Forest Health Protection
Pacific Southwest Region

Report No. R06-01

3420 Evaluation
March, 2006

Efficacy of Treating Live vs. Dead Stumps With Sporax® to Prevent Annosus Root Disease

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Introduction

Annosus root disease, caused by *Heterobasidion annosum* (Fr.) Bref., is a serious problem in California forests (Bega and Smith 1966, Parmeter and others 1978, DeNitto and others 1984, Smith and others 1966). The root disease is widespread and damaging in southern California recreation sites, resulting in conifer mortality which creates hazard trees, depletes vegetative cover and adversely effects wildlife habitat. The disease can easily be prevented by treatment of freshly cut conifer stumps with a registered borate compound. Sporax® is the currently registered material. Although borax (the common name for the active ingredient in Sporax®) is effective in preventing stump infection (Graham 1971), which is the primary infection court of the pathogen, the cost of treating numerous stumps following extensive fire or bark beetle-caused mortality is of concern. The increasing cost of treating freshly-cut conifer stumps with Sporax® to prevent infection by *H. annosum* has raised some recent questions and concerns as to why stumps created from cutting of dead or fire-killed trees need to be treated with borax. This evaluation provides some background information on the current recommendations, provides results of recent inoculation studies in southern California sites, and discusses suggested changes to the current recommendations for treating stumps of live and dead conifers with Sporax®.

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Background

Past observations, studies, and evaluations have indicated that *H. annosum* levels are high in southern California recreation sites (Wagener and Cave 1946, Wood and others 1979), and that treatment of freshly cut conifer stumps surfaces with a borate compound is effective in reducing infection by about 90%. Based on the above, Forest Service Manual direction (R-5 FSM 2303) requires treatment of all conifer stumps in California recreation sites with a registered borate compound. The same direction applies to other high value areas, such as progeny test sites, seed orchards, and locations with high value trees (R5 FSH 3409.11).

Recent drought and bark beetle attacks in southern California have resulted in extremely high numbers of dead and dying conifers. In some instances, these trees are removed without subsequent preventive stump treatment. Despite the Forest Service Manual directives on stump treatment with Sporax® (borax), the necessity of its use under these circumstances is being questioned. Are stumps of salvaged bark beetle killed trees susceptible to infection by *H. annosum*? Although limited research has been done, Meredith (1960) suggests that *H. annosum* is not a strong competitor in attacking dead tissue; the spores can germinate and penetrate dead tissue, but the pathogen rarely invades tissue occupied by other fungi.

During tree killing bark beetle outbreaks, the question of whether or not to treat stumps of salvaged trees with borax has forest health and economic implications. There is no experimental information from California about the susceptibility of freshly created stumps of dead trees to *H. annosum*. On one hand, if such tree stumps do not require borax treatment to prevent *H. annosum* infection, then salvage costs would be reduced, making it easier to solicit bids for such contracts. On the other hand, forest health can be severely impacted should salvage stumps remain susceptible to infection from airborne *H. annosum* spores. The following study addresses this issue for beetle-killed salvage operations conducted in southern California forests.

Methods

Two sites within the San Jacinto Ranger District, San Bernardino National Forest were selected as study areas. They were the South Ridge area east of the town of Idyllwild, and Camp Emerson, a Boy Scout camp west of Idyllwild. At each site, 30 pine trees were selected, 15 beetle-killed trees that had been dead at least 2 years or more and 15 live trees. Rationale for determining post-mortem age of trees was based on the complete absence of needles from the crowns of the trees. The sample size of 15 trees was determined by tabulating binomial confidence limits for several sample sizes ranging from 5 to 30. The sample size of 15 afforded the highest gain in efficiency relative to other sample numbers, based on upper confidence limits at p=0.05. The trees were cut with a chain saw at about a 2 foot height, the moisture content of surface tissues was measured with a Delmhorst® Model J-2000 wood moisture meter (10 readings taken randomly on the stump surface), and stumps were inoculated with a freshly prepared

water suspension of conidiospores of *H. annosum* within 2 hours after cutting (Figures 1 and 2). The trees at South Ridge were selected, cut, and inoculated May 24, 2005. The trees at Camp Emerson were selected June 13, 2005 and cut and inoculated the next day.

