Temperature × Water Potential Interactions on Growth and Sclerotial Germination of *Phymatotrichum omnivorum*

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ABSTRACT

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The response of *Phymatotrichum omnivorum* to the osmotic and vapor transfer-controlled components of the soil water potential under different temperatures was investigated. Mycelial growth on potato-dextrose agar (PDA) declined as the osmotically controlled water potential decreased from −4.5 to −50 bars at 20 and 28 C. At 35 C, colony growth reached a maximum between −8.5 and −15 bars. Growth was more rapid at 35 C than at 28 C at water potentials <−8 bars. Colony diameters at 20 C were less than those at 28 and 35 C at all water potentials. The use of sucrose instead of KCl to control water potential resulted in more growth and growth at lower water potentials. The three temperatures had a similar effect on the growth × water potential curves, whether the water potential was controlled osmotically or by vapor transfer, but there was a change to lower values of the optimal and minimal matric potentials for growth. Sclerotia of *P. omnivorum* germinated rapidly in culture over a range of water potentials including those expected in dry soil (<−10 bars). Germination percentages at 28 and 35 C were similar and higher than those at 20 C. Germination on PDA was greater and occurred at lower osmotic potentials than on water agar at all temperatures. Sclerotial germination responded similarly to osmotic- and vapor-transfer water potentials.

Additional key words: culture media, thermocouple psychrometry.

Phymatotrichum root rot of cotton, caused by the soilborne fungus *Phymatotrichum omnivorum* (Shear) Duggar, is widespread throughout the calcareous soil regions of the southwestern United States and northern Mexico (17). Soil moisture and soil temperature are the limiting factors for development and spread of the disease within this region (6,7,13,18). The disease is favored by fairly moist soils, but is affected unfavorably by either very dry or very wet soils (18). Rogers (13) observed that sclerotia and mycelial strands did not grow in Houston black clay soil at <8 or >35% soil moisture (oven-dry basis). Optimal mycelial growth occurred when soil moisture was depleted to 35% of the maximum water-holding capacity. Wheeler and Hine (19) found optimum strand formation in Gila silt loam at 22 and 30% moisture levels (oven-dry basis). Temperatures near 28 C are highly favorable for rapid strand growth, sclerotium formation, and disease development (10,13).

Previous research on the effect of soil water on *P. omnivorum* has related soil water content to fungal growth. Since growth of many organisms is strongly related to water potential, and since soil water content and soil water potential are not simply related, little quantitative information exists upon which to base comparisons of growth responses among soils or environments (2).

This work was undertaken to collect basic information related to possible environmental control of the growth of *P. omnivorum*. Accordingly, we determined the effects of the soil temperature × water potential interaction on germination of sclerotia and on mycelial growth of *P. omnivorum* under defined conditions.

MATERIALS AND METHODS

Production of sclerotia and mycelial inoculum. Sclerotia were produced in sterile soil culture by using the procedures of Lyda and Burnett (9). The sclerotia were recovered by wet sieving. Since there is a large difference in sclerotial size, only the sclerotia that passed through a 1.91-mm opening (U.S. Standard Sieve Series No. 10) and were retained on a 1.13-mm opening (U.S. Standard Sieve Series No. 16) were used for these experiments.

A culture of *P. omnivorum* was maintained on potato-dextrose agar (PDA). Agar plugs containing the mycelial inoculum were cut with a sterile cork borer (4-mm diameter) from the periphery of an actively growing 4-day-old culture.

Water potential and mycelial growth. *Osmotic potential effects.* Difco PDA was adjusted to various osmotic potentials by using KCl or sucrose of known molality. Actual osmotic potentials achieved were verified with Spanner-type thermocouple psychrometers (16). Plates were inoculated with a standard plug of *P. omnivorum* grown on 2-mm-thick PDA. The inoculated petri plates were sealed with Parafilm to prevent evaporation, placed in plastic bags, and incubated at 20, 28, or 35 C (± 1 C). Ten plates (replications) were used per treatment. Colony diameters were measured 3 and 7 days after inoculation.

