Effects of Physical and Chemical Factors on the Germination of Oospores of *Phytophthora capsici* in vitro

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ABSTRACT

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Oospores were produced by crossing two isolates of opposite mating type of Phytophthora capsici obtained from pepper on clarified V8 agar. Cultures were incubated in the dark at 24 C for 2 mo. Germination of oospores produced in the dark at 24 C was reduced by 78 or 90% when cultures were exposed to light (cool-white fluorescent, 40 µmol m⁻²s⁻¹) or incubated at 30 C for 1 wk before oospore collection and germination. Exposure of oospores to light during germination did not significantly affect the percentage of oospores that germinated, regardless of pregermination conditions. Germination of oospores in the dark was reduced by exposure to continuous light during oospore formation. Germination of oospores in sterile distilled water or soil extract showed a quadratic response to temperature, with maximum germination at 24 C. The mode and rate of germination of oospores was affected by incubation media. The predominant mode of oospore germination was via formation of sporangia. After 5 days, sporangia developed on germ tubes from approximately 94% of the oospores that germinated in soil extract, whereas sporangia developed on 33 or 8% of the oospores that germinated in root

extract or sterile distilled water, respectively. Oospores incubated in root extract and distilled water formed germ tubes that continued to elongate for several days before sporangia were formed. However, after 12 days, sporangia had formed on germ tubes from 82, 78, or 94% of the oospores that germinated in distilled water, root extract, or soil extract, respectively. The total percentage of oospores that germinated after 12 days was 29, 39, or 36% in distilled water, root extract, or soil extract, respectively. Treatment of oospores with Novozym effectively removed sporangia and mycelial fragments from suspensions, but it either increased or did not affect germination in two experiments, and decreased germination in two additional experiments. The total percentage of oospores that germinated differed among experiments using oospores of similar age produced under identical conditions. Germination was greatest (51%) when oospores were produced in the dark and germinated in soil extract at 24 C in the dark for 12 days. Oospores germinated predominantly by production of sporangia in all experiments.

Additional keywords: Oomycetes, Phytophthora blight, Phytophthora root and crown rot, Pythiaceae.

Phytophthora capsici Leonian is a widespread and destructive pathogen that causes root and crown rot, fruit rot, and foliar blight on many crops worldwide. Vegetable hosts of the fungus include pepper (Capsicum annuum), tomato, and cucurbits. Phytophthora blight has been reported in North Carolina, and severe losses have occurred in some pepper and cucurbit fields (8,38). P. capsici is a heterothallic fungus, and oospores are important in the life cycle both as primary survival structures (2,7,34) and as the site of genetic recombination (33,39). Oospores of P. capsici occur in naturally infected cocoa pods (25) and pepper plants (31), and both A1 and A2 mating types have been isolated from pepper and cucurbit fields in North Carolina and New Jersey (31,38).

Studies with oospores have been limited by the low frequency of germination. Studies to increase germination through manipulation of physical and chemical factors during oospore formation and germination have focused primarily on homothallic species of *Phytophthora* and *Pythium*. Although some success has been achieved, germination is usually low, and conditions for germination vary with each species (35). Recent work reported greater than 90% germination of oospores of *P. parasitica* after treatment of oospores with KMNO₄ (1). The percentage of germination reported for oospores of *P. capsici* is low and ranges from 0 to 40% (6,9,30,37,39).

Factors that influence germination of oospores of *Phytophthora* species include oospore age, nutrition, temperature, light, chemical

treatments, and treatment with enzymes (36). In general, oospore germination increases with increased age of oospores (1,13,19). Germination of oospores of *P. capsici* from 14- or 60-day old cultures was 3 or 20%, respectively (39). Oospore germination also may be affected by the substrate on which oospores are produced (39) and the availability of nutrients during germination (11,13). Temperature and light requirements for oospore germination vary among species of *Phytophthora* (36) but have not been defined for *P. capsici*. The enzymes helicase, cellulase, and glusulase have been used to separate oospores from mycelial mats and to increase germination (35,41). Germination of oospores of *P. capsici* was increased by treatment with helicase (36), t enzyme treatments may not be equally effective for different species or isolates of the same species (36).

