

Charcoal Stalk Rot of Sorghum: Effect of Environment on Host-Parasite Relations

G. N. Odvody and L. D. Dunkle

Former graduate assistant and associate professor, respectively, Department of Plant Pathology, University of Nebraska, Lincoln, 68583. Present address of second author: U.S. Department of Agriculture-Science and Education Administration, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Portion of a dissertation by the senior author submitted to the University of Nebraska. Published as Journal Series Paper 5554, Nebraska Agricultural Experiment Station, Lincoln. Research reported was conducted under NAES Project 21-20 and supported in part by the Rockefeller Foundation. The authors thank C.Y. Sullivan for valuable assistance and advice given throughout the course of this work.

Accepted for publication 6 October 1978.

ABSTRACT

ODVODY, G.N., and L.D. DUNKLE. 1979. Charcoal stalk rot of sorghum: Effect of environment on host-parasite relations. *Phytopathology* 69: 250-254.

The influence of high temperatures and low water potentials on the susceptibility of sorghum to *Macrophomina phaseolina*, infection phenomena, disease development, and response of the pathogen in culture was investigated. Roots of fertilized CK60 B (self-fertile) and nonfertilized CK60 A (male-sterile) sorghum lines differed in susceptibility to *M. phaseolina* when plants were subjected to drought stress at the soft dough stage. Only stressed fertile plants developed charcoal stalk and root rot. Root systems of stressed male-sterile plants had a high percentage of roots with latent infections but did not develop symptoms. Most root infections

of both fertile and male-sterile sorghums occurred only after the onset of stress conditions. Growth rates of *M. phaseolina* on potato-dextrose agar (PDA) declined as osmotic potential was decreased from -1.5 to -129 bars at 30 and 35 C. At 40 C, the highest growth rates occurred between -13 and -40 bars, although growth rates at all corresponding osmotic potentials were lower than at 30 and 35 C. At all temperatures, germination of sclerotia on PDA was $\geq 50\%$ from -1.5 to -90 bars. Mycelial growth and sclerotial germination occurred at lower osmotic potentials on PDA than on osmotically adjusted water agar.

Additional key words: stalk rot.

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is a prevalent stalk rot of corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* [L.] Moench). The disease occurs most often when sorghum plants are subjected to drought stress as grain approaches the soft dough stage. Factors for development of charcoal rot have been simulated in the greenhouse by various workers, and several techniques have been used to infect the host with *M. phaseolina* (4,7,10,17). The most successful method of achieving infection was developed by Hsi (7), who obtained a large percentage of charcoal-rotted plants with a combination of naturally-infested soil and simultaneous inoculation by the infested toothpick method.

Edmunds (4) defined the general factors necessary for charcoal rot development in greenhouse-grown sorghum by using pathogen-free soil and the toothpick method of inoculation. These factors included a used fertile sorghum plant (14–28 days after bloom) exposed to 35 to 40 C soil temperatures and 25% available soil moisture for 7 days prior to inoculation. In subsequent field studies, Edmunds et al (6) determined that drought stress (high soil temperatures and low soil moisture) caused a marked reduction in total stalk sugars which correlated with increased development of charcoal rot. Nonfertilized male-sterile sorghum plants subjected to drought stress were less susceptible to charcoal rot than were their isogenic fertile lines (5). Edmunds' work provided a basic system for studies of charcoal rot in the greenhouse. However, the toothpick method of inoculation circumvents the natural infection process and presents problems in inoculation timing, alteration of host physiology through wounding, and possible introduction of secondary organisms.

High temperatures (35 C) are favorable for growth of *M. phaseolina* in culture (10), but water potential-temperature interactions are not well known. Shokes et al (14) showed that, at 30 C, *M. phaseolina* grew best at a water potential of -17 bars and

sclerotial survival was deleteriously affected by a water potential of approximately -0.01 bars. However, temperatures other than 30 C were not used by Shokes et al and the effects of water potentials on sclerotial germination were not evaluated.

The objectives of this research were to determine: some factors affecting initial infection by and development of *M. phaseolina* in sorghum host tissues, time and location of initial infection(s), and effect(s) of osmotic potential-temperature interactions on *M. phaseolina* in culture and their possible relation to charcoal rot development.

