Effect of Furfural on the In Vitro Germination of *Peronosclerospora sorghi* Oospores

R. C. French and C. G. Schmitt

Plant physiologist and research plant pathologist, Plant Disease Research Laboratory, AR-SEA, U.S. Department of Agriculture, P.O. Box 1209, Frederick, MD 21701.

The authors greatly appreciate the technical assistance of Celeste Inez Maiorana.

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Accepted for publication 2 March 1980.

ABSTRACT

FRENCH, R. C., and C. G. SCHMITT. 1980. Effect of furfural on the in vitro germination of *Peronosclerospora sorghi* oospores. Phytopathology 70:877-880.

Methods of inducing germination and controlling contamination in oospores of *Peronosclerospora sorghi* were evaluated. Oospores centrifuged from autolysed sorghum leaf tissue had fewer contaminants than did those sieved from ground, dry tissue. Furfural, at 50–100 μl/L, was effective in stimulating germination and/or suppressing contamination. In the presence of furfural, dried spores commenced germination after 3 days on agar, reached a maximum at about 5 days, but were scarcely observable after 9 days at room temperature (~25 C) because germ tubes had disintegrated or were indistinguishable from mycelia of contaminants. Washed oospores (from autolysed leaves) that were not allowed to dry sometimes germinated within 2 days. Germination occurred at 16–30 C and was most rapid (3–5 days) at 26–28 C. Great variability in percent germination was attributed to the variability in contamination and in the proportion of mature viable oospores produced in the leaf tissue.

Additional key words: downy mildew of sorghum, *Sclerospora sorghi,* sorghum, germination stimulation, control of contamination.

Downy mildew is an important disease of sorghum, and has been discussed in reviews by Frederiksen et al (3) and Frederiksen and Renfro (4). Oospores of *Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw (formerly *Sclerospora sorghi* Weston and Uppal), the causal organism of sorghum downy mildew, are produced in mature leaf tissue of sorghum (*Sorghum bicolor* [L.] Moench) and represent the dormant or overwintering stage of the pathogen. Oospores are disseminated when infested leaf tissue is fragmented and dispersed into soil. The organism is an obligate parasite which has not as yet been grown in sterile culture. The diseased leaf tissue in which the oospores are produced is vulnerable to many parasitic and saphrophytic contaminants, including spores of other fungi, bacteria, and protozoans. Because of the extensive contamination of the experimental material, oospore germination is difficult to study. However, germination of the oospores of *P. sorghi* has been observed previously and has been reported by Weston and Uppal (19), by Safeeulla (17), and by Pratt (15). Pratt (15) has studied germination of oospores of *P. sorghi* in the presence of growing roots of host and nonhost plants. Pratt and Janke (14) studied the relationship of soilborne oospores to downy mildew in grain sorghum.

This report summarizes our studies to induce in vitro oospore germination by chemical and/or physical environmental factors and to control contamination in the environment of the germinating oospore.

MATERIALS AND METHODS

Oospores were obtained by crushing dried leaves of diseased sorghum plants by mortar and pestle. The fragments were ground in a ball mill, and the residue was passed through a 44-μm-mesh copper sieve.

Oospores also were obtained from autolysed 5- to 10-cm strips of infected sorghum leaf tissue that had been cut from diseased leaves and immersed in 10 ml of distilled water in glass petri plates for about 1 wk at room temperature (~25 C). The disintegrated tissue was stirred, and the oospores, which were contained in the brownish debris which settled out, were pipetted to 15-ml glass centrifuge tubes. These tubes were filled with distilled water and centrifuged at about 250 rpm to force the oospores to the bottom. The supernate was decanted and discarded. The oospores were suspended in sterile distilled water and the procedure was repeated five or six times until the supernatant liquid became clear. Oospores were then collected by vacuum on 8-μm Millipore filter disks, washed with 1 L of sterile distilled water, and allowed to remain under gentle vacuum until dry. Oospores were transferred by spatula to the surface of an agar germination medium (1% Bacto agar in distilled water, plus 100 μl/L furfural, total volume of 5 ml in 5-cm-diameter disposable plastic petri plates). Oospores were dispersed by adding a drop of sterile water and shaking the petri plate. Observations were made through the bottom of the unopened petri plates. The remaining oospores were transferred on the filter disk to a desiccator containing Drierite and held at room temperature for further experimentation.

