decades before they develop the primary wildlife tree attribute of internal heart rot (Manning 2008b; Hennon and Mulvey 2014). However, this natural decay process can be accelerated through fungal inoculation in order to recruit wildlife trees much more quickly than would otherwise occur through natural processes. For example, in Oregon, fungal inoculation trials have created trees with heart rot that are suitable for cavity excavation in less than 10 years, much earlier than would be expected from natural fungal colonization and decay (Parks 1996).

Some wildlife tree-dependent species in British Columbia, including Lewis's Woodpecker, Flammulated Owl, Western Screech-owl (*Megascops kennicottii macfarlanei*), and Williamson's Sapsucker (*Sphyrapicus thyroideus nataliae*) are considered at risk provincially and federally. These species are all native to the East Kootenay project area. Consequently, wildlife tree enhancement treatments using inoculation with native heart-rot decay fungi and mechanical stem modifications have been conducted since 2007 to increase the supply of critical habitat features. Some of the project treatment units are also undergoing thinning, pruning, and prescribed fire treatments to restore historic NDT-4 fire-maintained[1] open forest ecosystems.

Thirteen properties in the East Kootenay region, which are managed by several partners for ecosystem restoration, conservation of biological diversity, and maintenance or enhancement of wildlife habitat, were selected for wildlife tree treatments using fungal inoculation between 2007 and 2013. These include Hoodoo-Hofert, Rocks Pasture, Hebert, Wasa Mountain, Lazy Lake, Wolf Creek, Pine Butte, Dutch Findlay, Thunder Hill Ranch, Sheep Mountain, Foosey Pasture, Columbia Lake, and Perry River Road. Figure 1 shows the approximate location of these treatment areas.

Project partners included the Fish & Wildlife Compensation Program (FWCP), B.C. Ministry of Environment (MOE), B.C. Ministry of Forests, Lands and Natural Resource Operations (MFLNRO), Nature Conservancy of Canada (NCC), The Nature Trust of British Columbia (TNT), and Thunder Hill Ranch.

Project Goals and Objectives

The overall project goal was to enhance wildlife tree habitat supply and quality at each of the treatment area properties. The advantages and benefits of using fungal inoculation as a

1 Some NDT-4 ecosystems are characterized by historically frequent stand-maintaining disturbance events, typically low-intensity wildfire, which maintains a sparsely treed, open-canopy overstory and a vigorous herbaceous–shrubby understory.

