Effects of Cultural Practices and Biotic Soil Factors on Fomes annosus

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ABSTRACT

Field and laboratory studies were conducted to determine the influence of altered forest-soil environment on some activities of *Fomes annosus*. Cultural treatments applied to clear-cut slash pine *Fomes*-infested forestry plots were as follows: burned; burned and disked; and burned, disked, and seeded (one plot each) with lupine, oats, or rye. A control plot consisted of a natural forest stand. Samples of debris particles and soil were taken in January, April, and August and processed for microbial content or effect on *F. annosus*. Species of *Trichoderma* and *Penicillium* were the more prevalent colonizers of debris particles in natural forest soil, and the various cultural treatments caused these to either increase or decrease. *Fomes annosus* was not observed colonizing debris. Spores of *Fomes* germinated from 45 to 98% on sterilized soil, except that collected from the oats and rye plots in April, which revealed strong inhibition. Spores did not germinate on nonsterilized soil collected in January or August, but some germination occurred in April. Most severe inhibition was on oat-plot and burned-plot soils. Spore germination on agar containing plot-soil extracts varied greatly between soil sampling periods. Extracts of all plots inhibited germination below that on extract-free agar, most inhibition occurring with extracts from the oats and burned plots. Extracts in a basal medium had little effect on linear growth of *Fomes*. The highest percentage of microorganisms antagonistic to the pathogen was found in the oats and rye soils.

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Additional key words: spore germination, microbial populations, *Pinus elliottii*.

Fomes annosus (Fr.) Karst. causes a serious root and butt rot of coniferous trees in Europe and the United States. Little is known about the influence of biotic soil factors and other natural environmental conditions on the ecology of the pathogen. The fungus is a regular component of the air spora, and the spores are deposited on cut surfaces of stumps, where they germinate and establish sources of infection for adjacent trees. The apparent inability of the fungus to become established and grow extensively in soil free of living roots (3) has not been entirely clarified. It is generally believed that the pathogen cannot survive and grow successfully in competition with the soil microflora (2, 8); however, Rishbeth (9) has shown that basidiospores washed down into the soil remain viable for at least a short period, and stumps covered with soil can become infected.

This study had two objectives: (i) to determine whether the pathogen (*Oedocephalum* stage) may be found colonizing organic matter of a natural undisturbed forest floor or of adjacent areas altered by cultural practices; and (ii) to determine the influence of various cultural treatments on changes in the general microflora and associated fungistatic capacity of forest soil.

MATERIALS AND METHODS.—Six disease-study plots (ca. 75 × 175 ft) were established

on a *F. annosus*-infested site at International Paper Company's Southlands Experiment Forest in Decatur County, Georgia. Five of the six plots, representing various cultural practices, were located in a clear-cut slash pine (*Pinus elliottii* Engelm.) plantation. The sixth plot served as a control, and was located in an area of standing trees adjacent to the clear-cut area; this plot received no treatment during the period of study.

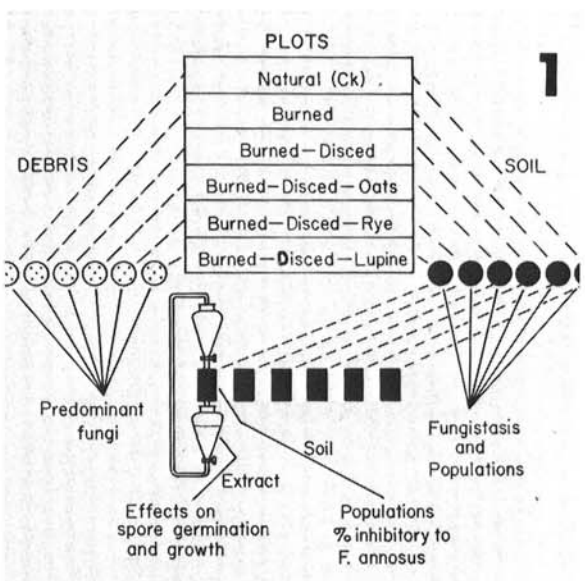
Logging slash on the clear-cut area was burned, and one plot was retained in this condition (burned). All other clear-cut plots were then disked between the rows of stumps, and one plot in this condition was retained (burned and disked). In the fall, the three remaining plots were sown with oats, rye, and lupine, respectively.

