Borax: Its Toxicity to Fomes annosus in Wood and its Diffusion, Persistence, and Concentration in Treated Stumps of Southern Pines

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ABSTRACT

Growth of *Fomes annosus* was prevented in wood of loblolly pine by 660 ppm of total anhydrous borax in oven-dry wood (300 ppm in wet wood). In freshly cut stumps, this concentration of borax diffused to its maximum depth more rapidly than the fungus would germinate and grow from the point of application. Borax diffused at toxic concentrations to a depth of only 2.1-5.1 cm. Thus, logging scars at the base of stumps should be treated with borax to afford complete protection. The chemical persisted uniformly at a toxic concentration 5.1 cm below the stump surface for at least 8 weeks. Twenty-six months after treatment, borax had leached to subtoxic levels throughout the upper 0.3 cm of stumps, but at a depth of 1.2 cm, 38% of the cross-sectional area still contained toxic amounts. Borax raised the pH of wood from 4.8 to 7.6-8.1; this effect is not important in preventing colonization of the stump surface by *F. annosus* since a supratoxic concentration of borax was required to achieve it. Phytopathology 61:269-274.

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Borax (U.S. Borax, sodium tetraborate decahydrate, granular technical) prevents colonization of cut stump-surfaces of southern pines by *Fomes annosus* (Fr.) Karst. (1, 2, 3). It is not, however, a highly lethal fungicide in vitro (7). Others (6) suggested that small amounts of this chemical could interact with wood to produce toxic substances, but analytical determinations of the concn of borax required to prevent growth of *F. annosus* in wood have not been published. Investigations reported here determined levels of borax toxic to the fungus in wood of loblolly pine (*Pinus taeda* L.), diffusion rates and persistence of borax in stumps of loblolly and slash pine (*P. elliottii* Engelm. var. *elliottii*), and the effect of borax on the pH of wood of both species.

MATERIALS AND METHODS.—*Assay and detection of boron.*—Boron (B) was assayed by a procedure developed in the Soils Laboratory, Forestry Sciences Laboratory, Research Triangle Park, N.C., and slightly modified (the centrifugation step) for wood samples. Aid-dried samples were ground in a Wiley mill to pass an 040-mesh screen. This ground wood was oven-dried at 60 C for 1 day, and 1 g was placed in a 30-ml beaker; 5 ml of saturated CaOH were added to prevent volatilization of boron during ashing, and the sample was dried for 4 hr at 105 C. The sample was ashed at 450 C for 4-8 hr, cooled to room temp, taken up in 10 ml of 0.4 N HCl, and centrifuged for 10 min at 10,000 *g*. Two ml of supernatant were placed in a 50-ml Erlenmeyer flask. When necessary for spectrophotometric considerations, dilutions were prepared from this supernatant with 0.4 N HCl. Ten ml of concd H_2SO_4 were added and mixed; 10 ml of carmine reagent [0.91 g carmine/liter H_2SO_4 (8)] were added and mixed. Transmittance was measured on a Coleman universal spectrophotometer at 585 mμ 1-3 hr after the previous step. Standards of borate (sodium tetraborate decahydrate, Fisher, AR) at 0, 0.5, 2, 5, and 8 ppm B in 0.4 N HCl were run simultaneously. Borate concn in wood were calculated from these data and are expressed as *anhydrous* sodium tetraborate in oven-dry wood throughout this paper except where noted.

B was also detected colorimetrically in oven-dried wood from slash and loblolly pine stumps or discs from stems with single drops of each reagent (solutions I and b) for detecting B as described by Wilson (12). Colorimetric reactions were rated visually from 0-4, based on the relative intensity of the hue, red, independent of any background coloration. Preliminary trials showed that *F. annosus* failed to grow when the colorimetric rating was approx midway between 3 and 4. Therefore, this value was used as an index of *the upper toxic-threshold concn of borate* ($[Borate]_T$). Based on three experiments to be reported elsewhere, the average $[Borate]_T$ of discs with this rating was 680 ppm.

pH Measurement.—The pH of the wood was measured by (i) estimating it visually to the nearest 0.3 unit from the color developed (5) with bromothymol blue, chlorphenol red, or thymol blue (4, p. 1613) applied as sprays or drops; or (ii) reading with a meter the pH of 10 ml of distilled water in which 2-g samples of ground wood had been boiled (11).

