THE INFLUENCE OF EXPERIMENTAL CONDITIONS ON A TEST OF BORAX AND SODIUM NITRITE AS STUMP TREATMENTS AGAINST INFECTION BY *FOMES ANNOSUS*

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Frontispiece. Sporophores of *Fomes annosus* produced on an infected red pine stump. The litter has been removed from around the stump to expose these structures fully.

ABSTRACT

Borax provided excellent protection for red pine and jack pine stumps against infection by *Fomes annosus*. Sodium nitrite failed to give adequate protection under the experimental conditions imposed but did reduce the intensity of infection. Because of the much lower spore load under natural operational conditions, sodium nitrite is possibly a satisfactory stump protectant in southern Ontario. The dissimilarity between the natural and experimental situations and the influence of these differences on the performance of stump protectants are discussed.

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Cover photo shows resupinate sporophores of *Fomes annosus* formed on small roots on the wall of a soil pit in an infection center.

INTRODUCTION

Root rot caused by *Fomes annosus* (Fr.) Karst is a difficult disease to control once it has become established in a forest plantation. Efforts to limit the damage caused by this fungus, therefore, have been based primarily on preventive measures. A freshly exposed stump top is the most frequent point of infection; hence, the disease is usually found in thinned plantations. Stump treatments to avoid infection have been developed and can be incorporated into the thinning operation. Myren and Punter (1972) conducted tests in Ontario to evaluate methods of treating stumps that had been found useful elsewhere. Of the six methods tested, dry borax and sodium nitrite provided the best protection to fresh red pine (*Pinus resinosa* Ait.) stump surfaces, the borax treatment being superior to sodium nitrite. Although sodium nitrite did not perform well in these tests, it has been used quite successfully in Great Britain (Phillips and Greig 1970). This chemical is being used now for stump treatment in Ontario as well; therefore, a further test of the relative effectiveness of borax and sodium nitrite in preventing infection of red pine and jack pine (*Pinus banksiana* Lamb.) stumps by *F. annosus* was initiated in 1971.

MATERIALS AND METHODS

A red pine plantation established in 1924 and a jack pine plantation established in 1928 in Station No. 2 of the St. Williams Forest Station were selected in the fall of 1971 as experimental areas. Both areas were unthinned and free of *F. annosus*, but there were infected plantations nearby.

Four rectangular blocks, 8 m x 30 m,[1] were laid out in the red pine plantation, each separated from adjacent blocks by unthinned isolation strips 5 m wide. Twenty-seven trees were selected in each block, and one of three treatments was assigned randomly to each tree, resulting in nine representatives of each treatment in each block. The test in jack pine was handled in a similar manner, the blocks being 21 m x 30 m with 6-m isolation strips. The red pine and jack pine blocks were thinned on September 28 and 29, respectively. Immediately after a tree was felled, the appropriate treatment was applied to its stump. All stumps were inoculated with a combined suspension of *F. annosus* conidia and basidiospores 24 hr later.

The treatments used were as follows:

a) The stump top was covered with dry, granular borax ($Na_2B_4O_7 \cdot 10\ H_2O$) of 40/100 mesh.

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[1] 1 meter = approximately 3.28 feet.

b) The stump top was sprayed with a 10% (w/v) aqueous solution of sodium nitrite ($NaNO_2$). Rhodamine B extra was added as a marker dye.

c) (control) Nothing was applied to the stump top.

Inoculum was prepared from basidiospores collected in petri plates placed beneath active sporophores during the night preceding inoculation, and from conidia washed from 21-day-old cultures of *F. annosus* growing on malt agar. Aqueous suspensions of the spores were prepared several hours prior to inoculation and were applied to the stump tops with an atomizer. Each red pine stump received approximately 2.2×10^6 basidiospores and 4.1×10^5 conidia. Each jack pine stump received approximately 4.9×10^5 basidiospores and 2.7×10^5 conidia.

