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Saprophytic Growth and Pseudothecia Production by *Pyrenophora tritici-repentis* in Plant Tissue Held at Controlled Water Potentials

W. F. Pfender, C. A. Pacey, and W. Zhang

Assistant professor, research assistant, and graduate research assistant, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan 66506.
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ABSTRACT

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Mycelial growth rate and production of pseudothecia by *Pyrenophora tritici-repentis* in dead wheat leaves at a range of water potentials was measured. In sterilized, reinoculated leaves the fungus grew maximally at the wettest level tested (−0.5 MPa = −5 bars) and was able to grow at a slow rate at −10.5 MPa. In infected nonsterile leaves, growth rates were slower than in sterilized leaves at all water potentials, and growth was detected at −8.5, but not at −10.5 MPa. Pseudothecia production in nonsterile leaves was equivalent to that in sterilized leaves. Production of pseudothecia was maximum at −0.5 MPa (wettest level tested), was significantly reduced at −1.6 MPa, and was limited to a very few, small ascocarps at −2.4 MPa; none were produced at −3.8 MPa.

Additional keywords: tan spot, *Triticum aestivum*, wheat.

The ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker) causes tan spot, a foliage disease of wheat (*Triticum aestivum* L.) (8). In several regions of the world, including the Central Plains area of the United States, this disease is becoming increasingly prevalent due to the widespread use of conservation-tillage farming, in which crop residue is retained on the soil surface between seasons to reduce erosion losses. Because *P. tritici-repentis* overwinters and produces its ascospores (primary inoculum) on wheat straw, wheat grown continuously under conservation tillage can be exposed to high levels of primary inoculum.

In a previous study, Pfender and Wootke (16) noted that *P. tritici-repentis* survives poorly in straw that is buried or placed

directly on the soil surface. In contrast, it survives well in straw that is slightly above the soil surface, as in standing stubble or within a mulch layer. Our working hypothesis is that residue on the soil is more often moist than that above the soil, and that this wetter environment results in more intense microbial activity and competitive interactions detrimental to *P. tritici-repentis*. Under this hypothesis, the influence of residue water potential on pseudothecia production and saprophytic growth of *P. tritici-repentis* is an important factor to consider. Further, although the saprophytic aggressiveness of *Pyrenophora* in the presence of the straw microflora has a major bearing on its capacity to produce inoculum from infected straw, such competitive saprophytic growth has not yet been demonstrated.

Fungi clearly differ in their tolerance to low water potential (7). Further, they often show different threshold moisture levels for different life stages or processes (11). For example, sporulation is commonly more sensitive than growth to low water potentials (1,6). The growth response of *P. tritici-repentis* to osmotic water potential was investigated by Norman (13), who detected growth at −7.8 MPa (−78 bars) but not at −9.5 MPa. Ascocarp production by *P. tritici-repentis* as a function of water potential has not been investigated, but the related ascomycete *Venturia inaequalis* was observed to produce ascocarps on infected apple leaf disks at 100%, but not at 98% relative humidity (9).

We report here experiments to measure saprophytic growth and pseudothecia production by *P. tritici-repentis* in dead wheat leaves held at various water potentials.

MATERIALS AND METHODS

Biological material. Nonsterile infected leaves were produced by inoculating the second- and third-youngest leaves of greenhouse-grown wheat seedlings (cultivar TAM 105) at 5 wk of age. Inoculum consisted of comminuted mycelium of isolate 6R180 of *P. tritici-repentis*, which had been grown on clarified V-8 juice agar (18). The advancing edge of a 5-day-old colony and a thin layer of underlying agar were removed from the culture plate and mashed by passing repeatedly through a 3-cc syringe (with no needle attached). With a sterile spatula a thin layer of this mycelium/agar mixture was applied in a band 2–3 mm wide across the width of the upper surface of a leaf, approximately 4 cm above the leaf base. Inoculated plants were placed in a mist chamber at 25 C for 48 hr, then removed to the greenhouse bench at 25 C. About 24 hr later, when the agar had dried, it was removed from the leaf. Thereafter, plants were watered only on alternate days to hasten senescence of leaves, and water was not permitted to wet the leaves. Leaves were monitored daily, and infected leaves were harvested when they became yellow and started to dry. Harvested leaves were pressed between paper towels to dry for at least 1 wk before being used in experiments. Immediately before use, these leaves were cut to approximately 60-mm length, then moistened between wet paper towels for various periods of time (15–120 min) as required to bring their water potentials to approximately the desired levels (W. F. Pfender and C. A. Pacey, *unpublished*). Leaves finally were cut to 50-mm length by trimming the ends and placed into chambers of controlled relative humidity.