Determination of the toxic concn of borate.—For toxicity tests, discs approx 0.6 cm thick were cut from stems (3.8-5.1 cm diam) of saplings of open-grown loblolly pine and numbered consecutively for each sapling. Discs in all growth and B assays were assigned to treatments so as to minimize errors due to possible positional effects within a stem. Discs from each tree that received the same treatment were sterilized together in Kraft paper bags either at 55 C for 4 hr with ethylene oxide or for 2 hr in a steam autoclave. Gas-sterilized discs were aerated in the bags at 25 C for 40 hr after sterilization. Twelve to 15 discs were soaked simultaneously for 10 min with intermittent agitation

in 1 liter of autoclaved solutions of (i) 0, 0.25, 0.5, 1.0 or 2%; (ii) 0, 0.009, 0.018, 0.09, 0.18, 0.9 or 1.8%; (iii) 0, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.4 or 1.8%; or (iv) 0, 0.4, 0.6, 0.8, 1.0, 1.4 or 1.8% borate in distilled water. The pH of concn series (i) was adjusted to 5.0-5.6 in three experiments, but was unadjusted in other experiments and in other series. Discs were placed in sterile petri dishes and inoculated with 1 ml of a conidial suspension of *F. annosus* isolate 23, 128, or 133 grown and prepared as previously described (7). Inoculated discs were incubated at 25 C, and fungal growth was rated at 7, 11, and 14 days. Growth was recorded as the percentage of the upper disc-surface covered by mycelium. Absence of growth in these and other test-discs or test-sections was confirmed by microscopic examination for the *Oedocephalum* conidiophores of *F. annosus*.

Diffusion of borax.—The rate of diffusion of B in stumps was measured by applying ca. 3 lb. borax/50 ft^2 (1.36 kg/4.64 m^2) of cut surface area of 12-year-old slash pine of the codominant and intermediate crown classes at Ft. Bragg, N.C., in January 1969. The amount of borax applied was determined by weighing amt applied similarly to paper shields of measured area. The stumps, about 27 cm high, were treated as the trees were felled. Groups of 25 treated and 5 non-treated control stumps were harvested at 1, 2, 4, and 8 weeks. The average depth of the pH reaction was measured from the stump surface on the longitudinal surface of one-half the split stumps with sprayed indicators. Three blocks approx 3.8 cm × 3.8 cm × height of the harvested stump were split from the other half of each stump. The blocks were sawn into sections ca. 2.5 cm thick (which were then separated by waxed paper to prevent diffusion of borate), oven-dried for 2 days at 55-60 C, and measured for penetration of B with Wilson's reagents applied as drops to 10-12 spots on the lower cross-sectional surface.

Short-term diffusion of B was measured by applying borax in December 1968 to the stump surface of 17-year-old loblolly pine of the suppressed and intermediate crown class and 10.2 cm in stump diam growing at Research Triangle Park, N.C. After discarding the surface 0.15 cm disc, cross-sectional discs 0.30 cm thick were cut from two stumps to a distance of 5.1-8.6 cm from the stump surface at 0, 1, 2, 4, and 8 days. The depth of the pH change was measured with the indicator and of B diffusion with Wilson's reagents.