MATERIALS AND METHODS

Sorghum culture. Seeds of sorghum lines CK60 B (fertile) and CK60 A (male-sterile), obtained from the Foundation Seed Division, University of Nebraska, were planted in 11-L plastic pots filled with a sand-vermiculite (1:1) mixture. Plants received water or nutrient solution which contained per liter: 734 mg KNO₃, 988 mg CaNO₃, 338 mg super phosphate (45%), 256 mg MgSO₄·7H₂O, 86 mg NH₄NO₃, 22 mg Fe-Sequestrene (CIBA-Geigy Corp., Greensboro, NC 27409), and 1 ml of a micronutrient stock solution containing 1.55 g H₃BO₃, 0.85 g MnSO₄·HOH, 0.27 g ZnCl₂, 0.13 g CuSO₄·H₂O, and 0.0184 g (NH₄)₂Mo₇O₂₄·4H₂O per liter. Plants (one per pot) were placed 60 cm apart between rows and 30 cm apart within rows. Data were not collected from the border rows and outside pots of each row. Male-sterile inflorescences were bagged upon emergence to assure complete head-sterility.

Environmental manipulation. All plants were subjected to the same favorable growth conditions until fertile plants were at the soft dough stage. Greenhouse air temperatures were increased (30–35 C maximum) and stress was induced in randomly-selected plants by withholding water. Corresponding nonstressed controls received only water to preclude a nutrient advantage over stressed plants. Humidity and air temperature were monitored with a hygrothermograph. Occasionally, temperature fluctuations within the soil profile were determined with a mercury thermometer. Plant

stress was monitored by measuring leaf water potential (13) and diffusive resistance (8) of the leaf below the flag leaf of stressed and nonstressed plants.

Inoculum production. Sclerotia produced in culture by an isolate of *M. phaseolina* (obtained from infected sorghum stalk tissue) were used to inoculate 9-cm-diameter petri plates containing 5 ml of Difco potato-dextrose broth (PDB). Sclerotia were collected from 5-day-old plates incubated at room temperature (25 C). The high surface area to volume ratio of the medium plus rapid fungal growth and water evaporation resulted in minimal mycelial growth and the production of large quantities of sclerotia. Sclerotia were removed from the plates, placed on aluminum foil to dry, and pulverized in a mortar and pestle to give small sclerotial aggregates.

Infestation of soil. Sclerotia (0.4 g) were incorporated in the middle third of the potting soil medium.

Treatments. Four basic treatments included the fertile and male-sterile sorghum lines grown in infested (MP) or noninfested (O) soil. Plants in these soils were then either stressed (S) or not stressed (NS). Two sampling times were used, either at the onset of stress (collection I; NS plants), or after 15 days of stress (collection II; S and NS plants). At each sampling time, leaf water potentials and diffusive resistances were determined for each of six replicate plants to be collected.

Pre-stress evaluation of viability of sclerotia in soil and detection of latent root infections by *M. phaseolina*. Prior to the onset of stress conditions (collection I), weekly or bi-weekly collections of soil infested with *M. phaseolina* and of roots of sorghum plants growing in this soil were made to determine sclerotial viability and to detect infections by *M. phaseolina*, respectively. For each sorghum genotype three samples of soil (5–10 g/pot) and roots (five per plant) were collected from the upper 15 cm of soil from pots containing extra plants not used for further collection of data.

Sclerotia were isolated from infested soil and from the surface of roots by washing the roots or soil through a 0.25 mm (60-mesh) screen onto a 0.047 mm (300-mesh) screen. Sclerotia retained on the 0.047 mm screen were collected on filter paper in a Büchner funnel and either left untreated or surface-sterilized by immersing in 1% NaOCl for 30–60 sec and rinsing with sterile distilled water. The untreated and surface-sterilized sclerotia were incubated on water agar (WA) at 30 C for 4 days. On WA germinating sclerotia of *M. phaseolina* produced colonies typified by the presence of sclerotia. Surface-sterilization was used when determining the viability of sclerotia.

Collected roots (all symptomless) were surface-sterilized by the procedure described above except that roots were treated with 1% NaOCl for 4–5 min to kill all sclerotia of *M. phaseolina* on the root surface. The surface-sterilized roots were incubated on WA at 30 C for 4 days. Preliminary experiments determined that under these conditions latent infections by *M. phaseolina* in roots could be detected by the production of sclerotia in the infected root tissue.

