

Growth of Cereal Root-Rot Fungi as Affected by Temperature-Water Potential Interactions

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ABSTRACT

Fusarium roseum 'Graminearum', *F. roseum* 'Culmorum', and *Gaeumannomyces graminis* var. *tritici* all required progressively drier conditions on osmotically-adjusted agar media for maximal growth, when the temperature of incubation was raised from 10 C to 35 C. An isolate of Graminearum from corn, and one from wheat, both grew optimally at -10 to -28 bars at 20 to 30 C and at -28 to -55 bars at 35 C. Three isolates of Culmorum from different areas each grew optimally at -8 to -14 bars at 20 to 30 C and at -28 bars at 35 C. *Gaeumannomyces graminis* grew optimally at

-8 to -12 bars at 30 C, but did not grow at 35 C, regardless of the water potential. All optimal water potentials were lower than normal in common culture media (-1 to -2 bars). The responses recorded for the three pathogens match their ecological distribution in nature; i.e., Graminearum and Culmorum both cause foot rot of wheat in hot, dry soil, with Graminearum being associated with slightly hotter and drier soil than Culmorum. *Gaeumannomyces graminis* causes foot rot of wheat in cool, wet soils.

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Root and foot rot of wheat caused by *Fusarium roseum* Lk. ex Fr. 'Culmorum' [= *F. culmorum* (W. G. Smith) Sacc.] and *F. roseum* 'Graminearum' (= *F. graminearum* Schwabe) is most severe in the Pacific Northwest, USA, in the 20- to 35-cm annual precipitation, stubble-mulched, wheat-fallow area (6). The problem areas include a large section of eastern Washington, north-central Oregon, and the Camas Prairie of Idaho. Culmorum predominates, and Graminearum is thus far confined to isolated fields in the hottest, driest part of Washington (Franklin, Benton, Grant, and Adams Counties). Graminearum also has been found in an occasional irrigated wheat or barley field in the Columbia Basin or southern Idaho; both of these areas have high summer temperatures. Take-all, on the other hand, caused by *Gaeumannomyces graminis* (Sacc.) Arx et Olivier var. *tritici* Walker, is confined in the Northwest almost entirely to the irrigated areas and to wheat or barley fields west of the Cascade Mountains where rainfall is high (approximately 100 cm/year) (7). This pattern of *Fusarium* root and foot rot of cereals in warm, dry areas and take-all in cool, wet areas is a well-known pattern to cereal pathologists throughout the world.

On solid media, growth of *G. graminis* var. *tritici* at different osmotic water potentials was prevented at -45 to -50 bars, and that of Culmorum at -85 to -90 bars (8). This greater capacity of Culmorum to grow at low water potentials (dry environments) may account, at least partly, for the greater incidence of disease caused by Culmorum in dryland wheat fields. The growth rate of Culmorum was actually stimulated by a slight (-8 bars)

osmotic water stress (8). This stimulation may be particularly significant, since osmotic forces presumably dominate while the fungus is within the host. However, the observation (8) was based on experimentation at only one temperature, 20 C. Fungal response to water potential may vary with different temperatures (1, 11, 12). Information on temperature responses obtained at only one water potential are equally ambiguous. Thus, *G. graminis* var. *tritici* apparently grows optimally at 19 to 24 C, depending on the isolate (9), and Graminearum at 24 to 28 C, depending on the pH of the medium (10), but these studies were made with potato-dextrose agar (PDA), which has a water potential generally above -2 bars. Just as water potential optima may vary with temperature, so temperature optima may vary with water potential.

This study attempts to clarify the influence of different water potential-temperature combinations on growth of the three pathogens.