Immediately after the inoculations, six disks each (1 cm thick and 8 cm in diameter)[2] were cut from a red pine and a jack pine tree, and sprayed with the inoculum to check spore viability. Five additional disks had been exposed in each experimental block during the 12 hours preceding inoculation to determine the deposition rate of natural inoculum. All of the disks were incubated in petri plates and after 10 days were examined under a dissecting microscope at 10X for the conidial stage of *F. annosus*.

Sixty days after thinning, a section 15 cm thick was cut from the top of each stump. These sections were cut transversely into three disks, each 5 cm thick. The top disk was labelled A, the middle one B, and the bottom one C. Each disk was quartered and the quarters were wrapped individually in moist paper towelling. The four quarters from each disk were then incubated in one plastic bag. Sample preparation took place over a 7-day period during which disks were stored at 5°C[3] with a maximum storage time of 6 days. After an incubation period of 7-10 days at room temperature, the quarter sections were examined for the conidial stage of *F. annosus*. Disks from stumps treated with borax revealed very little infection after 7 days; therefore, 72 of the quarter sections from red pine stumps treated with borax were incubated for an additional 7 days, and 432 of the quarter sections from jack pine stumps treated with borax were incubated for an additional 12 days.

All of the samples were stored at 5°C following the initial incubation periods and were reexamined in 10 days to determine the kinds of fungi, other than *F. annosus*, that were developing on them. These were broadly classed as stain fungi, *Penicillium* spp., *Trichoderma* spp., or other.

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[2] 1 centimeter = approximately 0.39 inches.

[3] $t°C = 9t/5 + 32°F$.

Isolations were made from borax-treated disks to determine if *F. annosus* was present in the tissue but that borax had prevented conidiophore development. The isolations were made by taking small wood chips from two quarters of disk A from each of 20 supposedly uninfected borax-treated stumps of each tree species. Isolations were also made from infected disks from the stumps receiving the other two treatments. Four chips from each quarter section were placed in a petri plate containing a culture medium selective for *F. annosus* (Kuhlman and Hendrix 1962).

RESULTS

The conidial stage of *F. annosus* (*Oedocephalum* sp.) developed on all of the pine disks which were sprayed with the inoculum suspensions in the field, proving that viable spores had been used. The natural deposition rate of viable spores of *F. annosus* during the 12 hours following the thinning operations was 2.6 spores per 100 cm^2 per hr in the red pine stand and 1 spore per 100 cm^2 per hr in the jack pine stand.

The best protection for both red pine and jack pine stumps was provided by borax (Tables 1 and 2). Sodium nitrite furnished some protection when used on jack pine stumps. When the data are examined from the standpoint of the number of infected quarter sections per disk, Tables 3 and 4 show that borax gave the best protection but that sodium nitrite also provided statistically significant protection. The number of infected quarter sections found in disks B and C indicated that *F. annosus* had penetrated into the stumps to a depth of 5-10 cm during the 60-day test period. Extending the incubation period for quarter sections from stumps treated with borax did not result in any additional infections being located.

During sampling it was noted that the bark separated readily from disk A of the stumps treated with sodium nitrite. This is probably an indication of phytotoxicity and was not observed on stumps treated with borax or on stumps in the control group.

Results of the classification of the fungi that developed on the disks are presented in Tables 5 and 6. The greater incidence of saprophytic fungi in the disks from stumps treated with sodium nitrite is quite noticeable, particularly in disks A and B. A high percentage of the disks were colonized by stain fungi but their influence on the intensity of infection cannot be determined from this test. The stain fungi colonizing the jack pine stumps were not inhibited by the borax treatment as they were in disk A of the red pine stumps similarly treated. This suggests that different species of fungi were involved or that the environment created in a jack pine stump treated with borax is quite different from that found in a similarly treated red pine stump. The absence of *Penicillium* spp. from all of the jack pine disks and its

Table 1 Treated and inoculated red pine stumps yielding *Fomes annosus* 10 weeks after thinning

Treatment[a]	% of stumps yielding *F. annosus*				
	Block No. 1	Block No. 2	Block No. 3	Block No. 4	Average
$Na_2B_4O_7 \cdot 10\ H_2O$ (borax)	0	0	0	11	2.8[b]
$NaNO_2$ in H_2O (sodium nitrite)	78	100	67	89	83.5
Control	100	100	89	100	97.2

[a] Borax was applied as a powder. The sodium nitrite was 10% (w/v) aqueous solution with rhodamine B extra added as a marker.