Temperature gradient plate. A temperature gradient plate was used in an additional experiment to more accurately quantify the temperature/osmotic potential interaction at high temperatures. The gradient plate (1.0 × 1.8 m) was insulated on both sides with Styrofoam and was placed in a constant temperature room at 27 C. Temperatures across the plate were determined with thermocouples and ranged from 29.5 to 37.5 C. The temperature gradient across the petri dishes varied from 0.2 to 1.0 C. Basal medium PDA (−4.5 bars) was used alone or adjusted to an osmotic potential of −17 bars by addition of sucrose. Plates were inoculated with a standard plug of *P. omnivorum* grown on 2-mm-thick PDA and sealed with parafilm. Four dishes (replications) for both osmotic potentials were placed at random on each of 15 isothermal lines on the gradient plate. Colony diametes were measured 3 and 6 days after inoculation.

The effect of different water potentials in a single substrate were also determined by using the vapor-transfer (isopiestic) technique described by Shokes et al (14). Sterile filter paper disks, 2.4 cm in diameter, were impregnated with PDA containing 200 ppm streptomycin sulfate. Disks were placed on Van Tieghem cells that were embedded in equilibrating substrates (ES) in square petri plates (100 × 15 cm). The ES consisted of water agar (WA) amended with KCl. Petri plates were sealed with Parafilm, wrapped in aluminum foil, and allowed to incubate at 20, 28, or 35

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C (± 1 C) for 2 wk. Then disks on the Van Tieghem cells received a standard plug of *P. omnivorum* grown on 1-mm-thick PDA. Ten replications were used per treatment. Plates were resealed and returned to the incubators for 30 hr. Colony diameters were measured with a calibrated eyepiece micrometer at ×10. Disk water potentials were determined at the time of inoculation and checked again at the time of colony diameter measurements with an HR33 dew point microvoltmeter (Westcor, Logan, UT 84321).

Water potential and sclerotial germination. Osmotic potential effects on sclerotial germination were evaluated by placing sclerotia produced in culture on PDA or WA, both osmotically adjusted with KCl. Five plates, each containing 20 sclerotia, were used per treatment. Germination percentages were determined after incubation at 20, 28, or 35 C (± 1 C) for 1 and 4 days.

Relative humidity effects were evaluated by placing sclerotia on PDA-impregnated filter paper disks previously equilibrated to the desired potential as described in the preceding section. Germination percentages were determined after incubation at 20, 28, or 35 C (± 1 C) for 1 and 2 days. Ten plates with five sclerotia each were used per treatment.

RESULTS

Water potential and mycelial growth. The growth response of *P. omnivorum* to decreases in water potential at three temperatures is shown in Fig. 1. In general, growth was inhibited by decreases in water potential at either 20 or 28 C regardless of the growth medium. Quantitatively, however, growth was more extensive at all potentials on PDA amended with sucrose compared to that amended with KCl. When grown at 35 C, the most extensive growth occurred at water potentials between −10 and −20 bars. The response of growth to water potential at 35 C was qualitatively the same for both media, but mycelial growth was more extensive on sucrose-amended PDA at all water potentials. Greater growth on sucrose-PDA occurred at water potentials 5–10 bars lower than on KCl-PDA.

Growth on PDA-impregnated disks generally followed the same pattern with regard to water potential and temperature as shown in Fig. 2. The small size of the disks precluded a direct comparison of growth with respect to time (30 hr versus 72 hr in Fig. 1). Though the effect was small at 30 hr, a shift in optimum water potential to lower values at the highest temperature was still apparent (Fig. 2). Greater growth at 35 C occurred between −20 and −30 bars.