The objective of this study was to investigate the effects of chemical and physical factors on germination of oospores of *P. capsici* in vitro. The effects of culture medium, light, temperature, treatment with enzymes, and treatment with root exudates, root extracts, or soil extracts on germination in vitro were investigated to define optimum conditions for production and germination of oospores of *P. capsici* and to gain insight into possible behavior of the pathogen in soil. A preliminary report has been published (17).

MATERIALS AND METHODS

Two isolates of *P. capsici* were used in these studies. Isolate Sc2A (A1) was isolated from pepper in Sampson County, NC (38), and isolate ATCC 15399 (A2) was obtained from D. Mitchell,

University of Florida (originally isolated from pepper in New Mexico). Cultures were maintained on Difco cornmeal agar (Difco Laboratories, Detroit, MI) slants at room temperature and transferred periodically to V8 agar (200 ml of V8 juice, 2 g of CaCO₃, 15 g of agar, 800 ml of distilled water).

Oospores were produced by placing two V8 agar disks with mycelium of each isolate 2-3 cm apart on clarified V8 (CV8) agar (V8 juice clarified by centrifugation for 10 min at 4,340 g before preparation of the medium). Cultures were wrapped in aluminum foil and incubated in the dark at 24 C for approximately 2 mo. Oospores formed in a distinct band between the two mating types. Agar blocks containing oospores were removed from five petri dishes and comminuted in 250 ml of sterile distilled water for 90 s in a blender. Oospores were concentrated by three successive centrifugations at 1,935 g for 10 min. Agar and mycelial fragments were removed with a pipette, and the oospore pellet was resuspended in sterile distilled water. The final concentration of oospores in the suspension was determined with a hemacytometer. Oospores were used immediately after preparation of the suspension unless otherwise stated.

Two culture media were tested for production of germinable oospores. Oospores were grown on either V8 or CV8 agar in the dark at 24 C for 1 mo and collected as described previously. Approximately 3×10^3 oospores were incubated in 3 ml of sterile distilled water in glass petri dishes (60 mm in diameter) at 24 C in the dark. The percentage of oospores that germinated was determined microscopically after 12 days.

Effects of temperature and light on oospore germination were evaluated in experiments with a completely randomized design and a factorial treatment arrangement. Three pregermination conditions and two incubation conditions were evaluated. Oospores were produced in cultures grown on CV8 agar at 24 C in the dark for 11 wk. Cultures were either incubated in the dark at 24 or 30 C, or exposed to continuous light (cool-white fluorescent light, 40 μ mol m⁻²s⁻¹) at 24 C for 1 wk before oospore collection. Approximately 4×10^4 oospores were spread on water agar amended with rifampicin (10 ppm) and incubated for 3 days at 24 C under continuous light (as described above) or dark conditions.

The effect of continuous exposure to light during oospore formation on subsequent germination of oospores was investigated with oospores grown on CV8 agar for 63 days at 24 C under either continuous light (cool-white fluorescent light, 40 μ mol m⁻²s⁻¹) or dark. Approximately 3×10^3 oospores were incubated in 3 ml of sterile distilled water in glass petri dishes at 24 C in the dark. Repeated measurements of the percentage of oospores that germinated were recorded over a 14-day period.