Persistence of borax.—The persistence of borax was measured by assaying for B in loblolly pine stumps located at Aulander, N.C. Trees at Aulander had been cut in February, May, and August 1966, and January 1967, as part of an experimental thinning, and treated with approx 1 lb. of borax/50 ft^2 (0.46 kg/4.64 m^2) of stump area. Untreated stumps were not available because they were usually rotted and unsuitable for controls. The upper 15.1-30.5 cm were cut from each of 40 stumps for each harvesting date. Three blocks approximately 3.8 cm × 3.8 cm × the height of the harvested stump were split from along a radial line through the center of each stump. These blocks were cut into four series of cross sections beginning at the stump surface: (i) 0-0.3 cm; (ii) 0.3-1.2 cm; (iii) 1.2-3.8 cm; and (iv) 3.8-5.1 cm. Sections in series i, ii, and iv were placed in petri dishes so that the upper surface of series i sections, the lower surface of series ii, and the upper surface of series iv sections faced upward. The blocks were autoclaved for 1 hr and inoculated with 1 ml of a conidial suspension of culture 128 and incubated at 25 C. Sections were examined for growth of *F. annosus* after 2 weeks. The pH of upper and lower surfaces of sections in series iii were tested with bromothymol blue and then for B with Wilson's reagents. Visual ratings of B levels were correlated with growth of *F. annosus* on the series ii and iv sections adjacent to the appropriate face of series iii sections.

RESULTS.—*Toxic concn of borate.*—Results of three preliminary tests with gas-sterilized discs soaked in solutions of 0, 0.25, 0.5, 1.0, and 2.0% borate showed that *F. annosus* grew on 93% of 56 discs soaked in 0.5% solution, but never on discs with > 1% solutions (Table 1). This generally confirmed previous results, but calculation revealed that a 10-g disc should absorb not more than 0.0004 ml of a 0.5% solution to give the 0.2 ppm quantity calculated to produce the toxic effect reported to occur in wood of red pine (6). In three tests with 105 discs from 13 trees, discs absorbed an average of 0.174 g water (range .138-.197 g) per g dry wt of wood, or an average of 1.39 g/disc, which suggested a species difference or a mathematical error in the cited work.

TABLE 1. Percentage of the number of gas-sterilized pine discs completely overgrown by *Fomes annosus* 2 weeks after discs were soaked in given concentrations of sodium tetraborate decahydrate and inoculated with a conidial suspension of *F. annosus*

Borate concn	Test 1[a]	Test 2[a]	Test 3 pH adjusted[a]	Test 3 pH unadjusted	Total no. discs	Weighted avg, %
%	*Discs covered with mycelium, % of the no.*					
0	100	100	100	100	60	100
0.25	100	100	100	100	60	100
0.50	75	92	100	89	56	93
1.00	0	0	0	0	58	0
2.00	0	0	0	0	57	0

[a] pH of borax solutions was adjusted to 5.0-5.6.

TABLE 2. Growth of *Fomes annosus* and boron analysis of gas-sterilized pine discs soaked for 10 min in various concentrations of sodium tetraborate decahydrate, then inoculated with a conidial suspension of *F. annosus*

Borate concn	Growth	Anhydrous borate concn
%	%[a]	*ppm*[b]
0	100	34.9
.4	100	389
.5	60	553
.6	40	586
.7	7	646
.8	0	806

[a] Expressed as the average of the estimated percentage of the area covered by mycelium at each concn 1 week after inoculation for 12 pine discs.

[b] Expressed as ppm of anhydrous borate in oven-dry wood for 12 discs at each concn.

To determine $[\text{Borate}]_T$ more accurately, a test was conducted with discs cut from three loblolly pine saplings and distributed, four from each tree, to each treatment as in the previous experiment. In this test, some growth occurred at intermediate concn; therefore, growth was evaluated by estimating the percentage of the disc surface covered by *F. annosus* 1 week after inoculation (Table 2). Although 7% of the total surface of discs was covered by fungal growth on 12 discs treated with 0.7%-borate solutions, the fungus failed to grow on half of these discs. Plotting data in Table 2 revealed that $[\text{Borate}]_T = 660$ ppm.