Oospores also were added to the surface of agar by gently pressing oospores from wet Millipore filter disks against the agar and then removing the disks, or by transferring aqueous suspensions of oospores to the agar surface. In some experiments, oospores were floated on water solutions in 5-ml plastic-stoppered glass vials.

To evaluate effects of chemicals on germination of oospores, the compounds were dissolved or suspended in 1% water agar for petri plate tests, or in distilled water in 5-ml plastic-stoppered glass vials. Oospores were transferred to the surface of these media as described above.

To study the effects of temperature on germination, two petri plates of oospores on 1% tap-water agar plus 100 μl/L furfural were placed in incubators ranging from 7 to 33 C, and were examined at intervals from 3 to 20 days.

RESULTS AND DISCUSSION

Because of the extensive contamination of the experimental material, the initial approach in these studies was to induce germination as rapidly as possible before the microbial saprophytes obscured the oospores. We examined first a group of volatile compounds, most of which are components of food flavors or aromas, and which had been shown effective in stimulating germination of a broad spectrum of biological propagules (5–8,16). Nonanal, β-ionone, octyl cyanide, 6-methyl-5-hepten-2-one, and numerous related compounds, which selectively stimulate germination of some 28 species of rust and smut spores,

two species of Penicillium (11), pine pollen (9), and certain weed seeds (10) were tested.

Of the various chemicals tested, furfural was the most effective in stimulating germination. Germination, as compared to controls, was significantly increased by 50 and 100 μl/L furfural (Table 1). At higher concentrations, germination was not observed and contamination was extremely low. As the concentration of furfural decreased below 50 μl/L, germination decreased and contamination increased. In most experiments, no germination was observed in the absence of furfural. Oospores also germinated well when floated on the surface of distilled water containing 100 μl/L furfural in stoppered glass vials. Contamination also decreased with increasing furfural levels in the liquid medium.

Other furane derivatives generally were less effective than furfural. Also, other germination stimulators and plant hormones, such as IAA, gibberellin, KNO_3, etc., were tested but produced no effect.

Chemical methods of controlling contamination and breaking dormancy, such as the use of potassium permanganate and sodium hypochlorite (12), likewise were investigated without success. Many antibiotics were studied as potential means of selectively controlling contamination, also without success.

In addition to chemical stimulation, physical environmental factors were studied, including heat shock, cold shock, light quality, leaching, and soaking in solvents to remove possible inhibitors. None had clearly discernable effects.

Temperature, of course, had a direct effect on germination. Oospores germinated over a temperature range of 16–30 C (Table 2). However, they germinated much more rapidly at 23–30 C. The optimum temperature range for germination was 26–28 C. The most rapid germination using dried spore samples was observed in 3 days. At 23 C or higher, germination could be observed only up to 9 days. After that time microbial contamination usually obliterated the germ tubes. The degree and rate of contamination varied considerably among samples, even those taken from the same preparation of oospores. Thirteen to 20 days were required before germination could be observed at 16 C. At that temperature, the growth of contaminating microorganisms was very slow.

With dried, ground material, 1% water agar and 100 μl/L furfural was the best medium for germination of oospores. Autolysed tissue, in which diseased leaves were soaked in water for 7–10 days, yielded oospores which were less contaminated. The autolysing technique seemed to be most effective with our experimental material. A technique that uses the lysing activity of soil extracts has been used by Ayers and Lumsden (1,13) to obtain preparations of oospores of *Pythium* spp. relatively free of contaminants and debris. Dried oospores required a minimum of 3 days at 26 C to germinate. Moist oospores separated from autolysed tissues and placed immediately on agar sometimes germinated in 2 days. When contamination was controlled for several days, newly germinated oospores could be observed daily during this time period.

The germinating oospore of *P. sorghi* produces a germ tube densely packed with globular, oily, and dense cytoplasm (Fig. 1A to E). Color of germinating oospores may vary from pale yellow to dark brown, but differs little from those not germinating. The germ tube rapidly elongates, and the contents become much less dense, with a few yellowish "oily" bands distributed at irregular intervals behind the tip, which usually remains rather dense (Fig. 1F–J). Some germ tubes have grown over 3.5 mm after 48 hr at 25 C. Occasional branching has been observed (Fig. 1K–M). A few oospores with two germ tubes have been observed. (Fig. 1N).