[b] Significantly different from the control and the sodium nitrite treatment at the 1% level.

Table 2 Treated and inoculated jack pine stumps yielding *Fomes annosus* 10 weeks after thinning

Treatment[a]	% of stumps yielding *F. annosus*				
	Block No. 1	Block No. 2	Block No. 3	Block No. 4	Average
$Na_2B_4O_7 \cdot 10\ H_2O$ (borax)	0	0	0	0	0.0[b]
$NaNO_2$ in H_2O (sodium nitrite)	89	55	33	33	52.5[c]
Control	100	100	100	100	100.0

[a] Borax was applied as a powder. The sodium nitrite was 10% (w/v) aqueous solution with rhodamine B extra added as a marker.

[b] Significantly different from the control and the sodium nitrite treatment at the 1% level.

[c] Significantly different from the control at the 1% level.

Table 3 Intensity of infection in treated and inoculated red pine stumps based on the number of quarter sections yielding *F. annosus* from each disk

Treatment[a]	Avg. no. of quarter sections yielding *F. annosus*		
	Disk A	Disk B	Disk C
$Na_2B_4O_7 \cdot 10\ H_2O$ (borax)	0.3[b]	0.0[b]	0.1[b]
$NaNO_2$ in H_2O (sodium nitrite)	2.4[c]	2.6[c]	0.8[d]
Control	3.6	3.4	1.4

[a] Borax was applied as a powder. The sodium nitrite was 10% (w/v) aqueous solution with rhodamine B extra added as a marker.

[b] Significantly different from the control and the sodium nitrite treatment at the 1% level.

[c] Significantly different from the control at the 1% level.

[d] Significantly different from the control at the 5% level.

Table 4 Intensity of infection in treated and inoculated jack pine stumps based on the number of quarter sections yielding *Fomes annosus* from each disk

Treatment[a]	Avg. no. of quarter sections yielding *F. annosus*		
	Disk A	Disk B	Disk C
$Na_2B_4O_7 \cdot 10\ H_2O$ (borax)	0.0[b]	0.0[b]	0.0[c]
$NaNO_2$ in H_2O (sodium nitrite)	1.1[c]	1.0[c]	0.1[c]
Control	3.9	3.8	3.3

[a] Borax was applied as a powder. The sodium nitrite was 10% (w/v) aqueous solution with rhodamine B extra added as a marker.

[b] Significantly different from the control and the sodium nitrite treatment at the 1% level.

[c] Significantly different from the control at the 1% level.

Table 5 Fungi detected on treated red pine stumps 12 weeks after inoculation with *Fomes annosus*

Fungi by disk[b]	Treatment[a]		
	Borax	Sodium nitrite	Control
	% incidence		
Disk A			
Trichoderma spp.	6.5	57.1	5.0
Penicillium spp.	0.0	31.4	2.5
Stain producers	19.3	100.0	80.0
Other	12.9	17.1	7.5
None	77.4	0.0	0.0
Disk B			
Trichoderma spp.	9.6	42.9	2.5
Penicillium spp.	0.0	37.1	2.5
Stain producers	70.9	94.3	77.5
Other	29.0	42.9	15.0
None	22.6	2.9	0.0
Disk C			
Trichoderma spp.	9.6	14.3	7.5
Penicillium spp.	0.0	14.3	0.0
Stain producers	90.3	94.3	75.0
Other	29.0	25.7	10.0
None	3.2	0.0	12.5

[a] Borax ($Na_2B_4O_7 \cdot 10\ H_2O$) was applied to the stump top as a powder. Sodium nitrite ($NaNO_2$) was applied as a 10% (w/v) aqueous solution with rhodamine B extra added as a marker. Nothing was applied to the control stumps.