Sterilized leaves were prepared by treating leaves infected by *Pyrenophora* (described above) with propylene oxide gas. Leaves were first cut to about 60-mm length, with the lesion approximately centered, and moistened between wet paper towels for 0.5 hr. They then were placed in a screw-cap jar (0.5-L capacity) containing a cotton plug moistened with 1 ml of the sterilant, and the jars were sealed. After 24 hr, leaves were removed and aerated in a sterile hood. They then were remoistened between wet, sterile paper towels before being inoculated with conidia of *D. tritici-repentis*. Inoculum for these leaves consisted of a 3-μl drop of conidial suspension (15 propagules [conidia + conidiophores] per 3 μl) prepared from agar-plate culture as described previously (15). The inoculum drop was placed on the site of the original lesion and was permitted to dry on the leaf in a sterile hood for 30 min. These leaves then were placed in closed petri dishes on a bed of moist, sterile vermiculite for 24 hr to induce spore germination and establishment of growth. Finally, leaves were placed into chambers of controlled relative humidity.

Humidity chambers. Leaf water potential during the experiments was maintained at selected levels by isopiestic equilibration with an atmosphere of known relative humidity (RH). For this purpose, humidity chambers were constructed from canning jars (1-pint capacity, Kerr Glass Mfg. Corp., Los Angeles, CA). Jars were placed on their sides and contained a semi-rigid polypropylene screen (plastic needlework canvas) supported by drops of silicone rubber caulk applied to the jar walls (Fig. 1). The removable screen was positioned horizontally about half-way between upper and lower chamber surfaces. Test leaves rested on the screen. A glycerol solution (25 ml) of known water activity was placed in the bottom of the chamber. Three sheets of pleated bibulous paper (each 10 × 15 cm) were placed in the glycerol solution to aid equilibration between the solution and the chamber atmosphere. An additional piece of screen was positioned 0.5 cm below the first to ensure that the glycerol-soaked paper in the bottom of the chamber would not contact the leaves or their screen.

To avoid oxygen depletion and/or carbon dioxide accumulation inside the closed chambers, each was fitted with an autoclaved, gas-permeable cap cemented into the jar lid. These caps (Kimble test tube closure no. 73670) are impermeable to water vapor but permit oxygen and carbon dioxide to diffuse at rates of 0.08 and 0.2 cc/24 hr, respectively (calculated by the authors from manufacturer's data). From data of Knapp et al (10), we calculated that microbial respiration from four leaves (about 0.02 g dry wt) would require approximately 0.1 cc of oxygen per day during maximum respiration, and an average of 0.06 cc/day over 10 days. To ascertain that aeration was sufficient, air samples occasionally were withdrawn from the chambers through a serum-bottle septum cemented into the jar lid. Air samples were taken with a 10-ml gas-tight syringe (Glenco, Houston, TX), and their oxygen levels were estimated by a gas absorption method (Burrell Manual for Gas Analysts, Burrell Corp., Pittsburgh, PA), which we modified for small sample volumes. A measured sample volume was passed repeatedly through a 45% (w/w) KOH solution, which absorbs water vapor and carbon dioxide. Then a measured volume of the remaining gas was passed repeatedly through a solution of alkaline pyrogallate (Fisher Scientific, Pittsburgh, PA), which absorbs oxygen. The volume of each gas can be determined by measuring the reduction in gas volume that occurs after passage through the appropriate solution.

