# Some Effects of Pine-Needle or Grass Smoke on Fungi

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Accepted for publication 11 June 1974.

ABSTRACT

Spore germination or mycelial growth of several fungi was reduced on cellophane previously exposed to smoke from burning pine needles. Prior exposure of bean plants and Monterey pine seedlings to smoke reduced the amount of bean rust or gall rust following inoculation. The possible implications of fungus inhibition by smoke from wildfires or prescribed burns is discussed.

Phytopathology 65:28-31

*Additional key words;* forest diseases, fungal ecology.

Wildfires and fires set by Indians were integral features of many native plant communities (1, 2, 3, 6, 10, 12). Biswell (1) suggests that fires were widespread each year in the Sierra Nevada. He suggests further (3) that wood smoke was a natural feature of the environment. Since smoking has long been used to protect foodstuffs from microbial attack, it seems likely that smoke might also affect microorganisms in natural plant communities.

The studies reported here were undertaken to determine how smoke might affect the activities of fungi, and to obtain preliminary information on possible implications of smoke in plant communities subject to frequent burning.

MATERIALS AND METHODS.—Two smoke chambers were used. Air-dried grass or dead needles of Monterey pine were burned in a ventilated garbage can and smoke was piped through a chamber containing test materials. The first chamber was a simple 51 × 51 × 62-cm wooden box with a smoke inlet, screen top, and a screen shelf. A second, more elaborate, chamber was built in which potted plants or large numbers of petri dishes could be smoked (Fig. 1). A baffle above the smoke inlet promoted even distribution of smoke, a window allowed observation of smoke density and circulation, and a series of drawers allowed insertion or removal of materials without opening the entire chamber.

The small chamber was used to smoke sheets of cellophane for spore germination studies. Because materials could not be removed quickly, different exposure times were obtained by hanging cellophane vertically in the chamber and allowing smoke to enter for specified times. The chamber was then allowed to clear for 2-4 min and the cellophane was then removed. Thus exposure times included the length of time smoke was allowed to enter the chamber plus the 2- to 4- minute period in which the smoke was allowed to clear.

The large chamber was used to smoke plates or plants for studies on mycelial growth or infection. Because the drawer arrangement permitted rapid insertion and removal of materials, smoke was run into the chamber continuously and plates or plants were exposed for specified period and then removed. Thus, exposure times for studies on mycelial growth and infection represent exact periods of exposure to smoke.

Fuel moisture levels were not recorded and the speed of burning was not controlled. Thus, while dense smoke was produced in each study, the chemical character of the smoke may have varied from one study to the next. Antifungal effects were obtained in all tests, and results were generally reproducible despite lack of close control of fuel moistures and burning rates.

Temperatures inside and outside the chamber were monitored in preliminary tests. Inside temperatures did not exceed ambient air temperatures by more than 2 C. These results indicated that chamber heating was slight and was not likely to have had any significant effect on test materials.

For spore germination studies, small pieces of smoked cellophane were placed on drops of water on microscope slides and sprayed with a spore suspension of the test fungus. Slides were then placed across small glass rods in petri dishes containing moist filter paper and were incubated at room temperature until germination was observed on unsmoked cellophane controls. Percent germination was determined by counting 100 spores in random microscope fields.

For studies of mycelial growth, 9-cm diameter disks were cut from uncoated cellophane and sterilized by autoclaving. The discs were then placed on potato-dextrose agar (PDA) in petri dishes. The dishes, with lids removed, were exposed to smoke for various periods and the lids were replaced. Within one day, each plate was inoculated with a plug of mycelium and agar cut from fresh PDA cultures with a 3-mm diameter cork borer. Dishes with unsmoked cellophane served as controls. Colony diameters, minus diameter of inoculum plug, were recorded in two, premarked directions at 1- to 2-day intervals following initiation of growth. Five-dish replicates were used for each treatment.

For bean infection studies, 12- to 17-day-old bean plants were exposed to smoke from burning grass for various periods, removed from the chamber, and sprayed with a suspension of uredospores of the bean rust fungus *Uromyces phaseoli* (Pers.) Wint. Inoculated plants were kept in a mist chamber for 24-48 hours and then transferred to a bench in the greenhouse. Amount of infection was determined by counting the numbers of rust pustules on leaf disks cut with a 23-mm diameter cork borer. A single disk as close to the leaf apex as possible was taken from each inoculated leaf.

For pine infection studies, 4-year-old Monterey pine seedlings were exposed to smoke from burning needles, removed from the chamber, and sprayed with a suspension of aeciospores of the western gall rust fungus.