Charcoal rot evaluation. At the onset of stress (collection I) and after stress (collection II), stalks and roots of collected plants (six per treatment) were evaluated for visible charcoal rot symptoms (presence of sclerotia). If the primary, adventitious (buttress) roots were symptomless, they were evaluated for latent infections by *M. phaseolina* using the method described above except that incubation of roots after surface-sterilization was on moist sterile filter paper. After sclerotia developed in these roots, indicating infection by *M. phaseolina*, the plants were evaluated using a scale of 0 to 4 where 0 = no infection and 4 = most of the roots infected (10–15 roots evaluated per plant).

Osmotic potential-temperature studies. Difco potato-dextrose agar (PDA) and WA were adjusted to various osmotic potentials using KCl or sucrose (12). Plates were inoculated with 5-mm diameter plugs of *M. phaseolina* from a PDA culture and incubated at 30, 35, or 40 C for 7 days. Every 24 hr, colony diameter was measured and observations of sclerotial production and growth habit were made. Also, the fungus was incubated at the same temperatures in osmotically-adjusted PDB in Erlenmeyer flasks (50 ml medium per 250-ml flask). Dry weights of mycelia were determined after 7 days of growth. The effect of osmotic potential-temperature interactions on sclerotial germination was

evaluated by placing large numbers of culturally-produced sclerotia on osmotically-adjusted PDA and WA followed by incubation at 30, 35, or 40 C.

RESULTS

Effect of stress on sorghum. At the onset of simulated drought stress, male-sterile plants had slightly lower (about 3 bars lower) leaf water potentials than the fertile plants, but diffusive resistances were similar (Table 1). After 15 days of stress, fertile and male-sterile plants had very high diffusive resistances ($> 60 \text{ sec cm}^{-1}$) and leaf water potentials of less than -25 and -20 bars, respectively. Nonstressed fertile and male-sterile plants had slightly reduced (about 4–5 and 2–5 bars lower, respectively) leaf water potentials and lower (about 4 and 2–9 sec cm^{-1} lower, respectively) diffusive resistances after the 15-day exposure to high temperatures. Although leaf water potentials of male-sterile plants in collection II were somewhat erratic, high ($> 60 \text{ sec cm}^{-1}$) and low (4.0–10.0 sec cm^{-1}) diffusive resistance measurements were indicative of stressed and nonstressed plants, respectively.

Charcoal rot development. Charcoal stalk rot and charcoal root rot developed only in stressed, fertile plants grown in infested soil (Table 1). Although only 17% (1/6) of the stressed fertile plants collected from infested soil developed charcoal stalk rot, 83% (5/6) developed symptoms of charcoal rot in most buttress roots. If the 20 extra, similarly treated plants were included in the analyses, 10% developed charcoal stalk rot and 87% developed charcoal root rot. Neither visible symptoms of charcoal rot nor latent infections by *M. phaseolina* were detected on roots of plants of collection I (onset of stress); and in infested soil, only a few latent root infections were detected on nonstressed male-sterile and fertile plants of collection II (after 15 days of stress). Roots of stressed male-sterile plants from infested soil were symptomless in the greenhouse but had a high percentage of latent infections by *M. phaseolina*.

Viability of sclerotia in soil and time and site of initial root infection. Prior to the initiation of stress in our experiment, sclerotia isolated from soil did not germinate when incubated on WA in the presence of the contaminating sand-vermiculite particles. High bacterial populations occurred on these plates but we were unable to demonstrate the fungistatic properties of any bacteria isolated from them. Sclerotia germinated readily ($> 50\%$) and bacterial populations were substantially reduced if the sclerotia-sand-vermiculite mixture was surface sterilized prior to incubation on WA or if single sclerotia were removed from the untreated mixture and incubated on WA or PDA.

Prior to the onset of stress no root infections were detected on plants of either genotype. Although buttress roots were not included in these early collections, the absence of any infected buttress roots in collection I indicates that infected root sites were not omitted in previous collections. Almost all root infections (visible and latent) occurred after sorghum plants of both genotypes had been subjected to moisture and temperature stress, but a few latent root infections were detected in nonstressed plants in the later stages of maturity.