Novozym 234 (80% a.i., batch PM2415, Novo BioLabs, Danbury, CT), an enzyme preparation from Trichoderma harzianum with cellulase, protease, and chitinase activity, was used to remove sporangia and mycelial fragments from oospore suspensions. The effect of Novozym on oospore germination was evaluated using oospores grown on CV8 for 9 wk in the dark. Oospores were collected as described previously, and the final concentration of the oospore suspension was adjusted to 10⁵ oospores per milliliter of distilled water. A 10-ml aliquot of the oospore suspension was treated with 200 mg of Novozym and incubated on a shaker for 24 h at 28 C. Untreated oospore suspensions were incubated similarly. Oospores were concentrated by centrifugation at 1,935 g for 10 min and washed in sterile distilled water three times to remove the enzyme. Approximately 3×10^3 oospores in a 50- μ l drop were added to 3 ml of filter-sterilized soil extract and incubated at 24 C in the dark. The germination of oospores was observed after 10 days.

The effect of temperature on oospore germination in vitro was investigated with oospores grown on CV8 agar at 24 C in the dark for 63 days. Approximately 3×10^3 oospores were incubated in 3 ml of sterile distilled water in glass petri dishes in the dark at 16, 20, 24, 28, or 32 C. Oospores were examined after 9 days to determine the percentage of oospores that germinated. The experiment was repeated two times with oospores treated with Novozym and incubated in sterile soil extract for 6 or 9 days.

Experiments were conducted to evaluate the influence of root extract, root exudate, and soil extract on oospore germination. Methods used to prepare pepper root extract and root exudate were modified from those of Förster et al (13). A susceptible cultivar of pepper (Keystone Resistant Giant) was grown in Metro Mix 220 (W. R. Grace and Co., Cambridge, MA) in a greenhouse for 4 wk and fertilized with a slow release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA). Root extract was prepared by homogenizing washed roots in distilled water (10 g of roots/ 100 ml of water). The homogenate was centrifuged at 6,780 g for 5 min, and the supernatant was diluted 1:9 with distilled water. Root exudates were collected from pepper plants grown axenically. Pepper seeds were surface-disinfested in 10% bleach (0.5% sodium hypochlorite) for 20 min, rinsed in sterile distilled water, and germinated on water agar. Germinated seeds were transferred aseptically to nylon screens in sterile glass petri dishes containing 50 ml of sterile distilled water. A bent glass rod was placed in each dish to hold the screen above the water. Plates were sealed with Parafilm, and seedlings were allowed to grow for 1 wk at room temperature. Water containing root exudates was collected and concentrated with a Rotavapor (Büchi 011, Flawil, Switzerland). Soil extract was prepared by adding 100 ml of distilled water to 100 g of air-dried field soil (Gilead loamy sand, pH 5.6). Soil was stirred vigorously and allowed to settle overnight. The supernatant was filtered through two layers of cheesecloth, centrifuged at 1,935 g for 15 min, and filtered through coarse filter paper. Root and soil extracts and root exudates were sterilized by filtration (0.45 μ m). Oospores were collected from 64-day-old cultures grown on CV8 agar as described previously. A 50-µl drop of oospore suspension containing approximately 3×10^3 oospores was added to 3 ml of sterile distilled water, root extract, root exudate, or soil extract in glass petri dishes and incubated at 24 C in the dark. The percentage of germinated oospores was recorded after 12 days.

The influence of root and soil extracts on the mode of oospore germination was investigated. Oospores were collected from 55-day-old cultures and treated with Novozym to remove sporangia and mycelial fragments. Approximately 3×10^3 oospores in a 50- μ l drop were added to 3 ml of sterile distilled water, root extract, or soil extract in glass petri dishes and incubated at 24 C in the dark. Oospore germination was recorded after 5, 9, and 12 days in the first experiment and after 5, 7, and 9 days in the second and third experiments.

All experiments were performed at least twice and were conducted using a completely randomized design with five replicate petri dishes per treatment. Germination data were based on microscopic observations of 100 oospores per dish. Data were tested for homogeneity of variance before transformation. Data ranging from 0 to 30% were transformed with the square root transformation (square root of (Y + 0.5), where Y = percent germinated oospores), and data ranging from 0 to 100% were transformed with the arcsine square root transformation before analysis of variance with the Statistical Analysis System version 6.04 (SAS Institute Inc., Cary, NC). Treatment means were compared with either a t test or a least significant difference test. Temperature data were analyzed by regression analysis. Multivariate repeated measures analysis of variance was used for analysis of data recorded over time (14,29). Tests of the effect of time and time × treatment interactions were performed using Wilks' lambda, which is a likelihood ratio test statistic for multivariate analysis of variance (22).