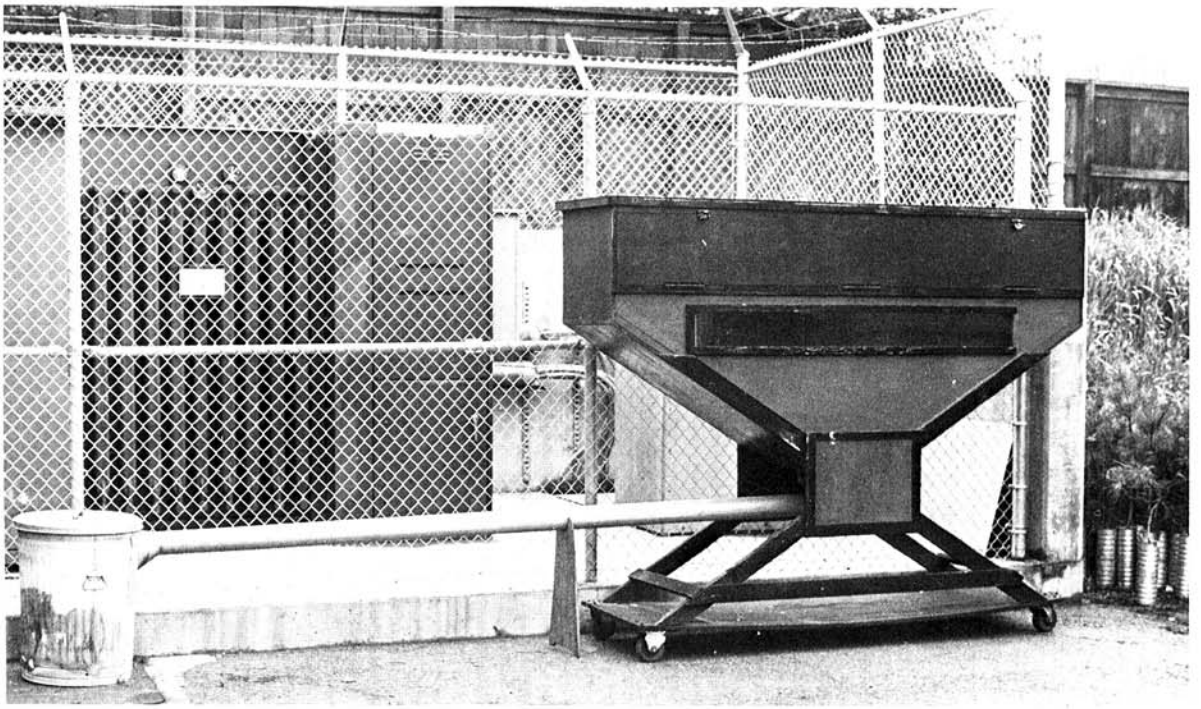

**Fig. 1.** Chamber for smoking test materials. Fuels were burned in the garbage can (left) and smoke was piped into the inlet at bottom of chamber.

Inoculated plants were kept in a mist chamber for 36 hours and then transferred to a lathhouse. Numbers of galls were counted after 8 months.

RESULTS.—*Spore germination.*—Aeciospores of *Peridermium harknessii* Moore, collected from sporulating galls at various times through the spring of 1971, were tested on cellophane exposed to approximately 2, 4, 8, 16, 32, or 64 minutes of smoke. Spore germination on six pieces of unsmoked cellophane averaged 61%. No spores germinated on any smoked cellophane. A second series of tests was made with cellophane exposed to approximately 15, 30, 45, 60, 75, 90, or 105 seconds of smoke. Spore germination on six pieces of unsmoked cellophane averaged 50%. Again, no germination occured on any of the smoked cellophane. A third series of tests was made with 0 and approximately 2.5, 5, and 10 seconds of smoke. Spore germination was 82, 24, 25, and 0%, respectively. This series was repeated with germination percentages of 58, 7, 8, and 0, respectively.

Further tests were made with conidia of five additional fungi (Table 1). Spores of all five fungi were sensitive to smoke deposits, but the degree of sensitivity varied. *Botrytis gemella* (Bon.) Sacc. and *Fusarium lateritium* Nees failed to germinate or germinated rarely on cellophane exposed to one or more minutes of smoke. *Penicillium expansum* Link and *Fomes annosus* (Fr.) Cke. still showed some germination on cellophane exposed to 16 minutes of smoke, and the *Trichoderma* sp. showed increased germination on cellophane smoked for periods less than 16 minutes.

*Mycelial growth.*—Colony growth of four fungi was invariably reduced on plates of cellophane exposed to pine-needle smoke (Table 2). Sensitivity varied among fungi and among tests on any one fungus. Growth of *F. annosus, Verticicladiella wagenerii* Hughes, and *Pholiota adiposa* (Fr.) Kummer was completely inhibited at 7 days by 16 or 32 minutes of smoke. *Trichosporium symbioticum* Wright was inhibited by 16 or 32 minutes of smoke in two tests, but made appreciable growth in a third test.