A test was established to determine whether the method of applying the borate solution influenced $[\text{Borate}]_T$, and whether there was a difference in the activity of borate against *F. annosus* in fresh wood vs. sterile wood. Three discs from each of three trees were distributed to each treatment as in previous tests. Results are presented in Table 3. Plotting data from Table 3 for both fresh and gas-sterilized discs indicated $[\text{Borate}]_T = 660$ ppm. The six soaked discs with the lowest percentage of their surface covered by *F. annosus* ($\bar{x} = 2.3\%$) were ranked in order of increasing borate content. The six discs with the lowest concn of borate and which showed no growth of *F. annosus* were similarly arranged. Four discs with the greatest borate concn and showing growth contained 546-600 ppm ($\bar{x} = 580$); four with the lowest concn and without growth had the same range of concn ($\bar{x} = 600$ ppm). When the concn was $\geqslant 710$ there was no growth. Similar ranking of six discs receiving 1 ml of borate solution revealed that three discs with growth ($\bar{x} = 2.0\%$) contained an average of 780 and three without growth averaged 790 ppm borate. Thus, $[\text{Borate}]_T$ for discs treated with 1 ml of solution of a given concn was about 15% higher than it was for those soaked in solutions of the same concn. Apparently the extra water ($\bar{x} = 1.39 - 1.00 = 0.39$ g/disc) absorbed by the soaked discs adversely affected growth of the fungus. The adverse effect of excess water is also suggested in the soaked, fresh discs on which the excess water prevented growth of *F. annosus* at even the lowest concn of borate, although inhibition from microbial competitors may have been more vigorous on these discs.

From data in Tables 2 and 3, the average $[\text{Borate}]_T$ for soaked discs was 660 ppm. The average "borate" in control treatments was 35.4 (range: 34.9-36.0). Therefore, in terms of actual borate added, approx 628 ppm totally inhibited the fungus.

Diffusion of borax.—In preliminary tests, $[\text{Borate}]_T$ diffused to a depth of 1 cm in 10 min from a 1.8% solution. This was determined by soaking stem cylinders 10 cm in length, cutting them into discs 1 cm thick, and testing for a colorimetric rating of $\geqslant 3.5$ with Wilson's reagents.

In stumps harvested and tested for borax after 2, 4, and 8 days, $[\text{Borate}]_T$ diffused a depth of 1.75 cm, 2 days after treatment. Diffusion of this concn progressed only to 2.07 cm depth at the longer sampling times. Diffusion was quite uniform in most stumps, although it was irregular in some infected with *Cronartium fusiforme*.

In slash pine stumps treated with granular borax at Fort Bragg, $[\text{Borate}]_T$ was present at its max depth (5.1 cm) in 1 week (Table 3). Some borate could be detected in this same time 25 cm below the cut surface of the stump.

Persistence of borax.—In 26 months, borax was leached to subtoxic levels in 88% of 120 test sections from the surface 0.3 cm of treated loblolly pine stumps at Aulander, N. C. At 1.2 cm depth, however, the chemical was present at a toxic concn in 38% of the sections at 26 months, 38% at 30 months, 13% at 33 months, and 2% at 36 months. Thus, although borax was leached drastically from the surface, a fairly appreciable amt remained at the 1.2 cm-depth 30 months after treatment.

The average colorimetric rating of inoculated sections of the surface-0.3 cm of 26-month-old stumps showing even microscopic growth was 1.3, while discs without growth were rated 3.1. Inoculated discs from the 0.3-1.2 cm depth with more than faint growth of *F. annosus* from stumps of all ages were rated colorimetrically. Of 104 discs tested, all were rated < 3.0 ($\bar{x}$: 26 months, 1.5; 30 months, 1.3; 33 months, 0.6; and 36 months, 0.5).

pH In borax-treated wood.—Fewer stem discs soaked in borate solutions in which the pH had not been adjusted supported growth of *F. annosus* (experiment 3, Table 1) than in discs treated with adjusted solution, and the growth was less luxuriant on other discs receiving the first treatment. This suggested that borate might be acting by altering the pH of wood, and thus the effect of borate on this variable was explored. Nine days after inoculation, the pH of sections used in this test was 6.9 for solutions soaked in $\leqslant 1.0\%$ borate and 7.3 for discs at 2.0%. The pH of other discs exposed for 10 min to borate solutions from 0.018-1.8% was measured with the pH indicators 2 days after treatment; wood soaked in 0.9 and 1.8% solutions had a pH of 7.3; discs soaked in $\leqslant .18\%$ solutions was 6.5. Six days after drying, however, the pH of discs soaked even in the higher concn was $\leqslant 6.0$. Apparently, upon drying,