Samples of organic debris and of soil were collected from 10 areas within each plot in January when the crops were immature, and again in April when they were mature. In May, the crops were plowed under and final samples were taken in August. Debris particles (organic particles remaining on a 4.76-mm mesh screen) were collected from the clear-cut plots by screening soil taken from the top 15 cm (6 inches). The amount of soil screened to provide the debris particles varied with the plots and sampling dates. Debris particles from the control plot were collected in a similar manner after removal of the A_{00} horizon. Soil samples were collected from the top 15 cm with a soil tube; the A_0 horizon was removed from the control plot before soil samples were taken. An equal number of soil cores was taken at each sampling area within each plot. Samples of debris and of soil were composited for each plot and transferred to the laboratory in plastic bags for processing.

A diagram of experimental procedures is presented in Fig. 1. Debris particles were serially washed (6 times for 15 min) in flasks of distilled water on a mechanical wrist-action shaker to remove organisms not actually colonizing the particles. The first three washings contained 0.01% Igepal CO-710 as a wetting agent; all washings were discarded. The washed particles were partially air-dried on paper towels overnight, chopped in a laboratory mill, screened to a uniform particle size (ca. 1 mm), and placed on acidified malt-extract agar in petri dishes. For each plot, six particles were placed in each of 50 dishes. These were incubated 4 days at 24 C, and the developing fungal colonies were examined microscopically for generic identification.

Composite soil samples were screened and thoroughly mixed with a Vortex mixer. For each plot, 50 g of soil (oven-dry weight equivalent) were placed in each of six sterile petri dishes for a test of fungistasis against laboratory-grown conidia of *F. annosus*. Three dishes/plot were sterilized by autoclaving; the other three were untreated; all were then brought to water-holding capacity with sterile demineralized water. The filter paper-water agar disc procedure of Jackson (6) was used to measure fungistasis. The control consisted of spore suspension on water agar discs resting on wet filter paper instead of on soil. Dishes were incubated for 48 hr at 26 C and percentage spore germination was determined; from 200 to 500 spores were counted on nine agar discs/treatment.

To study soil extracts, 200 g of soil (oven-dry weight equivalent) from each plot were mixed with



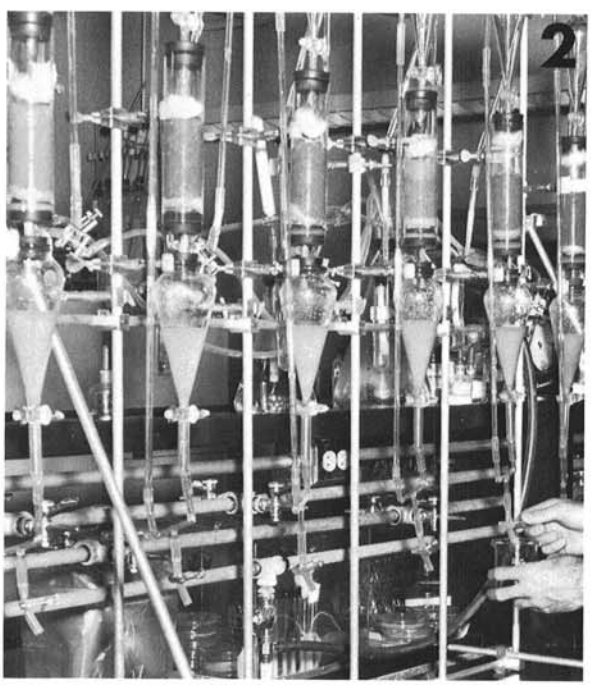


Fig. 1-2. 1) Diagram of general procedures for studying the influence of cultural treatments on *Fomes annosus*. **2)** Perfusion apparatus in series used for obtaining extracts from forest-plot soils.

