Impact of Temperature, Osmotic Potential, and Osmoregulant on the Growth of Three Ectotrophic Root-Infecting Fungi of Kentucky Bluegrass

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ABSTRACT

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Two isolates each of *Magnaporthe poae*, *Gaeumannomyces incrustans*, and *Leptosphaeria korrae* were grown at 25°C in liquid shake culture in minimal salts medium (–0.12 MPa) or minimal salts medium adjusted to –0.5 MPa with KCl, MgCl₂, or polyethylene glycol (PEG). Fungal dry weight of all three species was greater in minimal salts medium amended to –0.5 MPa with MgCl₂ than in nonamended medium, and dry weight in medium amended with PEG was not different from dry weights in nonamended medium or medium amended with KCl. Fungi were incubated at varying temperatures on a minimal salts solid-agar medium (–0.12 MPa) adjusted to osmotic potentials ranging from –0.5 to –5.0 MPa with KCl or MgCl₂. Optimum growth of *M. poae*, *G. incrustans*, and *L. korrae* on nonamended medium occurred at 30, 30, and 25°C, respectively. At optimum temperatures for each species, fungal growth was greatest at the higher osmotic potentials tested (–0.5 to –1.0 MPa) and decreased in a linear manner as osmotic potential decreased. In most cases, growth was detected at the lowest osmotic potential measured (–5.0 MPa). The relationship of fungal growth to osmotic potential depended on both temperature and osmoregulant. At temperatures optimal or nearly optimal for fungal development, the growth of all three fungi declined more rapidly with decreasing osmotic potential when grown on medium amended with MgCl₂ than on medium amended with KCl. At the highest temperature evaluated for growth of *M. poae* and *L. korrae* (35 and 30°C, respectively), growth on medium amended with KCl was curvilinear and peaked at osmotic potentials of –2.5 to –3.0 MPa. Furthermore, between osmotic potentials of –2.0 and –5.0 MPa, *M. poae* grew best at 35°C. When maintained on nonamended minimal salts medium (–0.12 MPa) in liquid culture at 25°C or on nonamended solid-agar medium at temperatures optimal for growth, *M. poae* grew at a faster daily rate than *L. korrae*.

Patch diseases, caused by ectotrophic root-infecting fungi, represent a newly recognized, destructive group of turfgrass diseases. Since the late 1970s, there has been a rapid expansion of knowledge pertaining to this group of fungi. As a result, several species of ectotrophic fungi have been implicated in patch disease development (13). The influence of environmental factors, particularly soil moisture, on disease development is still poorly understood, however.

Leptosphaeria korrae J.C. Walker & A.M. Sm. is the causal agent of necrotic ring spot, a disease of *Poa* and *Festuca* species (19, 26). This pathogen also is associated with spring dead spot, a patch disease of bermudagrass (*Cynodon dactylon*) (13). *L. korrae* infects turfgrass roots and crowns during cool, wet weather (20). *Magnaporthe poae* Landschoot & Jackson, originally identified as *Phialophora graminicola* (Deacon) J. Walker (19), is the causal agent of summer patch (15). Summer patch is a devastating root and crown rot of both *Poa* and *Festuca* species; infection typically begins during late spring during warm, wet weather (20). *Gaeumannomyces incrustans* Landschoot & Jackson (14) is a pathogen of fine fescue (12) and possibly other cool-season grasses (14). This fungus grows optimally at 30°C (16).

Utilizing half-strength potato dextrose agar (PDA), Smiley et al. (21) reported that *M. poae* and *L. korrae* attained an optimum rate of growth in vitro at 27 to 31 and 20 to 25°C, respectively. They further determined that the maximum growth of *M. poae* (reported as *P. graminicola*) and *L. korrae* at 20°C occurs at a high osmotic potential (–0.1 MPa) (21). For *M. poae*, growth was reduced by 50% at –0.6 MPa and was completely inhibited at –2.2 MPa, whereas growth of *L. korrae* was reduced by 50% at –2.5 MPa and was completely inhibited at –7.0 MPa. Because the osmotic studies were conducted at only one temperature (20°C), it is not possible to ascertain from this research the osmotic potential(s) at which optimum growth of *M. poae* and *L. korrae* occurs in vitro.