The inoculum consisted of a conidial suspension of spores of *H. annosum* isolates collected from the San Bernardino National Forest. The suspension was prepared by flooding 9 day old cultures growing on 0.5 % malt broth agar plates. Distilled water was added to the plates and spore concentration adjusted to 1 million to 1.2 million spores/ml. Prior to field applications, spore concentrations were estimated using hemocytometer counts from cultures of identical age, isolate, and water dilutions to that used in the field. Spore suspensions were applied to each stump with a mist sprayer until saturation occurred (approximately 50-100 ml per stump). Each stump was numbered with a metal tag nailed to the stump, marked by placing a surveyor's metal pin adjacent to the stump, and mapped.

Eight weeks after treatment, 1 to 2 inch thick discs were cut from the surface of each stump, placed in plastic bags, and taken to a laboratory for incubation at room temperature. The remaining stumps in the field were cut to ground level and immediately treated with Sporax® according to label direction (Figures 3, 4, 5).

After 7 to 9 days of incubation at room temperature, the upper and lower surfaces of the discs were examined under stereoscopes at 40 to 80X magnification for the presence of the asexual state (*Spiniger meineckellus*) of *H. annosum* (Figure 6).

Results

At South Ridge, the asexual state of *H. annosum* was found on 11 of the 15 (73%) stump surfaces from the live pines. None of the 15 surfaces from the dead pines were infested (see Table 1). Results were similar at Camp Emerson, with 10 of 14 (71%) surfaces from live pines (one of the 15 live tree samples was missing), and none of the surfaces from dead pines infested (see Table 2).

The moisture readings taken with the moisture meter had no apparent relationship to live vs. dead tissue or to colonization by *H. annosum*. The 10 readings taken on each stump often varied widely. Many of the stumps from the at least 2 year old dead trees had water-saturated tissues, as evidenced by water being pushed to the stump surface when stumps were cut. Much of the wood tissue from the at least 2 year old dead trees was decayed or stained.

Conclusions

The data would suggest that the current recommendation for treating stumps in recreation and other high value sites in California could be changed from treating all conifer stumps

to treating stumps of conifers with any needles remaining, with little risk of increasing annosus root disease. The same direction could apply to trees in general forest areas.

Literature stating that spores of *H. annosum* do not germinate on dead tissue colonized by saprophytic fungi (Meredith 1960) has been confirmed in this study. However, any live host tissue on stump surfaces could be colonized by *H. annosum* spores. The "treat all" part of the statement in the Forest Service Manual was based on the high value of the trees in recreation sites. The trees still have high value, but because of the questionable efficacy of treating dead tissues and the high cost of treatment, the line officer could be given some leeway when the decision to apply Sporax® to dead trees with no needles remaining is made.

The existing Forest Service Manual direction for the Pacific Southwest Region ("To perpetuate the forest environment in and around developed recreation sites, treat all freshly cut coniferous tree stumps to prevent introduction and spread of Fomes annosus.") could, based on the studies discussed here and combined with results elsewhere (Meredith 1960), be changed to "To perpetuate the forest environment in and around developed recreation and other high value sites, treat freshly cut coniferous tree stumps of all trees with any needles remaining to prevent introduction and spread of *Heterobasidion annosum*."

Recently insect-killed or fire-killed trees, as indicated by the presence of off-color, yellow or brown needles remaining on the tree, were not studied here. As it is likely that trees that appear to be dead by the condition of the needles may have live wood tissue at the stump surface not colonized by saprophytic fungi, stumps of these types of trees should continue to be treated with Sporax® until more research information is available. Further tests are needed to determine the efficacy of Sporax® treatment on recently insect or fire-killed conifers with needles remaining.

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Appendix

Table 1. Recovery of *H. annosum* from inoculated stumps at South Ridge, San Jacinto Ranger District, San Bernardino National Forest.