The RH (expressed as a fraction, 0–1.00) of the atmosphere in equilibrium with an aqueous solution at a given temperature is equivalent to the water activity of the solution, and an aqueous glycerol solution of known water activity can be prepared by adjusting its concentration. We first determined the concentration

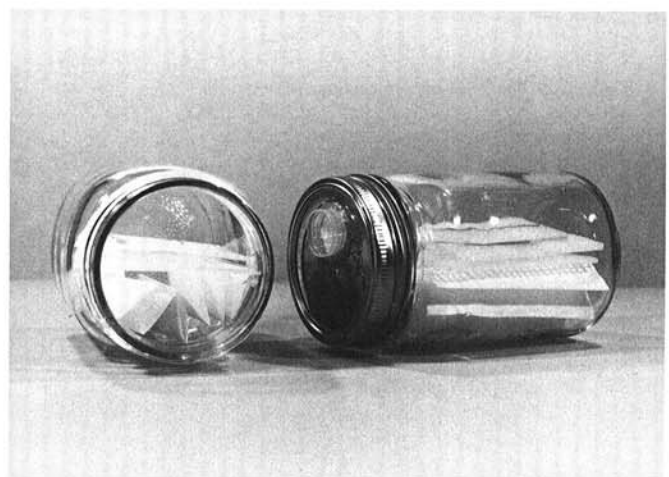

Fig. 1. Humidity chamber used for isopiestic control of leaf water potential. Gas-permeable cap (upper) and septum for withdrawing gas sample (lower) are visible in capped jar; jar without cap shows folded paper and two polypropylene screens.

of purchased glycerol (Sigma Chemical Co., St. Louis, MO; glycerol approx. 99%) by using an Abbe refractometer (Bausch & Lomb, Rochester, NY) to measure the refractive index, and from these data obtained the percent glycerol (w/w) from tabulated values (17). Solutions of the desired water activity then were prepared by mixing this stock glycerol with distilled water in desired proportions (w/w); water activity is related to percent glycerol in equations given by the American Society for Testing and Materials (2). Water activities of prepared solutions then were measured with a thermocouple psychrometer (Decagon, Pullman, WA).

To reduce the possibility of thermal gradients in the chambers that would disrupt water vapor equilibrium, the assembled chambers were placed in open-top boxes constructed of 2.5-cm-thick Styrofoam and covered with a clear plastic top. Air exchange was provided by raising the clear tops slightly. Experiments were conducted on a laboratory bench at 24 ± 1 C.

Leaf water potential measurement. Water potential of leaves was measured with a thermocouple psychrometer device having multiple sample holders and designed to wet the thermocouple junction by dipping into liquid water, rather than by collecting condensate (Decagon, Pullman, WA). Because only a single leaf was to be measured in each sample cup (14 mm deep × 14 mm diameter), the device was modified by placing cylindrical teflon inserts (7 mm high × 14 mm diameter) into each stainless steel sample cup to reduce its volume. To reduce the rapid loss of moisture to the atmosphere that can occur from small, wet materials such as our leaves, sample processing (including removal of leaves from jars) was done inside a Plexiglas box (51 × 36 × 29 cm) lined internally with wet paper towels and having sheathed openings for the operator's hands. Each leaf was cut transversely into strips about 1 mm wide, and placed into the modified sample cup, which was, in turn, inserted into the psychrometer instrument. A 20-min equilibration period was allowed before taking readings.

Experimental design and data collection. In experiments to determine the effect of water potential on ascocarp production, there were four leaves per chamber and two chambers (replicates) per treatment. Ascocarp counts were averages for the four leaves in a chamber. Treatments were a factorial of leaf type (nonsterile precolonized versus sterile reinoculated) and glycerol water activity (1.000, 0.995, 0.990, 0.980). One month after the beginning of the experiment, leaves were removed from the chambers, and the water potential and number of ascocarps were determined for each leaf. Ascocarp sizes were measured with the aid of a stereo microscope fitted with an ocular micrometer. The experiment was repeated once.

The design for growth-rate experiments was similar to that for ascocarp studies, except that the experiments were performed separately for nonsterile and sterilized leaves, rather than as a factorial. In these experiments, both an initial and final estimate of fungal location was needed to calculate growth rate. For experiments with nonsterile leaves, the starting location of *Pyrenophora* could not be determined with certainty from the extent of the visible lesion; before the start of the experiment, each dry leaf was split longitudinally and the identity of these two halves was retained during the experiment. One of the halves of each leaf was removed from the humidity chamber 24 or 48 hr after the start of the experiment. This was cut transversely into 2-mm segments that were plated sequentially on plates of water agar amended with chloramphenicol (200 mg/L) and triphenyltin hydroxide (0.42 mg a.i./L) and incubated at 20 C under 12-hr fluorescent photoperiod to induce sporulation of *D. tritici-repentis*. The location of the fungus (and any of the several other hyphomycetes that often occurred on these leaves) at this time could be determined. At an appropriate later time (determined in preliminary experiments), the second halves of the leaves were removed from the jar and similarly plated. At both initial and final assessment times, a half-leaf of an additional leaf was removed and processed immediately to determine water potential, which was taken to indicate water potential of all leaves in the jar.