30 g acid-washed sand and placed in soil columns of six units of a modified Audus-type (1) perfusion apparatus mounted in series (Fig. 2). Each column was first perfused by gravity with 150 ml of sterile demineralized water to bring the soils to approximate water-holding capacity. The excess water was then discarded, and the soil columns were incubated 14 days at room temperature (24-27 C). The columns were aerated 10 hr/day by pulling filtered air through the system with a vacuum pump. After incubation, each column was perfused 10 times with 150 ml of sterile water. The leached extracts were passed through clarifying Seitz filters, concentrated to 50% original volume in a vacuum oven at 60 C, and sterilized by Seitz filtration.

Equal amounts of concentrated soil extract from each plot and a concentrated malt-extract agar (made with one-half the suggested volume of water) were mixed in each of four petri dishes. Final volume in each dish was 15 ml. Dishes were inoculated in the center with an agar disc of *F. annosus*, and diameter growth was measured over a period of several days to test for stimulation or inhibition by the soil extracts. Growth on standard malt-extract agar served as the control.

Other portions of the extracts were mixed with water agar in a 1:1 ratio and tested for fungistasis against laboratory-grown *F. annosus* conidia. Three 7-mm discs of the extract-water agar were placed on a moist 4-cm^2 piece of sterile filter paper in a petri

TABLE 1. Number of colonies of genera identified on 300 debris particles obtained in January, April, and August from each of six *Fomes*-infested plots representing various cultural practices

Genera recorded	Plots[a]						
	Natural stand	Clear-cut slash pine area					Total
		Oats	Rye	Lupine	Burned only	Burned, disked	
				January			
Trichoderma	214	187	169	171	222	74	1,037
Penicillium	135	231	170	175	132	218	1,061
Aspergillus	4	107	13	5	0	10	139
Gliocladium	8	75	108	173	41	179	584
Fusidium	0	0	39	47	6	17	109
Mucor	91	0	0	1	15	2	109
Curvularia	0	16	12	3	2	7	40
Unident.[b]	60	16	10	3	11	10	110
Totals	512	632	521	578	429	417	3,189
				April			
Trichoderma	167	53	140	154	137	72	723
Penicillium	110	173	101	121	44	54	603
Aspergillus	7	48	23	68	67	225	438
Gliocladium	5	0	14	0	10	146	175
Fusidium	3	19	148	24	49	0	243
Mucor	19	0	1	1	0	0	21
Curvularia	1	1	0	2	0	0	4
Fusarium	0	14	5	2	1	2	24
Unident.	9	23	9	8	8	2	59
Totals	321	331	441	380	316	501	2,290
				August			
Trichoderma	240	90	125	96	116	27	694
Penicillium	79	88	72	114	25	70	448
Aspergillus	2	43	93	51	158	132	479
Gliocladium	0	7	2	10	5	4	28
Fusidium	2	39	137	0	14	6	198
Mucor	6	2	0	5	2	2	17
Curvularia	0	4	3	3	0	2	12
Fusarium	0	4	0	17	0	1	22
Unident.	5	1	4	27	7	0	44
Totals	334	278	436	323	327	244	1,942

[a]Natural stand was undisturbed; all other plots were clear-cut and burned. One plot was retained in this condition (burned only). Remaining plots were disked between the rows of stumps; one plot was retained in this condition (burned and disked). The three remaining plots were planted in the fall as indicated.

[b]Unidentified, nonsporulating; majority were white colonies.