Furfural was effective at levels ranging from 10 to 100 μl/L, but usually was most effective at 50 or 100 μl/L, which permitted very little fungal growth other than that of the germinating oospores. All germ tubes were of uniform width and structure; they grew from normal, mature oospores, and thus represented true germination. Furfural also was effective in combination with 10 μl/L streptomycin and penicillin (Fig. 1 O, P). In general, the germination response was quite variable, undoubtedly because of contamination and variation in the oospore material. Contamination also was extremely variable; it consisted of unidentified bacterial, fungal, yeast, and protozoan components. Some of these microorganisms might be expected to cause beneficial effects by destroying inhibitors or to cause inhibitory or toxic effects by producing an unfavorable pH or parasitizing the oospores. Considerable variability was noted in the oospores; some samples had much larger numbers of immature or aborted oospores, as described by Pratt (15). Some of the contaminants undoubtedly came from within the oospores, also described by Pratt (15).

Although furfural at 50–100 μl/L was beneficial to the germination of oospores, it is not known if this effect would be noted with uncontaminated oospores. If the role of furfural were merely that of controlling contamination, no stimulatory action would be expected with uncontaminated oospores. Since this is an obligate parasite and no technique has yet been developed to produce oospores in axenic culture, uncontaminated oospores are not likely to become available soon, hence the true role of furfural cannot be determined at this time. Furfural, in fact, may have no direct stimulatory effect on oospore germination, but nevertheless it is very useful in facilitating observation of germination, because decreased levels of contamination were noted when it was present.

Furfural has been reported active in stimulating germination of other fungal spores. Emerson (2) and Sussman (18) reported furfural to be effective in breaking dormancy in fresh ascospores of *Neurospora crassa* and *N. tetrasperma*.

Germination of oospores of *P. sorghi* in these experiments was observed in the complete absence of host plants. In experiments in which oospores were exposed to susceptible sorghum roots

TABLE 1. Effect of furfural on germination of *Peronosclerospora sorghi* oospores[a]

Furfural concentration (μl/L)	Germination (%)[b]
1,000	0
500	0
100	15.6**
50	19.2**
10	7.9
5	5.9
1	6.9
0	6.1

[a] Dried oospores from lysed tissues. Germination determined after 5 days at 25 C, 5.0 ml 1% agar in distilled water per 5.0-cm-diameter plastic petri plate.

[b] Avg of 800 spores, four replicates of 200 spores, all spores (including immature) were counted; ** indicates means significantly different, $P = 0.01$.

TABLE 2. Effect of temperature on germination of *Peronosclerospora sorghi* oospores on 100 μl/L furfural in 1% tap water agar[a]

Time (days)	Germination (%)[b] Temperature (C) 7	12	16	20	23	26	28	30	33
Exp. 1									
3	–[c]	0	0	0	0.2	3.5	4.4	5.5	–
5	–	0	0	0.1	6.9	7.5	8.2	6.8	–
20	–	0	3.6	4.1	†[d]	†	5.1	†	–
Exp. 2									
3	–	0	0	0	0.8	8.1	7.6	2.1	–
7	–	0	0	3.3	4.5	3.8	8.6	3.5	–
Exp. 3									
3	0	0	0	0	0	4.5	2.1	–	0
7	0	0	0	3.3	3.5	11.0	4.2	–	0
13	0	0	0.2	9.1	14.1	15.6	5.6	–	0

[a] Oospores from milled, dried tissue. Immature oospores included in counts.

[b] Average of 1,200 spores, 600 counted per plate, for Exp. 1 and 2; average of 600 spores, 300 counted per plate, for Exp. 3.

[c] –No experimental material.

[d] †Experimental material obliterated by contamination.