For growth-rate experiments with sterilized, reinoculated leaves, leaves were not split longitudinally. Instead, two leaves were removed for plating 24 or 48 hr after placing them in jars, and two additional leaves were removed and plated at an appropriate later time. The leaves, which had been inoculated centrally, were cut in half transversely at the inoculation site; one half was plated for growth determination, the other half was used for water potential measurement.

The RH treatments used for the growth rate experiments were 100, 98, 97, 95, 93, and about 78% (leaves allowed to air-dry before placing in the chamber, and pure glycerol used as the equilibrating solution). These experiments were repeated once.

For data analysis, the measurements from all leaves within a chamber were averaged to give a single value, and this value was used as a replicate datum. Values from two experiments were used in the analyses below.

RESULTS

Performance of the humidity chambers. Water potentials of leaves incubated in the chambers were reproducible. Accurate determination of leaf water potential depended on the use of the Plexiglas box for processing samples. When leaves were processed within about 10 min of opening the humidity chamber jars in the box, leaf water activity was 0.01–0.02 (about 1.4–2.8 MPa) lower than that of the glycerol solution. More exacting experiments, in which leaves were processed within 30–120 sec of removal from the jars, gave a regression equation by which leaf water activity could be estimated from glycerol water activity. From these data, leaves were determined to be consistently drier than the glycerol solutions by 0.6 MPa, with a 95% confidence interval of ± 0.3 MPa. There was only a slow drift toward more negative water potentials during the 30–120-sec interval, and we assumed that the 30-sec values were approximately correct for the leaves at 0 sec. In the data below, we report leaf water activities as calculated from measured glycerol solution water activities, using our derived regression equation.

Determination of oxygen and carbon dioxide levels within the chambers was only approximate (±3%). Although occasional readings as low as 17% oxygen were observed, statistical analysis of the chamber oxygen measurements (23% average, 0.95 confidence interval of 20–26%) indicated they were not significantly different from measurements of oxygen in ambient air samples. Carbon dioxide + water vapor measurements for chamber samples also were similar to values for water-saturated air.

Ascocarp production. Each of the two trials of this experiment was first analyzed separately; because there was no significant difference ($P = 0.05$) between them with respect to error variance, the two trials were combined for subsequent analysis. Covariance analysis indicated no significant difference ($P = 0.05$) in ascocarp production between infected, nonsterile leaves and leaves that had been gas sterilized and inoculated with a drop of spore suspension. Maximum ascocarp production occurred at the wettest level tested, −0.5 ± 0.3 MPa (Table 1); these ascocarps were mostly large (450–650 μm). The ascocarps produced at −1.6 MPa were predominantly small (<300 μm), and at −2.4 MPa there were very few ascocarps produced, all of them small. No pseudothecia were produced on any leaves held at −3.8 MPa.

The relationship of water potential to ascocarp production was tested by linear regression. The data from −3.8 MPa were deleted before analysis, since this moisture level is apparently below the lower threshold permissive for ascocarp production, i.e., it does not give an accurate estimate of the relationship. Ascocarp data were transformed to their square root values to reduce inequality of variances among water potential levels, and water potential values were transformed to the logarithm of their absolute value (Fig. 2). For total number of ascocarps, as well as for number of ascocarps >300 μm diameter, the linear regressions are significant (Table 1). The regression equations are: $(\text{total ascocarps})^{1/2} = 3.138 - 5.215 \times \log(-\text{water potential})$, and $(\text{large ascocarps})^{1/2}\ 2.354 - 6.404 \times \log(-\text{water potential})$.

Growth rate. Saprophytic growth rate was a function of leaf water potential and was also greatly influenced by the presence of other microorganisms (Fig. 3). In gas-sterilized, reinoculated leaves, maximum growth rates were about 5 mm/day, whereas in

nonsterile leaves the highest growth rates were less than 1.5 mm/day (Fig. 3A). *P. tritici-repentis* grew at −8.5 MPa, but not at −10.5 MPa in nonsterile leaves; in sterilized leaves, there was growth at −10.5 MPa.