RESULTS

Germination of oospores produced on CV8 agar was not significantly different from germination of oospores produced on V8 agar. Germination of oospores produced on CV8 or V8 agar was 17 or 11%, respectively, after incubation for 12 days in sterile distilled water. CV8 agar was used to produce oospores in subsequent experiments.

Germination of oospores produced on CV8 agar at 24 C in the dark was reduced (F value significant at P = 0.0001) by 78

or 90% by exposure of cultures to light at 24 C or incubation at 30 C in the dark, respectively, for 1 wk before oospore collection (Fig. 1). In contrast, exposure of oospores to light during germination did not significantly affect the percentage of oospores that germinated regardless of pregermination conditions (Fig. 1). Similar results were obtained in repeated experiments. Oospores germinated on water agar by production of sporangia in all treatments.

Significantly fewer oospores exposed to continuous light during oospore formation germinated than oospores produced in the dark (Fig. 2). Initially, average germination of oospores produced in the dark or light was less than 4% after incubation for 3 days in sterile distilled water at 24 C in the dark. However, after 14 days, approximately 18% of the oospores produced in the dark and 2% of the oospores produced in the light had germinated in the first experiment (Fig. 2). In the second experiment, germination of oospores produced in the dark or light was 17 or 10%, respectively, after incubation for 12 days in distilled water (Fig.

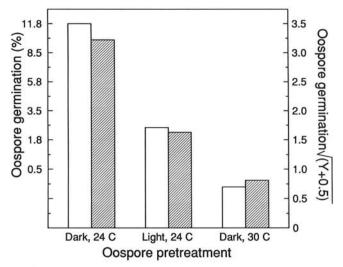


Fig. 1. Effect of light and temperature pregermination conditions on germination of *Phytophthora capsici* oospores in the light (open bars) or dark (crosshatched bars). LSD_{0.05} = 0.41 (square root-transformed scale) for comparison of means between bar pairs. Means within bar pairs not significantly different. Square root-transformed data are shown on the right y-axis and back-transformed values are shown on left y-axis.

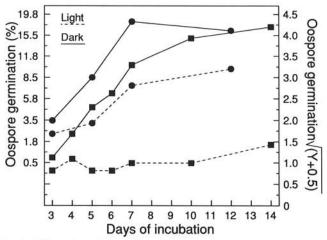


Fig. 2. Effect of continuous light or dark conditions during oospore formation on subsequent germination of *Phytophthora capsici* oospores in distilled water at 24 C in the dark. ■, Experiment one; ●, experiment two. Square root-transformed data are shown on the right y-axis and back-transformed values are shown on the left y-axis. The standard error of the change in germination from initial to final measurement times was 0.23 and 0.28 (square root-transformed scale) for experiments one and two, respectively.

2). Oospore germination increased significantly over time and the rate of increase was higher in the light than dark treatments (Wilks' lambda for time \times treatment interaction significant at P = 0.0048 and P = 0.0376 for each experiment, respectively).

Treatment of oospores with Novozym effectively removed sporangia and mycelial fragments from the oospore suspensions. However, the effect of Novozym enzyme treatment on oospore germination in soil extract was inconsistent among experiments when oospores of similar age were used (Table 1). Germination of oospores either increased or was not affected by treatment with Novozym as compared with untreated controls in two experiments, and decreased as compared with untreated controls in two additional experiments.

Temperature influenced oospore germination in distilled water and soil extract (Fig. 3). Germination of oospores showed a quadratic response to temperature (P=0.0001) and maximum germination occurred at 24 C. Germination of oospores was predominantly by production of sporangia in distilled water and soil extract at all temperatures evaluated. Oospore germination was higher at all temperatures in soil extract than in distilled water.

