Limiting Influence of Low Water Potential on the Formation of Sporangia by Phytophthora drechsleri in Soil

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Supported by National Science Foundation Grant GB 32809. The author thanks Ms. H. D. Zumwalt for technical assistance.

Accepted for publication 26 April 1975.

ABSTRACT

The formation of sporangia by *Phytophthora drechsleri* was examined in soil at water potentials (ψ) between 0 and −150 bars. Soil and mycelial disks were air-dried separately to the desired ψ values and placed together in the chambers of thermocouple psychrometers. The psychrometers were subsequently used to measure and maintain ψ at nearly constant values. Crocks of soil equipped with thermocouple psychrometers were also dried to various ψ values before mycelia were buried in the soil. Sporangia were counted 4-12 days after mycelial disks were placed in soil. Large numbers of sporangia formed at ψ values between −0.3 and −4 bars, whereas few or no sporangia formed at $\psi \simeq 0$ or at ψ values lower than −4 bars. Variation in the period of time mycelial disks were in soil or the use of sterilized as opposed to unsterilized soil did not alter the influence of ψ on the formation of sporangia. Exposure of mycelial disks to ψ values less than −8 bars in soil inhibited subsequent formation of sporangia in water. *P. drechsleri* remained viable in soil at all ψ values down to −40 bars.

Phytopathology 65:1089-1093

Most of the literature on soil-borne *Phytophthora* spp. suggests free water is required for, or is at least highly stimulatory to, the formation of sporangia (13). However, the exact water requirements for sporangia to form are unknown and the author knows of only one previous study on the influence of soil water potential (ψ) on the formation of sporangia by a *Phytophthora* sp. In that study Sneh and McIntosh (10) found that *P. cactorum* formed numerous sporangia at matric potentials (ψ_m) of −0.1 and −0.3 bar but few or no sporangia at $\psi_m = -3.0$ bars. Unfortunately, Sneh and McIntosh did not examine the influence of ψ_m values between −0.3 and −3.0 bars. The influence of ambient humidity on the formation of sporangia by *P. infestans* is known (6). However, the water status of fungi at plant-air interfaces is influenced by plant ψ values as well as ambient humidity and the actual ψ value of *P. infestans* on aerial plant parts is unknown. Therefore, the humidity data for sporulation by *P. infestans* cannot be applied directly to the soil. The most precise studies (1, 11) to date on the water relations of soil-borne *Phytophthora* spp. examined the influence of ψ on the growth of mycelium and did not examine sporulation. Because the influence of soil moisture on the formation of sporangia by *Phytophthora* spp. is of probable significance in disease epidemiology, the influence of soil ψ values over the entire range of 0 to −150 bars on the formation of sporangia by *Phytophthora drechsleri* Tucker was examined quantitatively.

MATERIALS AND METHODS.—The isolate of *P. drechsleri* used in all experiments was the A2 mating type originally isolated from safflower (*Carthamus tinctorius* L.). Mycelium was grown on lima bean agar made by boiling 200 g of frozen lima beans in distilled water for 10 minutes and then blending the cooked beans in the water. The resulting suspension was brought to 1,000 ml with distilled water, and 10 g dextrose, 10 g yeast extract, and 15 g agar added before autoclaving at 121 C for 15 minutes. Petri plate cultures were incubated at 25 C in the dark where *P. drechsleri* produced an abundance of aerial mycelium with few swellings and no sporangia. When mycelia were to be placed in soil, a 7-mm diameter cork borer was pressed against the agar surface and disks of aerial mycelia lifted from the agar with forceps. A preliminary experiment showed that mycelial disks from 3– to 30-day-old cultures of *P. drechsleri* yielded similar numbers of sporangia in soil, and disks (28-35 mg fresh weight) were taken from 14– to 21-day-old cultures for the soil experiments. Mycelial disks were submerged in a solution of fluorescent brightener (0.4 mg/ml Calcofluor® White M2R New, American Cyanamid Co.) for 3 hours. Disks were subsequently rinsed three times in water and blotted dry between several layers of filter paper for 10 seconds. Before being placed in soil, disks were placed on a mesh support and allowed to dry in the laboratory at 20-30% relative humidity (RH) for the periods of time specified for each experiment.