Growth responses to temperature. The effects of temperature on mycelial growth are shown in Fig. 3. The temperature optimum was between 32 and 33 C for both water potentials, but the optimum was less sharply defined for the −4.5 bar water potential. At 3 days on the −4.5 bar medium, growth was more extensive for temperatures up to about 34 C. Above 34 C, more growth occurred on media having a water potential of −17 bars. Even though the

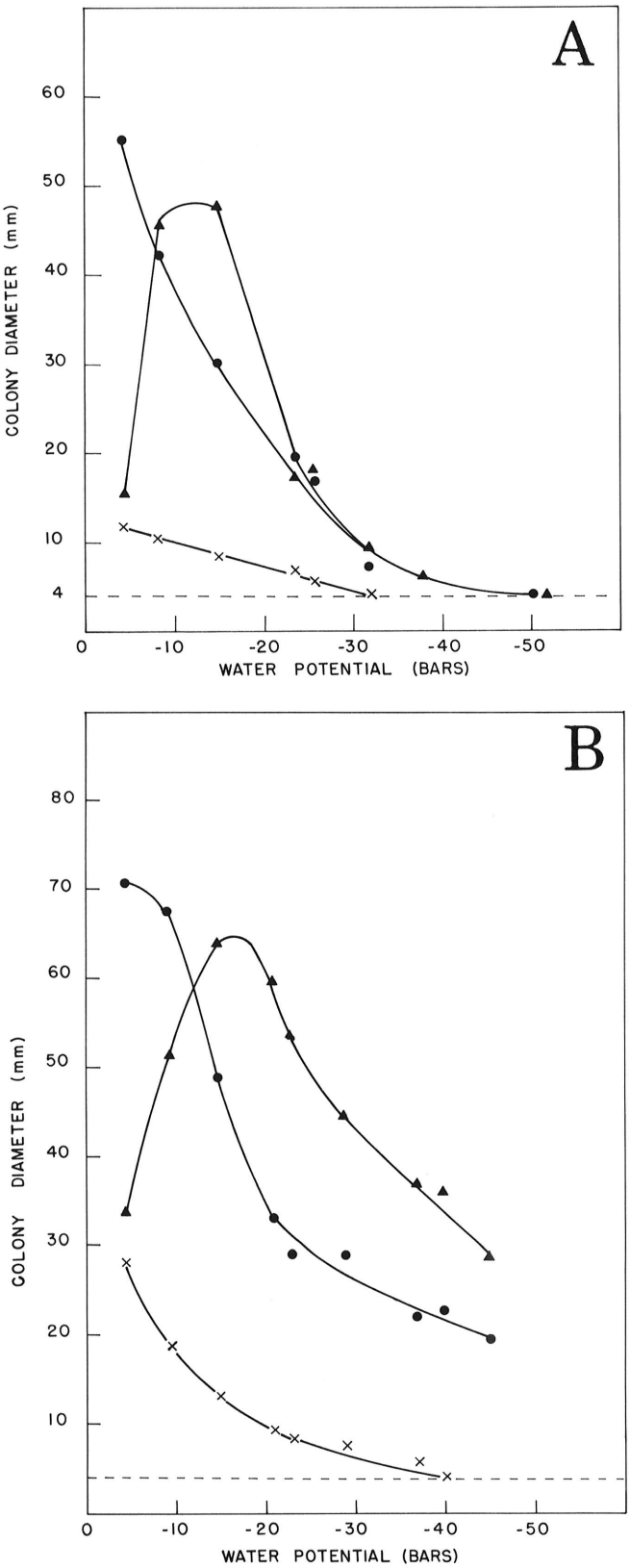

Fig. 1. Response of mycelial growth of *Phymatotrichum omnivorum* expressed as colony diameter 72 hr after inoculation, to water potential of a PDA medium **A**, with KCl and **B**, with sucrose for three temperatures, 20 (×), 28 (●), and 35 (△) C.

