Growth of *Magnaporthe poae* and *Gaeumannomyces incrustans* as Affected by Temperature-Osmotic Potential Interactions

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ABSTRACT

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Two isolates of *Magnaporthe poae* and one isolate of *Gaeumannomyces incrustans* were grown at 20, 25, 30, and 35 C on a basal salts medium (−0.12 MPa) adjusted with KCl to obtain osmotic potentials from −0.12 to −2.35 MPa. Isolates of *M. poae* produced maximal growth at the highest osmotic potentials (−0.12 to −0.35 MPa) at 25 and 30 C. At 20 C, no significant ($P = 0.05$) shift in optimal growth was observed between −0.35 and −1.03 MPa. Significantly reduced growth of *M. poae* occurred at 35 C with an optimum between −1.03 and −1.47 MPa. Optimal growth of *G. incrustans* generally occurred at osmotic potentials between 0.23 and 0.68 MPa lower than that of the basal medium. Maximal growth at 20 C occurred between −0.80 and −1.3 MPa, whereas at 25 and 30 C maximal growth occurred between −0.35 and −0.80 MPa. There was no difference in growth of *G. incrustans* among any of the osmotic potentials tested at 35 C. *G. incrustans* produced more growth than the isolates of *M. poae* at all temperatures except 35 C. Based on these results, growth of *M. poae*, causal agent of summer patch of Kentucky bluegrass (*Poa pratensis*), would be restricted by drought at temperatures supraoptimal for the growth of Kentucky bluegrass, and summer patch is most likely to be severe at temperatures >25 C when moisture is not limiting.

Magnaporthe poae Landschoot & Jackson and *Gaeumannomyces incrustans* Landschoot & Jackson are two fungi associated with the roots of Kentucky bluegrass (*Poa pratensis* L.) and other grasses exhibiting symptoms of summer patch (5–7). Summer patch is one of two or more new diseases formerly included with Fusarium blight based on symptomatology (11).

In 1984, Smiley and Craven Fowler (12) reported the isolation, identification, and successful completion of Koch's postulates in the field with two new organisms found on Kentucky bluegrass exhibiting Fusarium blight symptoms. They identified these organisms as *Leptosphaeria korrae* Walker & Smith, the causal agent of the disease named necrotic ring spot by Worf et al (19), and *Phialophora graminicola* (Deacon) Walker, the causal agent of the disease they called summer patch. It was reported that summer patch occurred under the same cultural and environmental conditions and produced symptoms indistinguishable from those reported for Fusarium blight (12).

In 1987, Landschoot (5) conducted an extensive survey of the ectotrophic root-infecting fungi associated with the roots of Kentucky bluegrass and showed that isolates designated as *P. graminicola* and reported as the causal agent of summer patch by Smiley and Craven Fowler (12) were actually *M. poae*. The teleomorph (*M. poae*) produces an anamorph in the genus *Phialophora* that is morphologically and physiologically distinct in vitro from the true *P. graminicola*, whose teleomorph is *Gaeumannomyces cylindrosporus* Hornby, Slope, Gutteridge, & Sivanesan. It is now known that the organism that Smiley and others (12–15) referred to as *P. graminicola* actually is *M. poae* (5). Additionally, Landschoot (5) isolated and identified another previously unidentified ectotrophic fungus with a conidial state of *Phialophora* from the roots of plants that were in various stages of recovery from summer patch. Landschoot and Jackson (6) have named this organism *Gaeumannomyces incrustans*. *G. incrustans* was mildly pathogenic on both 8-wk-old and 7-yr-old Kentucky bluegrass (5). The importance of this fungus as a turfgrass pathogen requires further study (6).

Smiley et al (14) conducted studies on temperature and osmotic

potential effects on the growth of *M. poae* (reported as *P. graminicola*) in vitro; however, in those studies, temperature and osmotic potential were investigated individually, not as they interact. They assessed growth at various water potentials at one temperature and at various temperatures at one water potential. In instances where temperature and water availability have pronounced effects on the growth of the pathogen, it is very difficult to separate the relative importance of one variable without taking the other into account (10). Landschoot (5) conducted temperature studies on the in vitro growth of *G. incrustans*, but no water potential studies were conducted. The objective of our study was to investigate the effect of the interaction of temperature and osmotic potential on the growth of *M. poae* and *G. incrustans* in vitro.