Sporangia formed in soil were observed with the aid of fluorescence microscopy (12). Mycelial disks from the soil were blended in 5 ml water for 10 seconds in a microcontainer on a Waring Blendor at medium speed. The suspension was centrifuged at 1,600 *g* for 10 minutes, and the pellet resuspended in water on a vortex mixer to give a final volume of 0.5 ml. A drop of the final suspension was placed under a cover glass supported by small pieces of glass on a slide to give a sample of constant depth. Either 100 sporangia or the sporangia in 50 fields (0.4-mm field diameter) were counted on a slide, depending on which number was reached first, and the total number of sporangia calculated for each mycelial disk. The blending and centrifugation procedure did not alter the appearance or viability of sporangia. The viability of sporangia was examined by spreading the suspension used for counting on a selective medium. Sporangia with germ tubes were counted after 16 hours of incubation on the medium. The viability of *P. drechsleri* was also examined by placing mycelial disks from the soil directly on the medium. Selective medium contained 17 g cornmeal agar (Difco), 10 mg pimaricin (Delvocid®, Gist-Brocades nv, Delft, Holland), 300 mg vancomycin hydrochloride (Vancocin®, Eli Lilly & Co.), 100 mg pentachloronitrobenzene (Aldrich), 50 mg penicillin G (1,585 units/mg, Calbiochem), and 50 mg streptomycin sulfate (Sigma) per 1,000 ml distilled water. The antibiotics were added to autoclaved medium after it had cooled to 50 C.

The influence of soil ψ on the formation of sporangia

was examined by enclosing soil and mycelial disks in thermocouple psychrometers of the type shown in Fig. 1. Soil was prepared by mixing two volumes of sandy loam with one volume of sieved (1.4 mm openings) peat. Some of the soil mix was sterilized by autoclaving at 121 C for 1 hour on two successive days. After thorough watering, soil was allowed to dry in the laboratory and soil samples were periodically put into the psychrometers. A standard procedure was used to pack soil to a depth of 5 mm in the bottom of the sample chambers (Fig. 1) and the isopiestic technique of Boyer and Knipling (4) was used to measure soil ψ values. Briefly, the isopiestic technique involves placing water and various solutions of known ψ value on the measuring junction (Fig. 1) until a nearly zero reading is obtained. Psychrometers were allowed to equilibrate for 2-3 hours before each reading and finally a reading was obtained with the measuring junction dry to correct for possible heat of respiration (3). Obtaining soil samples in the psychrometers at the desired ψ values was done by trial and error over the course of several days. In an attempt to bring mycelia to the same ψ values as the soil samples, mycelial disks were air-dried for 0-41 minutes before they were placed in the psychrometers. Unless stated otherwise, the disks were placed 0.5 mm below the soil surface. Soil ψ value was measured within 9 hours after mycelial disks were put into the psychrometers and remeasured just before the disks were removed to count sporangia. Nearly isopiestic solutions were maintained on the measuring junctions between ψ value measurements, and the plunger heat sinks (Fig. 1) were lifted from the psychrometers for 15 minutes each day to facilitate aeration. The relationship between soil ψ value and water content was determined by returning the soil from which mycelial disks were removed to the psychrometers and allowing the psychrometers to equilibrate for 3 days. After measuring soil ψ value, water content was determined by drying the soil to a constant weight at 105 C.

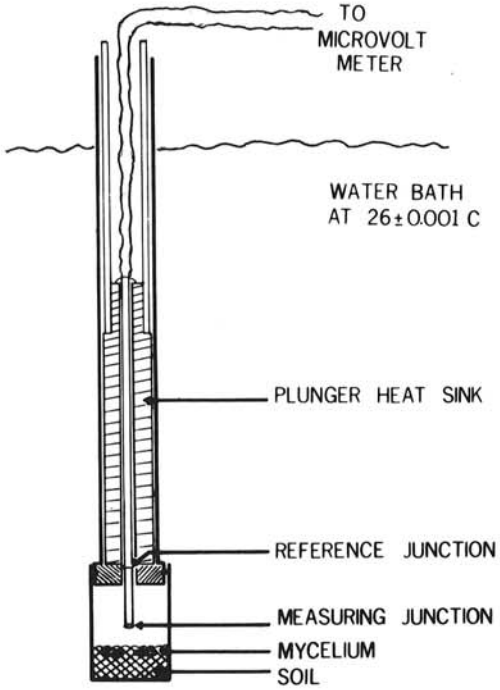

Fig. 1. Thermocouple psychrometer for isopiestic determinations of water potential. The measuring junction consists of a 1.5 mm diameter silver ring supported by 0.1 mm diameter chromel and constantan wires which are connected to 1 mm diameter copper wires at the reference junction. The copper wires are embedded in epoxy in the plunger heat sink. The heat sink is made of brass and the surrounding top assembly and botton sample chamber are made of copper. A plastic handle permits the plunger heat sink and thermocouple junctions to be lifted from the top assembly without disturbing the sample chamber or its position in the water bath. The inside of the sample chamber is coated with petrolatum and is 2 cm in diameter and 2.5 cm high.