MATERIALS AND METHODS.—All studies were conducted on agar media adjusted to different water potentials with salts. In the earlier study (8), the response of Culmorum was the same, whether measured as colony diameters on osmotic agar media, or as linear hyphal growth in wheat straws in soil amended with salts. Moreover, the response was generally the same, regardless of whether NaCl, KCl, or a mixture of KCl, NaCl, and Na₂SO₄ was used. This clearly suggested that the response was a function of water potential, and not of specific ion effects. It was also evident in the earlier study (8) that the salts in high concentrations (sufficient to give

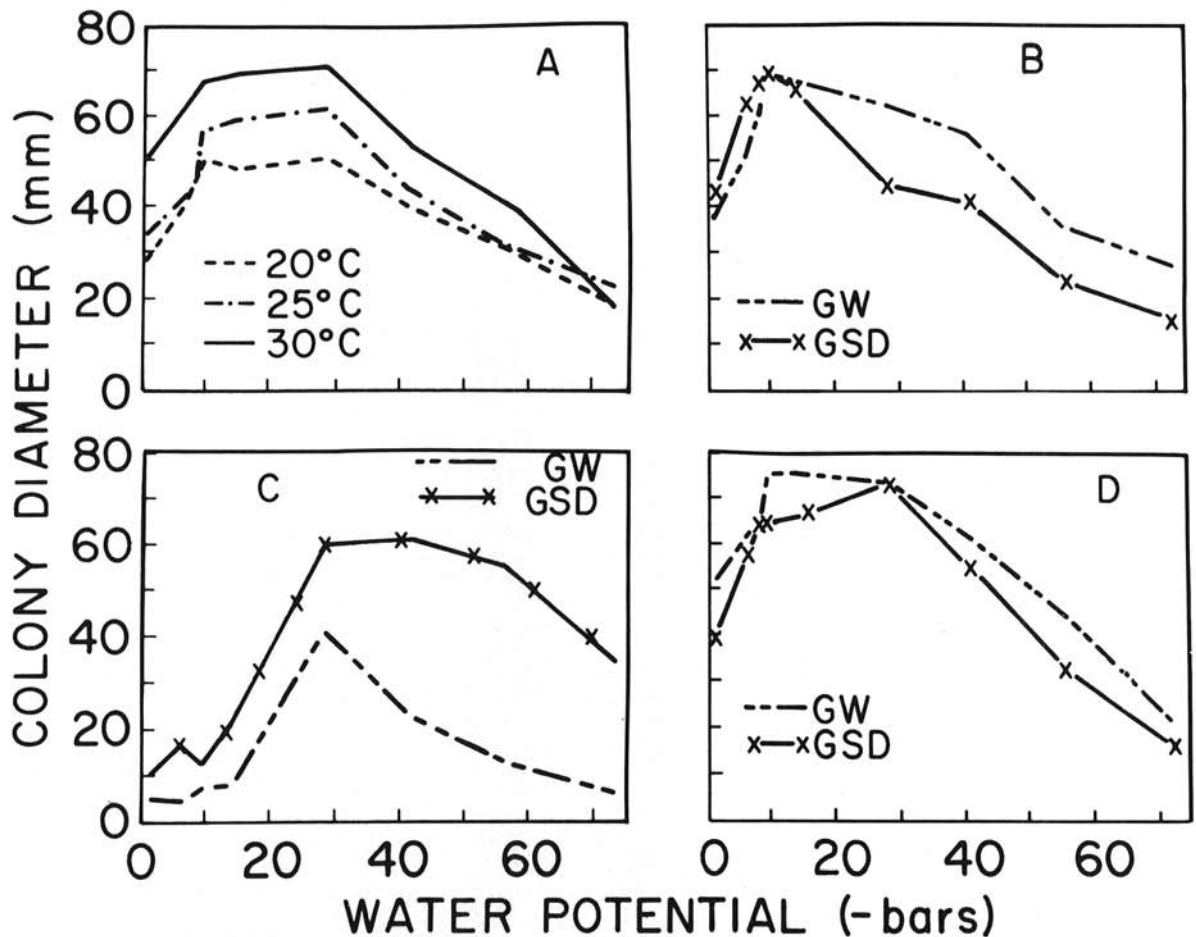


Fig. 1-(A to D). Colony diameters of isolates of *Fusarium roseum* 'Graminearum' after 98-104 hours at various temperatures on osmotically-adjusted media. A) Isolate G_{SD} on basal medium with KCl; B) Isolates G_{SD} and G_W on basal medium at 23 C; C) same as (B), but at 35 C and read at 30 days; D) same as (B), but at 29 C.