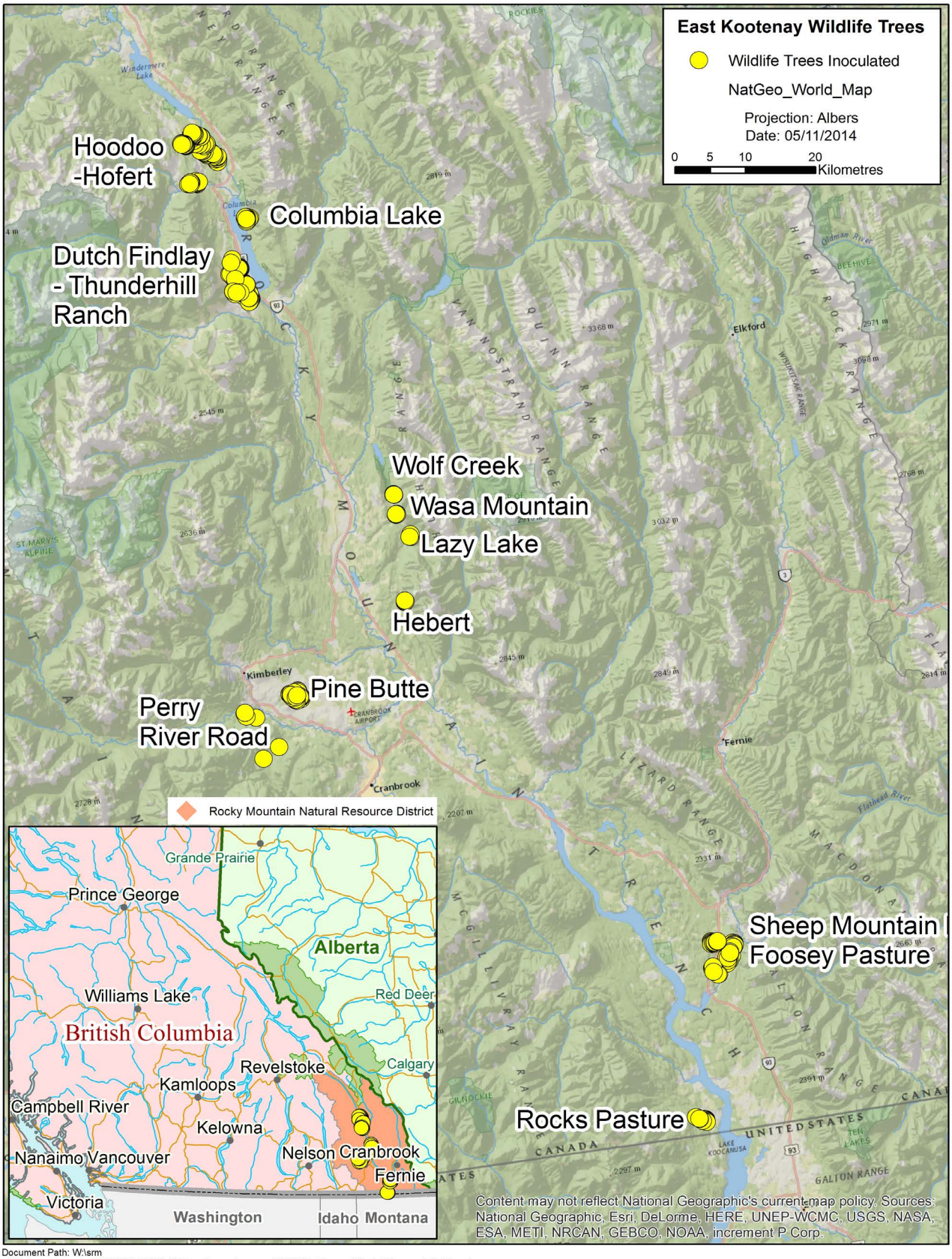

FIGURE 1 *Approximate locations of project treatment areas in the East Kootenay region of British Columbia.*

wildlife-tree creation technique have been described by various researchers in the Pacific Northwest (Bull and Partridge 1986; Parks et al. 1996; Lewis 1998; Brandeis et al. 2002; Manning 2008a, 2008b, 2009; Filip et al. 2011; Bednarz et al. 2013; Manning 2014; Hennon and Mulvey 2014).

Specific project objectives were to:

(a) enhance wildlife tree habitat supply in areas that currently lack wildlife trees; and
(b) increase the abundance of wildlife trees in areas that have high habitat capability for Lewis's Woodpecker, Flammulated Owl, and other cavity-dependent wildlife.

Field Methods

Tree inoculation treatments

Trees intended for treatment were pre-selected, measured, and marked by the authors or by FWCP staff prior to wildlife tree inoculation treatments. All trees selected for inoculation were live, relatively defect-free coniferous trees prior to treatment; this means that except for only a few trees, they did not have dead or broken tops, forked tops, large stem scars or cracks, or any visible evidence of internal decay or root disease.

Three types of wildlife tree creation treatments were applied, either solely or in combination, to candidate trees. The first treatment, a "window treatment" (Figures 2, 3, and 7), involved limbing (pruning)[2] and superficially scarring a 2– to 3–m section in the middle–upper portion of the bole (generally 10–15 m above ground), and then applying a partial stem girdle (i.e., approximately half the bole circumference) to stress the tree and reduce sapflow in this part of the stem but not kill the tree. This section of the tree was then inoculated with a native heart-rot fungus[3] that had been previously cultured in the lab, first on agar-medium petri plates, then onto 8 cm (3 in) × 1.3 cm (0.5 in) softwood dowels (Figure 4). Limbing and scarring the tree in this manner had the added function of introducing a visual stimulus to woodpeckers because it simulated tree damage and potential decay in this part of the stem.

FIGURE 2 *Stem "window treatment" applied at mid-bole in a large, high-value western larch tree on the Thunder Hill Ranch property.*

The second treatment, a "dead top treatment," consisted of removing the original live tree growth leader and leaving a 2– to 3–m de-limbed section as the remaining top (Figures 5 and 7). The tree was completely girdled beneath this section to kill this upper part of the tree, which was then inoculated with the heart-rot fungus *Fomtopsis pinicola* or *Ganoderma applanatum*. These species of fungi prefer to colonize dead woody tissue (Allen et al. 1996). In some cases, trees received a combination of window and dead top treatments.

FIGURE 3 *Douglas-fir partially girdled and scarred in mid-stem section (at red arrows). Fungal inoculant is applied between these positions.*

FIGURE 4 *Fungi being established on wooden dowels inside sterile bags in the laboratory.*

2 In 2007, at Hoodoo-Hofert, the "window treatment" did not include limbing (pruning) in the mid–upper bole.
3 *Phellinus pini* was used for the "window treatment" application at the Hoodoo-Hofert property in 2007. In all subsequent years and locations, *Fomitopsis officinalis* was used for this type of treatment.