To examine the influence of air drying mycelia on the subsequent formation of sporangia in soil, mycelial disks were dried for 0-65 minutes and placed in soil at $\psi_m = -0.3$ bar. Soil ψ_m was maintained constant by using Büchner funnels with fritted glass disks of fine porosity (Kimble 28400-90F) as tensiometers (7). Soil mix was packed to a depth of 1 cm on top of the fritted glass, and mesh envelopes containing mycelial disks were buried 5 mm below soil surface. Tops of the Büchner funnels were covered with loosely fitting plastic bags to retard evaporation.

The influence of soil ψ value on the formation of sporangia was also examined in 2-liter crocks of Yolo fine sandy loam. Soil was taken directly from the field without drying or sterilization and used to fill glazed crocks with drain holes. The ceramic tip of a thermocouple psychrometer (Type PT51, Wescor, Inc., Logan, Utah) was positioned 2 cm below the soil surface at the center of every crock, and 1-week-old safflower (*Carthamus tinctorius* L.) seedlings transplanted into the soil. The thermocouple psychrometers were used with a Peltier cooling current and were calibrated in NaCl solutions (3). (To reduce thermal noise to an acceptable level it was necessary to bury 60 cm of psychrometer lead wire in the soil.) Wood pot labels were pushed into the soil around each psychrometer, and the crocks placed in a controlled environment (26 ± 0.1 C, 80 ± 5% RH, and 85 Wm^{-2} of light at 300-700 nm for 16 hours/day) and watered daily. Soil ψ was measured twice daily, and after 2 weeks, the crocks were watered differentially. Soil was kept saturated by standing one crock in water, and ψ values between −0.3 and −1.0 bar were obtained by watering one crock daily. Water was withheld from the remaining crocks. After 3-5 days, soil had dried to desired ψ values and mycelial disks were placed in the soil. Mycelial disks were air-dried for 10 minutes, coated with a thin layer of soil ($\psi \simeq -1$ bar), and enclosed in small envelopes of fiberglass window screen. The envelopes were stapled to wood pot labels which were subsequently used to replace the pot labels already in the soil. Mycelial disks had a final position 2 cm below the soil surface and 2 cm from a thermocouple psychrometer. After all disks were in place crocks were maintained in the dark in a saturated atmosphere. Two mycelial disks were periodically taken from each crock for counting sporangia.

RESULTS.—The results of one experiment in which soil and mycelial disks were enclosed in thermocouple psychrometers are shown in Fig. 2. Maximum numbers of sporangia formed at initial ψ values between −2.1 and −3.5 bars, whereas fewer sporangia formed at an initial ψ

value of −1.6 bar. More importantly, almost no sporangia formed at $\psi \leqslant -4$ bars in both sterilized (Fig. 2-A) and unsterilized soil (Fig. 2-B). Air drying mycelial disks to the ψ values used in the psychrometers (Fig. 2) did not have a detrimental effect on their formation of sporangia in soil at $\psi_m = -0.3$ bar (Fig. 3). The relationship between water content and ψ for the soil used in the psychrometers (Fig. 2) is shown in Fig. 4.

The methods of a second psychrometer experiment differed from the first in that mycelium was placed 2-3 mm rather than 0.5 mm below the soil surface. Except for variation at ψ values between −2.3 and −9.4 bars, the influence of ψ on the formation of sporangia in the second experiment (Fig. 5) was similar to that in the first experiment (Fig. 2). In addition to using mycelial disks from the second experiment for counting sporangia formed in soil, one disk from each psychrometer was placed in 1 mm of water and one was transferred to selective medium. With one exception, all disks which had been at $\psi \geqslant -8$ bars formed an abundance of new sporangia when left in water for 48 hours, whereas disks which had been at $\psi < -8$ bars did not form sporangia in water (Fig. 6). Mycelium grew from all but the −150-bar sample on selective medium (Fig. 6).