-90 to -110 bars) were not toxic to the test fungi, since the progressively lower growth with progressively higher salt concentrations matched a reduced growth measured in a soil system under matric control. In general, the test fungi made better growth with a given osmotic compared with matric water potential, but this difference related to the growth stimulation that occurred at -8 to -10 bars osmotic water potential. Of all the salts, KCl has been easiest to use. Thus, we used the synthetic agar medium of Sommers et al. (15), or corn meal agar (Difco) adjusted to different water potentials with KCl, although in one study Na_2SO_4 was used.

Two isolates of Graminearum were used: G_{SD} , isolated from a corn plant with stalk rot from South Dakota; and G_W , isolated from a wheat plant with foot rot from Washington. Three isolates of Culmorum were used: C_I from Ireland from a wheat head with scab; C_{ND} from a spring wheat plant with root rot from North Dakota; and C_W from a wheat plant with foot rot from Washington. The only isolate of *G. graminis* used was from a diseased wheat plant from Washington. All were fresh isolates at the start of the study.

In a typical study, test isolates were grown first on

PDA. Disks (5-mm diameter) were cut with a cork borer from the advancing margin of a colony and placed in the center of petri dishes of osmotically adjusted or basal media. Each plate (plastic, 9-cm diameter) contained 20 ml of medium. At least three replicate plates were used per isolate \times water potential \times temperature of incubation. All inoculated plates of a given isolate \times temperature group were placed together in a plastic bag that was sealed with tape, and were incubated in an inverted position. The results shown are for colony diameters measured when colonies in the most growth-conductive treatments were near the edge of the plate. These periods were 74-78 hours for Culmorum, 98-104 hours for Graminearum, and 7 days for *G. graminis* var. *tritici*. An exception was the 33 to 35 C treatments, which were measured at 19-20 days for Culmorum and 28-30 days for Graminearum.

Water potentials were varied at -1.2 bars (basal medium) to -90 bars, and temperatures ranged from 10 to 35 C.

RESULTS.—Growth at different water potential-temperature combinations.—Three consecutive and similar experiments were conducted using C_W and G_{SD} . *Gaeumannomyces graminis* var. *tritici* was included in

the first experiment, but not in the second or third experiment. The synthetic agar medium was adjusted with KCl to -6.0 , -8.2 , -9.3 , -14.0 , -28.1 , -41.4 , -55.8 , -72.9 , and -91.0 bars. The inoculated plates in the first experiment were incubated at 10, 15, 20, 25, and 30 C; in the second and third experiments, using only C_W and G_{SD} , at 20, 25, and 30 C.

There was little evidence of a growth response interaction between temperature and water potential at either 10 or 15 C. All three fungi grew slowest at 10 C, regardless of water potentials, and there was only a slight response to osmotic stress (-5 to -10 bars), compared to growth on basal medium without KCl (-1.2 bars). After 8 days, C_W and G_{SD} still had not grown at -72.7 bars at 10 C