Plates were kept for at least 2 weeks. Generally, if at least a trace of growth did not occur within 7 days, it did not occur at 2 weeks. The only exception was one test with

TABLE 1. Germination of conidia of five fungi on cellophane exposed to various periods of smoke prior to inoculation

| Fungus species | Conidial germination (%) following smoke exposure (min)[a] of: 0 | 1 | 4 | 16 |
|---|---|---|---|---|
| *Botrytis gemella* | 86 | 0 | 0 | 0 |
| *Penicillium expansum* | 58 | 24 | 13 | 4 |
| *Fusarium lateritium* | 76 | 0 | 1 | 0 |
| *Trichoderma* sp. | 18 | 73 | 36 | 12 |
| *Fomes annosus* | 60 | 38 | 23 | 1 |

[a]Smoke was introduced into chamber for indicated periods and then allowed to clear for 2-4 minutes before cellophane was removed.

[b]Average of two tests in which at least 100 spores per test were counted in random microscope fields.

TABLE 2. Average colony diameter of four fungi after 6-7 days on cellophane exposed to pine-needle smoke for various periods prior to inoculation[a]

| Fungus species | Growth (colony diameter, mm) following exposures to smoke (min) of: | | | | |
|---|---|---|---|---|---|
| | 0 | 4 | 8 | 16 | 32 |
| *Fomes annosus* | 35 A | 20 B | 6 C | 0 D | 0 D |
| *Verticicladiella wagenerii* | 22 A | 10 B | 1 C | 0 D | 0 D |
| *Trichosporium symbioticum* | 77 A | 68 A | 35 B | 22 BC | 11 C |
| *Pholiota adiposa* | 14 A | 8 B | 4 C | 0 D | 0 D |

[a]Each figure represents the average of two-to-four tests, five plates per test. Adjacent means followed by the same letter in a line did not differ significantly by LSD test, $P = 0.01$.

TABLE 3. Average numbers of rust lesions on sample discs from bean leaves exposed to various periods of grass smoke prior to inoculation.

| Test No. | Average number of lesions per disc[a] following exposure to smoke (sec) for | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 40 | 80 | 160 | 320 | 640 |
| 1 | 42 A | 20 B | 7 C | 11 D | | |
| 2 | 76 A | 35 A | 10 B | 8 B | 0 C | 0 C |

[a]Lesions were counted on five discs in Test 1 and on 10 discs in Test 2. Adjacent means with the same letter on the same line did not differ significantly by LSD test, $P = 0.01$. Leaves were damaged by smoke at 320 and 640 seconds, but no rust lesions were found on uninjured leaves, or on the green areas of injured leaves.

*V. wagenerii* on cellophane smoked for 32 minutes in which traces of growth appeared at 10 days.

*Infectivity of bean rust on smoked plants.*—The numbers of rust lesions declined with increased exposure of plants to smoke prior to inoculation (Fig. 2). Numbers of lesions were variable (Table 3), but in two separate tests, average numbers of lesions on unsmoked leaves were consistently about twice those on leaves smoked for 40 seconds and about 4-8 times those on leaves smoked for 80 or 160 seconds. Exposure to 320-640 seconds of smoke severely injured leaves on some plants in one test (Fig. 2), but no lesions were found on uninjured leaves or on green areas of injured leaves.

*Infectivity of gall rust on Monterey pines.*—A single test with Monterey pine seedlings exposed to smoke for 0, 4, 8, 16, or 32 minutes gave respectively 12, 1, 0, 0, 0, and 0 galls on the five trees in each treatment.

DISCUSSION.—Fire was a frequent and integral feature of many natural ecosystems. Some of the direct effects of burning on such ecosystems have been documented (10, 11). The possible effects of smoke from burning plant materials have received little attention aside from sociological implications (3, 9). Metcalf (8) suggested that smoke may reduce white pine blister rust. Melching et al. (7) have shown that cigarette-, cigar-, and pipe smoke inhibit the germination of spores of several plant pathogenic fungi. If such inhibition was common, it could produce important effects on fungal activity.

All of the fungi we tested, with the exception of *Trichoderma* sp., showed a decline in spore germination with increased exposure of the substrate to smoke. Sensitivity varied among fungi. With western gall rust fungus and *B. gemella,* exposure to as little as 30 seconds of smoke greatly reduced the suitability of the substrate for spore germination. Spores of *F. annosus, Trichoderma* sp., and *P. expansum* showed considerable tolerance to smoke deposits, and germination of *Trichoderma* spores was increased by exposure of the substrate to up to 4 minutes of smoke. If antifungal materials deposited from smoke were common in natural ecosystems, fungi active throughout the growing season might be expeceted to have evolved tolerance to such materials; whereas fungi such as the western gall rust fungus, which infects new growth in early spring before fires are likely, would not evolve such tolerance. The limited observations reported here are inadequate to test this hypothesis, but it appears worthy of exploration.