In both psychrometer experiments (Fig. 2 and 5), the appearance of sporangia from the soil did not vary with ψ values higher than −5 bars and 55-65% of the sporangia contained cytoplasm and had not germinated. In samples with at least 10^4 sporangia per disk, 87-93% of the full sporangia were viable. Sporangia in some of the remaining samples were also viable, but only small numbers of sporangia were found on the plates of selective medium. At $\psi > -3$ bars, 3-10% of the sporangia in soil had germinated directly, whereas none had germinated at $\psi < -3$ bars.

Results of the experiment conducted with crocks of soil are shown in Fig. 7. A maximum of 3.2×10^4 sporangia was formed per mycelial disk at ψ values between −0.3

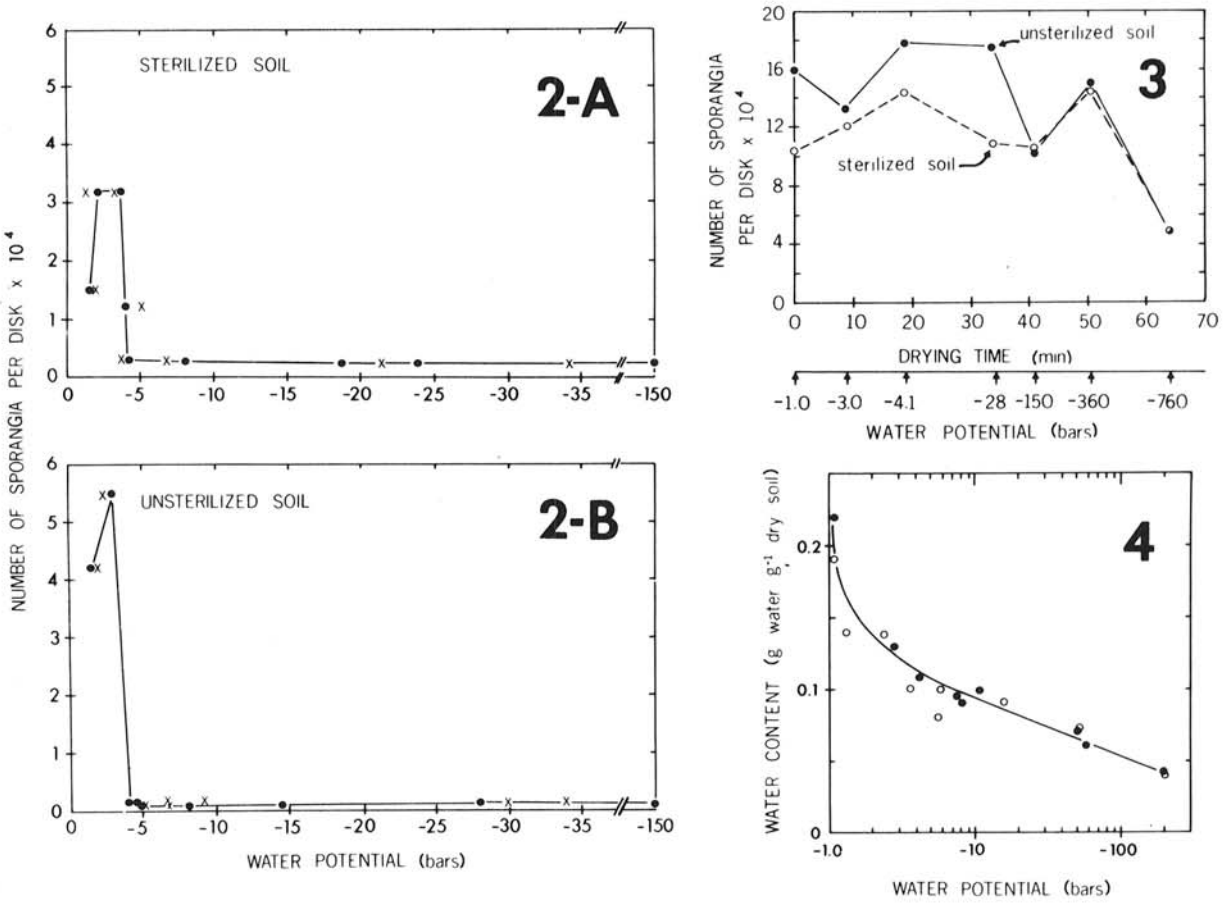

Fig. 2-4. 2) Effect of water potential of sterilized (A) and unsterilized soil (B) on the number of sporangia formed per mycelial disk in thermocouple psychrometers. Circles and crosses, respectively, represent ψ values determined just after mycelial disks were put into a psychrometer and just before the disks were removed from a psychrometer to count sporangia. Numbers of sporangia are averages for four mycelial disks which were in a psychrometer for 4-5 days. 3) Effect of air-drying mycelial disks for various periods of time on the formation of sporangia in sterilized and unsterilized soil in tensiometers at $\psi_m = -0.3$ bar. Arrows on the bottom axis indicate the times at which parallel samples were placed in tensiometers and in a thermocouple psychrometer (Fig. 2). Water potentials were measured shortly after mycelial disks were placed in soil in a psychrometer, or in the case of −360 and −760 bars, after mycelial disks alone were placed in a psychrometer. Numbers of sporangia are averages for two mycelial disks which were in tensiometers for 4-5 days. 4) Relationship between water content and water potential for sterilized (open circles) and unsterilized soil (closed circles).