MATERIALS AND METHODS

Fungal isolates. Two isolates of *M. poae* and one of *G. incrustans* were evaluated. The isolates of *M. poae* were designated AT and PA, and the isolate of *G. incrustans* was designated SS. Isolate AT of *M. poae* was obtained from the American Type Culture Collection in Rockville, MD, where it was designated as *P. graminicola* ATCC 60239. This isolate was deposited by R. W. Smiley, who isolated it from diseased Kentucky bluegrass roots in New York (12); it subsequently was identified as *M. poae* by Landschoot (5). Isolate PA of *M. poae* was collected from the roots of *Poa annua* L. exhibiting symptoms of summer patch on a golf course putting green in Glenn Dale, MD. Isolate SS of *G. incrustans* was collected from the roots of Kentucky bluegrass (cv. Sydsport) exhibiting symptoms of summer patch at the University of Maryland Turfgrass Research and Education Center in Silver Spring, MD. Both PA and SS were isolated by the procedure of Smiley et al (15). Identity of these isolates was confirmed by the induction of perithecia produced by pairing opposing mating types (obtained from Landschoot) on either side of surface-disinfested wheat seedling roots (17). Landschoot has confirmed that ascospore measurements and other morphological characteristics are within the range reported for these species (6,7). Cultures were maintained on potato-dextrose agar (PDA) in slants at 5–10 C. Inocula for this study were produced on PDA in petri dishes and consisted of disks (6 mm in diameter) cut aseptically with a cork borer from the advancing margin of actively growing colonies. These disks were placed in the center of petri dishes containing osmotically adjusted or basal media.

Osmotic potential-temperature treatments. A minimal salts agar medium (16) was adjusted to different osmotic potentials by the addition of KCl (18). This medium has a high osmotic potential of −0.12 MPa when unamended and has been used in similar studies (3,4,14,16). Other researchers have found that the growth response to media adjusted with various salts of the closely related fungus *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* and other unrelated fungi (*Phytophthora* spp. and *Fusarium culmorum* (Wm. G. Sm.) Sacc.) was a function of water potential and not of a specific ion (3,4,16). Therefore, only KCl was included for osmotic adjustment in this study. Based on preliminary studies, media of seven different osmotic potentials were prepared. The calculated osmotic potentials of these media at 25 C were −0.12, −0.35, −0.58, −0.80, −1.03, −1.47, and −2.35 MPa. Twenty milliliters of the appropriate medium was dispensed into each petri dish, and the dishes were left at room temperature for 3 days before seeding to allow evaporation of any free moisture. All seeded dishes of a given isolate × osmotic potential × temperature group were placed together in a plastic bag that was sealed with tape and placed in an inverted position in a dark incubator maintained at 20, 25, 30, or 35 C. Colony diameters were measured in two perpendicular directions, and the mean was recorded every 48 hr until hyphae from the treatment with the fastest growing colony had crossed the plate.

Experimental design and analysis. The three fungal isolates were subjected to seven osmotic potentials in four experiments differing in the temperature of the incubator. The experimental design within each incubator was a completely randomized, four-replicate, 3 × 7 factorial for the factors' isolate and osmotic potential. The experiments were analyzed by day as 3 × 7 factorials combined over incubators (8) by the SAS ANOVA procedure (SAS Institute, Inc., Cary, NC) (9). Mean separation for significant factors or interactions having two or more degrees of freedom was calculated with a Bayes least significance difference multiple