FIGURE 5 *This ponderosa pine at Sheep Mountain Ranch was topped and full-ring girdled (at red arrow), and inoculated above the girdle in the remaining uppermost section. A live tree with a dead, decaying top will result.*

FIGURE 6 *This larger-diameter ponderosa pine was topped and completely girdled (at red arrow) below the lowest live limbs. This will become a dead, tall stub tree.*

FIGURE 7 *Two treated Douglas-fir trees at the Hoodoo-Hofert property. The tree in the left foreground received a "window treatment" involving fungal inoculation and limb pruning to "open up" the appearance of the stem in the area where inoculation occurred (red arrow). The tree in the right background was topped (green arrow), then completely girdled and inoculated above the girdle to create a live tree with a dead and decaying top section.*

A third treatment, the "tall stub treatment" (Figure 6), was applied to only some of the larger-diameter ponderosa pine and Douglas-fir (i.e., trees >55 cm diameter at breast height [dbh]). This consisted of completely girdling the tree below the lowest live limbs, and inoculating above this position with either *F. pinicola* or *G. applanatum,* or *Stereum sanguinolentum* for some of the pine only. These trees were also topped and completely girdled at 2–3 m below the remaining top; *F. pinicola* was then applied in this uppermost section. This treatment was intended to kill the tree and produce a moderate-height standing dead stem containing both sap-rot and heart-rot decay, which would provide both short- and long-term feeding and nesting substrate.

Dowel insertion – drilling technique

In conjunction with the respective topping or stem limbing and girdling treatments, all trees were inoculated twice for each treatment type by inserting two cultured dowels into holes drilled into the stem at the desired positions. Inoculation holes were drilled into the heartwood of the live tree to a minimum depth of 15 cm, and were located at the same height on the east-facing and north-facing sides of the tree bole. All drill holes, with inoculant dowels inserted, were left open to the atmosphere. The tree will subsequently seal over this wound and create a dark, low-aerobic environment, which is thought to be more amenable to fungal colonization and growth post-inoculation (Boddy 2001; Manning 2013, 2014). This procedure mimics the natural wounding and infection of trees by airborne fungal spores, which most commonly enter the stem through broken branches and other stem wounds (Hunt and Etheridge 1995). This procedure is a modification of methods previously used by Manning (2008b) and others (Parks et al. 1996; Filip et al. 2011),

where a PVC tube was inserted into the drill hole to purposely keep the wound open to light and atmospheric oxygen.

A Stihl model BT45 gasoline-powered drill with a 9/16-inch wood auger bit was used for drilling holes into the stem. Manning (2008a, 2009) provides a more detailed discussion of relevant laboratory and field methods.

Results

From 2007 to 2013, 369 trees (217 Douglas-fir, 139 ponderosa pine, and 13 western larch) were inoculated and mechanically modified to create wildlife tree habitat in the 13 treatment areas. All trees were live and relatively healthy pre-treatment; fewer than 10 trees had forked tops, stem crooks, or small stem scars. Pre-treatment tree heights were variable but ranged from approximately 12 to 40 m. Mean dbh (all trees) was 54.3 cm, with diameters ranging from 32 to 124 cm.

Table 1 shows the number of trees treated by each treatment type. Most trees received the window treatment (69%) or topping treatment (15%).

TABLE 1 *Number of trees treated by each treatment type at 13 East Kootenay properties from 2007 to 2013*

Property name	Treatment year	Treatment type[a] and number of trees treated						Subtotal	Additional comments
		WI	TI	WI+TI	I	TS	OTH		
Hoodoo-Hofert	2007	94	0	13	0	0	0	107	In 2007, the "WI" treatments did not include limb removal (pruning) in the mid–upper bole
Rocks Pasture	2009	12	0	0	2	0	0	14	Three trees that received "WI" treatment also had additional chainsaw scarring on the stem to simulate lightning strike
Hebert/Wasa Mountain/ Wolf Creek	2009	8	7	0	0	0	0	15	
Pine Butte	2009	26	18	0	3	0	0	47	
Dutch Findlay	2010	45	23	0	0	6	1	75	OTH: tree FWT16 received a second inoculation treatment with *Ganoderma applanatum* at an existing stem scar
Thunder Hill Ranch	2010	8	4	0	0	6	1	19	Tree FWT034 (western larch) had existing significant use as a nest tree, with eight cavities present in the lower bole (2–6 m height). This tree had two window treatments applied. OTH: tree THR004 had WI and TI treatments applied
Sheep Mountain/ Foosey Pasture	2011	44	0	3	0	8	0	55	
Perry River Road	2013	3	0	0	0	4	0	7	
Lazy Lake	2013	4	0	1	0	0	0	5	
Columbia Lake	2013	12	3	1	0	9	0	25	Three trees had two opposing partial stem girdles applied, with four inoculant dowels inserted
Total		256	55	18	5	33	2	369	

a WI = window treatment with partial stem girdle + inoculation
TI = topped, full ring-girdled + inoculation
WI+TI = combination of both treatments
I = inoculation only, no girdling or other chainsaw modifications. These trees had been previously killed by wildfire.
TS = full ring-girdle below lowest live limbs + top treatment (TI) + inoculation at both positions to create a tall stub
OTH = other treatment

Effectiveness evaluation – destructive sampling of trees

To determine if the inoculation and mechanical modification treatments were producing internal decay (sap rot and heart rot), a limited destructive sampling program was conducted in 2010 at the Hoodoo-Hofert property using trees that had been inoculated in 2007; the program was repeated in 2013 at the Dutch Findlay property using trees that had been inoculated in 2010. Five trees were destructively sampled at each location. The purpose of this exercise was to determine (1) the effectiveness of the inoculation/mechanical modification treatments 3 years post-treatment, (2) if the original fungal inoculant was still viable, and (3) if any early heart-rot decay resulting from the treatments was evident.