The effects of temperature and osmotic potential on growth of *M. poae* and *G. incrustans* in vitro were investigated both independently and interactively, using KCl as an osmoregulant (9). Both temperature and osmotic potential regulated the growth of these fungi. At 25 and 30°C, growth of *M. poae* was greatest at –0.12 MPa and decreased as osmotic potential was reduced. At 35°C, isolates of *M. poae* produced maximal growth at lower osmotic potentials (–1.03 to –1.49 MPa). The growth response of *M. poae* to various osmotic potentials was temperature dependent. This type of study has not been conducted for *L. korrae*. Because Kackley et al. (9) used only one osmoticum (KCl) to adjust osmotic potential, the possible effect of osmoregulant on fungal growth was not addressed. Brownell and Schneider (3) demonstrated that osmoregulant affected the growth of *Fusarium oxysporum* on minimal nutrient medium amended with either MgCl₂ or KCl. If this phenomenon were true for ectotrophic fungi such as *M. poae*, *G. incrustans*, and *L. korrae*, then their growth on an amended basal medium could vary considerably at different osmotic potentials and temperatures.

The objectives of this study were to evaluate the effects of temperature and osmotic potential on the growth of *M. poae*, *L. korrae*, and *G. incrustans* in vitro. These parameters were examined alone and interactively to determine the optimum conditions required for growth of each fungus. Because solutes in growth medium may influence fungal growth through nutritional or fungitoxic means, three solutes were used as osmoregulants to determine the impact of osmotic potential on fungal growth.

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MATERIALS AND METHODS

Fungal isolates. Two isolates each of *M. poae* (American Type Culture Collection [ATCC], Rockville, MD, 64411 and 64412), *G. incrustans* (ATCC 64416 and 64417), and *L. korrae* (PJL1 and PJL2) were maintained on half-strength PDA (Difco Laboratories, Detroit) at 25°C and subcultured regularly. Both cultures of *L. korrae* were isolated by P. J. Landschoot (The Pennsylvania State University, University Park) from Kentucky bluegrass exhibiting symptoms of necrotic ring spot.

Impact of osmoregulant on fungal growth in liquid culture. Osmoregulant treatments were prepared by amending a minimal salts medium with a low organic nutrient content and a baseline osmotic potential of –0.12 MPa (22) to –0.5 MPa, us-

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ing KCl, MgCl₂, or polyethylene glycol (PEG). Calibrated Peltier (Logan, UT) thermocouple psychrometers were utilized to determine the osmotic potential (2) of the nonamended and amended minimal salts media.

Each fungal isolate described above was grown for 4 days at 25°C on PDA plates overlaid with sterile cellophane. One 6-mm

Table 1. Summary of combined analysis of variance for growth (fungal dry weight) of *Magnaporthe poae*, *Gaeumannomyces incrustans*, and *Leptosphaeria korrae* at 25°C in liquid shake culture containing minimal salts medium (–0.12 MPa) or minimal salts medium adjusted to –0.5 MPa with MgCl₂, KCl, or polyethylene glycol

Source of variation	df	MS ^y	F ^z
Experiment (EXP)	1	3.1	3.3
Fungus (F)	2	131.0	140.3****
EXP × F	2	0.1	0.1
Fungal isolate (EXP × F)	6	5.0	5.3***
Osmoregulant (OS)	3	19.1	20.5****
OS × EXP	3	0.1	0.1
OS × F	6	1.3	1.4
EXP × F × OS	6	2.2	2.4*
Error	115	0.9	

^y Mean square.

^z **** = significant at $P = 0.0001$; *** = significant at $P = 0.001$; and * = significant at $P = 0.05$.

Table 2. Summary of combined analysis of variance for growth (mean colony diameter) of *Magnaporthe poae*, *Gaeumannomyces incrustans*, and *Leptosphaeria korrae* on minimal salts agar medium adjusted to osmotic potentials ranging from –0.5 to –5.0 MPa with MgCl₂ or KCl at 20, 25, 30, and 35°C (*M. poae* and *G. incrustans*) or 15, 20, 25, and 30°C (*L. korrae*)

Source of variation	df	MS ^y	F ^z
Fungus (F)	2	4,046	52.7****
Experiment (EXP)	1	212	2.8
F × EXP	2	146	1.9
Fungal isolate (EXP × F)	6	4,340	56.6****
Osmoticum (OS)	1	51,036	664.9****
OS × EXP	1	3,759	50.0****
OS × F	2	6	0.1
Temperature (T)	4	16,651	216.9****
T × F	8	16,088	209.6****
T × EXP	4	632	8.2****
T × OS	4	7,085	92.3****
Osmotic potential (OP)	5	58,041	756.2****
OP × F	10	2471	32.2****
OP × EXP	5	287	3.7**
OP × OS	5	1,592	20.8****
OP × T	20	3,867	50.4****
Error	1,946	77	

^y Mean square.