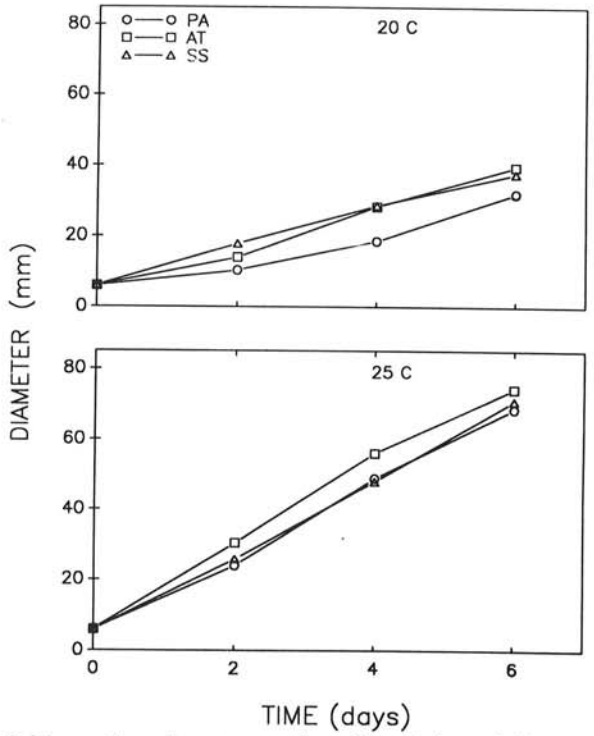

Fig. 1. Mean colony diameter over time of two isolates of *Magnaporthe poae* (PA and AT) and one isolate of *Gaeumannomyces incrustans* (SS) grown on a minimal salts medium (osmotic potential = −0.12 MPa) at 20 or 25 C. Each point represents the mean of four replicates.

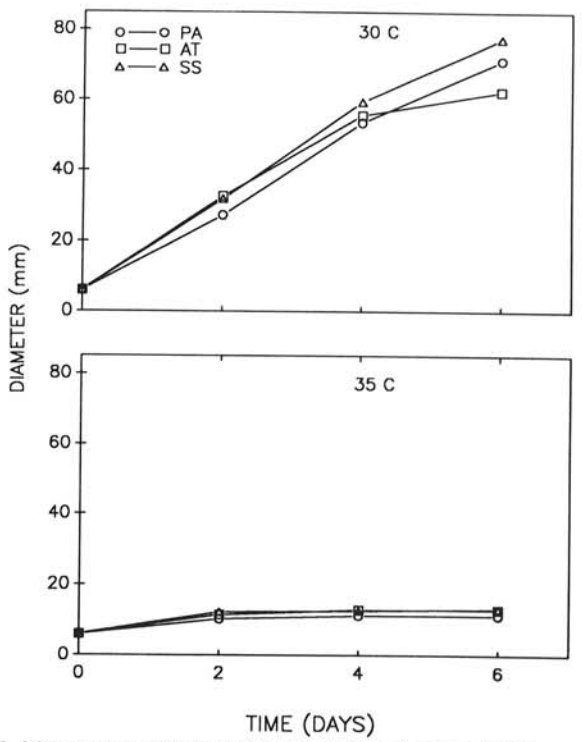

Fig. 2. Mean colony diameter over time of two isolates of *Magnaporthe poae* (PA and AT) and one isolate of *Gaeumannomyces incrustans* (SS) grown on a minimal salts medium (osmotic potential = −0.12 MPa) at 30 or 35 C. Each point represents the mean of four replicates.

comparison test. Data on the response to temperature and osmotic potential was highly correlated (r^2 ranged from 0.83 to 0.92) with baseline data collected from preliminary trials.

RESULTS

All control treatments, except 35 C, were in log growth at day 4; therefore, subsequent analyses were performed on day 4 data (Figs. 1 and 2). An analysis of variance of treatment colony diameters indicated that all factors and interactions differed significantly ($P = 0.0001$) (Table 1). We therefore examined the highest order interaction (temperature × osmotic potential × isolate) and those factors with the highest F values, as these factors accounted for the majority of variation within the experiment.

The factor responsible for the greatest amount of variation in colony diameter was temperature, followed by osmotic potential of the medium, and the fungal isolate (Table 1). In general, maximal growth occurred at 30 C for all isolates and osmotic potentials tested. Growth in response to osmotic potential averaged over isolates and temperature was greatest at −0.35 MPa, followed in decreasing order by −0.58, −0.12, and −0.80 MPa. Overall, the isolate SS of *G. incrustans* produced the most growth, followed by isolates AT and PA of *M. poae*. However, because there was a significant temperature × osmotic potential × isolate interaction, diameter means of each isolate were compared within each osmotic potential × temperature combination (Figs. 3 and 4).