and −1.0 bar. Many sporangia were also formed at ψ values of −2 to −5 bars, whereas fewer than 1×10^4 sporangia were formed at $\psi \leqslant -5$ bars. Almost no sporangia were formed in completely saturated soil at $\psi \simeq 0$. Numbers of sporangia remained nearly constant between 4 and 12 days after mycelial disks were put into the crocks of soil. After 12 days in soil, mycelia from all treatments grew when transferred to selective medium.

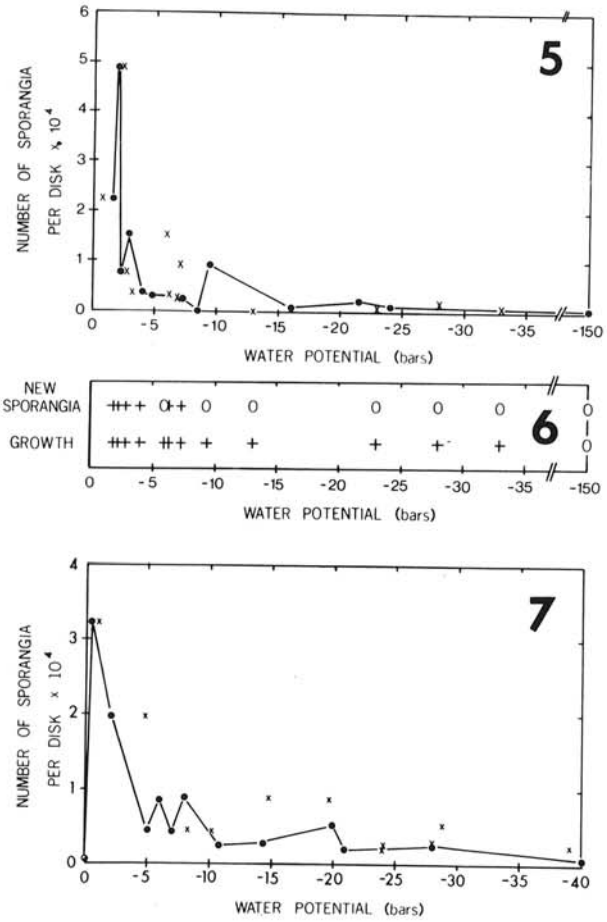

Fig. 5-7. 5) Effect of water potential of unsterilized soil on the number of sporangia formed per mycelial disk in thermocouple psychrometers. Circles and crosses, respectively, represent ψ values determined just after mycelial disks were put into a psychrometer and just before the disks were removed from a psychrometer to count sporangia. Numbers of sporangia are averages for two mycelial disks which were in a psychrometer for 7-8 days. **6)** Effect of water potential of unsterilized soil on the formation of sporangia in water and growth on selective medium by mycelial disks after removal from thermocouple psychrometers. A plus or zero indicates the presence or absence of new sporangia or growth. The position on the horizontal axis indicates the minimum ψ value observed during the 7-8 days mycelial disks were in soil in a psychrometer (Fig. 5). **7)** Effect of water potential of soil in crocks on the number of sporangia formed per mycelial disk. Circles and crosses, respectively, represent ψ values determined when mycelial disks were put into the soil and, when disks were removed from the soil to count sporangia. Numbers of sporangia are averages for two mycelial disks which were in soil for 4-5 days.