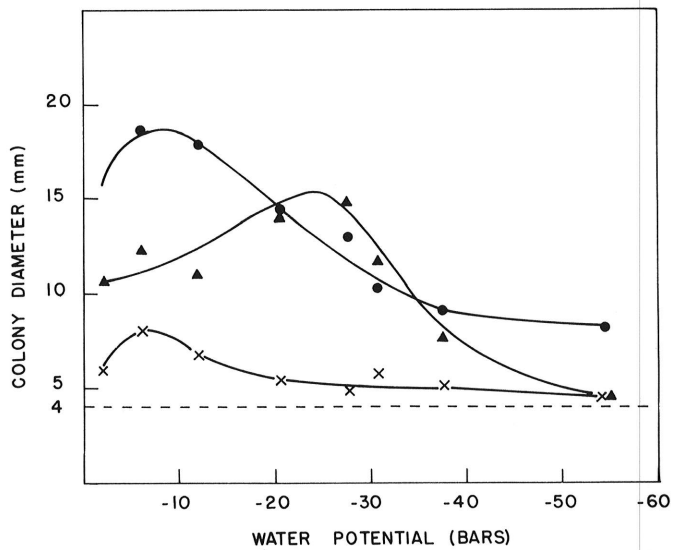

Fig. 2. Mycelial growth of *Phymatotrichum omnivorum* on PDA-impregnated filter paper disks after 30 hr at 20 (×), 28 (●), and 35 (△) C. Water potentials established by vapor transfer as described in Materials and Methods.

colonies grew to the margin of the plates by 6 days at most temperatures, the beneficial effects of low water potential (−17 bars) remained evident at temperatures above 35 C. The maximum temperature that would support mycelial growth was near 38 C regardless of water potential.

Water potential and sclerotial germination. The effects of decreasing water potential on germination of sclerotia at three temperatures are illustrated in Fig. 4. In general, low water potentials inhibited sclerotial germination at all temperatures, but germination rates and temperature effects were media-specific. Germination on water agar (Fig. 4A) was complete (>98%) within 24 hr for all three temperatures at high potentials (−15 bars). The water potentials associated with 50% germination were −12 to −14 bars for 20 C, and −16 to −19 bars for the two higher temperatures. There was no additional germination during the second 24-hr period and germination was prevented at water potentials <−30 bars.

The remaining portions of Fig. 4 illustrate responses on PDA medium with water potential adjusted either by KCl addition (C and D) or vapor transfer (E and F). PDA generally supported more rapid and complete germination at higher temperatures and lower water potentials compared with water agar. Germination was more rapid and more extensive on KCl-PDA at 20 C, but germination rates were similar at water potentials <−25 bars at 35 C regardless of how the potentials were established. Germination on PDA was prevented by water potentials between −40 and −50 bars.

DISCUSSION

The use of sucrose to control the water potential resulted in greater growth of *P. omnivorum*. This effect probably is due to the improved available carbon source of the medium. The same nutrient/water potential interaction has been observed by Sommers et al (15) for *Phytophthora cinnamomi, P. megasperma,* and *P. parasitica* and by Adebayo and Harris (1) for *Alternaria tenuis*.

The observation that more growth occurred at higher temperatures at lower osmotically-controlled water potential is not unique. The same response was shown by Cook and Christen (4) for *Fusarium culmorum, F. graminearum,* and *Gaeumannomyces graminis* var. *tritici* and by Manandhar and Bruehl (11) for *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium albo-atrum*. This water potential-temperature interaction may be an adaptive mechanism in the organisms needed to meet a common situation, namely, a dry environment when temperatures are high or vice versa (4). A basal medium of a higher water potential should be used to see whether colony growth of *P. omnivorum* increases or decreases in osmoregulated water potentials −4.5 bars. Several authors observed increases in radial growth of fungi and streptomycetes with a slight reduction in osmotic but not matric potential. The reason for this response is unknown.

Direct comparisons between osmotic and vapor transfer-controlled water potential effects on mycelial growth of *P. omnivorum* are difficult because the filter paper disks were overgrown within 3 days. Colony diameters were measured over different periods (30 and 72 hr) (Table 1). Growth starts after an initial lag period. Consequently, the lag period has a stronger influence in the total growth over a 30-hr period than 72 hr (Figs. 1 and 2). Growth was greater at lower potential in PDA substrates when water potential was controlled with vapor transfer than when sucrose was used as the osmoticum. At 20 C, the maximum growth was −4.5 bars and at 28 and 35 C at water potentials −10 and −21 bars, respectively.