TABLE 1. Abbreviated analysis of variance table for the variable colony diameter of two isolates of *Magnaporthe poae* and one isolate of *Gaeumannomyces incrustans* incubated on an osmotically adjusted basal salts medium for 4 days

Source of variation	df	F value	P
Temperature (Temp)	3	1741.72	0.0001
Isolate (Isol)	2	161.00	0.0001
Osmotic potential (Molal)	6	432.00	0.0001
Isol × Molal	2	7.10	0.0001
Temp × Isol	6	55.32	0.0001
Temp × Molal	18	96.44	0.0001
Temp × Isol × Molal	36	6.72	0.0001

At 20 C (Fig. 3), all isolates produced maximal growth at an osmotic potential lower than the unamended medium (−0.12 MPa). Growth of isolate AT of *M. poae* peaked at −0.35 MPa, whereas isolate PA produced maximal growth between −0.58 and −1.03 MPa. Growth of isolate SS of *G. incrustans* was inhibited at −0.35 and −0.58 MPa, when compared with the unamended basal medium, but then peaked between −0.80 and −1.03 MPa. All isolates produced maximal growth at osmotic potentials ≥ −1.03 MPa. Whereas the growth peak of PA was shifted to the more negative end of the scale, both isolates of *M. poae* produced somewhat similar growth.

Growth of all isolates at 25 C generally was greater than growth at 20 C, particularly at osmotic potentials greater than −1.5 MPa (Fig. 3). Maximal growth of isolate AT of *M. poae* occurred between osmotic potentials of −0.12 MPa (unamended basal medium) and −0.35 MPa; and as osmotic potential decreased further, so did the growth of AT. Isolate PA of *M. poae* exhibited a slight but nonsignificant peak in growth at −0.35 MPa. Growth of this isolate steadily declined with a further decrease in osmotic potential. Isolate SS of *G. incrustans* exhibited reduced growth at −0.12 MPa (unamended medium) compared with its growth peak, which occurred between −0.35 and −0.80 MPa. Only at osmotic potentials less than −1.03 MPa was growth less than that produced on the basal medium. Growth of *G. incrustans* was significantly greater ($P = 0.05$) than that of the isolates of *M. poae* in all instances except on basal medium.

Growth at 30 C (Fig. 4) of the isolates of *M. poae* generally declined with a decrease in osmotic potential. *G. incrustans* (isolate SS) exhibited a pronounced peak in growth between −0.35 and −0.58 MPa. All isolates produced their greatest overall growth at 30 C, with isolate SS of *G. incrustans* producing the most growth. This superior growth by SS was especially pronounced at the lower osmotic potentials.

The growth of all isolates was greatly reduced at 35 C (Fig. 4). Growth at 35 C was reduced even more than at 20 C at osmotic potentials greater than −1.0 MPa. Both isolates of *M. poae*

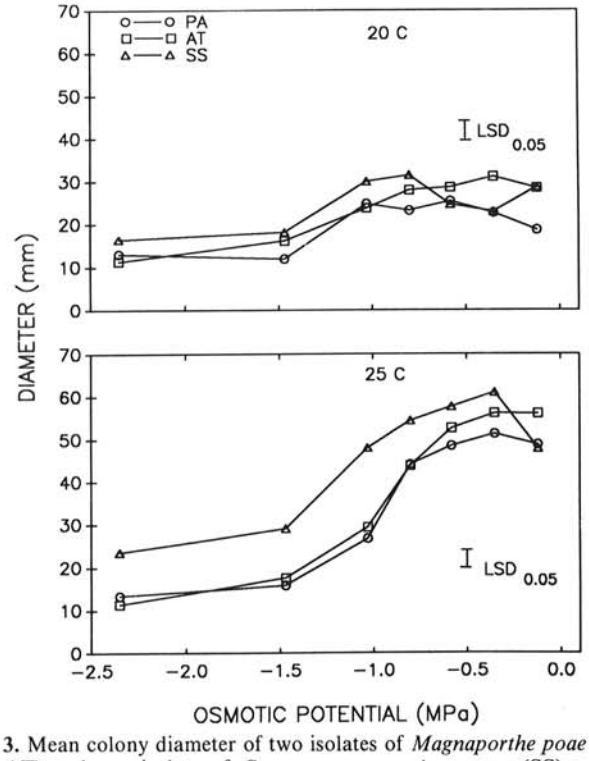

Fig. 3. Mean colony diameter of two isolates of *Magnaporthe poae* (PA and AT) and one isolate of *Gaeumannomyces incrustans* (SS) grown on an osmotically adjusted basal salts medium at 20 or 25 C at day 4. Each point represents the mean of four replicates.