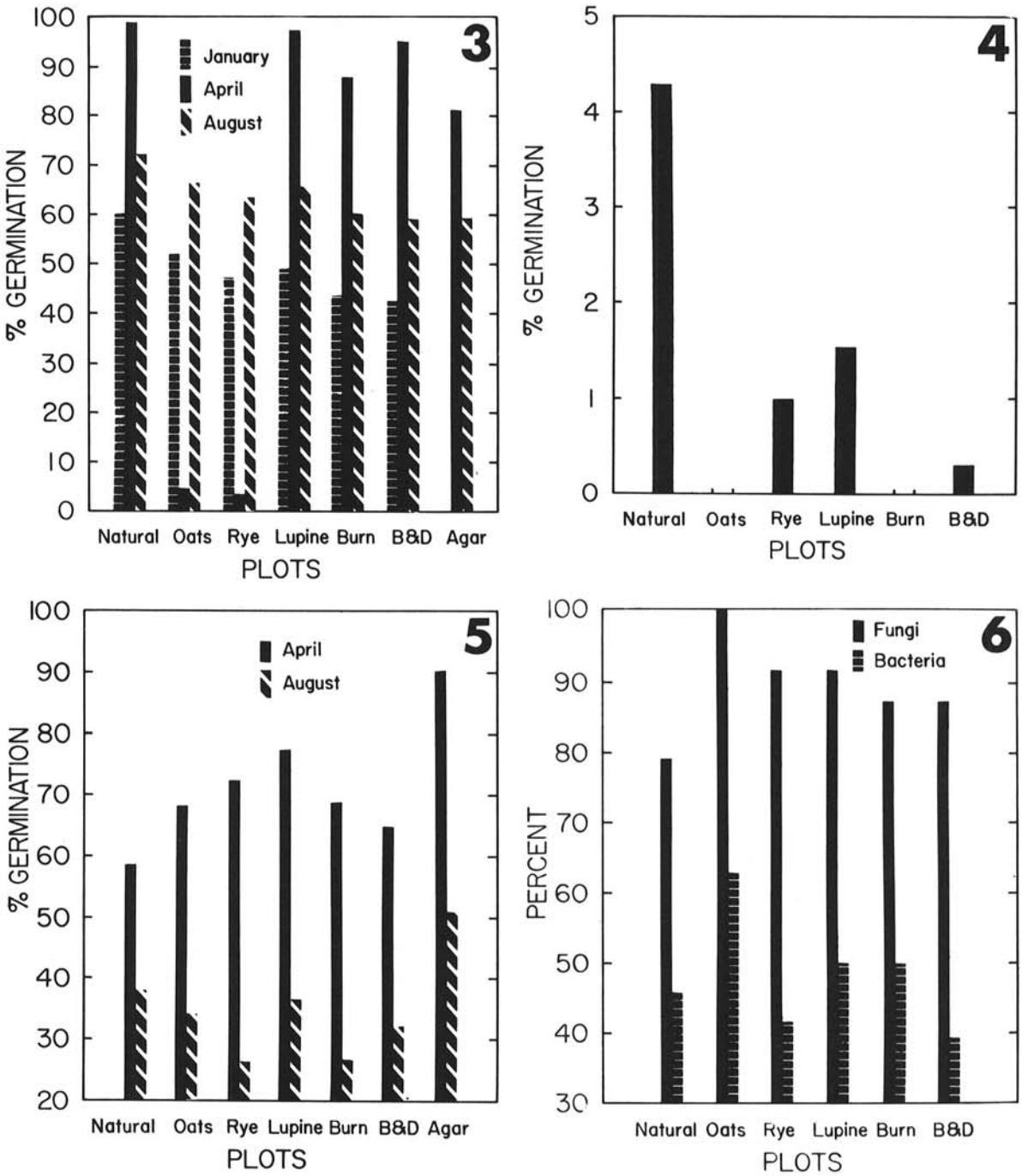


Fig. 3-6. Influence of cultural treatments on germination of *Fomes annosus* conidia and on microbial populations. **3)** Germination of conidia on sterilized soil from plots at three sampling dates. **4)** Germination of conidia on nonsterilized soil from plots in April. **5)** Germination of conidia on agar with filter-sterilized soil extracts from plots sampled in April and August. **6)** Percentage of soil microbial populations antagonistic to *Fomes annosus* in plate assay, April samples.

dish. Three dishes/plot were used. A drop of spore suspension was placed on each disc and incubated for 48 hr at 26 C, and germination percentages were determined. The control consisted of spores on water agar discs placed on moist filter paper.

Relative microbial populations of soil in the perfusion columns were determined before and after incubation by standard dilution plating. Peptone-dextrose-rose bengal agar plus streptomycin (7) was used for fungi; and Thornton's standardized medium (10), for bacteria and actinomycetes. After 5 days' incubation, colonies on dilution plates were

counted, and isolates representative of morphologically different types were assayed on malt-extract agar by standard procedure for antagonism against *F. annosus*. Because of its slow growth, the pathogen was grown on test plates 3 days prior to inoculation with the soil isolates. Antagonism was evaluated and recorded after an additional 3-day incubation period at 26 C. Only a definite toxic-zone effect or stoppage of *F. annosus* growth by contact of contending organisms was rated as antagonism.