Effect of water potential on growth rate, and the difference in growth rates on sterilized and nonsterile leaves, was examined by linear regression and analysis of covariance. Variances of growth rates among treatments were first equalized by transformation to the fourth root. The data from −35.0 MPa were deleted, since this moisture level was considered too low to be relevant to defining the quantitative relationship between moisture and growth. Data from −0.5 MPa were also deleted, since they were identical with growth rates at −3.5 MPa, and, in any transformation we used, these data points had a disproportionate influence on the location and slope of the regression line in relation to the data between −3.5 and −10.5 MPa. Over this latter range, there was a good linear fit of the transformed growth rate to water potential (Fig. 3B). For sterilized as well as nonsterile leaves, the regressions were significant at $P = 0.001$, and the adjusted r^2 values were 0.934 and 0.770, respectively.

TABLE 1. Pseudothecia production by *Pyrenophora tritici-repentis* on dead wheat leaves as affected by water potential

Leaf water Potential (MPa)	Leaf type[a]	Ascocarps[b] Total	Large[c]
−0.5 ± 0.3	NS	25	20
	S	22	20
−1.6 ± 0.3	NS	9	4
	S	5	1
−2.4 ± 0.3	NS	1	0
	S	1	0
−3.8 ± 0.3	NS	0	0
	S	0	0
Regression[d]			
Adjusted r^2		0.648	0.785
Probability $> F$		0.001	0.001
Intercept (standard error)		3.138 (0.241)	2.354 (0.211)
Slope (standard error)		−5.215 (0.792)	−6.404 (0.695)

[a] NS = nonsterile leaves infected with *P. tritici-repentis*; S = gas-sterilized leaves inoculated with a drop of spore suspension of *Drechslera tritici-repentis* (anamorph of *P. tritici-repentis*).
[b] Number of pseudothecia per 5-cm leaf.
[c] Ascocarps >300 μm in diameter.
[d] Linear regression analysis of (ascocarps)$^{1/2}$ on log (−water potential) for water potentials from −0.5 to −2.4 MPa.

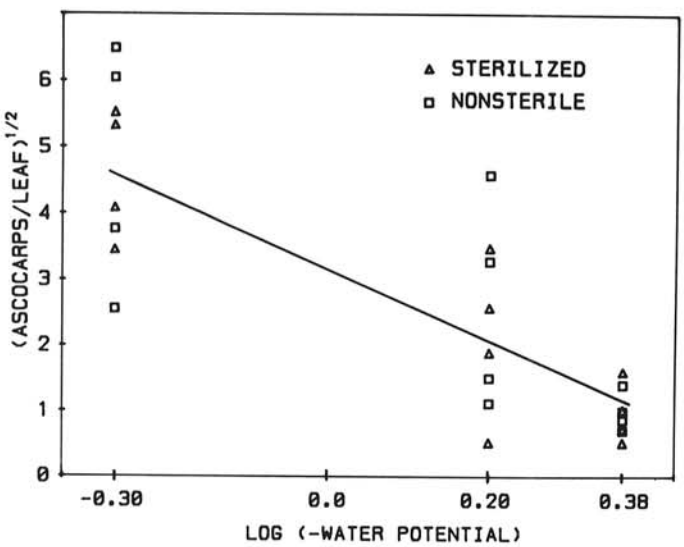

Fig. 2. Linear regression relating total ascocarps of *P. tritici-repentis* per leaf to water potential of the leaf. Both variables are transformed to reduce variance heterogeneity and to improve fit of linear model. Response of the fungus in sterilized, reinoculated leaves was not significantly different from that on nonsterile infected leaves. The regression equation for the combined data is: (ascocarps)$^{1/2}$ = 3.138 × 5.215 × log(−water potential); adjusted $r^2 = 0.785$.

The regression equation for sterilized leaves is: (growth rate)$^{1/4}$ = 1.794 + 0.0926 (water potential); for nonsterile leaves it is: (growth rate)$^{1/4}$ = 1.281 + 0.0704 (water potential). Covariance analysis (4) showed that these regression lines differed ($P = 0.01$) in location (intercept) but did not differ significantly ($P = 0.01$) in slope. Although the between-replicate and between-experiment variability was similar for sterile and nonsterile leaves (Fig. 3A), the leaf-to-leaf variability within experiments was greater for nonsterile than for sterilized leaves, especially at the higher moisture levels. This variability probably was due to the variable presence and location of other saprophytes (e.g., *Alternaria* sp., *Epicoccum* sp., *Cladosporium* sp.) on the nonsterile leaves; in these leaves *P. tritici-repentis* generally made its greatest growth where there were relatively low levels of other fungi, as assessed by observation of the isolation plates (data not shown).

