Factors Affecting Production and Germination of Oospores of Phytophthora drechsleri

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ABSTRACT

Compatible mating strains of *Phytophthora drechsleri* produce oospores abundantly in paired cultures on different seed extract media and safflower plant material. Development of oospores on cornmeal agar was best at 15, 18, 21, and 24 C, with most rapid development occurring at 24 C. Paired cultures incubated in continuous darkness were very productive, whereas, cultures in continuous light produced only few oospores.

Oospores produced on safflower stems germinated better than those from cultures on liquid or agar media. Oospores germinated at temperatures between 15 and 30 C, and between pH 4.5 and 9.5. Germination was best at 24 C and pH 7.5, and increased with age of cultures. Incubation of cultures at 33 C for 2-8 days prior to assays stimulated germination, whereas, chilling at 3 C did not alter germination. Light or darkness did not influence germination of oospores previously exposed to light in culture, but light was required for good germination of oospores produced in continuous darkness. Phytopathology 60:1738-1742.

Genetic studies of *Phytophthora* have been hindered because oospores are difficult to germinate in adequate numbers. Few attempts have been made to determine environmental effects or other influences on oospore germination of *Phytophthora* species other than *P. cactorum* (3) and *P. infestans* (19, 21). Several workers have reported that light and light quality affect production (4, 11, 14, 16) and germination (2, 13, 14, 15, 16, 19, 20) of oospores of some *Phytophthora* species. Various factors which influence spore germination of fungi (5, 10, 22), however, need to be evaluated for their effect on the germination of oospores of *Phytophthora*.

Little is known about the effects of environment or other factors on the germination of oospores of *P. drechsleri* Tucker, incitant of root rot of safflower (*Carthamus tinctorius* L.) (6). These oospores have been germinated successfully (1, 8, 9), however, and evidence is reported (8, 9) of genetic recombination. Erwin et al. (8) encountered a germination of 20-80% among oospores from aged cultures which were incubated with some variation in light and temp. Galindo & Zentmyer (9) germinated oospores of a species designated as *P. drechsleri*, apparently without specific treatment to enhance germination. Various media (8, 9, 12, 17) are suitable for sexual reproduction in compatible strains of *P. drechsleri*.

This study was done to seek a method of producing large quantities of germinable oospores; to determine the effect of temp and light on production and germination of oospores, and effect of pH on germination; and to evaluate pretreatments of cultures which might enhance oospore germination. Such knowledge would be helpful toward understanding the role of the oospore in survival of the fungus in nature and in advancing genetic studies of this species.

MATERIALS AND METHODS.—The compatible mating strains of *P. drechsleri* used were isolated from infected roots of safflower plants. Sexual reproduction of strains A201 and A265 was studied in paired cultures in liquid and agar media prepared with different seed extracts and on autoclaved safflower stems. Seed-extracts were obtained by steeping or boiling 50 g of seed in a liter of distilled water. Cultures were incubated at 21 and 24 C. Stems from 4- to 6-week-old Nebraska 10 safflower plants were defoliated and cut into 130-mm sections. Stem sections were placed on moistened Whatman filter paper in 150 × 25-mm glass petri dishes and autoclaved for 1.5 hr at 15 psi. The autoclaved stems (10 g in each dish) were inoculated with 5 ml of suspension containing 5,000 zoospores of each strain/ml, and incubated at 24 C. Cultures received a 10-hr photoperiod daily with low (25 ft-c) white fluorescent light intensities.

Relation of temp and light to production of oospores was shown in paired cultures on 10 ml cornmeal agar (CMA) in closed plastic petri dishes. The CMA consisted of 17 g commercial Difco cornmeal agar in a liter of distilled water. Agar discs from compatible-strain cultures were placed equidistant in each dish. For temp studies, paired cultures were incubated at 24 C for 48 hr in continuous fluorescent light (580 ft-c) to allow contact of margins of mycelial growth in the dishes, then transferred to incubators at 3 degree intervals from 3 to 39 C before sexual bodies began to form. Paired cultures were incubated at 21 C for 4 days in total darkness or fluorescent light (580 ft-c) and under alternate dark-light conditions to show effect of light on oospore production. Relative numbers of oospores formed at different temp and light conditions were counted in a 4-mm agar disc taken at midpoint between the discs seeded in each dish and compressed on a glass slide.