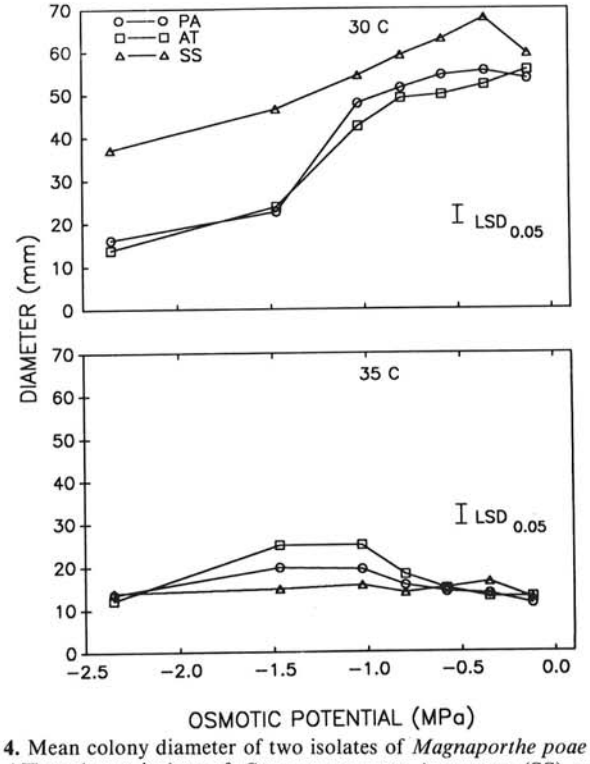

Fig. 4. Mean colony diameter of two isolates of *Magnaporthe poae* (PA and AT) and one isolate of *Gaeumannomyces incrustans* (SS) grown on an osmotically adjusted basal salts medium at 30 or 35 C at day 4. Each point represents the mean of four replicates.

exhibited a peak in growth at osmotic potentials of −1.03 to −1.47 MPa, whereas growth of *G. incrustans* peaked at −0.35 MPa. Growth of all isolates was very similar at osmotic potentials greater than −0.80 and at −2.35 MPa. However, at −1.03 and −1.47 MPa, the diameter of AT was significantly ($P \geq 0.05$) greater than the diameter of PA, which was significantly greater than that of SS. This was the only temperature treatment where growth of *G. incrustans* was less than that of the isolates of *M. poae*.

DISCUSSION

A significant temperature × osmotic potential × isolate interaction occurred in the growth of *M. poae* and *G. incrustans*. At temperatures of 25 and 30 C, the growth of isolate AT of *M. poae* decreased with a decrease in osmotic potential; however, at 35 C, maximal growth occurred at more negative osmotic potentials (−1.47 to −1.03 MPa). At 20 C, there was an apparent shift in the growth peak, but this was not significantly different ($P = 0.05$) from growth on the unamended basal medium. Although growth of isolate PA of *M. poae* differed slightly from that of isolate AT, it exhibited a very similar overall pattern of growth. *G. incrustans*, however, showed a consistent pattern of maximal growth at osmotic potentials lower than the basal medium, except at 35 C, where there was no significant difference ($P = 0.05$) in growth at any osmotic potential. These results are consistent with previous studies with the related organism *G. g. tritici* and two species of *Fusarium* (3). Cook and Christen (3) demonstrated that a lower osmotic potential was required for maximal growth of these pathogens at a higher temperature. The shift to a lower optimal water potential for growth at higher temperatures may be an adaptive mechanism in fungi, because high temperatures and dry conditions generally occur simultaneously (3).

Smiley et al (14) and Landschoot (5) conducted studies on the effects of temperature on growth of *M. poae* (also reported as *P. graminicola*) and *G. incrustans*. In those studies, all isolates were grown on half-strength PDA, whose osmotic potential was reported as −0.3 MPa (14). Smiley et al (14) found that maximal growth of their isolates of *M. poae* (reported as *P. graminicola*) occurred at 27–31 C. Landschoot (5) found that optimal growth of three isolates of *M. poae* (including ATCC 60239) occurred at 30 C, whereas two other isolates produced maximal growth at 35 C. Landschoot (5) also found that one isolate of *G. incrustans* grew optimally at 25 C, whereas another isolate exhibited optimal growth at 30 C. The response to temperature of our isolates followed the same pattern.