^z **** = significant at $P < 0.0001$; and ** = significant at $P < 0.01$.

disk was aseptically cut from the margin of each cellophane colony with a cork borer and ground with a sterile mortar and pestle in 1 ml of amended or nonamended minimal salts medium. The fungal slurry was added to 3 ml of the same medium in a sterile 15-ml test tube and incubated at 25°C on a shaker (180 rpm). After 8 (*M. poae* and *G. incrustans*) or 10 (*L. korrae*) days, fungal dry weight was determined by placing a preweighed filter-paper disk in a Buchner funnel and suctioning off the liquid medium. The disks were dried for 48 h at room temperature and reweighed. Each osmoregulant-fungal isolate combination was replicated four times, and the entire experiment was repeated.

Differences in fungal dry weight were analyzed as a factorial design by analysis of variance (ANOVA) with experiment, osmoregulant, fungus, and fungal isolate as main effects (Table 1). The impact of osmoregulant on the dry weight of each fungal isolate was assessed by one-way ANOVA, and the least-squares means separation procedure was used to analyze differences in treatment means. SAS (SAS Institute, Inc., Cary, NC) was used for all statistical computations.

Impact of temperature and osmotic potential on fungal growth. Each fungal isolate was grown for 5 days at 25°C on minimal salts (–0.12 MPa) solid-agar medium. Fungal plugs (6 mm) were removed with a sterile cork borer from colony margins and placed in the center of petri dishes containing 20 ml of nonamended minimal salts agar medium or on the same medium adjusted to different osmotic potentials (–0.5, –1.0, –2.0, –3.0, –4.0, or –5.0 MPa) with KCl or MgCl₂. Four replicate petri dishes for each isolate-osmoregulant-osmotic potential combination were prepared, and all plates were placed in a sealed plastic bag and incubated in an inverted position in the dark at one of four temperatures. *M. poae* and *G. incrustans* were incubated at 20, 25, 30, and 35°C. *L. korrae* was incubated at 15, 20, 25, and 30°C due to its lower temperature requirement for optimum growth (23). To assess fungal growth, two perpendicular measurements of colony diameter were recorded and averaged every 48 h.

Measurements continued for each species until mycelium in the treatment with the fastest growth had reached the margin of the plate. The experiment was repeated.

Differences in mean colony diameter after 6 (*M. poae* and *G. incrustans*) or 14 (*L. korrae*) days were analyzed as a factorial design by ANOVA, with experiment (trial), fungus, fungal isolate, osmoregulant, temperature, and osmotic potential as main effects (Table 2). The relationship between osmotic potential (independent variable) and fungal growth (dependent variable) for each osmoregulant at each temperature was quantified by linear regression analysis. For each fungus, slopes and intercepts of regression lines between isolates, osmoregulants, and temperatures were compared by analysis of the heterogeneity of slopes.

RESULTS

Effect of osmoregulant on fungal growth in liquid culture. Because fungal dry weight did not differ between experiments ($P > 0.05$) (Table 1), data from both trials were analyzed collectively. Dry weight of *M. poae*, *L. korrae*, and *G. incrustans* ATCC 64417 was greater in minimal salts medium amended to –0.5 MPa with MgCl₂ than in nonamended medium or medium amended with PEG (Table 3). Differences in fungal growth among osmoregulant treatments for *G. incrustans* ATCC 64416 were not significant. Growth of *M. poae* on medium amended with KCl did not differ from growth on medium amended with MgCl₂; the opposite was true for *L. korrae* and *G. incrustans* ATCC 64417. For all species, fungal dry weights in medium amended with PEG was not different from dry weights in nonamended medium or medium amended with KCl. For this reason, and because agar fortified with PEG does not solidify (3), PEG was deleted from further consideration. In liquid culture at 25°C, *M. poae* grew at a faster daily rate than *L. korrae* and *G. incrustans* (0.98, 0.52, and 0.56 mg/day, respectively) (Table 4).