[b] Each 15-cm stump section sampled was cut transversely into three 5-cm disks, the top disk being A and the bottom C.

Table 6 Fungi detected on treated jack pine stumps 12 weeks after inoculation with *Fomes annosus*

Fungi by disk[b]	Treatment[a]		
	Borax	Sodium nitrite	Control
	% incidence		
Disk A			
Trichoderma spp.	2.9	79.4	17.5
Penicillium spp.	0.0	0.0	0.0
Stain producers	91.4	97.1	67.5
Other	17.1	44.1	12.5
None	17.1	0.0	0.0
Disk B			
Trichoderma spp.	19.4	70.6	23.1
Penicillium spp.	0.0	0.0	0.0
Stain producers	100.0	100.0	71.8
Other	19.4	50.0	5.1
None	0.0	0.0	0.0
Disk C			
Trichoderma spp.	16.7	44.1	22.5
Penicillium spp.	0.0	0.0	0.0
Stain producers	100.0	91.2	67.5
Other	25.7	32.3	0.0
None	0.0	0.0	0.0

[a] Borax ($Na_2B_4O_7 \cdot 10\ H_2O$) was applied to the stump top as a powder. Sodium nitrite ($NaNO_2$) was applied as a 10% (w/v) aqueous solution with rhodamine B extra added as a marker. Nothing was applied to the control stumps.

[b] Each 15-cm stump section sampled was cut transversely into three 5-cm disks, the top disk being A and the bottom C.

presence in the red pine disks cut from stumps that received the sodium nitrite treatment results in a similar conclusion.

Fomes annosus was not recovered from any of the 40 "A" disks of borax-treated stumps from which isolations were made. The fungus was recovered from infected quarters of "A" disks from stumps treated with sodium nitrite and from stumps which received no chemical treatment.

DISCUSSION

Data presented in Tables 1 and 2 indicate that sodium nitrite did not prevent red pine and jack pine stumps from becoming infected by *F. annosus*, although the intensity of the infection was significantly reduced relative to that in the controls (Tables 3 and 4). Borax, on the other hand, gave excellent protection.

These results require some explanation since sodium nitrite is the standard stump protectant used in southern Ontario at the present time. One must remember the conditions imposed for these experiments. The inoculum load used was from 500 to 2000 times that which could be expected to occur naturally in a 24-hr period in southern Ontario. Certainly the inoculum used would not exhibit 100% viability, but undoubtedly the viable spore load substantially exceeded what would be considered normal in nature. Possibly sodium nitrite is simply not sufficiently toxic to prevent infection when such a large number of spores is present. This tends to be substantiated by the fact that in jack pine, where the inoculum load was less than that used in inoculating the red pine, sodium nitrite did provide some stump protection. Furthermore, the intensity of infection was reduced more than in the red pine. It is conceivable that sodium nitrite could offer satisfactory stump protection under natural conditions of relatively low infection intensities. Inoculations were carried out in southern Ontario to assure the presence of spores of *F. annosus*, as natural spore deposition rates are quite variable and hence unreliable for experimental purposes. The failure of sodium nitrite to protect red pine stumps from infection by *F. annosus* reported in two previous studies (Hadfield 1968; Myren and Punter 1972) could also be due to the above-mentioned factor.