To obtain oospores for germination studies, 35-day-old oospore-bearing agar cultures were homogenized for 2 min or less in sterile tap or distilled water in a Waring Blendor and strained in a Tyler screen (80 0.175-mm meshes/inch). Oospores were incubated in dishes at 24 C in alternate light (10 hr) and dark (14 hr) conditions. Safflower stem cultures were homogenized 1 to 2 min in 200 ml sterile tap water, pH 7.5, in a Waring Blendor. Homogenized material was strained

in a Tyler screen (twenty 0.833-mm meshes/inch) and the plant pulp was rinsed by blending, and discarded. The suspension of oospores and plant cell components was centrifuged 2 min in a clinical centrifuge. The residue was resuspended in tap water by blending 15 sec, and centrifuged. After the second centrifugation, the oospores (with some plant cell components) were resuspended in 150 ml sterile tap water, pH 7.5, and 10 ml were pipetted into petri dishes containing a thin layer of tap water agar at pH 7.5. To avoid contamination during preparation procedures, blender accessories, screens, centrifuge tubes, and beakers were rinsed with hot water.

Germination tests were run with oospores from stem cultures at pH 7.5 and incubated at 24 C under a regime of alternating fluorescent white light (45 ft-c for 10 hr) and darkness (14 hr), or as otherwise stated. Six dishes with oospores were incubated for each experiment or treatment.

Germination data represent oospores germinated within 72 hr after preparation. Counts of 600 to 1,200 oospores/experiment or treatment at 72 hr included those with early germ tube stage; germ tubes bearing sporangia; evacuated oospores in oogonial envelopes with germ tubes bearing evacuated sporangia; and ungerminated oospores judged to be viable and germinable.

RESULTS.—*Oospore development in relation to medium, temp, and light.*—Two compatible mating strains of *P. drechsleri* produced abundant oospores on autoclaved safflower stems and on liquid and agar media containing extracts from safflower, sunflower, hemp, pea, and lima bean seed.

The number of oospores developed on CMA within 2 days was greatest at 24 C (Fig. 1), although 3 days later the quantity was about the same in paired cultures at 15, 18, 21, and 24 C. Development of oospores decreased at 9, 12, 30, and 33 C, and none was evident within 14 days at 3, 6, 36, and 39 C.

The average numbers of oospores formed per 4-mm agar discs were, respectively, 2 and 152 from cultures in continuous light or darkness. Cultures incubated 2 days in light followed by 2 days of darkness had 144 oospores/sample disc, compared with 22 under a reverse light sequence.

Oospore germination.—Germination of oospores taken from 30- to 50-day-old cultures on agar media was slow, generally less than 5%. Oospores from cultures on safflower stems began to germinate within 48 hr, and among such oospores germination at 72 hr was consistently 10% or higher. The germ tube of the germinating oospore usually penetrates the oogonial wall or may pass through the oogonial stalk, and terminates with formation of a sporangium and release of zoospores.

Factors affecting oospore germination.—The effect of temp on germination was determined with oospores from 35- and 102-day-old cultures. Oospores incubated at 15 to 30 C at 3 degree intervals germinated within 72 hr at all temp. Optimum for germination was at or near 24 C (Fig. 2). Percentage germination at all temp was higher among oospores from the older culture.

The pH of an oospore suspension from 86-day-old cultures was adjusted from a beginning level of 7.5 to levels varying from 4.5 to 9.5 with 0.1 N HCl and 0.1 M NaOH in petri dishes. Suspensions were tested with pH paper at 12, 24, and 36 hr; pH changed little in 72 hr. Among 600 oospores counted/pH level, the percentage germination was highest at pH 7.5, with a slight decrease at pH 8.5 and 9.5 (Fig. 3). The germination pattern of younger oospores was similar for the same pH values.

Fourteen-day-old oospore-bearing cultures were transferred from 24 C to 3 C for aging and to preclude new oospore formation and growth of mycelium. In four germination tests during and at the termination of the 42-day aging period, the percentage germination increased in each successive test (Fig. 4).

Oospore cultures, incubated at 21 C for 30 days after inoculation, were treated by altering moisture and incubation temp to determine whether germination would be affected thereby. Cultures on stems in lot A were gradually air-dried by transferring at 4-day intervals to higher temp (at 3 degree intervals) from 21 to 33 C. Thereafter, the cultures were similarly transferred from 33 to 3 C, then back up to 15 C, and treatment was terminated after 4 days at 15 C (Fig. 5). Drying of stem tissue was essentially completed at 33 C, and the cultures remained dehydrated until 66 days. They were rehydrated with 15 ml of sterile tap water/culture dish upon transfer to 15 C during the chilling process. Cultures on stems in lot B were not treated, but remained moist at 21 C. Oospores were germinated from untreated cultures at 30 days, and thereafter from A and B cultures at 50, 66, 74, 86, and 102 days. Dry cultures were rehydrated before homogenizing at 50 and 66 days. Oospores from A cultures had consistently higher germination (11 to 17% greater) than B cultures (Fig. 5). Increase in germination was highest in A (22%) and B (18%) from 66 to 74 days. Thereafter, percentage germination continued to increase in B but leveled off in A. Highest germination of 51% was obtained with oospores from 86- and 102-day-old A cultures. Chilling did not enhance germination in this experiment, whereas, an effect of aging was indicated.