In earlier studies, Landschoot (5) and Smiley et al (14) showed that growth rates varied among isolates within a species. This variability also was demonstrated in the studies reported herein. Cook (3) notes, however, that because the optimal temperature for growth can vary with osmotic potential of the medium, it may become necessary to reassess the presently accepted temperature optima of pathogens by use of agar media osmotically adjusted to ranges expected in nature.

Smiley et al (14) conducted studies on the growth of two isolates of *M. poae* (reported as *P. graminicola*) with the same minimal salts basal media at various osmotic potentials at 20 C. They found that maximal growth occurred at the highest osmotic potential tested (−0.1 MPa) and was reduced to 50% of maximum by potentials of −0.6 MPa or less. Growth at approximately −0.3 MPa was nearly equal to growth at −0.1 MPa. Our results were different from those of Smiley et al (14). At 20 C, isolates AT and PA produced maximal growth at osmotic potentials lower than −0.12 MPa. Further, instead of a 50% reduction in growth at −0.6 MPa, the isolate AT exhibited growth at −0.6 MPa equal to growth on unamended media, and isolate PA exhibited significantly ($P = 0.05$) greater growth than was measured on unamended basal medium. A significant ($P = 0.05$) reduction in growth was demonstrated only at osmotic potentials lower than −1.03 MPa. This disparity was attributed to physiological differences among isolates.

Summer patch occurs only during the summer when air, thatch, and soil temperatures are high (11). Smiley and Craven Fowler (12) observed the presence of ectotrophic hyphae on roots, stolons, and rhizomes of Kentucky bluegrass before symptom development. Summer patch symptoms did not develop until the imposition of summer stress conditions. *M. poae* grew through Kentucky bluegrass sod at 21–24 C, but disease development was slow at these temperatures (13). Summer patch severity was amplified by raising soil temperatures to 29 or 30 C (13). This is the same temperature range that is optimum for the growth of *M. poae* in vitro (Figs. 3 and 4).

Cook (2) suggested that drought may affect plant diseases caused by soilborne fungi by affecting growth of the pathogen, pathogen-antagonist interactions in the rhizosphere, or host-pathogen interactions. Based on our in vitro research, it seems unlikely that drought would be involved in increasing the development of summer patch by enhancing growth of the pathogen. Drought, however, may alter pathogen-antagonist interactions or adversely affect the host's defense mechanisms. Severe summer patch occurs at soil temperatures of 25–30 C (13). In vitro growth of our isolates of *M. poae* decreased as osmotic potential became more negative at 25 and 30 C. For example, growth of isolate AT was reduced significantly at −0.80 MPa, compared with growth on unamended basal medium, and was reduced to less than 50% of maximum at −1.47 MPa at 25 and 30 C. Because root growth of Kentucky bluegrass is limited by temperatures in excess of 22 C, higher soil temperatures could provide the pathogen a competitive advantage over the host (14). Growth of Kentucky bluegrass remains unstressed if irrigated when soil water potentials drop to approximately −0.045 MPa and is considered by some researchers to be under water stress at −0.40 MPa (1). Because *M. poae* is capable of growth at osmotic potentials of −0.80 MPa at temperatures ranging from 20–35 C, the fungus could gain an advantage over the host at water potentials less than −0.40 MPa.

The role that *G. incrustans* plays in the development of summer patch is unclear. Although *M. poae* is most frequently isolated from plants affected by summer patch, Landschoot (5) reported that *G. incrustans* is most frequently isolated from patches in various stages of recovery. Some of Landschoot's (5) isolates were obtained from turf exhibiting a decline in quality but not necessarily showing patch symptoms. *G. incrustans* apparently is only mildly virulent on Kentucky bluegrass (5). Perhaps its faster growth at lower water potentials gives this less virulent pathogen a competitive advantage only under conditions less favorable for the growth of the host and the more virulent *M. poae*.

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