DISCUSSION

Methodology for determining the influence of water potential on fungal activity presents some problems. In many natural

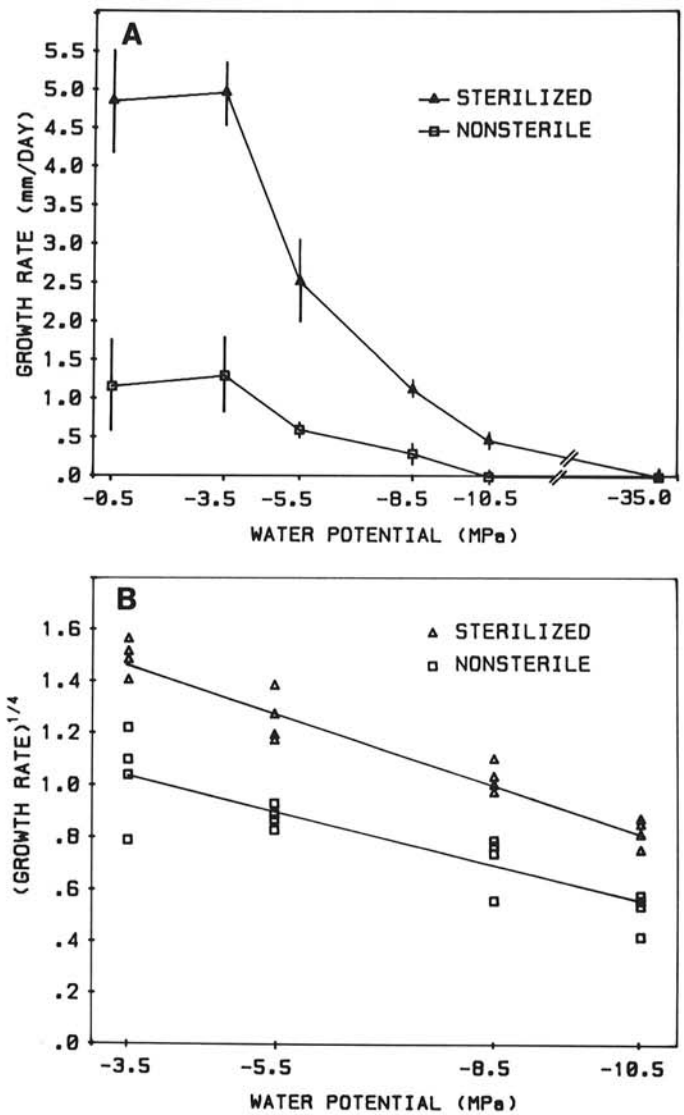

Fig. 3. Growth rate of *Pyrenophora tritici-repentis* in dead wheat leaves held at controlled water potentials. Leaves were either nonsterile (previously infected by *P. tritici-repentis*), or gas sterilized and reinoculated with a drop of spore suspension. **A,** Arithmetic plot of data over entire experimental range of water potential values. Each data point is the mean of two experiments, with two replicates per experiment and two to four leaves per replicate. Bars represent ± standard error of the mean. **B,** Linear regression of transformed growth rate values on water potential over the range −3.5 to −10.5 MPa. Adjusted r^2 values for sterilized and nonsterile leaves are 0.934 and 0.770, respectively.

situations, matric components of water potential predominate, but laboratory studies commonly employ osmotically controlled water potential because the latter usually can be more precisely controlled. As reviewed by Cook and Duniway (3), the effects of matric and osmotic water potential may differ; generally, fungi are more tolerant of low osmotic potentials than low matric potentials. In a recent study of straw-colonizing soilborne saprophytes, Magan (12) found that spore germination rate and germ-tube growth were more sensitive to matric than to osmotic water potential stress, and that germination was less on straw leaf sheaths than on agar adjusted to corresponding water potentials. Another complicating factor in interpreting the effects of water potential from studies on growth media is the interaction of nutrient availability with water potential; fungi may tolerate lower water potentials in the presence of abundant nutrients than they can under nutrient-poor conditions (5).