RESULTS AND DISCUSSION.—*Debris particles.*—Table 1 shows the relative frequency of occurrence of fungal genera on plated debris particles from each plot. Most prominent in the natural forest organic matter were species of *Trichoderma* and *Penicillium*, which are well known to possess the attributes for competitive saprophytic colonization. Except for *Mucor* spp., relatively few fungi of other genera were found in the control area. Fungal colonization varied considerably in treated plots. For some genera, frequency of occurrence was greater in treated plots than in the natural forest debris; in other cases, the frequency was reduced; in still others, there was little difference. In no case was *Trichoderma* appreciably increased as a result of any cultural treatment, as compared with the natural control. The total number of all fungi found in all plots was highest in January and lowest in August. Possibly, the turning under of crops in May increased microbial activity (particularly bacteria) that suppressed fungal colonization of organic matter before the August sampling.

Fomes annosus was not found colonizing debris particles from any of the plots. This is not surprising, in view of the criteria set forth by Garrett (5) for competitive colonization of a substrate; inoculum density or dosage is of primary significance in determining an organism's inoculum potential or energy of growth for substrate colonization. Species of *Trichoderma* and *Penicillium*, already established on or near organic particles, should have a competitive advantage to the exclusion of colonization from airborne spores.

Soil assays.—Conidial germination was tested on both sterilized and nonsterilized soil from the plots in January, April, and August. With the exception of the oats and rye plots, percentage spore germination on sterilized soil was highest in April (Fig. 3). Stimulation in April and August was indicated as compared with germination on water agar; excessive drying of the agar discs precluded the counting of control plates in January; thus, a similar comparison on that sampling date was not possible. The fungistatic effect of soil is usually eliminated by sterilization, but the pronounced inhibition on soil from the oats and rye plots in April suggests effect of a heat-stable principle. Heat alteration of the nutritional status of soil, however, may also result in inhibition or stimulation.

Spores did not germinate on nonsterilized soil collected in January or August, and little germination occurred in April (Fig. 4). Germination was totally inhibited on soil from the oats plot and from the burned plot. Though germination on natural control soil reached only 4.3%, a differential influence of cultural treatments was indicated.

Linear growth of *F. annosus* (data not shown) was little affected on malt-extract agar containing extracts from water-perfused soil. Filter-sterilized soil extracts in water agar inhibited spore germination (Fig. 5) as compared with extract-free agar, but no direct relation to results on nonsterilized soil was observed. There was a great difference between results for April and August. Least germination was recorded for the rye and burn treatments. Effects in these tests were influenced to some extent by the dilution factor of the agar media, and possibly by loss of some inhibitory or stimulatory components of the extracts on the sterilizing filters. Curl & Arnold (4) showed that extracts from a mineral soil, in which *Trichoderma* and a bacterium had previously grown, stimulated growth of *F. annosus*.

Soil microbial populations varied with the sampling date. Fungal populations usually decreased during incubation of soil in the perfusion columns, whereas bacterial populations increased, but no correlation with fungistasis or other tests was evident. Tests for antibiosis revealed that percentages of antagonistic fungal isolates were considerably higher than those of bacteria from each test period. Data for the April sampling are presented in Fig. 6. A very high percentage of antagonistic microorganisms was generally associated with the oats plot; the August tests showed a higher percentage of antagonistic bacteria in rye plots. Thus, some correlation with fungistasis results is indicated.

Definite conclusions with practical implications cannot be made from a 1-year study of field plots with many uncontrolled variables, but some points of ecological significance were made. Since *Fomes annosus* probably cannot colonize natural organic matter, various cropping and burning practices may have little value as controls. However, such practices apparently do influence the fungistatic nature of soil, which may contribute to the dormancy of spores. This effect, and the primary microbial groups (fungal or bacterial) associated with it, fluctuate with season and kind of organic matter in the soil. The value of oat culture and oat residues in particular should be studied further in relation to growth and survival of *F. annosus*.

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