Several authors observed that fungi appear to be more sensitive to matric than to osmotic water potential stress (1,5,11). In some species the minimum osmotic potential permitting growth is even twice as low as that for matric potential. Adebayo and Harris (1) attribute this to a reduction in solute diffusion, concurrent with a reduction in matric potential. The results from our study show exactly the opposite, namely a greater sensitivity to osmotic than to vapor transfer-controlled potential at lower water potentials. The reason for this response is not clear.

Germination of sclerotia was greater on PDA and occurred at lower water potentials than on WA at the three temperatures. The same response was found by Odvody and Dunkle (12) for sclerotial germination of *Macrophomina phaseolina*. Bandara (3) observed a higher percentage germination of sclerotia of *Sclerotium rolfsii* at −9 to −11 bars. No germination was observed at −1/3 bar in soil culture and this was attributed to enhanced microbial antagonism at high water potential. The availability of nutrients plays an

TABLE 1. Relative water potential relations and growth of *Phymatotrichum omnivorum* after 72 hr in osmotic (KCl)-controlled versus 30 hr in relative vapor transfer-controlled PDA systems

Water potential (bars)	Colony diameter (mm)[a]					
	20 C		28 C		35 C	
	KCl osmotic	Vapor transfer	KCl osmotic	Vapor transfer	KCl osmotic	Vapor transfer
−5	12	8	55.5	18.5	15.5	12
−10	10	7.5	39	18	47.5	11.5
−20	7	5.5	22.5	14.5	32	14.5
−30	4.5	5	10.5	10.5	10.5	13
−40	ND	5	5.5	9	5.5	7.5
−50	ND	4.5	4	8	4	5

[a] "KCl-osmotic" and "vapor transfer" designate the method of water potential control as interpolated from Figs. 1 and 2. ND means not determined.

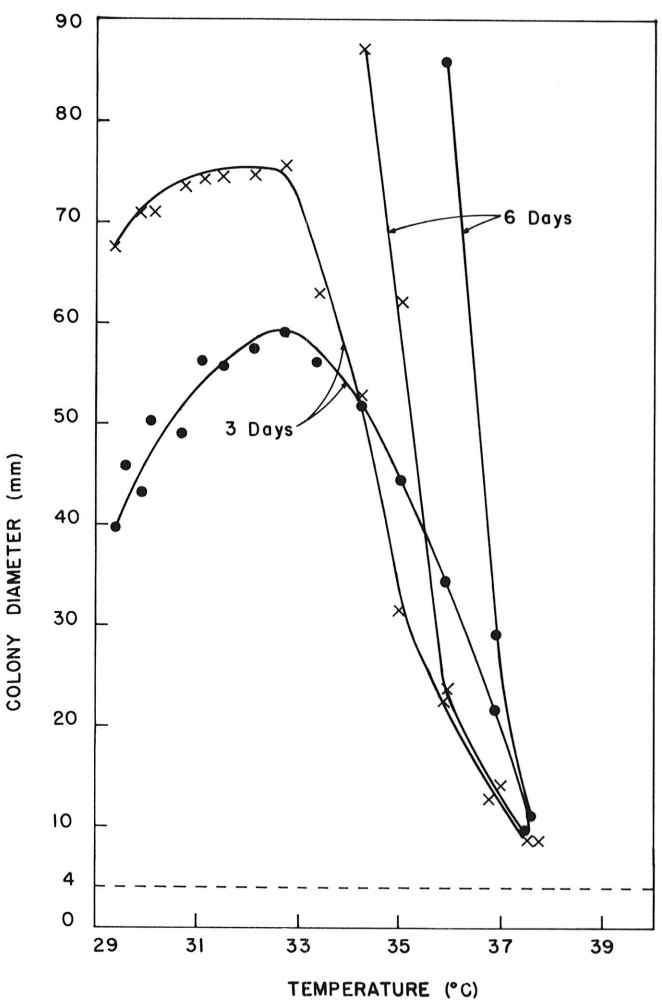

Fig. 3. Temperature response curves for mycelial growth of *Phymatotrichum omnivorum* expressed as colony diameter 3 and 6 days after inoculation, for two water potentials. Water potential of the basal PDA medium (×) was −4.5 bars, while water potential of the basal PDA medium amended with sucrose (●) was −17.0 bars.