Destructive sampling of these trees involved climbing and "falling out" the portion of the stem that had been treated in either 2007 or 2010, and cutting it into short sections to determine if any internal decay was present in association with the inoculation treatment. If any evidence of decay was observed (e.g., wood staining/discoloration, wood softening, mycelial filaments), a cross-sectional "cookie" was removed (Figure 8) and taken for laboratory analysis to determine if fungal organisms were still present in the wood and if they were the original treatment fungus. Small wood-tissue samples were also extracted from near the points of original inoculation for each of the 10 sampled trees, and returned to the laboratory for analysis.

Subsequent to destructive sampling, and depending on the initial treatment type, a 10–12 m tall stub was left standing on site. This stub was then re-inoculated about 2 m below the remaining top with *F. pinicola,* and will function as a future medium-height wildlife tree (Figure 9).

Laboratory analysis of wood samples

Fungal isolations from the "cookie" samples taken from the destructively sampled trees at Hoodoo-Hofert and Dutch Findlay were cultured in the laboratory. From the interior surfaces of each wood sample, tissue that had not been exposed to the outside environment was collected using a flame-sterilized scalpel. Tissue from each sample was then plated on malt-extract agar according to methods described by Bridson and Brecker (1970). After approximately 2 weeks of growth, multiple subcultures were made on malt-extract agar in an effort to obtain pure cultures of any fungi growing from the initial wood samples. The following presents the overall results of this work; a more detailed summary of wood condition is presented in Table 2.

FIGURE 8 *Cutting a "cookie" from a destructively sampled section of an inoculated tree for subsequent lab analysis.*

FIGURE 9 *A tall stub wildlife tree left standing after the top portion was removed for analysis.*

Hoodoo-Hofert

All examined sections from the five treated trees showed clear evidence of staining and mycelial filaments, indicating the onset of decay in the treated trees. *F. officinalis* was successfully isolated and cultured (Figure 10) from the inoculated section of Tree #70 (Douglas-fir). However, wood samples taken from the other four trees did not produce re-isolates in the lab. This was likely due to the small mass of woody tissue collected in the field and to possible contamination of these samples with bacteria during handling and transport to the laboratory.

Dutch Findlay

All examined sections from the five treated trees showed clear evidence of staining. One tree (#3, ponderosa pine) had staining and mycelial filaments (Figure 11). Tree #12 (ponderosa pine) had mycelial filaments and significant onset of sapwood and heartwood decay post-inoculation, and stem girdling (Figure 12). Except for one tree (#26, ponderosa pine), all the inoculant dowels had completely rotted away.

F. officinalis, F. pinicola, or *S. sanguinolentum* were successfully re-isolated and cultured from the treatment trees (Figure 13), indicating that the respective inoculant fungi were still viable in the trees at the time of destructive sampling, 3 years post-treatment.

TABLE 2 *Condition of stem wood in relation to fungal inoculation treatments applied in 2007 and 2010 at the Hoodoo-Hofert and Dutch Findlay properties, respectively*

Tree #	Species	Treatment type	Evidence of heart rot decay
Hoodoo-Hofert 2007			
24	Douglas-fir	*Fomitopsis officinalis* at mid-bole window treatment and ½ stem girdle	Noticeable fungal mycelia present in area of dowel, and advanced staining
70	Douglas-fir	*Fomtopsis pinicola* at top treatment and *F. officinalis* 2 m below top (no stem girdle)	Staining and mycelia present; some localized spongy decay
147	Douglas-fir	*F. pinicola* at mid-bole window treatment	Early staining evident that extended vertically along the tree bole ~18 cm above and 14 cm below the inoculum dowel; some mycelia present
163	Douglas-fir	*F. officinalis* at mid-bole window treatment and ½ stem girdle	Some staining near inoculum dowel but less toward the exterior of the stem
162	Ponderosa pine	*F. officinalis* at mid-bole window treatment and ½ stem girdle	Some staining and mycelia evident
Dutch Findlay 2010			
3	Ponderosa pine	*S. sanguinolentum* at mid-bole window treatment and ½ stem girdle	Reddish purple staining and white mycelial filaments present adjacent to the drill holes; both drill holes had grown/sealed over to the atmosphere
10	Douglas-fir	*F. officinalis* at mid-bole window treatment and ½ stem girdle	Minor staining adjacent to drill holes
11	Ponderosa pine	*Stereum sanguinolentum* at mid-bole window treatment and ½ stem girdle	Minor staining adjacent to drill holes
12	Ponderosa pine	*F. pinicola* at top and mid-bole treatments with full-ring girdle below inoculation points to create a tall stub	Mycelial filaments and brown cubical decay present; clear onset of advanced sap rot and heart rot in the inoculated tree sections
26	Ponderosa pine	*F. officinalis* at mid-bole window treatment and ½ stem girdle	Minor staining; inoculant dowels had not decayed and were completely encased by pitch