Tree Number	Pine Species	Tree Condition	Stump Diameter (cm)	Mean Moisture Reading	*H. annosum* colonization at 8 weeks
1	Jeffrey	live	46.9	32.51	Yes
2	Sugar	live	49.1	30.47	Yes
3	Coulter	live	18.0	34.13	Yes
4	Jeffrey	live	30.4	31.12	No
5	Jeffrey	live	48.0	34.33	Yes
6	Sugar	live	26.6	32.42	No
7	Jeffrey	live	30.3	37.24	No
8	Sugar	live	36.3	28.61	Yes
9	Jeffrey	live	25.9	32.93	Yes
10	Jeffrey	live	35.2	37.7	Yes
11	Jeffrey	live	34.9	32.35	Yes
12	Jeffrey	live	27.2	32.78	No
13	Jeffrey	live	22.3	35.99	Yes
14	Jeffrey	live	26.5	31.37	Yes
15	Coulter	live	50.3	29.38	Yes
16	Sugar	dead	70.0	35.28	No
17	Sugar	dead	96.5	20.21	No
18	Sugar	dead	49.2	32.8	No
19	Sugar	dead	84.0	28.27	No
20	Sugar	dead	68.2	26.42	No
21	Coulter	dead	76.8	15.02	No
22	Sugar	dead	70.6	28.95	No
23	Sugar	dead	77.5	35.4	No
24	Sugar	dead	49.6	36.18	No
25	Coulter	dead	47.0	23.8	No
26	Coulter	dead	63.0	24.38	No
27	Coulter	dead	45.1	33.2	No
28	Ponderosa	dead	76.1	35.98	No
29	Coulter	dead	80.4	20.82	No
30	Coulter	dead	69.1	20.01	No

Table 2. Recovery of *H. annosum* from inoculated stumps at Camp Emerson, San Jacinto Ranger District, San Bernardino National Forest.

Tree Number	Pine Species	Tree Condition	Stump Diameter (cm)	Mean Moisture Reading	*H. annosum* colonization at 8 weeks
31	Jeffrey	live	47.1	35.32	No
32	Jeffrey	live	74.0	31.85	Yes
33	Jeffrey	live	48.8	20.62	No
34	Jeffrey	live	41.5	25.40	MISSING
35	Jeffrey	live	60.5	30.98	Yes
36	Jeffrey	live	60.0	35.02	Yes
37	Jeffrey	live	37.5	34.44	Yes
38	Jeffrey	live	46.5	36.06	No
39	Jeffrey	live	53.5	34.66	Yes
40	Jeffrey	live	43.5	34.64	No
41	Jeffrey	live	59.0	35.16	Yes
42	Jeffrey	live	64.2	35.01	Yes
43	Jeffrey	live	32.0	32.60	Yes
44	Jeffrey	live	15.5	21.1	Yes
45	Jeffrey	live	51.0	35.54	Yes
46	Jeffrey	dead	59.5	25.74	No
47	Jeffrey	dead	56.5	34.48	No
48	Jeffrey	dead	61.0	39.10	No
49	Jeffrey	dead	38.5	35.72	No
50	Jeffrey	dead	58.5	31.04	No
51	Jeffrey	dead	49.5	38.54	No
52	Jeffrey	dead	40.5	39.22	No
53	Jeffrey	dead	35.4	26.34	No
54	Jeffrey	dead	25.8	34.30	No
55	Jeffrey	dead	32.0	34.70	No
56	Jeffrey	dead	54.0	36.81	No
57	Jeffrey	dead	50.7	30.92	No
58	Jeffrey	dead	14.6	22.28	No
59	Jeffrey	dead	68.0	37.00	No
60	Jeffrey	dead	32.5	34.98	No

Fig. 1. Moisture readings of freshly cut stump surfaces were taken.

Fig. 2. A spore suspension of *H. annosum* was applied.

Fig. 3. A 1-2 inch thick slab was cut from the surface 8 weeks after inoculation.

Fig. 4. The stump was then cut as flush to the ground as possible.

Fig. 5. Sporax was applied to the freshly cut surface immediately after cutting.

Fig. 6. Slabs were examined in the lab after 7 to 9 days for fruiting of *H. annosum.*