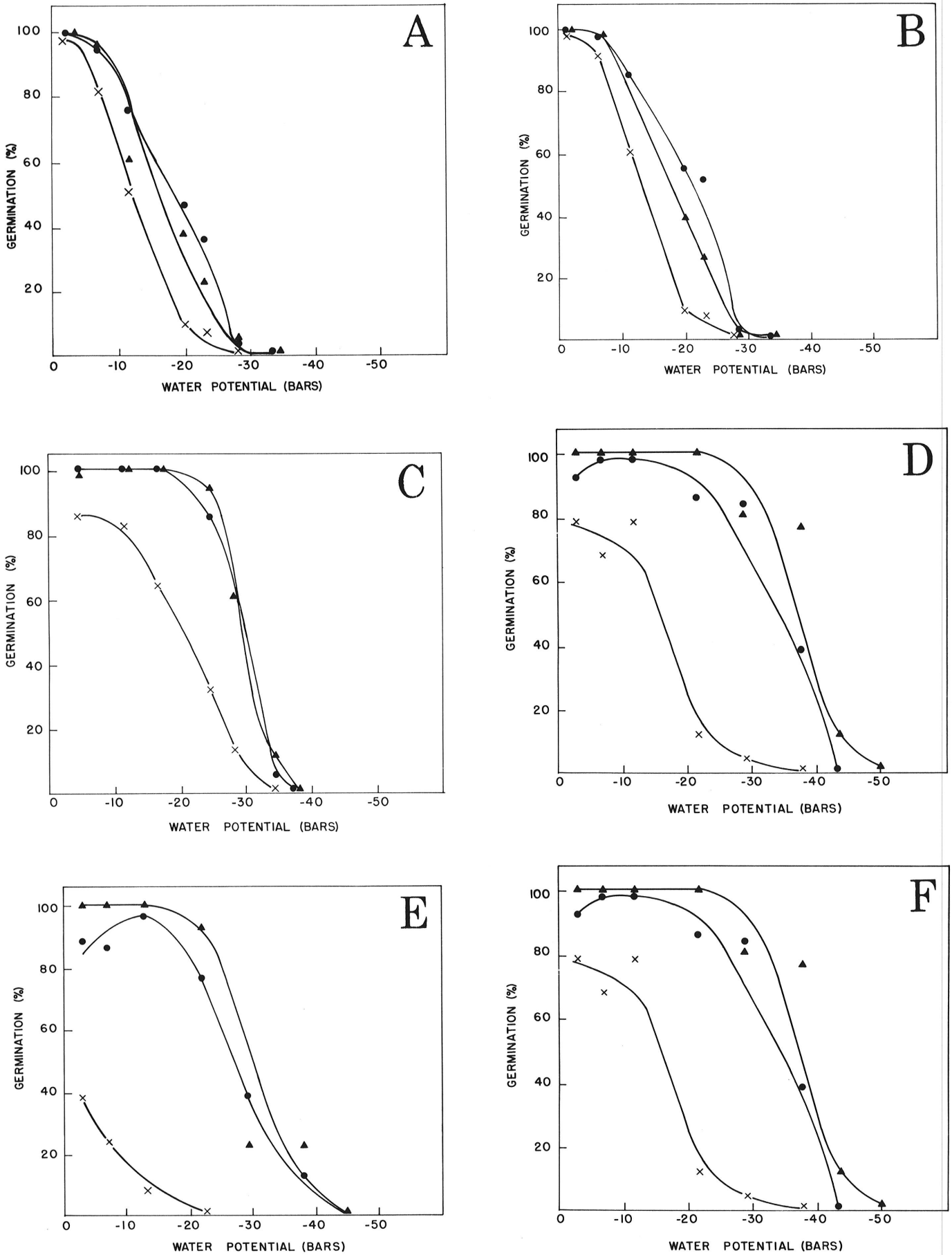

Fig. 4. Effects of water potential and temperature on *Phymatotrichum omnivorum* sclerotial germination at 20 (×), 28 (●), and 35 (△) C. Germination on water agar amended with KCl after **A,** 24 and **B,** 96 hr. Germination on PDA amended with KCl **C,** 24 and **D,** 96 hr. Germination on PDA-impregnated filter paper disks after **E,** 24 and **F,** 48 hr. Water potential of disks adjusted by vapor transfer as described in Materials and Methods.