Data (Fig. 5) suggest that drying of cultures may affect germination. Because of varied temp treatments, however, the role of temp was not clear. Oospore cultures incubated at 24 C for 49 days were gradually dried for 7 days at 24 and 33 C. Moist cultures were maintained at both temp and at 3 C. Dry cultures were rehydrated before homogenizing. Percentage germination was similar among oospores from dry and moist cultures at 24 C, and moist cultures at 3 C (Fig. 6). Germination of oospores from moist cultures incubated at 33 C was 38% among 1,200 oospores, and 28% among those from cultures dried at 33 C. In other experiments, germination of oospores from moist cultures was 29% for those incubated at 24 C for 42 days and then at 33 C for 7 days, and 19% for those treated similarly except that the 7-day incubation was at 36 C (Fig. 7). Germination of oospores increased with incubation time at 33 C (Fig. 8).
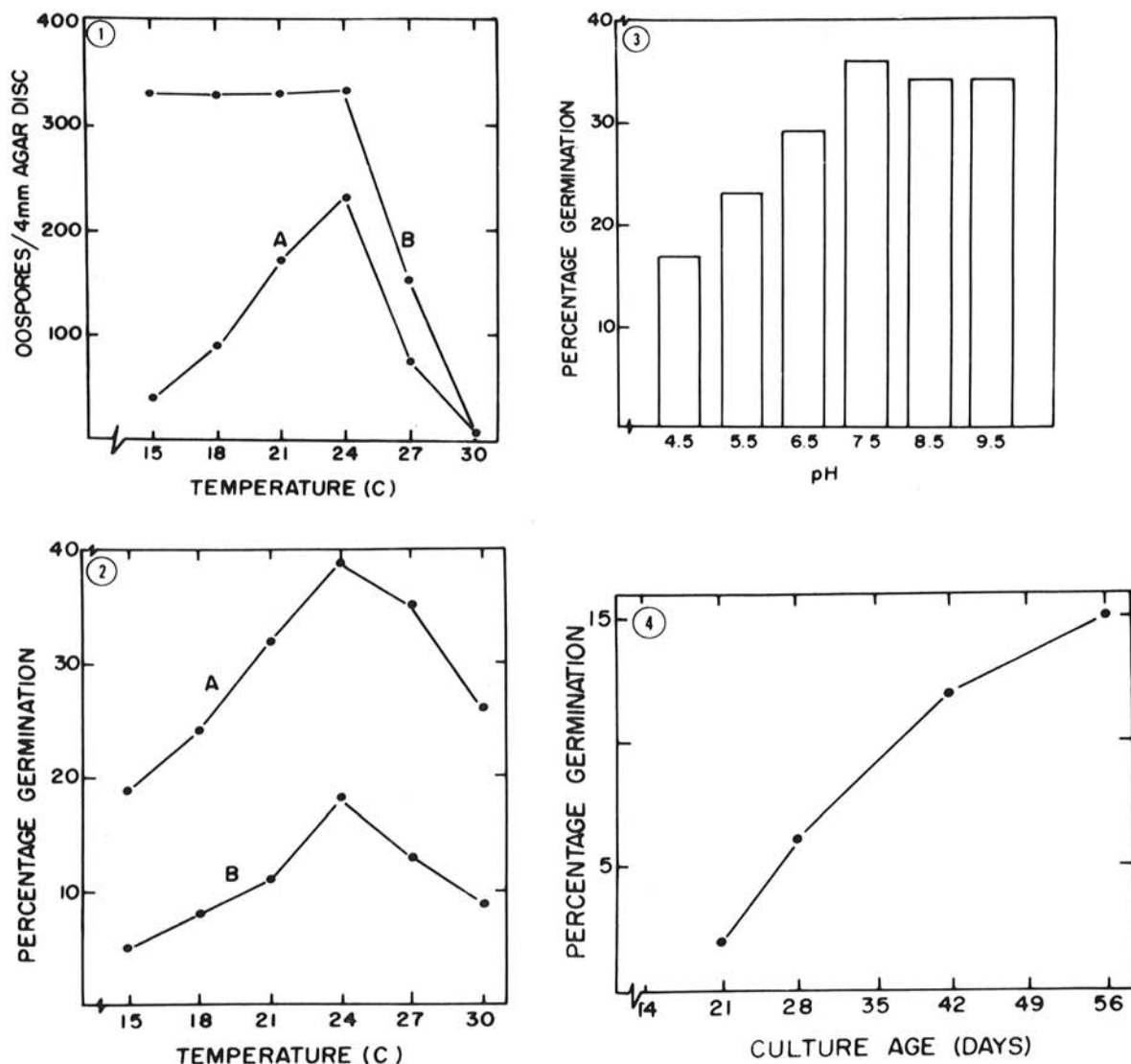
Fig. 1-4. 1) Effect of temp on development of oospores of *Phytophthora drechsleri* on cornmeal agar. (A) Number of oospores after 2 days of incubation; (B) number of oospores after 5 days of incubation. 2) Effect of temp on germination of *P. drechsleri* oospores. (A) Percentage germination of oospores from 102-day-old cultures. (B) Percentage germination of oospores from 35-day-old cultures. 3) Germination of *P. drechsleri* oospores in relation to pH at 24 C. 4) Germination of *P. drechsleri* oospores aged at 3 C.