Observations of infected roots at various stages of disease development revealed that most infections apparently were initiated in the buttress roots within the upper 15 cm of soil. Soil temperatures in the upper 15 cm and especially upper 5 cm of soil varied with the ambient air temperature. Soil temperatures recorded during the stress period in all treatments were generally 30 C or lower but occasionally temperatures up to 34 C were recorded. Fungal growth proceeded up infected buttress roots and entered the stalk tissue at or above the crown. Disease development in roots was visible initially as a progressive red or pink rot of cortical tissue followed by extensive formation of sclerotia on the remnant walls of the vascular cylinder and cortex.

Effect of osmotic potential-temperature interactions on *M. phaseolina* in culture. All data presented were obtained from cultures grown on media osmotically adjusted with KCl, although the response of *M. phaseolina* was similar when osmotic potentials were adjusted with sucrose. At 30 and 35 C, the growth rate of *M. phaseolina* declined as osmotic potential was decreased from -1.5

(nonadjusted) to -129 bars on PDA (Fig. 1) and from -0.8 (nonadjusted) to -62 bars on WA (Fig. 2). At 40 C, growth was less at all corresponding osmotic potentials than at 30 and 35 C, and growth was best between -13 and -40 bars on PDA and between -9 and -13 bars on WA. At all temperatures, growth occurred at lower osmotic potentials on PDA than on WA. Sclerotial production was delayed at all temperatures as osmotic potential was decreased; however, at 40 C no sclerotia were produced on PDA at -1.5 bars but were produced at -9 bars and lower. When growth was determined by dry weights of mycelia in osmotically-adjusted liquid media, trends were similar to those based on colony diameter on agar media. However, growth at 40 C was relatively less in liquid than in solid media, indicating that growth in liquid media was limited by additional factors not limiting on solid media. Germination of sclerotia on PDA was $\geq 50\%$ from -1.5 to -90 bars and $\leq 50\%$ at -110 bars at all temperatures. Sclerotial germination on osmotically adjusted water agar was $\geq 50\%$ from -1.2 to -40 bars with little germination occurring below -60 bars.

DISCUSSION

Our results with a sclerotia-soil inoculation procedure showed that *M. phaseolina* infected and colonized living sorghum tissue that was subjected to drought stress. Edmunds (4) obtained similar results with a toothpick-stalk inoculation method, but our procedure allowed differentiation between initial infection phenomena and continued development of the pathogen within host tissues. Corroborating Edmunds' results (4), neither drought-killed nor nonstressed stalk tissue of male-sterile or fertile plants supported growth of *M. phaseolina* when inoculated with pathogen-infested toothpicks. Roots of nonstressed male-sterile and fertile plants apparently remained resistant to infection by *M. phaseolina* throughout the growth period, because only a few infected roots were detected late in plant maturity. Most root infections occurred after the onset of stress, indicating that stress is required for initial infection of host tissue. The drought stress indicators (diffusive resistance and leaf water potential) were erratic but generally indicated that fertile plants developed lower water potentials when exposed to stress than did sterile plants (Table 1). In both noninfested and infested soil, stressed male-sterile plants had less necrotic tissue in leaves, stalk, and roots than did comparable fertile plants. This differential sensitivity to stress may, in part, explain why infection by *M. phaseolina* occurred in drought-stressed roots of both sorghum genotypes, but fungal

development, symptom expression, and charcoal stalk rot occurred only in fertile plants. However, all of our data were taken on greenhouse-grown plants and these differential responses may not be the same in field-grown sorghum. The leaf water potential of -21 bars reported (15) for field grown sorghum under severe drought