important role in germination. Griffin (8) mentioned that germination occurs at lower water potentials if nutrients are readily available and if other environmental factors, especially temperature, are optimal. There was an increase in the latent period for germination with decreasing water potential on PDA, but not on WA (Fig. 4A–D). This phenomenon may be related to nutrient availability.

LITERATURE CITED

1. Adebayo, A. A., and Harris, R. F. 1971. Fungal growth responses to osmotic as compared to matric water potential. Soil Sci. Soc. Am. Proc. 35:465-469.
2. Adebayo, A. A., Harris, R. F., and Gardner, W. R. 1971. Turgor pressure of fungal mycelia. Trans. Br. Mycol. Soc. 57:145-151.
3. Bandara, J. M. R. S. 1980. Effect of soil water potential on the survival of *Capsicum annuum* root rot fungus in soil. Plant Soil 56:331-334.
4. Cook, R. J., and Christen, A. A. 1976. Growth of cereal root rot fungi as affected by temperature-water potential interactions. Phytopathology 66:193-197.
5. Cook, R. J., Papendick, R. G., and Griffin, D. M. 1972. Growth of two root rot fungi as affected by osmotic and matric water potentials. Soil Sci. Soc. Am. Proc. 36:78-82.
6. Ezekiel, W. N. 1942. Cotton root rot, weather and cotton yields. Trans. Texas Acad. Sci. Proc. 25:1-6.
7. Ezekiel, W. N. 1945. Effect of low temperatures on survival of *Phymatotrichum omnivorum*. Phytopathology 35:296-301.
8. Griffin, D. M. 1978. Effect of soil moisture on survival and spread of pathogens. Pages 175-197 in: Water Deficits and Plant Growth. Vol. V. T. T. Kozlowski, ed. Academic Press, New York.
9. Lyda, S. D., and Burnett, E. 1970. Sclerotial inoculum density of *Phymatotrichum omnivorum* and development of Phymatotrichum root rot in cotton. Phytopathology 60:729-731.
10. Lyda, S. D., and Burnett, E. 1971. Influence of temperature on Phymatotrichum sclerotial formation and disease development. Phytopathology 61:728-730.
11. Manandhar, J. B., and Bruehl, G. W. 1973. In vitro interactions of Fusarium and Verticillium wilt fungi with water, pH, and temperature. Phytopathology 63:413-419.
12. Odvody, G. N., and Dunkle, L. D. 1979. Charcoal stalk rot of sorghum: Effect of environment on host-parasite relations. Phytopathology 69:250-254.
13. Rogers, C. H. 1939. The relation of moisture and temperature to growth of the cotton root rot fungus. J. Agric. Res. 58:701-709.
14. Shokes, F. M., Lyda, S. D., and Jordan, W. R. 1977. Effect of water potential on the growth and survival of *Macrophomina phaseolina*. Phytopathology 67:239-241.
15. Sommers, L. E., Harris, R. F., Dalton, F. N., and Gardner, W. R. 1970. Water potential relations of three root-infection Phytophthora species. Phytopathology 60:932-934.
16. Spanner, D. C. 1951. The peltier effect and its use in the measurement of suction pressure. J. Exp. Bot. 2:145-168.
17. Streets, R. B. 1937. Phymatotrichum (cotton or Texas) root rot in Arizona. Arizona Agric. Exp. Stn. Tech. Bull. 71:299-410.
18. Taubenhaus, J. J., and Dana, B. F. 1928. The influence of moisture and temperature on cotton root rot. Texas Agric. Exp. Stn. Bull. 386:1-23.
19. Wheeler, J. E., and Hine, R. B. 1972. Influence of soil temperature and moisture on survival and growth of strands of *Phymatotrichum omnivorum*. Phytopathology 62:828-832.