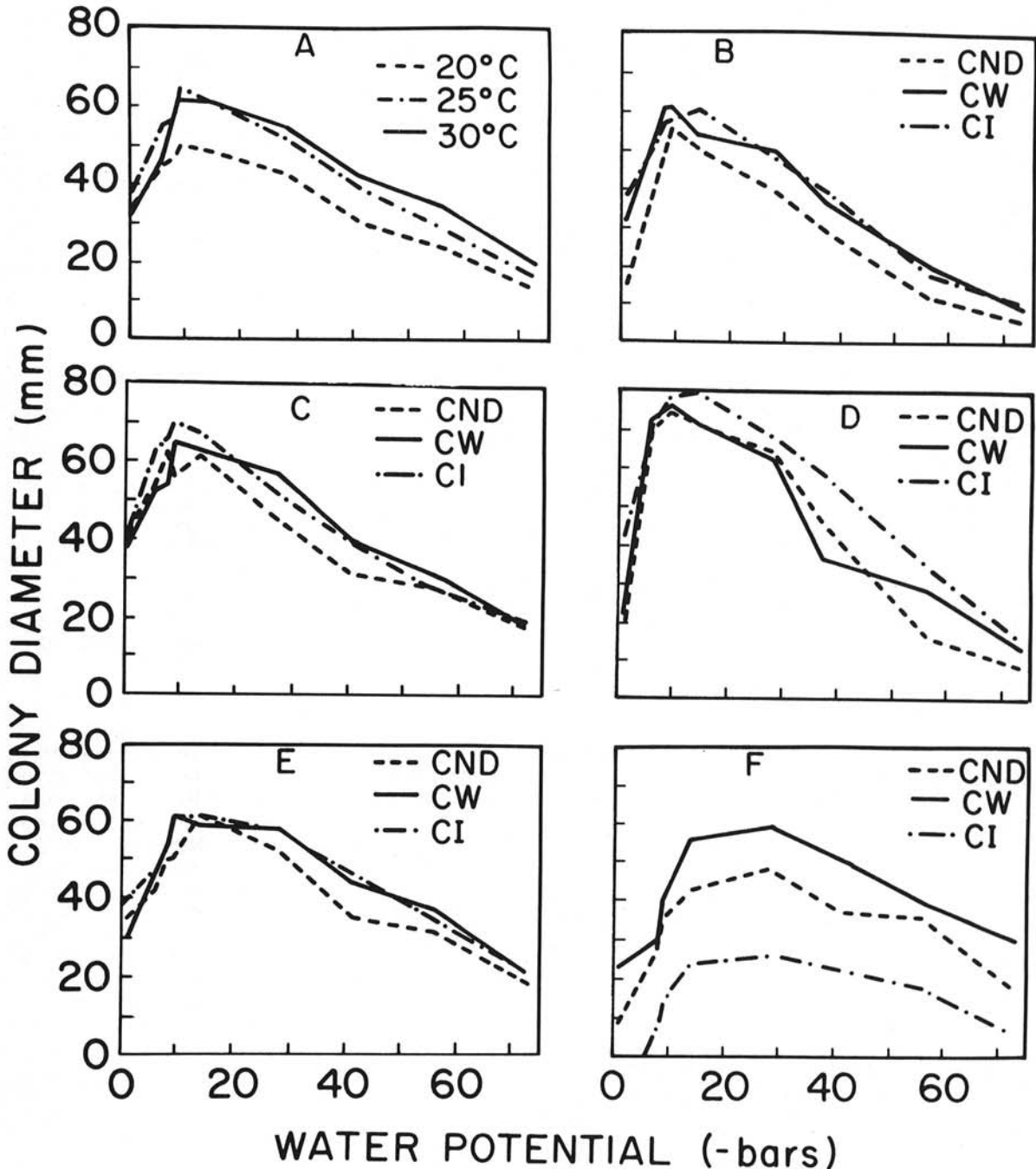


Fig. 2-(A to F). Colony diameters of isolates of *Fusarium roseum* 'Culmorum' after 74-78 hours at various temperatures on osmotically-adjusted media. A) Isolate C_W on basal medium with KCl; B) Isolates C_{ND} , C_W and C_I on corn meal agar (Difco) with KCl at 20 C; C) Isolates C_{ND} , C_W and C_I on basal medium with KCl at 23 C; D) same as (B) but at 25 C; E) same as (C) but at 29 C; F) same as (C) but at 35 C and read at 20 days.

(some growth was made by 20 days) or at -91 bars at 10 C (growth was not evident even after 20 days). *G. graminis* var. *tritici* grew slightly at -41.4 bars, but not at -55.8 bars at either 10 or 15 C, throughout the 30 days of the experiment.