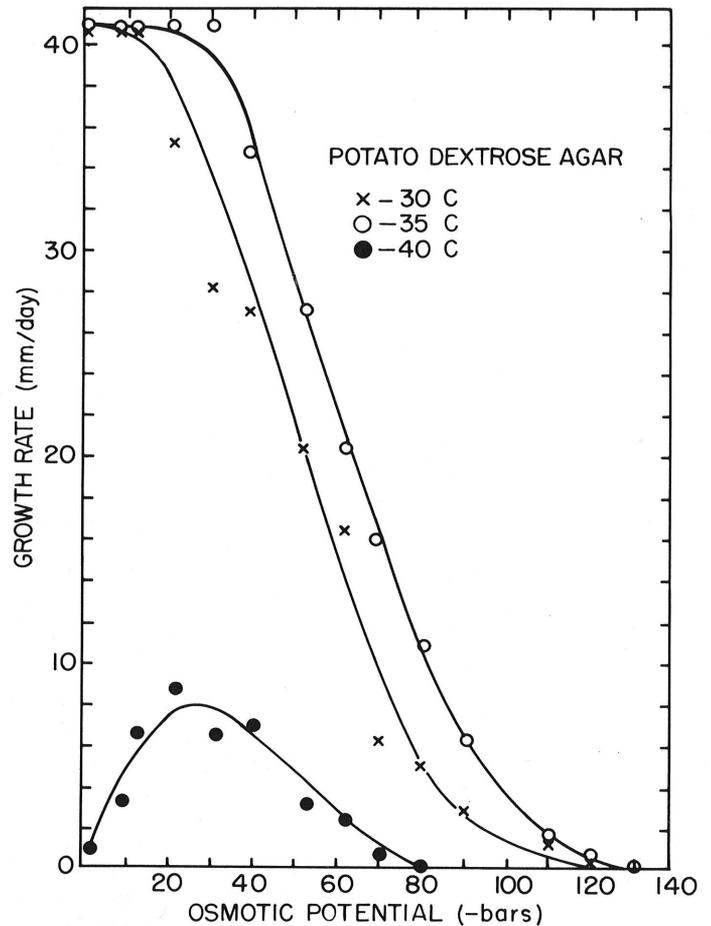


Fig. 1. Effect of osmotic potential-temperature interactions on growth of *Macrophomina phaseolina* on potato-dextrose agar.

TABLE 1. Effect of stress on development of charcoal rot caused by *Macrophomina phaseolina* in fertile and male-sterile sorghum

Collection ^a	Treatment ^b	Leaf water potential (bars)	Diffusive resistance (sec cm ⁻¹)	Roots infected by <i>M. phaseolina</i> ^c	
				With charcoal rot symptoms (% of plants)	Symptomless (0-4)
I	B NS MP	-12.9	12.0	0	0
	B NS O	-12.9	12.5	0	0
	A NS MP	-15.8	13.4	0	0
	A NS O	-15.8	11.7	0	0
II	B NS MP	-16.8	8.1	0	2
	B NS O	-18.0	7.7	0	0
	B S MP	-25.6	>60	83	4 ^d
	B S O	<-28	>60	0	0
	A NS MP	-18.4	4.0	0	1
	A NS O	-21.3	9.6	0	0
	A S MP	-20.8	>60	0	3
	A S O	-27.0	>60	0	0

^a Collection I was taken at the onset of stress conditions and collection II was taken 15 days later.

^b B = CK60 B (fertile), A = CK60 A (male-sterile), NS = nonstressed, S = stressed, MP = soil infested with sclerotia of *M. phaseolina*, and O = no *M. phaseolina*.

^c Root systems were evaluated for charcoal rot symptoms (sclerotia) in the greenhouse. If root systems contained only symptomless roots, all buttress roots (10-15) were collected from each plant, surface-sterilized (to kill surface sclerotia) and incubated on moist sterile filter paper at 30 C for 4 days. After sclerotia developed in these roots, indicating infection by *M. phaseolina*, the plants were evaluated using a scale of 0 to 4 in which 0 = no infection; 1 = one infected root per plant; 2 = two to four infected roots per plant; 3 = five to 10 infected roots per plant; and 4 = most roots infected per plant.

^d Roots of plants from B S MP were not evaluated in the laboratory but received an average rating of 4 based on charcoal rot symptoms in the greenhouse.

stress near the completion of grain fill is similar to the leaf water potentials we obtained from stressed sorghum plants in the greenhouse. The erratic nature of the leaf water potentials measured in the greenhouse may have been due in part to the limited area for root growth in our plastic containers.