TABLE 3. Growth of *Fomes annosus* on and boron analysis of fresh and gas-sterilized pine discs previously soaked in solutions of sodium tetraborate decahydrate or topically treated with 1-ml volumes of given concentrations and inoculated with a conidial suspension of *F. annosus*

Borate concn, %	% Growth[a]				Anhydrous borate concn, ppm[b]	
	Fresh		Gas sterilized		Gas sterilized	
	Soak	1 ml	Soak	1 ml	Soak	1 ml
0.0	100, C[c]	90, C	100	100	35.7	36.0
0.4	0, C	100, C	21	100	455	
0.6	0, C	75, C	1	100	652	
0.8	0, C	45, C	0	30	810	
1.0	0, C	5, C	0	11		448[d]
1.4	0, C	2, C	0	2		659[d]
1.8	0, C	0, C	0	<1		848

[a] Expressed as the average of the estimated percentage of the surface area covered by mycelium 1 week after inoculation for three discs from each of three pines.
[b] Analyses of four to nine discs for each treatment; expressed as ppm of anhydrous borate in oven-dry wood.
[c] C = Contaminated with *Penicillium*.
[d] Concentration calculated from the amt analyzed in discs treated with 1 ml of 1.8% solution.

B is adsorbed to the wood and then is not free to alter pH upon rehydration.

In stumps of trees of the suppressed and intermediate crown class, the pH in the surface 0.6 cm was raised to ⩾ 7.6 within min after treatment. The pH effect extended to a depth of 3.1 cm 2 days after treatment, but extended no further with increasing time. The percentage of the cross-sectional area which registered a pH of ⩾ 7.6 was 30, 50, and 25% for discs at the 2.07 cm depths at 2, 4, and 8 days, respectively. The percentage of the cross-sectional area with a pH of ⩾ 7.6 increased sharply above and decreased below this depth for all three sampling dates. The pH change was not uniform radially or vertically within the stump; at 0.80, 1.43, 2.07, 2.70, and 3.34 cm depths, 90, 80, 25, 5, and 2% of the respective cross-sectional area was at a ⩾ pH 7.6 after 8 days.

The pH in slash pine stumps harvested at Fort Bragg, N.C., was ⩾ 8.0 just below the surface of nearly all stumps 1 week after treatment (Table 5); the percentage of stumps with pH ⩾ 8.0 decreased to 70% after 4 weeks and to 0% 8 weeks after treatment. The depth of the zone with a pH of ⩾ 7.6 was nearly the same for the 8-week period. By 3 months, however, the pH in 4 of 10 stumps had returned to ⩽ 6.0 and the zones in the remaining 6 were not as deep, nor were the hues as intense, as those in stumps treated earlier.

The pH just beneath the surface of 10 loblolly pine stumps at Aulander, N.C., 26 months after treatment with borax was 7.2. At 36 months it was 6.8 for non-resin-soaked wood; the pH in resin-soaked wood was 6.0 or less in all cases. At 1.2 and 3.6 cm depths, the average pH varied from ⩽ 6.0-6.4 for the 40 trees from four sampling dates. The elevated pH at the surface of these stumps, however, was not due to high concn of borax, as borax had leached from this zone.

Correlation of borate concn with pH in stumps.—Stumps from six trees cut and treated with borax in February 1969 were tested for pH and diffusion of borax. In 1 week, the pH of wood rose from 4.8 to ⩾ 7.6 to an average depth of 4.5 cm, while borax was detected with turmeric to an average depth of 100 cm. A comparison of Tables 4 and 5 reveals that in stumps treated 1-8 weeks previously, the region with pH ⩾ 7.6 is delimited by a colorimetric rating of ⩾ 3.5.