Our method for maintaining natural substrata at controlled water potentials was shown to be adequate for determining quantitative response of *P. tritici-repentis* to moisture. Control of water potential is not as precise as that obtainable with osmotically adjusted media; the imprecision of the described method is primarily a shortcoming of measurement technology. Because water potential of the leaves only can be measured after they are removed from the humidity chambers, we can not be absolutely certain that we had an accurate assessment of the water potentials to which the fungus was exposed. Thus, the calculated depression in leaf water potential compared with glycerol water potential (0.3–0.9 MPa) cannot be proved conclusively; however, we are confident that it is essentially correct. The reason for this depression is unknown. Leaves, which are darker than other components in the jar, may absorb a small amount of long-wave radiation from the environment and, thus, have a slightly elevated temperature in comparison with the glycerol solution. Additionally, the required indirect method for measuring growth rate is less precise than direct observation of hyphal extension on agar plates. Despite its relative imprecision, however, the described method may be superior to osmotically adjusted agar growth media because matric (rather than osmotic) components of water potential predominate, and because the fungus is exposed to nutritional conditions that more closely represent those in nature. For this particular fungus, growth-rate response to matrically adjusted water potential was generally similar to that previously reported for osmotically adjusted water potentials (13).

The response of ascocarp production to water potential was very distinct; moisture of −0.5 MPa was required for maximum pseudothecia production, and essentially no ascocarps were produced below −2.5 MPa. Thus, the response of *P. tritici-repentis* to water potential was similar to that of *V. inaequalis*, which produced ascocarps from infected leaves held in an atmosphere of 100% RH, but not at 98% RH (−2.8 MPa at 24 C) (9). Within the range of permissive water potentials for ascocarp development by *P. tritici-repentis*, water stress affected ascocarp size. Whereas ascocarps produced on straw in the field are from 200–700 μm in size (authors, *unpublished observations*), those produced in our experiments at −1.5 MPa were predominantly <300 μm. Preliminary experiments indicated that small ascocarps produced under dry conditions would not increase in size under those conditions even after a 6–8-wk incubation. In a previous in vitro study (15), we found that small ascocarps produced under conditions of nutrient stress did not produce ascospores. In the current study, we did not determine whether small ascocarps produced under water stress could enlarge and produce ascospores if subsequently given high moisture. In *V. inaequalis*, a period of low moisture during pseudothecia development can have a deleterious effect on subsequent maturation, even after conditions conducive to ascocarp development are reestablished (14).

It is noteworthy that ascocarp production was not significantly affected by the presence of other microorganisms in the substrate. *P. tritici-repentis* produced no fewer ascocarps when competing with other fungi for the leaf substrate than when it was the sole occupant of presterilized leaves.

The growth rate experiments showed that *P. tritici-repentis* is capable of saprophytic growth in competition with other fungi. Thus, after pathogenesis, continued colonization of crop residue by this pathogen can be a significant aspect of its life history and the disease cycle. However, its reduced growth in nonsterile leaves, compared with its growth in sterilized leaves, indicates that it is quantitatively affected by competition of some nature.

These findings add to our knowledge of the relationship of *P. tritici-repentis* to the physical and, indirectly, the biotic aspects of its habitat. Periods of high moisture are required for ascocarp production. Although this fungus is capable of growth over a moderately wide range of water potentials (down to about −10 MPa), it is not unusually tolerant of low moisture. Other fungi, such as *Aspergillus* and *Penicillium*, are capable of growth at lower water potentials (7). In a previous descriptive study of fungal communities in no-till wheat straw (16), we found that the habitat most favorable for survival of *P. tritici-repentis* was in straw that was slightly above the soil; in field studies (W. Zhang and W. F. Pfender, *unpublished*) we found that this habitat is wet less frequently and for shorter time periods than straw resting on the soil surface. However, results reported here indicate that survival of *P. tritici-repentis* in above-soil straw is due not simply to a xerotolerant physiology, but it appears to be related indirectly to detrimental factors in the wetter on-soil habitat. Our current hypothesis is that the detrimental factors are other microorganisms exerting competitive effects on *P. tritici-repentis*. Although our results do show a partial suppression of growth in the presence of other organisms, we saw no suppression of ascocarp formation in the presence of these other saprophytes. In the field, ascocarp production is greatly reduced or absent in the on-soil habitat (W. F. Pfender and S. L. Wootke, *unpublished*). Presumably, this suppression of ascocarps in the field is due to a different community of organisms than was present on our nonsterile leaves in the laboratory tests described here. Indeed, the fungi we commonly observed on the plated nonsterile leaves of the present study were common to the natural community of above-soil straw (where *P. tritici-repentis* produces ascocarps) and were distinctly different from those common in on-soil straw (where *P. tritici-repentis* appears to be suppressed).