In culture, *M. phaseolina* grew at high temperatures (35 C) and low osmotic potentials, especially if nutrients were present (Fig. 1 and 2). More importantly, sclerotia of *M. phaseolina* germinated readily in culture over a wide range of water (osmotic) potentials and temperatures, including those expected in soil during drought (<-10 bars). Although we have no indication of the response of *M. phaseolina* to the matric component of water potential in soil, Cook et al (3) determined that some pathogens of wheat responded similarly to either matric or osmotic potentials, which may also apply to *M. phaseolina*.

The implications of and relationships between our greenhouse results with charcoal stalk rot and our results demonstrating responses of *M. phaseolina* to osmotic potential and temperature are more apparent when considered with previous reports relating soil fungistasis (1,2,11), sclerotial activity of *M. phaseolina* (1,2,14,16), and incidence of charcoal rot diseases (14,16). Ayanru and Green (1) proposed a nutrient-dependent fungistasis for sclerotia of *M. phaseolina*, and Bhattacharya and Samaddar (2) determined that these sclerotia required exogenous nutrients for germination after long periods in soil. Our data demonstrating that nutrients increased sclerotial germination and mycelial growth at low osmotic potentials implicate a role for nutrients in overcoming soil fungistatic factors and promoting infection by *M. phaseolina*. Although we could not demonstrate bacterial fungistasis, the absence of bacteria was highly correlated with germination of sclerotia isolated from infested soil. Norton (11) reported that

Bacillus cereus greatly inhibited the growth of *M. phaseolina* in soil, and, conversely, Shokes et al (14) implicated reduced bacterial activity in dry soil as a factor promoting growth of *M. phaseolina* under these conditions and possibly increasing incidence of charcoal rot diseases. Because no root infections occurred prior to simulated drought stress in our experiments, perhaps drought stress in the field may alter soil microflora responses such that the nutrient-dependent fungistasis is inactive, inoperative, or its influence is considerably reduced. Exogenous substrates or nutrients for sclerotial germination may be provided by host root exudates as suggested by Bhattacharya and Samaddar (2) and Smith (16). Smith has reported that sclerotia of *M. phaseolina* germinated in nonsterile soil if it was amended with natural or synthetic root exudate from the host *Pinus lambertiana*. There also is evidence that root exudates may increase under drought stress (9). Sclerotia of *M. phaseolina* could have germinated on and grown on host roots without infecting them prior to drought stress in our experiments, but our results and those of others suggesting fungistatic factors seem to negate this possibility.

Most root infections were initiated in large buttress roots in the upper 15 cm of soil where highest temperatures and lowest water potentials would be expected. Early-season (pre-bloom) drought conditions may result in root infections which remain latent with little or no fungal development through the roots into the crown until proper host and environmental stress conditions favor disease development. However, our data demonstrate that root infections with *M. phaseolina* may be immediately followed by extensive fungal growth and charcoal rot development in susceptible tissue. In the field, charcoal stalk rot probably develops from root infections that are either latent or occur just prior to disease development.

Root infection phenomena are not well understood and may involve additional interactions of soil environment, soil microflora, host roots, and sclerotia of *M. phaseolina*. Although initial infection and subsequent disease development were favored by similar environmental conditions, root infections occurred independent of the susceptibility or resistance of the root tissue to further colonization by *M. phaseolina*. This indicates some fundamental differences between the phenomena influencing infection and those governing resistance or susceptibility of tissue to *M. phaseolina* after infection.