Sodium nitrite is believed to protect the stump by its toxicity to *F. annosus* and by encouraging the development of a variety of other fungi (Gundersen 1967). A toxic and competitive environment is created in the stump top, reducing the probability of successful colonization by *F. annosus*. The data presented in Tables 5 and 6 indicate that stumps treated with sodium nitrite did support a rapid development of saprophytic fungi. This development is probably due to the presence of additional nitrogen provided by sodium nitrite and to the toxicity of sodium nitrite to living stump tissues. Such tissues are an unsuitable

substrate for many saprophytic fungi. The increase in colonization by saprophytes was less pronounced in disk C than in disks A and B of the stumps treated with sodium nitrite, indicating a decrease in penetration of the chemical. Stumps treated with borax, which inhibits the growth of many fungi, showed an increase in fungi in the B and C disks as the concentration of this compound decreased. Little change was evident in the colonization of the three groups of disks from the untreated stumps. It should be mentioned that the data in Tables 5 and 6 do not reflect the situation that would be found in the field. Sectioning of the disks exposed surfaces of disks B and C to airborne and bark-inhabiting fungi and resulted in a more rapid colonization than would have occurred through penetration from the stump top. The data do reflect the ability of the substrate to support these fungi, however. Colonization of disk B by *F. annosus* and, in some cases, by blue-stain fungi, was observed to have resulted from penetration through disk A prior to the sectioning of the stumps.

Gundersen (1967) reported that two species of *Trichoderma* isolated from spruce and pine disks treated with sodium nitrite were highly antagonistic to *F. annosus*. In Tables 5 and 6 it can be seen that the development of *Trichoderma* spp. was enhanced in the red pine and jack pine stumps treated with sodium nitrite. Rishbeth (1963) has reported that some blue-stain fungi can restrict the growth of *F. annosus*, but that this is an erratic occurrence. Blue-stain fungi were encouraged by the sodium nitrite treatment and also by the borax treatment in the case of jack pine.

An assessment of the influence of other fungi on infection by *F. annosus* must be made in both the natural and the experimental situations. Stumps normally remain susceptible to infection for several weeks, during which time a variety of fungi, including *F. annosus*, can become established on the stump surface. The sodium nitrite treatment encourages development of saprophytes, while the untreated and the borax-treated stumps tend to exclude many of them, blue-stain fungi being a notable exception. Under natural conditions the competitive situation in the sodium nitrite stump would soon develop, reducing the area on which *F. annosus* could become established while also tending to limit its growth. Inoculating stumps with a substantial number of *F. annosus* spores 24 hr after the tree is felled, however, gives *F. annosus* a competitive advantage.

An unusually dry summer preceded the thinning date and this may have affected the trees, resulting in stumps that were more susceptible to infection by *F. annosus* than is typical. Possibly changes of this nature also decreased the effectiveness of sodium nitrite as a stump protectant. The influence of the above weather conditions and the influence of the inoculum density on the ability of sodium nitrite to protect red pine and jack pine stumps will be the subject of future research projects.

Under the experimental conditions of this study sodium nitrite did not prevent infection of red pine and jack pine stumps. However, this chemical did substantially reduce the intensity of infection. There is a definite need for a realistic evaluation of the efficiency of sodium nitrite and borax used operationally. Borax has been shown to be highly effective as a stump protectant for both red pine and jack pine stumps but, because of its tendency to preserve stumps, and supposedly the stumps' roots, it is suitable for use only in stands which do not have existing infection centers of *F. annosus*. A delay in the breakdown of the roots would result in their serving as a food base for *F. annosus* and as a vehicle for its spread for a longer than normal period of time. In stands having active infection centers, then, borax would not be the best protectant, at least not near the infected areas. Rather, sodium nitrite, which encourages the growth of saprophytic fungi and presumably would speed up stump breakdown, might be preferable.

Borax can be recommended for use by private individuals, such as cottage owners, who may be interested in cutting a few trees and wish to avoid problems with *F. annosus*. Pure borax can be purchased in many grocery stores and can be used directly; no solution need be prepared. The borax powder is simply applied so as to form a thin layer over the entire stump surface.

It is conceivable that a wider recommendation for the use of borax as an alternate method of treating stumps will be made at some future date. Further studies comparing the costs of treatment, methods of treatment, and the effectiveness of borax and sodium nitrite under operational conditions are required, however, before a final evaluation of each chemical's merits can be made and a recommendation formulated.

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