FIGURE 10 *Wood sample taken from Tree #70 near the original point of inoculation with* Fomitopsis officinalis *(left); the same fungus re-isolated as a pure culture on growth medium in the laboratory (right).*

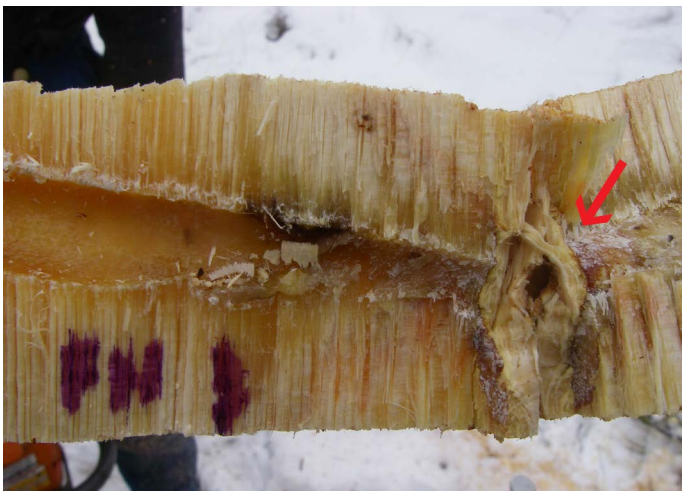

FIGURE 11 *Destructively sampled section from Tree #3 showing empty drill hole at left (dowel decayed away) and white mycelial filaments (red arrow).*

FIGURE 12 *Section of the stem taken from Tree #12 (ponderosa pine) showing white mycelial filaments and brown cubical decay in the heartwood surrounding the drill hole (at red arrow). Note that the inoculant dowel is no longer present and that extensive sap rot is also evident.*

FIGURE 13 *Tree #12 (ponderosa pine) from Dutch Findlay showing significant brownish heart-rot decay and white mycelial filaments (red arrow) in the stem post-inoculation and girdling. Also note the mycelial felt immediately below the pen, which appeared in the laboratory about 1 month after destructive sampling; these are thickened accumulations of vegetative fungal filaments that form once the fungus is well established within the wood tissue. The petri dish shows a thick mycelial layer of* F. pinicola*, which was successfully isolated from this wood sample and re-cultured on growth medium.*

Discussion

Effectiveness of the inoculation treatments

The results of this study shed new light on ways to improve the efficacy and application of the inoculation techniques, and are consistent with other assessments of fungal inoculation trials in British Columbia, Washington State, and Oregon (Filip et al. 2011; Manning 2011; Bednarz et al. 2013; Hennon and Mulvey 2014; Manning 2014). For example, in a follow-up destructive sampling program of live Douglas-fir and western hemlock (*Tsuga heterophylla*) trees in western Washington that had been inoculated 8–9 years previously with the heart-rot fungus *F. pinicola*, Bednarz et al. (2013) concluded that ". . . a higher proportion of treatment trees displayed *F. pinicola* conks (0.200) and mycelia (0.073) than did (non-inoculated) control trees." The authors also observed evidence of significantly more ($p = 0.01$) woodpecker excavations and sapsucker foraging holes associated with fungal inoculation treatments (6.2%) than at control trees (1.2%). Similarly, but in only 3–4 years post-treatment, at least three trees that had been inoculated and mechanically modified (dead top or tall stub treatments) at the East Kootenay Pine Butte and Dutch Findlay project areas showed recent woodpecker activity and nest cavity construction (Figure 14).

In a post-treatment destructive sampling assessment of Douglas-fir on Vancouver Island that had been inoculated 10 years previously with *Phellinus pini,* Manning (2014) found that reddish purple discoloration (staining) occurred in 10 of 12 inoculated trees; two control trees, which had a "blank" wooden dowel inserted, had no staining or any evidence of decay in the heartwood near the inoculant drill hole. Manning also found that two trees had significant heartwood decay and "white fleck" in the wood tissue near the inoculum dowels. The "loci of decay" in these two trees occurred where the original drill hole, which had been artificially kept open to the atmosphere at the time of inoculation using PVC tubing (as per methods described by Parks et al. 1996), had subsequently plugged up with pitch. This was also the case with the "pitch sealing" of the drill holes in Dutch Findlay Tree #3; this tree showed more visible decay (mycelial filaments) than other trees in the sample that had received the same window treatment but where the inoculant drill holes had not completely sealed over to the external atmosphere.