LITERATURE CITED

1. AYANRU, D. K. G., and R. J. GREEN, JR. 1974. Alteration of germination patterns of sclerotia of *Macrophomina phaseolina* on soil surfaces. *Phytopathology* 64:595-601.
2. BHATTACHARYA, M., and K. R. SAMADDAR. 1976. Epidemiological studies on jute diseases. Survival of *Macrophomina phaseoli* (Maubl.) Ashby in soil. *Plant Soil* 44:27-36.
3. COOK, R. J., R. I. PAPENDICK, and D. M. GRIFFIN. 1972. Growth of two root rot fungi as affected by osmotic and matric water potentials. *Soil Sci. Soc. Am. Proc.* 36:78-82.
4. EDMUNDS, L. K. 1964. Combined relations of plant maturity, temperature, and soil moisture to charcoal rot development in grain sorghum. *Phytopathology* 54:514-517.
5. EDMUNDS, L. K., and R. L. VOIGT. 1966. Role of seed production in predisposition of sorghum to charcoal rot. (Abstr.) *Phytopathology* 56:876.
6. EDMUNDS, L. K., R. L. VOIGT, and F. M. CARASSO. 1965. Charcoal rot induction and development in the field in Arizona. *Proc. Biennial Grain Sorghum Res. Util. Conf.* 4:47-53.
7. HSI, C. H. 1961. An effective technique for screening sorghum for resistance to charcoal rot. *Phytopathology* 51:340-341.
8. KANEMASU, E. T., G. W. THURTELL, and C. B. TANNER. 1969. Design, calibration and field use of a stomatal diffusion porometer. *Plant Physiol.* 44:881-885.
9. KATZNELSON, H., J. W. ROUATT, and T. M. B. PAYNE. 1955. The liberation of amino acids and reducing compounds by plant roots. *Plant Soil* 7:35-48.
10. LIVINGSTON, J. E. 1945. Charcoal rot of corn and sorghum. *Nebraska Agric. Exp. Stn. Bull.* 136.
11. NORTON, D. C. 1954. Antagonism in soil between *Macrophomina phaseoli* and selected soil inhabiting organisms. *Phytopathology* 44:522-524.
12. ROBINSON, R. A., and R. H. STOKES. 1955. *Electrolyte Solutions*.

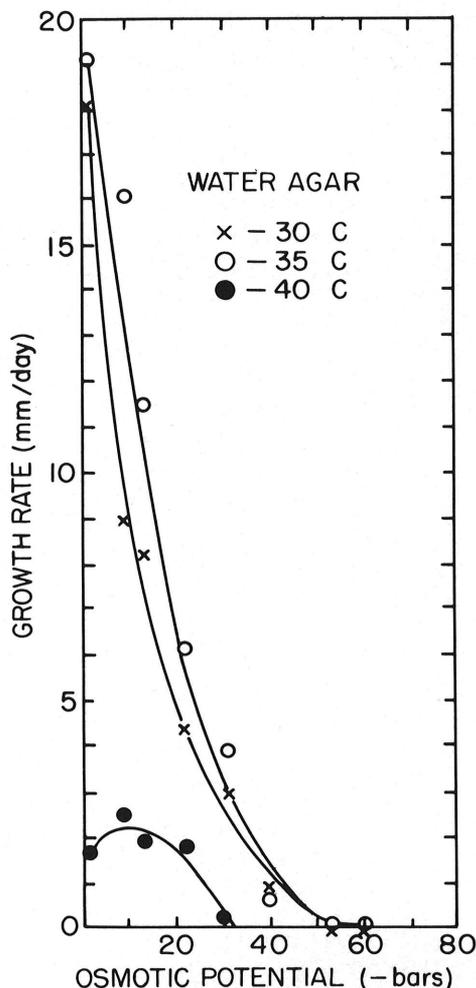


Fig. 2. Effect of osmotic potential-temperature interactions on growth of *Macrophomina phaseolina* on water agar.

- Butterworths, London. 512 pp.
13. SCHOLANDER, P. F., H. T. HAMMEL, E. D. BRADSTREET, and E. A. HEMMINGSEN. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
 14. SHOKES, F. M., S. D. LYDA, and W. R. JORDAN. 1977. Effect of water potential on the growth and survival of *Macrophomina phaseolina*. *Phytopathology* 67:239-241.
 15. SMITH, D., C. Y. SULLIVAN, and J. D. EASTIN. 1972. Leaf water potentials of grain sorghum during the grain filling stage under dryland production in southeast Nebraska. Pages 168-172 in *The physiology of yield and management of sorghum in relation to genetic improvement*. Annual Report No. 6, The University of Nebraska Crops Research Division, ARS, USDA, The Rockefeller Foundation.
 16. SMITH, W. H. 1969. Germination of *Macrophomina phaseoli* sclerotia as affected by *Pinus lambertiana* root exudate. *Can J. Microbiol.* 15:1387-1391.
 17. YOUNG, H. C., JR. 1943. The toothpick method of inoculating corn for ear and stalk rot. (Abstr.) *Phytopathology* 33:16.