Growth of G_{SD} and C_W at 20 , 25 , and 30 C, was similar in the triplicate trials; the results of the trials were thus combined (Fig. 1-A and 2-A). Optimal growth for G_{SD} was in the range of -10 to -28 bars at 20 , 25 , and 30 C (Fig. 1-A). Optimal growth for C_W was at -8 to -10 bars at 20 and 25 C and -12 to -14 bars at 30 C (Fig. 2-A). Neither *Fusarium* sp. made more than a trace of growth at -91 bars.

Gaeumannomyces graminis grew slightly better with an osmotic stress of -5 to -8 bars at 20 and 25 C, but response was variable (Fig. 3-A). In the earlier study conducted at 20 C, there was no response to an osmotic stress (8). At 30 C, however, growth was unmistakably best at -8 bars (Fig. 3-A). This fungus did not grow at -55.8 bars or lower at any of the temperatures tested.

The response of *G. graminis* was also tested on the synthetic agar medium at 20 , 25 , and 30 C by use of Na_2SO_4 as the osmoticum, but only over the range of -1 to -20 bars. As with KCl, there was a marked stimulation of growth by a slight osmotic stress at 30 C, but not at 25 or 20 C (Fig. 3-B). Moreover, although tests below -20 bars were not made, the trends suggested that growth would have been prevented at about -40 bars, as occurred with KCl.

Uniformity of response among isolates of Fusarium.—An experiment was made with all five *Fusarium* isolates C_W , C_I , C_{ND} , G_{SD} , and G_W grown on the synthetic agar medium amended with KCl and incubated at 20 , 23 , and 29 C. There was virtually no difference in responses at 20 and 23 C, thus the results at 20 C are not shown. The three Culmorum isolates responded (Fig. 2-C and E) as C_W did (Fig. 2-A). The two Graminearum isolates responded (Fig. 1-B and D) as G_{SD} did (Fig. 1-A). Moreover, the two Graminearum isolates again differed from the three Culmorum isolates by growing optimally at slightly lower water potentials, and by their tendency to grow optimally over a broader range of water potentials below zero. With all five isolates, the water potential required for optimal growth was lower at 29 C (Fig. 1-D and 2-E) than at 23 C (Fig. 1-B and 2-C).

The three Culmorum isolates were also grown on corn meal agar over the entire range of water potentials (KCl) at 20 and 25 C. The magnitude of response to osmotic stresses was particularly pronounced on this medium (Fig. 2-B and 2-D), but growth at 20 and 25 C was optimal at essentially the same water potentials as those recorded earlier for isolate C_W on synthetic agar media (Fig. 2-A and 2-C).

Growth at high temperatures.—Several experiments were conducted at 35 C (33 C in two cases, due to a faulty incubator) to determine if growth was possible at higher temperatures with osmotic water stress. *G. graminis* did not grow at 35 C, even after 30 days, regardless of the water potential used. Moreover, the five *Fusarium* isolates essentially did not grow at these temperatures on the media without salts. However, each *Fusarium* isolate grew typically, albeit slowly (3-4 weeks required to reach the edge of the plate compared with only 3-4 days at the lower temperatures) under osmotic stress (Fig. 1-C, 2-F).