Oospore germination was lower (P = 0.0019) in distilled water than in root extract, root exudate, or soil extract. The total percentage of oospores that germinated after 12 days did not differ significantly among root extract, root exudate, and soil extract in repeated experiments. Thirty-two percent of the oospores incubated in distilled water germinated, whereas 45, 51, or 51% of the oospores incubated in root extract, root exudate, or soil extract germinated, respectively.

TABLE 1. Effect of Novozym enzyme treatment on germination of oospores of *Phytophthora capsici* in sterile soil extract

Experiment	Oospore germination (%) ^a	
	Control	Novozym-treated
1	17.0 (±1.3) ^b	8.0 (±1.6)
2	$33.7 (\pm 2.9)$	$41.5 (\pm 2.4)$
3	$57.2 (\pm 1.5)$	$46.8 (\pm 3.9)$
4	25.7 (±2.6)	25.0 (±2.4)

^a Oospore suspensions containing 10⁵ oospores per milliliter were treated with Novozym (20 mg/ml) and incubated on a shaker for 24 h at 28 C, then washed and germinated at 24 C in the dark for 10 days.

^b Values in parentheses indicate the standard error. Statistical analyses were conducted on square root-transformed data. Means within experiments were separated by a t test and were significantly different at P = 0.003, 0.0324, and 0.0431 in experiments 1, 2, and 3, respectively. Means for experiment 4 were not significantly different.

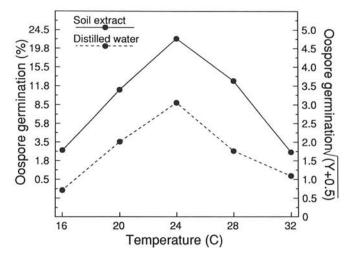


Fig. 3. Influence of temperature on germination of *Phytophthora capsici* oospores in distilled water or soil extract. Square root-transformed data are shown on the right y-axis and back-transformed values are shown on the left y-axis. The standard error of the mean was 0.19 or 0.25 (square root-transformed scale) in water or soil extract, respectively.

The mode and rate of germination of oospores was affected by incubation in root or soil extracts (Fig. 4). Oospores incubated in distilled water or root extract germinated via one or several germ tubes that continued to elongate for several days before sporangia were produced. Oospores germinated and produced sporangia more rapidly in soil extract than in either distilled water or root extract (Wilks' lambda significant for time X treatment interaction at P = 0.0202) (Fig. 4A). In the first experiment, after 5 days, sporangia were produced on approximately 94% of the oospores that germinated in soil extract, whereas sporangia were produced on 33 or 6% of the oospores that germinated in distilled water or root extract, respectively (Fig. 4A). However, after 12 days, sporangia were produced on long germ tubes on 82 or 78% of the oospores that germinated in distilled water or root extract, respectively (Fig. 4A). The percentage of oospores that germinated over time to form sporangia was greater in soil extract than in distilled water (F value significant at P = 0.0001and P = 0.0001) or root extract (F values significant at P =0.0341 and P = 0.0042) in two additional experiments.

The total percentage of oospores that germinated via either formation of germ tubes or sporangia in distilled water, root extract, or soil extract increased over time in each experiment (Wilks' lambda for time significant at P=0.0001) (Fig. 4B). Average total germination over time was lower in distilled water than in soil extract (Fig. 4B) in three experiments (F value significant at P=0.0171, 0.0001, and 0.0565), but differences in total levels of oospore germination over time between soil and root extract were not significant. The total percentage of germinated oospores after 12 days was 29, 39, or 36% in distilled water, root extract, or soil extract, respectively (Fig. 4B).