FIGURE 14 *Recent Pileated Woodpecker (*Dryocopus pileatus*) nest cavity (likely excavated in 2012) located near the top of Tree #903 at Pine Butte. This tree had been completely girdled below the top and inoculated with* Fomtopsis pinicola *in 2009. A nesting Northern Flicker (*Colaptes auratus*) was observed at the cavity entrance in 2013.*

In another post-treatment destructive sampling assessment of 20 ponderosa pine, Douglas-fir, and black cottonwood (*Populus trichocarpa*) in southwestern British Columbia that had been inoculated with *F. pinicola* 5 years previously (but not girdled), Manning (2011) found that while this species of fungus remained viable in all the sampled trees, the only significant amounts of decay occurred in the cottonwood. A few salient observations were made during that work:

- Inoculation with *F. pinicola* generally produced minor reddish purple staining in the wood tissue immediately surrounding the inoculum dowel in the Douglas-fir and pine, with abundant pitch generally encasing the dowel; this was particularly true with the highly resinous pine.
- The two sampled cottonwood trees showed significant staining and moderate to advanced decay (wood softening) radiating outward from the inoculum dowel and extending approximately 2 m longitudinally along the stem. White mycelial felts were evident within the cross-sectioned stem wood, an indication of well-established vegetative fungal growth within the tree at this position.

Manning (2011) suggested that partial stem girdling should be applied above and below the inoculation points to reduce sapflow and "pitch sealing." That was not done in the 2011 study nor in the 2007–2011 East Kootenay project treatments, and is particularly important when inoculating live, resinous coniferous tree species. Secondly, it appears that cottonwood is a good candidate species for fungal inoculation treatment since significant heart-rot decay was observed by Manning (2011) even though no stem girdling had been conducted, the drill wound hole had been kept open with PVC tubing, and there are other species of heart-rot fungi, such as *Spongipellis delectans*, that are more suitable for use as an inoculant fungus in live cottonwood.

The small sample size of 10 trees that were destructively sampled in this project does not provide sufficient data to enable statistically significant conclusions to be made about the efficacy of the inoculation treatments. Consequently, additional destructive sampling and monitoring will need to be conducted, particularly on trees that were inoculated along with window treatment, partial-girdling techniques in 2013. Yet it is apparent that heart-rot decay in its early to moderately advanced stages occurred within 3 years post-treatment. This timeframe is very rapid compared to natural heart-rot decay dynamics, which often require more than 100 years from initial tree wounding for significant decay to become established (Hunt and Etheridge 1995; Allen et al. 1996), and suggests that the revised drilling and girdling techniques employed during the latter part of the project period improved the efficacy of the inoculation treatments.

Some key aspects of fungal physiology and the complex relationships between biotic and abiotic variables that influence fungal establishment and wood decay are pivotal to a better understanding of the application of fungal inoculation techniques and the ultimate success of these treatments. A summary of this information is provided below.

Altered sapwood micro-environment

The responses by trees to injury and infection that lead to the genesis of heart-rot decay involve two major and complementary processes: (1) changes in the sapwood micro-environment (called the "reaction zone" [Shain 1979]), including moisture levels, nutrient availability, presence of volatile organic compounds, gaseous regime (CO_2 and O_2), pH, and ambient temperature (Etheridge and Craig 1976; Boddy 1983; Rayner 1986; Boddy 1992, 2001); and (2) succession in the faunal community post-wounding (e.g., Species A alters the micro-environment and is subsequently followed and replaced by Species B, etc.). Faunal succession can be based on interspecific competition, symbiosis, and physical/chemical changes in the woody micro-environment. Consequently, a combination of biotic and abiotic factors, physical and chemical changes, species interactions, and various cause–effect and synergistic relationships are necessary for decay to successfully develop in trees at any given location and time. A detailed discussion of such relationships is provided by Manning (2013) and illustrated conceptually in Figure 15.

In particular, changes in the sapwood micro-environment (called the reaction zone) upon injury strongly influence subsequent faunal succession and progression of internal decay. The reaction zone is also an area of "self-defence," where the tree attempts to resist infection and establish barriers to prevent or limit the onset of decay by fungal organisms (this latter process is referred to as "compartmentalization of decay" [Shigo 1991]). In turn, this compartmentalization often stimulates the onset of decay by various sapwood, then heartwood, fungi (Haddow 1938; Zeglen 1997; Boddy 2001).