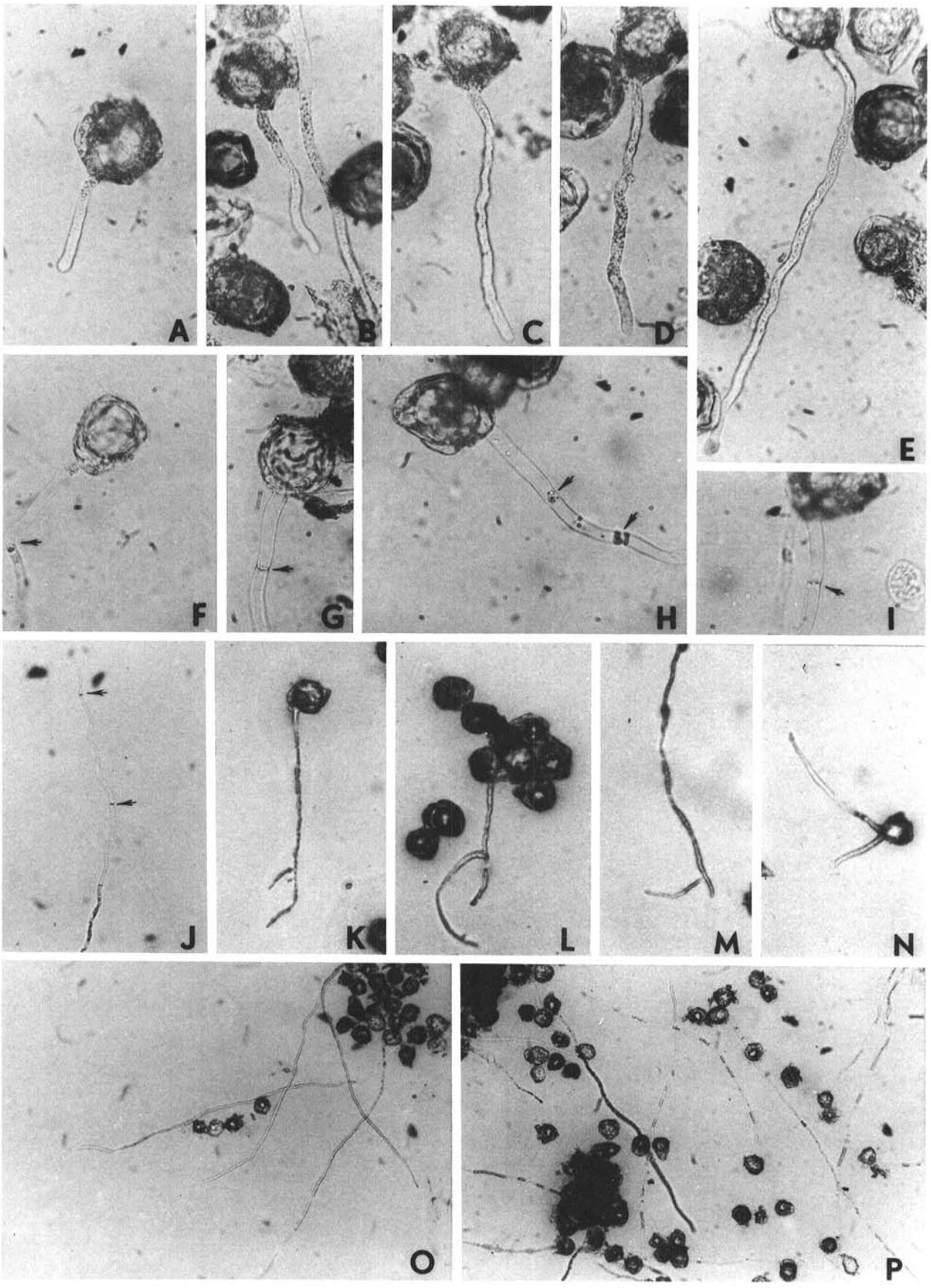

Fig. 1. A–E, Germination of single oospores of *Peronosclerospora sorghi;* 100 μl/ L furfural, 3 days, ×300. **F–I,** Oospores germinated for a longer time period, showing depletion of spore contents and banding or plugging of germ tubes (arrows); 100 μl/ L furfural (F–H), distilled water, in closed vials (I), 7 days, ×300. **J,** Section of germ tube showing two or three bands or plugs, with most of protoplasm migrated to tip of germ tube; 100 μl/ L furfural, ×150. **K–M,** Branch formation in germ tubes; 100 μl/ L furfural plus 10 μl/ L streptomycin and penicillin (K,L), 100 μl/ L furfural only (M), ×150. **N,** Oospore with two germ tubes (rare); 100 μl/ L furfural plus 10 μl/ L streptomycin and penicillin, ×150. **O,** Group of germ tubes; 10 μl/ L furfural, streptomycin, penicillin, ×75. **P,** Group of germ tubes; 50 μl/ L furfural, ×75.

(authors, *unpublished*) no strong stimulatory effect from such roots on oospore germination was observed. Our research describes the usefulness of furfural for induction of in vitro germination of oospores of *P. sorghi* and for control of contamination so that germination can be observed. This technique has not yet been adapted to studies of oospore germination in soil.

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