DISCUSSION

Oospores used in most of our experiments were collected from

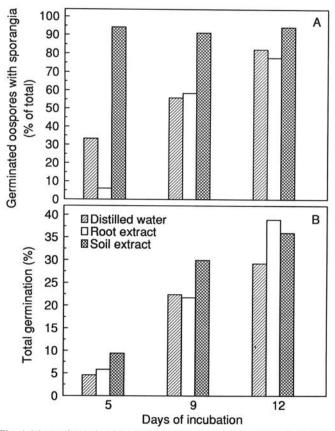


Fig. 4. Mode of germination of *Phytophthora capsici* oospores in distilled water, root extract, or soil extract. A, Percentage of germinated oospores that produced sporangia. B, Total percentage of oospores that germinated. Data were transformed with the arcsine square root transformation, and back-transformed values are shown on the y-axis.

cultures grown for approximately 2 mo. Oospore germination is reported to increase with increasing age of oospores (1,13,23,39). Recent studies with *P. capsici, P. megasperma* f. sp. *medicaginis, P. m. glycinea,* and *P. cactorum* indicated that oospores germinated only after nuclei fused within the oospore (19). The nuclear condition of the oospores used in studies described here was not determined; however, nuclear condition might be a useful criterion for the evaluation of oospore maturity in future studies.

Germination of oospores of P. capsici was greatest when oospores were produced and germinated in the dark. Exposure of cultures to either continuous light during oospore formation or a 1-wk light period before collection of dark-produced oospores suppressed subsequent germination. However, exposure to light during germination did not significantly affect the percentage or mode of germination. Exposure of oospores of P. m. glycinea to light during formation also inhibited germination, whereas light provided during the germination stage increased oospore germination (40). In contrast, a light requirement for oospore germination has been reported for several *Phytophthora* species (1,3,37). Oospores of P. parasitica did not germinate if light was not provided during oospore maturation and germination (1). Similarly, light was required for germination of oospores of P. cactorum produced in the dark (3). Photoactivation and stimulation of oospore germination by exposure to blue (400-480 nm) light also has been reported for P. citricola, P. palmivora, P. cactorum, and P. cinnamomi (3,18,32,37). Germination of oospores of P. capsici increased if blue or far-red light was provided during oospore maturation; however, germination in previous studies did not exceed 10% and exposure to cool-white fluorescent light had inconsistent effects on germination (37). Some authors have suggested that a flavin or carotenoid photoreceptor exists in oospores of Phytophthora species (4). Further studies on lightmediated germination responses in Phytophthora species are needed to fully explain the mechanisms of photoactivation and photosuppression of oospore germination.

The elimination of mycelial fragments and sporangia from oospore suspensions was necessary for subsequent studies on the ecology of oospores in soil. Preliminary studies showed that treatment of oospores with Novozym was more effective in eliminating other germinable propagules from oospore suspensions than glusulase (β -glucuronidase and aryl sulfatase) or pure cellulase (M. J. Hord, unpublished). However, the effect of Novozym on oospore germination was inconsistent among experiments. Others have found that Novozym reduced the viability of protoplasts of P. capsici (27). Germination of oospores of P. capsici was increased from less than 8% to greater than 50% by treatment with a 1% helicase solution, but germination of P. cinnamomi was not increased (36). Germination of oospores of P. m. medicaginis was not affected by treatment of oospores for 12 h with a mixture of β -glucuronidase and aryl sulfatase or cellulase, but prolonged treatment suppressed germination (11). Treatment with enzymes may affect oospore germination by causing alterations in the structure of the oospore wall. Dissolution of the thick inner wall of the oospore, composed primarily of insoluble β -glucans, occurs during the early stages of oospore germination (16). Enzymes that mobilize reserves stored in the oospore inner wall may trigger the germination process. However, prolonged exposure to enzymes may be detrimental to oospore germination.