Of the micro-environmental changes that can occur in the sapwood, moisture levels, CO_2 and O_2 levels, and ambient light levels are perhaps most significant, and all affect mycelial and reproductive fruiting-body development in fungi. For example, Chapela and Boddy (1988) found that the establishment and development of primary fungal colonizers in beech branches was strongly affected by the water content of the wood after sapwood tissue moisture drops. Simi-

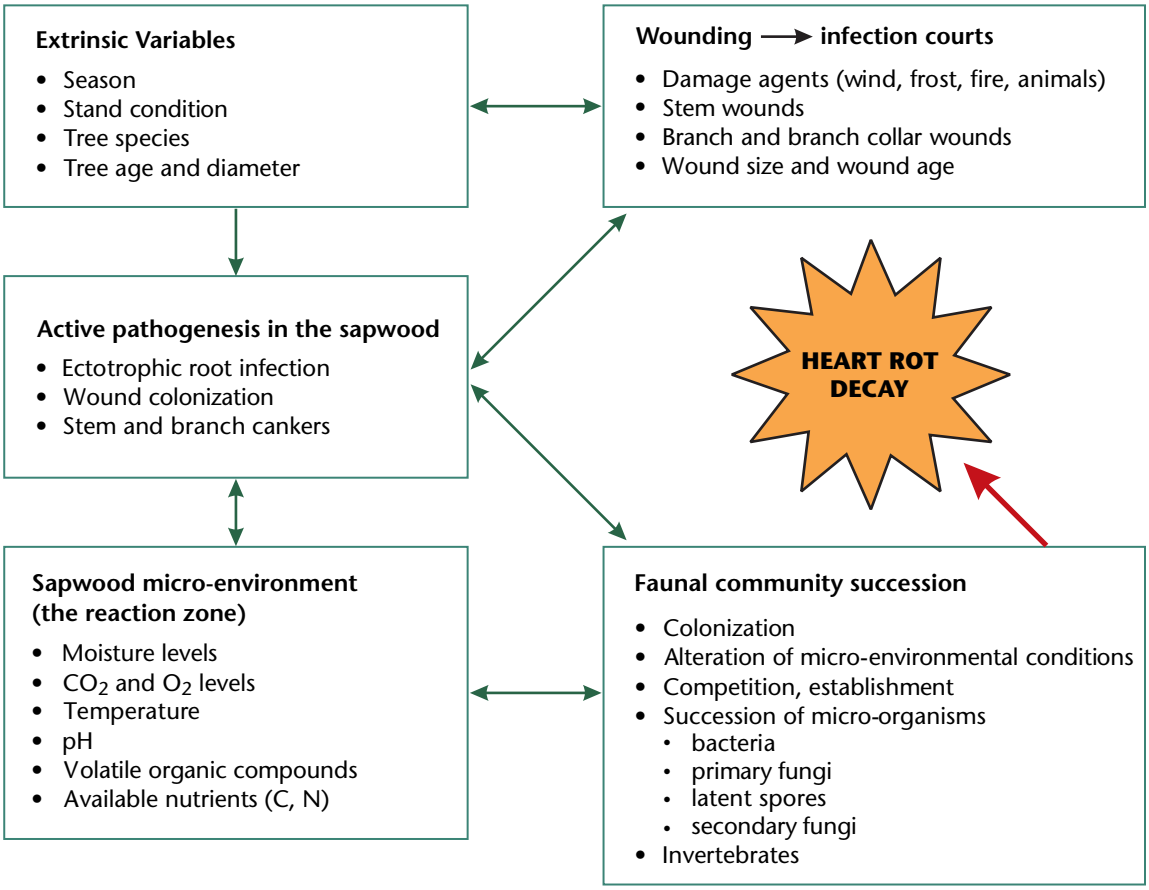

FIGURE 15 *Conceptual illustration showing the complex relationships between biotic and abiotic variables that cause internal tree decay (heart rot). Figure adapted from Manning (2013).*

larly, Stamets (2000) found that vegetative mycelial growth is favoured by micro-environments with prolonged high CO_2 levels up to 50 000 ppm (or 5%), high relative humidity often at 95–100% (but not high water content, as would be the case in live, functional xylem or phloem tissue), and low or nil ambient light levels. Conversely, high CO_2 levels for prolonged periods followed by a sudden drop in CO_2 to near-normal atmospheric levels (350 ppm or 0.035%), along with good aeration and increased light exposure, favour production of fruiting bodies (Stamets 2000; Moore et al. 2008). Such changes can occur as a result of stem wounds or branch breakage, and can quickly change micro-environmental conditions such as relative humidity and CO_2 levels in adjacent woody tissue.

Thus, it is very likely that the different inoculation and mechanical modification techniques that were (or were not) applied in the East Kootenay project area and in the other studies described in this section affected the efficacy of the respective inoculation treatments to varying degrees. For example, whether to keep the drill hole artificially open to the atmosphere, and whether to apply full or partial girdling above or below the drill hole, in turn affected moisture, CO_2 levels, and ambient light levels in the sapwood adjacent to the fungal dowels. As indicated, the resulting micro-environmental conditions in the sapwood strongly influence the ability of colonizing organisms to successfully establish and develop.