Three months after treatment at Fort Bragg, 10 stumps were harvested, split in half longitudinally, and sprayed with bromothymol blue. Only six stumps showed a well defined pH reaction of ⩾ 7.6; these and two stumps not treated with borax were selected for study. Blocks approximately 2.5 cm^3 were cut from three places 3.8 cm above, three places straddling, and three places 3.8 cm below the margin of the pH 7.6 zone from each of the six stumps. The average concn of borax above, straddling, and below the margin was 2145, 1115, and 550 ppm, respectively. The four remaining trees with very weak or without pH reactions were split, and 2.5 cm^3 blocks were cut from them and from two nontreated trees at 2.5-, 7.6-, 12.7-, 17.9-cm depths. Chemical analysis revealed that there was an

TABLE 4. Concentration of borax at increasing depths in stumps of intermediate and codominant slash pine at Fort Bragg, N. C., each at 1, 2, 4, and 8 weeks after treatment in January 1969, with 1.36 kg of borax/4.64 m^2 of stump surface[a]

Depth-cm[c]	Time after treatment, weeks			
	1	2	4	8
	Colorimetric rating,[b] average			
2.5	3.9	3.8	3.6	3.9
5.1	3.5	3.5	3.0	3.6
7.6	2.3	2.7	2.3	3.3
10.2	1.2	1.6	1.6	2.4

[a] Dotted line indicates the depth to which a toxic concn of borate had penetrated.
[b] Arbitrary scale from 0-4 based on the intensity of the hue, red. The average is based on three samples from each stump for each depth interval for each time period.
[c] Samples were taken at 2.55 cm intervals to a depth of 27.9 cm; colorimetric ratings decreased from 2.0 at the 12.7 cm depth after 8 weeks to 0 at all times at the 27.9 cm depth.

TABLE 5. Average depth of pH reaction of 7.6 in slash pine stumps at various times after treatment with 1.36 kg borax/4.64 m² of stump surface area in January 1969 at Fort Bragg, N. C.

	Time, weeks			
pH	1	2	4	8
	Average depth of pH reactions, cm			
7.6	4.0	4.2	4.3	3.7
>8.0	2.4	1.9	2.4	0.0
No. stumps	25	23	23	25

average of 535, 370, 165, and 60 ppm borax at the respective depths for the treated trees, and 26, 49, 70, and 28 ppm borax at the same depths in the controls.

DISCUSSION.—Growth of *F. annosus* was completely inhibited by a concn of approx 660 ppm anhydrous borate (borate + native boron) in oven-dry wood. If borax is distributed equally between water and wood in fresh wood, however, dry wt values need to be corrected for moisture content. When the moisture content ($\bar{x}$ = 120%) correction was applied, the average $[Borate]_T$ was 300 ppm, which in vitro reduces growth to about 20% of that in the control medium without added boron (*unpublished data*). Results have been expressed here as anhydrous values because the water of hydration in borax and borate would be lost upon solution of the boron-containing moiety, and the latter is the form which is related to toxicity and to the chemical and colorimetric analyses. Initial growth of *F. annosus,* judged by the length of time for mycelium to become visible, is slower on wood than on malt extract agar. These facts suggest that $[Borate]_T$ is likely to be less in wood than in vitro, because wood is a slightly less satisfactory substrate for growth of *F. annosus.* In any event, it seems unlikely that low amt of borax interact with southern pine wood to produce a toxic chemical (6). Other modes of action, such as enzyme inhibition or formation of chemical complexes, are still plausible (7).

Borax diffused rapidly into freshly cut stumps in quantities sufficient to protect adequately the cut surface from infection by *F. annosus* in suppressed, intermediate, and codominant trees. Diffusion began almost immediately, and within 2 days advanced below the distance to which the fungus would have grown in the same length of time. Continued diffusion afforded additional protection to a depth of at least 5.1 cm. Diffusion to this depth within the stump appeared fairly uniform in living wood, but not in dead tissues, such as old fusiform rust galls. Although borax would easily be washed from such nonresinous dead surfaces, these areas are not significant infection courts as they are usually already colonized by microbial competitors (3) and, in fact, are difficult to inoculate with *F. annosus* (C. S. Hodges, *personal communication*). At increasing depth, diffusion of borax was more irregular, with concn being higher in inverted conical pockets of wood than in surrounding areas. It is doubtful whether borax diffused either deeply or uniformly enough to protect stumps from infection through the bark at the ground line or through wounds created there by logging in stumps of a height normal in the southeastern United States. Thus, additional treatment of logging wounds with borax is recommended. Since studies to date (1, 2, 3) have evaluated the chemical's effectiveness after only 1-1.5 years, and since the present studies indicate that diffusion of $[Borate]_T$ is of limited depth, treated stands should be watched for several years to determine the frequency of infection of stumps by alternate routes.