The optimum temperature for germination of oospores of *P. capsici* was 24 C and is similar to the optimum temperature range (24–27 C) reported for sporangium formation from mycelial mats (9), but is lower than the optimum temperature range (26–32 C) reported for vegetative growth (38). Oospore germination in *P. cactorum*, *P. fragariae*, and *P. syringae* also is favored at temperatures lower than those optimal for vegetative growth (3,10,15). Oospores of *P. capsici* germinated to form sporangia in distilled water or soil extract at all temperatures tested between 16 and 32 C. In comparison, the mode of germination of sporangia of *P. capsici* is temperature-dependent, with germination by germ tube favored at 26 C and zoospore differentiation and release occurring at lower temperatures (24,39). Average soil temperatures in North Carolina during the early months of the growing season

range from 17 to 26 C. Germination of oospores to form sporangia rather than germ tubes alone within this range of soil temperatures may be a selective advantage to the pathogen and may increase initial inoculum densities present in soil for early-season infections.

Germination of oospores was reduced by incubation of cultures of oospores at 30 C for 1 wk before germination. In contrast, a period of incubation at 30–33 C increased germination of oospores of *P. capsici* from cacao, *P. m. medicaginis, P. palmivora*, and *P. drechsleri* (11,23,26). Recovery of propagules of *P. parasitica* from soil on selective media also was increased by heat treatment of soils (28). A chilling period stimulated germination of oospores of *P. cactorum* (5). The effect of heat on oospore germination may be cumulative. A critical number of degreedays above a threshold temperature may be required to stimulate oospore germination under field conditions as has been described for propagules of *P. parasitica* in soil (28). However, brief heat treatments on agar media appear to be detrimental to oospore germination of *P. capsici*.

Therefore, exogenous nutrients were not required for germination. Oospores of P. capsici germinated in distilled water in our study. However, germination was increased by incubation in root exudates, root extracts, and soil extracts. In contrast, exogenous nutrients were required for germination of oospores of P. parasitica (1). Increased oospore germination in root or soil extracts also has been reported for P. m. glycinea (20,42) and P. m. medicaginis (13). Germination of oospores of P. m. medicaginis was greater in root extract (13) than in distilled water, and the mode of oospore germination was related to the concentration of root extract (12). Oospores of P. m. medicaginis germinated via germ tubes and did not form sporangia in distilled water (13). In our studies, after 12 days, the predominant mode of germination of oospores of P. capsici was by production of sporangia in distilled water, root extract, or soil extract. However, the rate of sporangium formation differed among incubation media. Sporangia developed more rapidly from germinated oospores in soil extract than in distilled water or root extract. Soil extract is commonly used to induce sporangium formation from mycelial mats of Phytophthora species and may contain chemical factors that stimulate sporangium production (35).

Some oospores incubated in root extract germinated directly to form long germ tubes on which sporangia were produced after prolonged incubation. A similar response to plant exudates was observed with oospores of *Pythium aphanidermatum* incubated in soil amended with bean seed exudate (43). Oospores of *Pythium ultimum* germinated directly in soil and formed germ tubes oriented toward roots of cotton seedlings (21). Bimodal germination, which appears to be affected by exogenous nutrients, may be important to ensure the survival of the pathogen in soil.

Germination of oospores of *P. capsici* increased significantly with time of incubation in water, root extract, or soil extract, but did not exceed 51% in any of our studies. Asynchronous germination is typical of oospores of *Phytophthora* species (13,20,27) and could enhance survival by ensuring a continuing supply of infective propagules in soil.

In our studies, maximum germination of obspores of *P. capsici* occurred when oospores produced on CV8 agar at 24 C in continuous darkness were germinated in soil extract at 24 C in the dark. Exposure of cultures to light or high temperature before oospore collection caused a significant reduction in the subsequent germination of oospores. Oospore germination in distilled water, root extract, or soil extract was predominantly by formation of sporangia, but sporangia formed more rapidly on oospores germinated in soil extract. Germination was greatest when oospores were incubated in root or soil extract in the dark at 24 C for 12 days. These results should facilitate future research with oospores of *P. capsici* and provide useful information on the probable mode of germination of oospores in soil.

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