The effect of light on oospore germination was studied. Oospores from cultures incubated in alternate light and dark were incubated in continuous light (580 ft-c), and in continuous darkness during germination. In three experiments, germination of oospores was 10, 17, and 50% in light, and 9, 17, and 53% in darkness.

In two experiments, oospore-bearing cultures were kept in continuous darkness after inoculation and were homogenized in the dark. Oospore suspensions were incubated in the dark, and in 10 hr of light (45 ft-c) alternated with darkness during germination. Germination among oospores without light was 4 and 4%, respectively, compared with 25 and 23%, respectively, for those with light during germination. In the second

assay, germination of oospores which received light for 10 min, 4 hr, and 12 hr, respectively, was 15, 15, and 17%.

Oospore germination was 22, 19, and 18% in 2:1, 1:1, and 1:2 dilutions of sterile tap water and suspension, and 15% in undiluted suspension.

DISCUSSION.—Oospores of *P. drechsleri* are easily obtained for germination assays from paired cultures on various media. Plant material is preferred for producing germinable oospores because of low germination among oospores from liquid or agar cultures. The oospore suspensions obtained were free of contamination, but elimination of all plant cell components from the suspensions would improve the method.
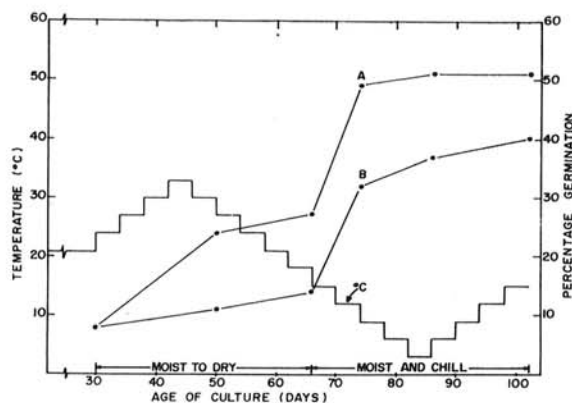
Fig. 5. Comparative germination of *Phytophthora drechsleri* oospores from (A) cultures incubated under conditions of altered temperatures and moisture; (B) cultures at constant 24 C and constant moisture.

An adverse effect of light on sexual reproduction in species of *Phytophthora* (4, 11, 14, 16) is evident also with *P. drechsleri*. Continuous darkness or darkness alternated with light is essential for sexual reproduction when the mycelia of compatible strains intermingle in growth. Since growth of mycelium of the two strains is comparable in light or darkness, the inhibitory effect of continuous light may be on the functional processes of sexual reproduction.

Light is reported to be required for germination of oospores and oogonia of some species of *Phytophthora*
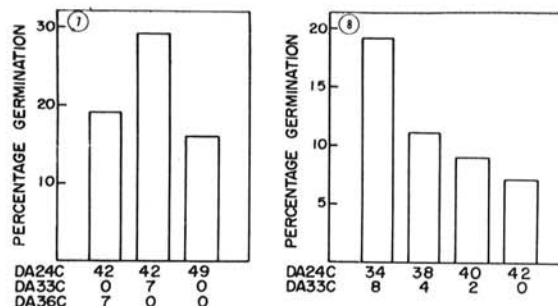

Fig. 6. Effect of chilling, drying, and incubation at 33 C on germination of *Phytophthora drechsleri* oospores. DA = days at degrees C; M = moist; D = dried during 7 days prior to germination assay.