$[Borate]_T$ persisted in all stumps for at least 8 weeks after treatment. Twenty-six months after treatment, the chemical leached from 88% of the upper 0.3 cm, but $[Borate]_T$ persisted in 38% of the cross-sectional area at the 1.2 cm depth. Because *F. annosus* rarely colonizes cut stump surface after 1 month (10), borate remains at effective levels longer than necessary. Borate could possibly prolong the period of susceptibility of parts of the stump other than the cut surface, although no observations made here support this conclusion. That borax persisted long enough to protect the cut surface is evidenced by the apparent lack of colonization of the stump surface by *F. annosus* 26-36 months after cutting, whereas 78% of untreated stumps on this same area were infected by the fungus (E. W. Ross, *personal communication*). Since the fungus was not rotting any of these stumps but could grow on sterilized wood from them, it appears that microorganisms were currently preventing growth of *F. annosus.*

The nearly equivalent values for $[Borate]_T$ in sterilized and nonsterilized wood (Table 3) suggest that $[Borate]_T$ in the field is similar to that in sterilized discs in the laboratory. This appears to be true even though *F. annosus* grows less vigorously on nonsterilized discs than on sterile ones. Thus, any natural resistance of living tissue does not seem to affect $[Borate]_T$. Growth ratings for sterilized and nonsterilized discs are not directly comparable because of the presence of fungal contaminants (competitors) on the fresh discs.

A red hue developing upon application of colorimetric reagents is insufficient evidence that $[Borate]_T$ is present. In oven-dried wood of southern pine, Wilson's reagents were much more sensitive for detecting B than has been described (12). A rather impressive color reaction developed at relatively low and certainly subtoxic concn. Thus, discs calibrated for borate content and some of these treated with colorimetric reagents and some inoculated should be used as references for evaluating the distribution of $[Borate]_T$. In the present studies, the choice of a colorimetric rating for $\geqslant 3.5$ as an index of the presence of $[Borate]_T$ appears justified, as the average borate content of discs colorimetrically rated at 3.5 was 680 ppm while $[Borate]_T$ in the toxicity tests was 660 ppm.

Near the stump surface, where concn is high, borax raises the pH to a point where pH per se would sharply restrict or prevent growth, and inhibit but probably not prevent germination (*unpublished data*). The concn of borate in the upper part of treated stumps is $> 3\times$ that of $[Borate]_T$. At the edge of the zone of high pH, borate concn (1,115 ppm) still would completely inhibit

the fungus. Although the elevated pH may not exclude *F. annosus*, it may explain altered succession of other microorganisms (3). Wood of high and normal pH is sharply delimited, but not necessarily uniform in distance from the stump surface. The borate concn 3.8 cm below the perimeter of the zone of altered pH was a subtoxic 550 ppm.

In these studies, sodium tetraborate decahydrate was employed. In another test (9), disodium octaborate was used. Sodium tetraborate persisted in wood of southern pines for an extended period, while disodium octaborate is usually undetectable in Scots pine after only 1 year (9). This difference could be related to differences in solubility of the chemical (4) or the amount of resin in the wood (9), or other factors affecting retention by the wood. Each chemical evidently functions differently, at least under the different conditions of use; stumps treated with borax remain sound up to 36 months and are essentially sterile for 3 months after treatment (3). Later they obviously contain, in addition to *Trichoderma* spp. (3), dematiaceous hyphomycetes. Scots pine stumps treated with disodium octaborate die in 8-10 months after treatment and decay rapidly (9). Neither chemical apparently diffused deeply enough into roots to prevent infection by or to kill *F. annosus* in roots (9). Although the chemicals may differ in even the most general aspects of their mode of action, they still are both apparently effective in controlling the disease in the field.

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