Conclusion, Recommendations, and Applications

Of the trees that were destructively sampled in the East Kootenay project area, Tree #12 at Dutch Findlay showed the most advanced heartwood decay. This ponderosa pine had the tall stub treatment: it received a combination of full girdling and inoculations. Not only did this treatment kill the tree, but it is likely that the resulting reduction in sapwood moisture from girdling and the maintenance of high CO_2 and low light levels, worked favourably in the rapid establishment of the heart-rot inoculum fungus

(*F. pinicola* in this case). Although not statistically significant because of limited sample size, this result and the findings from the other sampled trees in this study and similar studies support the following recommendations and applications, which are intended to improve the efficacy of fungal inoculation and mechanical stem modifications as a useful wildlife habitat enhancement tool.

1) For future inoculation treatments, use native heart-rot fungi that are host-tree specific. For example, *F. officinalis, F. pinicola,* and *S. sanguinolentum* have a range of coniferous hosts and have been shown to be the most effective fungi for use in inoculation treatments of Douglas-fir, western larch, and ponderosa pine (Filip et al. 2011).
2) Future inoculation treatments should also apply stem scarring and stem girdling techniques below and above the inoculation points to reduce sapflow (moisture content) to the region of the inoculum dowels. This should improve the sapwood microenvironment for fungal succession and lessen "resin pitching" around the inserted dowels. This is especially important when inoculating ponderosa pine and Douglas-fir, which have abundant sap/pitch flow. If inoculant dowels are inserted using the drilling method, do not keep the drill wound hole open to the atmosphere (i.e., do not insert PVC tubing); instead, partially plug the opening with a small branch or twig.
3) Insert at least three inoculated dowels at the same height (horizontal plane) on the treated tree stem. This will increase radial decay at this level and allow adjacent fungal cultures to coalesce into a larger internal decay column. This technique was also recommended by Filip et al. (2011).
4) The effectiveness of fungal inoculation as a wildlife tree enhancement treatment for black cottonwood looks very promising (Manning 2011). Future treatments involving this tree species should use a host-appropriate heart-rot fungus such as *G. applanatum* or *S. delectans.* Additional mechanical treatments such as stem girdling and scarring can be used in conjunction with inoculation. Enhancement of live cottonwood in this fashion can be used to increase denning or nesting habitat for target species such as fisher (*Pekania pennanti*) and Western Screech-owl.
5) Consider conducting supplementary wildlife tree creation treatments in areas that are scheduled for ecosystem restoration treatments (e.g., stand thinning and prescribed burning). Tall stub treatments are especially valuable in these locations, where there may be few existing standing dead trees. Such treatments will result in near-term feeding activity and longer-term nesting potential, particularly on larger-diameter coniferous stems. As well, removing the upper third or more of the treatment tree greatly reduces the likelihood of future windthrow and stem breakage.
6) Consider using the "rifle induction method" for inoculating preexisting standing dead trees that may have been killed by insects or by treatments such as prescribed fire, and that, because of worker safety concerns, preclude the use of standard climbing and drilling techniques. Filip et al. (2004) and Manning (2008b) provide additional information on using rifles to inoculate trees.
7) Continue ongoing and long-term effectiveness monitoring of inoculated trees to verify the presence and extent of internal decay and subsequent use by cavity-dependent wildlife.

Using the techniques described above, fungal inoculation treatments can provide accelerated creation, enhancement, and recruitment of wildlife trees in areas where there is a shortage of such habitat, and where management goals include increasing wildlife habitat supply and restoration of old forest-like structural characteristics.

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Glossary

Bole – the main stem or trunk of a tree.

Compartmentalization of decay in trees (CODIT) – a reactionary process involving formation of a reaction zone in the sapwood and up to four "walls" or barrier zones. According to the CODIT model, four types of walls form around the injury and infection site to compartmentalize the decay and associated organisms and limit their spread within the affected branch or tree trunk.

Conk – a reproductive fruiting body of a fungus. Typically a woody, bracket-shaped structure as found in basidiomycete fungi.

Heartwood – the inner layers of wood situated farthest away from the vascular cambium. In the living tree, the heartwood has ceased to conduct water or nutrients and contains no living cells, and the cells often become filled with various organic compounds, such as resins and tannins. Heartwood is typically darker than sapwood and is more resistant to attack by decay-producing organisms. Heartwood has an important structural function in early years, but the heartwood column may decay in later years (Dunster and Dunster 1996).

Mycelia – a collective term for fungal hyphae or filaments.

Sapwood – the outer layers of xylem tissue lying immediately interior to the cambium. In living trees, sapwood forms the active water-conducting system.

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