Fig. 7-8. 7) Comparative germination of *Phytophthora drechsleri* oospores from cultures incubated at 33 and 36 C for 7 days prior to germination assay, and untreated cultures. 8) Germination of *P. drechsleri* oospores treated to different incubation periods at 33 C. DA = days at degrees C.

(2, 13, 14, 15, 16, 19, 20), but some reports give little or no details on light conditions during incubation of cultures prior to incubation of oospores for germination. Romero & Erwin (15) found that oospores of *P. infestans* produced in the dark did not germinate in the dark, although 8-15% germinated in light. Light had the same effect on germination of oogonia in that species (16). Data indicate that light is required for germination of *P. drechsleri* oospores. Oospores produced in alternate light and dark germinated in the dark as well as in light, presumably because of exposure to light during culture incubation and preparation for assays. Light was required for good germination of oospores produced in darkness. The increase in germination among such oospores receiving a 10-min photoperiod suggests that the germination process in germinable oospores is rapidly activated by light. Since germination was best among oospores in light alternated with darkness, however, such light environment may be more favorable for germination than a short or long constant photoperiod.

In these experiments, germination was not improved by chilling at 3 C (Fig. 5, 6). A lower chilling temp or longer cold period may be required to influence oospore germination. An increase in germination of oospores from cultures stored at 3 C (Fig. 4) is attributed to aging. Erwin et al. (8) stored cultures of *P. drechsleri* for 90 days at 9 C, which may have served as a chilling treatment, although aging was probably the important factor. Treatment of *P. megasperma* at 1 C up to 48 hr did not increase germination (7). Blackwell (3) found that 1-2 weeks at slightly above freezing would break the dormancy of oospores of *P. cactorum*. Thawing and freezing had no effect on germination of oospores of *P. infestans* (21).

Aging has improved germination of oospores of some species of *Phytophthora* (2, 18, 20). Smoot et al. (21) found that aging oospores of *P. infestans* for 5-6 months did not affect germination, whereas Romero & Erwin (15) germinated relatively young oospores of the same species. Although aging improved the germination of oospores of *P. drechsleri*, long periods of aging were not necessary for germination to occur (Fig. 2, 4, 5).
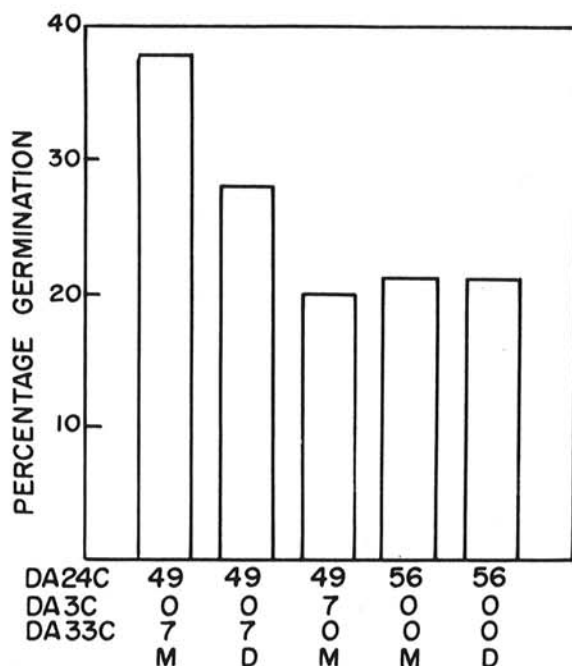
Germination data obtained with oospores from old cultures represent germination of a cumulative number of germinable oospores, unless the cultures are incubated or stored at a temp which inhibits production of new oospores.

Germination of *P. drechsleri* oospores was effectively stimulated by transferring cultures from 24 C to 33 C for 2-8 days prior to germination assays. It is logical to assume that dormancy and metabolic activity of the oospores are affected by high temp, and that germination is stimulated as in spores of other fungi (10, 22). The higher temp seems critical in view of a marked reduction in germination of oospores after incubation of cultures at 36 C for 7 days. At 30 C, some germination might occur in cultures. Decrease in germination among oospores from cultures dried at 33 C (Fig. 6) suggests that desiccation of some germinable oospores occurs. Presumably, oospores could survive some desiccation in nature, in view of the germinability of oospores from dried plant material.

The prolific oospore production of *P. drechsleri* strains A201 and A265 on safflower stems, the production and germination of oospores in a wide range of temp, and germination at different pH values support the view that this fungus can survive under less than ideal conditions and perpetuate itself in the soil through sexual reproduction, assuming that compatible strains are present.

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