Mycelial growth of all fungi tested, including two root disease fungi, two heartrot fungi, and one bark beetle symbiont, was reduced on cellophane exposed to smoke. This suggests that smoke deposits on dead branches or stubs, exposed wounds, and other plant surfaces might reduce the activities of important forest fungi, if smoke deposits are active on plant surfaces, as well as on cellophane.

The rate of infection of bean and pine seedlings by rust fungi was markedly reduced by exposure to smoke, indicating that smoke deposits on plant surfaces were inhibitory to fungi.

Studies reported here demonstrate that smoke inhibited spore germination, mycelial growth, and infection with several diverse fungi. With one fungus (*Trichoderma* sp.), spore germination was enhanced on surfaces briefly exposed to smoke. Since *Trichoderma* spp. are considered inhibitory to many pathogenic fungi, any increase in activity resulting from smoke might further reduce the incidence or rate of growth of other forest fungi, including pathogens.

Wildfires often result in dense palls of smoke that may drift for many miles through plant communities. The frequent occurrence of natural and man-caused fires in the early history of the West suggests that many plant

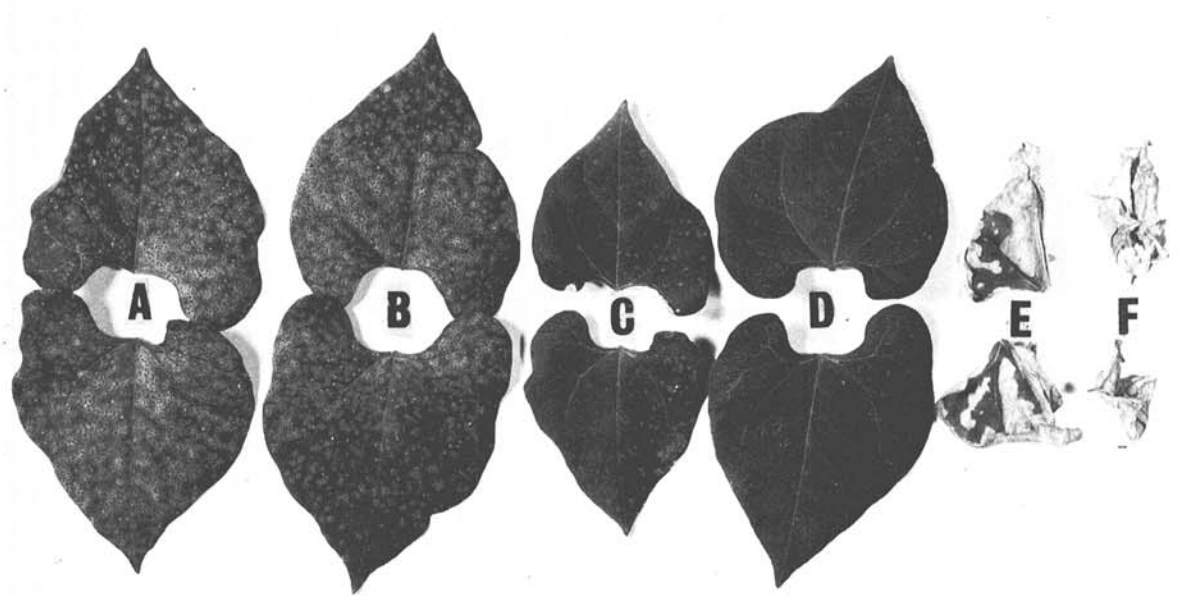


**Fig. 2.** Rust lesions on bean leaves exposed to smoke for 0, 40, 80, 160, 320, or 640 sec (left to right, labeled A, B, C, D, E, and F). Leaves E and F show smoke damage. Leaves for photographing were selected randomly. Leaf C is small by chance and not because of smoke treatment.

communities were repeatedly exposed to drifting smoke. Smoke from various fuels yields a wide variety of compounds (4, 5), many of which are antimicrobial (5). The limited data presented here are inadequate to assess the possible biological effects of repeated exposure to smoke, but current controversy over the advantages and disadvantages of agricultural and silvicultural burning suggests that we should learn as much as we can about effects of fire before deciding for or against such programs. Evidence presented here suggests that effects of smoke on microbial activity should be considered in evaluating the possible benefits of burning.

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