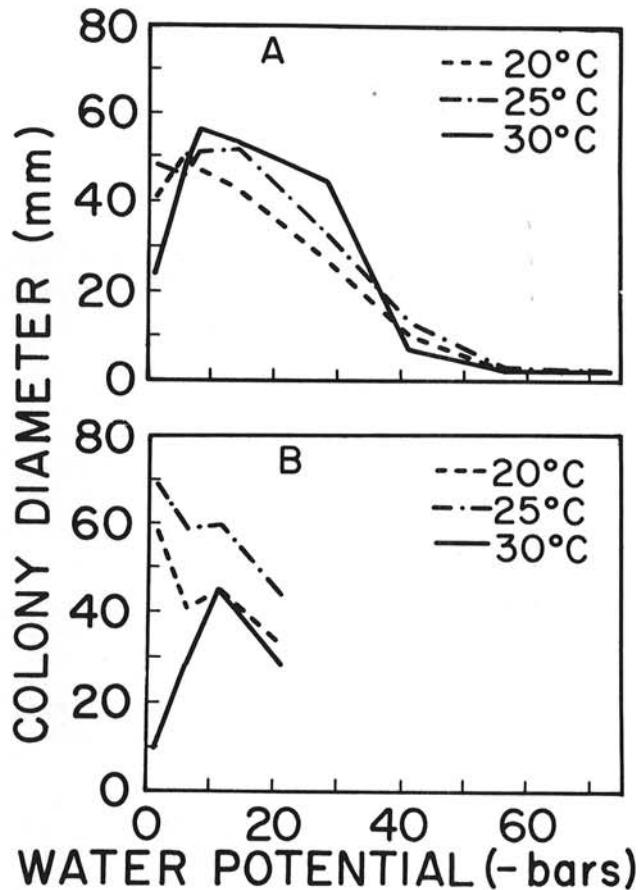


Fig. 3. Colony diameters of an isolate of *Gaeumannomyces graminis* var. *tritici* after 7 days at various temperatures on basal medium adjusted to various osmotic water potentials with A) KCl and B) Na_2SO_4 .

The three isolates of Culmorum all grew optimally at -28 bars (KCl) on both synthetic (results not shown) and corn meal agar (Fig. 2-F), the latter having been at 33 C. Of the two isolates of Graminearum on the synthetic medium with KCl, G_{SD} grew optimally over the range -28 to -55 bars, and G_W did best at -28 bars (Fig. 1-C). In another test at 35 C, the results of which are not shown, G_{SD} did not grow on synthetic agar after 30 days at -1.2 to -9.3 bars, made growth at -14 and -28 bars, and had a 40-mm colony diameter at -41 bars, 43 mm at -55 bars, and 27 mm at -72 bars. Growth in this test was thus optimal between -28 and -41 bars. At 33 C, G_{SD} and G_W produced colonies of 68 and 60 mm diameter, respectively, after 20 days at -28 bars, compared to 38 and 30 mm diameter, respectively, on the same medium without salts.

DISCUSSION.—These results show for all three pathogens that at the higher temperature, a lower water potential generally was required for maximal growth. Conversely, at the lower water potentials, the pathogen grew best at the higher temperature. Manandhar and Bruehl (12) showed the same response for *Verticillium albo-atrum* and *Fusarium oxysporum*. This response is probably an adaptive mechanism in the organisms needed to meet a common situation, namely, a dry environment

when temperatures are high or vice versa. The shift was for the optimal water potential, but not for minimal water potential; growth by a given fungus was prevented at the same water potential, regardless of the temperature.

The optimal temperatures for growth have been accepted at 19 to 24 C and at 24 to 28 C for *G. graminis* (9) and *Graminearum* (10), respectively, on PDA, which presumably had a water potential of about -1.5 bars. Our studies show a slightly higher optimum temperature if lower water potentials prevail. Cochrane (4) summarized that most plant pathogens grow optimally at about 24 C, but this was based on growth on common agar media. It may eventually be necessary to reassess the presently-accepted temperature optima of pathogens by use of agar media adjusted to more realistic water potentials.

Wheat tissues have water potentials of -15 to -20 bars in the juvenile stage in the field and may reach -30 to -40 bars by maturity (14). Even well-watered potato plants in a growth chamber have leaf water potentials of -4 to -8 bars (3). Culturing pathogens at -1 to -2 bars has perhaps led to misconceptions about their abilities and environmental requirements. The validity of physiological studies at -1 to -2 bars is also questionable, when in nature the pathogen probably does not encounter this situation until the plant is dead and its tissues have equilibrated with the water potential of the soil.