DISCUSSION.—Although the trend of the data from both psychrometer experiments is the same, the results of the first psychrometer experiment in Fig. 2 demonstrate the influence of ψ value on the formation of sporangia more clearly than do the results of the second experiment in Fig. 5. The results in Fig. 2 were obtained with mycelial disks placed just below the soil surface. Therefore, the ψ value at the soil surface, which is the value measured by the psychrometer (Fig. 1), would very nearly equal the actual ψ value of mycelia in soil. It proved impossible to dry mycelial disks precisely to a predetermined ψ value, and mycelia were sometimes as much as 3 bars wetter or drier than the soil in psychrometers into which they were placed. Because mycelial disks in the second psychrometer experiment (Fig. 5) were 2-3 mm below the soil surface, and because the hydraulic conductivity of soil decreases markedly with ψ (9), the soil ψ value measured by the psychrometer probably did not always represent the ψ value of mycelia. Lack of equilibration between mycelia and bulk soil was also a complication in the crock experiment at $\psi \leqslant -5$ bars (Fig. 7). Therefore, the data in Fig. 5 and 7 probably overestimate the ability of *P. drechsleri* to sporulate at low ψ values. In fact, the precision gained by placing mycelium near the soil surface in psychrometers (Fig. 2) illustrates the importance of measuring the actual ψ value of an organism when studying the influence of water on biological processes in soil where water equilibration can be very slow.

Maximum numbers of sporangia formed in the psychrometers (Fig. 2 and 5) and in crocks of soil (Fig. 7) were considerably less than the numbers of sporangia formed in tensiometers at $\psi_m = -0.3$ bar (Fig. 3). There was even disparity between the techniques when both used soil at 0.2 g water per g dry soil and $\psi \simeq -1$ bar. (The solute potential, ψ_s, of soil in tensiometers was −0.6 bar.) Two factors other than ψ value may have been somewhat limiting to sporangium formation in the psychrometers. First, the soil solution is highly stimulatory to the formation of sporangia, and mycelial disks in the psychrometers probably did not have the same intimate contact with the soil solution as did the disks in tensiometers. The soil in tensiometers was watered immediately after mycelial disks were in place, whereas no water was added to the soil after the disks were placed in psychrometers. Some provision was made for aeration in the psychrometers, but the psychrometers were closed most of the time and aeration in the psychrometers may also have been somewhat limiting to the formation of sporangia. Increases in the numbers of sporangia formed to a maximum with slight decreases in ψ in the psychrometers (Fig. 2 and 5) were associated with appreciable decreases in soil water content (Fig. 4) and, therefore, probably improved gas exchange in the soil. The results of the crock experiment also indicate the importance of aeration because almost no sporangia formed in saturated soil (Fig. 7). Even though conditions in the psychrometers were not optimum, with the exception of ψ value and associated soil factors, conditions in all the psychrometers were equivalent and the observed influence of ψ value on the formation of sporangia by *P. drechsleri* is probably a general occurrence in soil. Although there was limited replication within any one experiment, three different experiments

(Fig. 2, 5, and 7) in two different soils gave similar relationships between soil ψ and numbers of sporangia formed. Comparatively few sporangia were formed in crocks of soil (Fig. 7), but water extract (200 g moist soil per liter) of the field soil used in the crocks was also less stimulatory to the formation of sporangia than was water extract of the soil used in the other experiments. Interestingly, the similar influence of $\psi \leqslant -4$ bars in psychrometers with sterilized and unsterilized soil (Fig. 2) indicates the limiting influence of low ψ values on the formation of sporangia by *P. drechsleri* is not due to the activities of living soil microflora (5, 7). In addition, the results of one psychrometer experiment (Fig. 6) suggest low ψ values in soil not only limit the current formation of sporangia, but can also reduce subsequent formation of sporangia at a nonlimiting ψ value.