LITERATURE CITED

1. Alderman, S. C., and Beute, M. K. 1987. Influence of temperature, lesion water potential, and cyclic wet-dry periods on sporulation of *Cercospora arachidicola* on peanut. Phytopathology 77:960-963.
2. American Society for Testing and Materials. 1980. Maintaining constant relative humidity by means of aqueous solutions. Part 39:930-933.
3. Cook, R. J., and Duniway, J. M. 1981. Water relations in the life-cycles of soil-borne plant pathogens. Pages 119-139 in: Water Potential Relations in Soil Microbiology. J. F. Parr, ed. Special Publ. 9. Soil Sci. Soc. Am., Madison, WI. 151 pp.
4. Freund, R. J., and Minton, P. D. 1979. Regression Methods: A Tool for Data Analysis. Marcel Dekker, New York. 261 pp.
5. Griffin, D. M. 1981. Water potential as a selective factor in the microbial ecology of soils. Pages 141-151 in: Water Potential Relations in Soil Microbiology. J. F. Parr, ed. Special Publ. 9. Soil Sci. Soc. Am., Madison, WI. 151 pp.
6. Grogan, R. G., and Abawi, G. S. 1975. Influence of water potential on growth and survival of *Whetzelinia sclerotiorum*. Phytopathology 65:122-128.
7. Harris, R. F. 1981. Effect of water potential on microbial growth and activity. Pages 23-95 in: Water Potential Relations in Soil Microbiology. J. F. Parr, ed. Special Publ. 9. Soil Sci. Soc. Am., Madison, WI. 151 pp.
8. Hosford, R. M., Jr. 1982. Tan spot. Pages 1-24 in: Tan Spot of Wheat and Related Diseases Workshop. R. M. Hosford, Jr., ed. North Dakota State University, Fargo, ND. 116 pp.
9. James, J. R., and Sutton, T. B. 1982. Environmental factors influencing pseudothecial development and ascospore maturation of *Venturia inaequalis*. Phytopathology 72:1073-1080.
10. Knapp, E. B., Elliott, L. F., and Campbell, G. S. 1983. Microbial respiration and growth during the decomposition of wheat straw. Soil Biol. Biochem. 15:319-323.
11. Lacey, J. 1986. Water availability and fungal reproduction: Patterns of spore production, liberation and dispersal. Pages 65-86 in: Water, Fungi and Plants. P. G. Ayres and L. Boddy, eds. British Mycological

Society, Cambridge. 413 pp.
12. Magan, N. 1988. Effects of water potential and temperature on spore germination and germ-tube growth in vitro and on straw leaf sheaths. Trans. Br. Mycol. Soc. 90:97-107.
13. Norman, B. L. 1987. Selecting biological control agents to limit the saprophytic ability of *Pyrenophora tritici-repentis*. M.S. thesis, Kansas State University, Manhattan. 30 pp.
14. O'Leary, A. L., and Sutton, T. B. 1986. The influence of temperature and moisture on the quantitative production of pseudothecia of *Venturia inaequalis*. Phytopathology 76:199-204.
15. Pfender, W. F., and Wootke, S. L. 1987. Production of pseudothecia and ascospores by *Pyrenophora tritici-repentis* in response to macronutrient concentrations. Phytopathology 77:1213-1216.
16. Pfender, W. F., and Wootke, S. L. 1988. Microbial communities of *Pyrenophora*-infested wheat straw as examined by multivariate analysis. Microb. Ecol. 15:95-113.
17. Segur, J. B. 1953. Physical properties of glycerol and its solutions. Pages 238-334 in: Glycerol. C. S. Miner and N. N. Dalton, eds. Reinhold, NY. 460 pp.
18. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Co., Minneapolis, MN. 239 pp.