Graminearum occurs under warmer, drier conditions than *Culmorum*. In Washington State, for example, *Graminearum* predominates in southern Franklin and adjacent Benton Counties (mean July daily highs about 25 C; mean annual precipitation, 20-25 cm). *Culmorum* predominates in Adams and Lincoln Counties (mean daily highs in July about 21 C; mean annual precipitation, 25-35 cm). *Culmorum* predominates in England (5) and *Graminearum* in Australia (2). *Gaeumannomyces graminis*, on the other hand, requires cool, wet soil to produce severe disease (13). Dickson (10) concluded that seedling blight of wheat and corn caused by *Graminearum* was most severe at temperatures more unfavorable for the plant than for *Graminearum*. This same concept can be applied to water potential. The common -25 to -30 bars recorded for maturing healthy, nonstressed wheat (15) are surely more limiting to *G. graminis* (growth reduced by more than one half) than to wheat. Indeed, -40 bars in wheat tissues should essentially prevent growth by *G. graminis*. This prevention can account at least partly for the absence of take-all in wheat in dry years. In contrast, *Fusarium* is well equipped to grow in wheat tissues at -35 to -40 bars; it grows well at water potentials in this range if temperatures are near 35 C. However, if severe disease resulted from simple water-potential effect on the fungus, then disease should also be severe at higher water potentials (-20 to -25 bars), at 20-30 C, but it is not (14). Instead, although -35 to -40 bars may suppress the

Fusarium pathogens slightly, these water potentials probably suppress cells of the wheat plant relatively more, which gives *Fusarium* the advantage, and results in severe disease.

LITERATURE CITED

- BRUEHL, G. W., and B. CUNFER. 1971. Physiologic and environmental factors that affect the severity of snow mold of wheat. *Phytopathology* 61:792-799.
- BURGESS, L. W., A. H. WARING, and T. A. TOUSSOUN. 1974. Quantitative studies on *Fusarium* crown rot of wheat in eastern Australia. *Aust. J. Exp. Agric. Anim. Husb.* (In press).
- CAMPBELL, G. S., and M. D. CAMPBELL. 1974. Evaluation of a thermocouple hygrometer for measuring leaf water potential in situ. *Agron. J.* 66:24-27.
- COCHRANE, V. W. 1958. *Physiology of fungi*. John Wiley, New York. 524 p.
- COLHOUN, J., and D. PARK. 1964. *Fusarium* diseases of cereals. I. Infection of wheat plants, with particular reference to the effects of soil moisture and temperature on seedling infection. *Trans. Br. Mycol. Soc.* 47:559-572.
- COOK, R. J. 1968. *Fusarium* root and foot rot of wheat in the Pacific Northwest. *Phytopathology* 58:127-131.
- COOK, R. J., D. HUBER, R. L. POWELSON, and G. W. BRUEHL. 1968. Occurrence of take-all in wheat in the Pacific Northwest. *Plant Dis. Rep.* 52:716-718.
- 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.
- DAVIS, R. J. 1925. Studies on *Ophiobolus graminis* Sacc. and the take-all disease of wheat. *J. Agric. Res.* 31:801-825.
- DICKSON, J. G. 1923. Influence of soil temperature and moisture on the development of the seedling-blight of wheat and corn caused by *Gibberella saubinetii*. *J. Agric. Res.* 23:837-870.
- GRIFFIN, D. M. 1970. Effect of soil moisture and aeration on fungal activity: an introduction. Pages 77-80 in T. A. Toussoun, R. V. Bega, and P. E. Nelson, ed. *Root diseases and soil-borne pathogens*. University of Calif. Press, Berkeley. 252 p.
- MANANDHAR, J. B., and G. W. BRUEHL. 1973. In vitro interactions of *Fusarium* and *Verticillium* wilt fungi with water, pH, and temperature. *Phytopathology* 63:413-419.
- MC KINNEY, H. H., and R. J. DAVIS. 1925. Influence of soil temperature and moisture on infection of young wheat plants by *Ophiobolus graminis*. *J. Agric. Res.* 31:827-840.
- PAPENDICK, R. I., and R. J. COOK. 1974. Plant water stress and development of *Fusarium* foot rot in wheat subjected to different cultural practices. *Phytopathology* 64:358-363.
- 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.