The influence of soil ψ on the formation of sporangia by *P. drechsleri* appears to be in agreement with study by Sneh and McIntosh (10) in which *P. cactorum* was found to form numerous sporangia at $\psi_m = -0.3$ bar but few or no sporangia at $\psi_m = -3.0$ bars. In the present study, solution extracted from the soil mix on a pressure plate at 0.8 to 1.0 bar had solute potentials (ψ_s) between -1.1 and -1.3 bar. Because $\psi = \psi_s + \psi_m$ (7, 9), soil mix at $\psi_m = -3.0$ bars would have a ψ value less than -4 bars and would limit the formation of sporangia by *P. drechsleri*. However, the influence of ψ_m values between -0.3 and -3.0 bars on the formation of sporangia by *P. cactorum* is unknown (10), and the possibility remains that the formation of sporangia by *Phytophthora* spp. other than *P. drechsleri* may have water requirements which differ from those reported here.

The sharp decrease in the formation of sporangia by *P. drechsleri* when the soil ψ value decreased to -4 bars (Fig. 2) differs from the more gradual influence of decreasing ψ value on many soil fungi (5, 7). In fact, mycelial growth by *Phytophthora* spp. is not markedly reduced until ψ values are below -20 bars (1, 11) and *Pythium ultimum* evidently can form some sporangia at ψ values as low as -10 bars (2). Zoosporogenesis by *Aphanomyces euteiches* in a nonpenetrating solute (8) is confined to the same high ψ values as is the formation of sporangia by *P. drechsleri*. However, results obtained with fungi in solution at various ψ_s values may not apply directly to the soil where ψ_m is generally the predominate component of ψ (1, 7, 9).

In conclusion, the formation of numerous sporangia by *P. drechsleri* requires that the soil be not completely saturated with water but wetter than $\psi = -4$ bars. Unfortunately, these moisture requirements for *P. drechsleri* correspond to the moisture regime of many agricultural soils. Of course, given sufficient time between irrigations or periods of rainfall, soils, and particularly the upper layers of soils, dry to ψ values which limit the formation of sporangia by *P. drechsleri*. For example, in the absence of rain, soil at a depth of 5 to 15 cm in a safflower field at Davis, California, dried to $\psi \leqslant -4$ bars in 12 to 42 days after surface irrigations, the exact time depending on factors such as location in the field, size of the plants, and weather (Duniway, *unpublished*).

LITERATURE CITED

1. ADEBAYO, A. A., and R. F. HARRIS. 1971. Fungal growth responses to osmotic as compared to matric water potential. Soil Sci. Soc. Am. Proc. 35:465-469.
2. BAINBRIDGE, A. 1970. Sporulation by Pythium ultimum at various soil moisture tensions. Trans. Br. Mycol. Soc. 55:485-488.
3. BARRS, H. D. 1968. Determination of water deficits in plant tissues. Pages 235-368 *in* T. T. Kozlowski, (ed.). Water deficits and plant growth, Vol. I. Academic Press, New York. 390 p.
4. BOYER, J. S., and E. B. KNIPLING. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc. Nat. Acad. Sci., USA 54:1044-1051.
5. COOK, R. J., and R. I. PAPENDICK. 1972. Influence of water potential of soils and plants on root disease. Annu. Rev. Phytopathol. 10:349-374.
6. DE WEILLE, G. A. 1964. Forecasting crop infection by the potato blight fungus. K. Neth. Meteorol. Inst. Meded. Verhandl. 82, 144 p.
7. GRIFFIN, D. M. 1972. Ecology of soil fungi. Syracuse University Press, Syracuse, New York. 193 p.
8. HOCH, H. C., and J. E. MITCHELL. 1973. The effects of osmotic water potentials on Aphanomyces euteiches during zoosporogenesis. Can. J. Bot. 51:413-420.
9. SLATYER, R. O. 1967. Plant-water relationships. Academic Press, New York. 366 p.
10. SNEH, B., and D. L. MC INTOSH. 1974. Studies on the behavior and survival of Phytophthora cactorum in soil. Can. J. Bot. 52:795-802.
11. SOMMERS, L. E., R. F. HARRIS, F. N. DALTON, and W. R. GARDNER. 1970. Water potential relations of three root-infecting Phytophthora species. Phytopathology 60:932-934.
12. TSAO, P. H. 1970. Applications of the vital fluorescent labeling technique with brighteners to studies of saprophytic behavior of Phytophthora in soil. Soil Biol. Biochem. 2:247-256.
13. ZENTMYER, G. A., and D. C. ERWIN. 1970. Development and reproduction of Phytophthora. Phytopathology 60:1120-1127.