Relationship of temperature, osmotic potential, and osmoregulant to fungal growth on agar medium. Mean colony diameter was not different between experiments ($P = 0.10$) (Table 2), so data from

Table 3. Dry weight of *Magnaporthe poae*, *Gaeumannomyces incrustans*, and *Leptosphaeria korrae* isolates grown at 25°C in liquid shake culture containing minimal salts medium (–0.12 MPa) or minimal salts medium adjusted to –0.5 MPa with MgCl₂, KCl, or polyethylene glycol (PEG)

Osmoregulant	<i>M. poae</i> ^y		<i>G. incrustans</i> ^y		<i>L. korrae</i> ^y	
	ATCC 64411	ATCC 64412	ATCC 64416	ATCC 64417	PJL1	PJL2
MgCl ₂	8.45 a	9.84 a	7.60 a	6.88 a	5.90 a	6.68 a
KCl	7.75 ab	8.65 ab	7.35 a	4.97 b	5.27 b	5.17 b
PEG	7.15 b	8.31 b	5.47 a	4.69 b	4.90 b	5.04 b
Nonamended medium ^z	7.59 b	8.04 b	5.25 a	4.23 b	5.07 b	5.34 b

^y Fungal dry weights (milligrams) were determined for each osmoregulant treatment 8 (*M. poae* and *G. incrustans*) or 10 (*L. korrae*) days after inoculation. Means within a column followed by the same letter are not significantly different ($P < 0.05$, least squares means).

^z –0.12 MPa.

both trials were combined prior to further analysis. After 6 days, the temperature that was optimal for growth of *M. poae* and *G. incrustans* on nonamended minimal salts agar medium (-0.12 MPa) was 30°C (84 and 69 mm, respectively) (Fig. 1). For both fungi, mean colony diameter at 35°C was reduced by approximately 80% and was intermediate at 20 and 25°C . Alternatively, growth of *L. korrae* after 14 days was greatest at 25°C and poorest at 30°C (63 and 11 mm, respectively) (Fig. 1). When maintained on minimal salts agar medium at an optimal temperature for growth, *M. poae* grew at a faster daily rate than the other fungi evaluated (Table 4).

Regardless of incubation temperature, growth of all fungal species on minimal salts agar medium amended with either KCl or MgCl_2 was affected by changes in osmotic potential in a linear or curvilinear manner (Figs. 2 through 4). In most cases, mean colony diameter was greatest between -0.5 and -1.0 MPa and decreased as osmotic potential decreased. At very low osmotic potentials (-4.0 to -5.0 MPa), fungal growth at all temperatures was minimal or nonexistent.

The relationship of fungal growth to osmotic potential depended on temperature and osmoregulant (Table 2; Figs. 2 through 4). Within each isolate of *G. incrustans* and *L. korrae* grown on medium amended with KCl or MgCl_2 , the y-intercept of regression lines differed with varying temperature ($P < 0.001$) based on analysis of the heterogeneity of slopes. Alternatively, y-intercepts did not differ for the two *M. poae* isolates grown at 25 or 30°C on medium amended with MgCl_2 ($P > 0.2$), nor did they differ for *M. poae* ATCC 64411 grown at 20 or 35°C on medium amended with KCl ($P = 0.4$). For both isolates of *M. poae*, mean colony diameter declined more rapidly with decreasing osmotic potential at 20 and 25°C when grown on medium amended with MgCl_2 than when grown on medium amended with KCl ($P < 0.04$) (Fig. 2). This same trend was observed at 30°C for *G. incrustans* ATCC 64416 ($P < 0.001$) (Fig. 3); at 20, 25, and 30°C for *G. incrustans* ATCC 64417 ($P < 0.05$); at 20°C for *L. korrae* PJJ1 ($P < 0.001$) (Fig. 4); and at 15, 20, and 25°C for *L. korrae* PJJ2 ($P < 0.05$).

When maintained on medium amended with KCl, maximum growth of *M. poae*

and *L. korrae* occurred at osmotic potentials of approximately -2.5 to -3.0 MPa at the highest temperatures tested (35 and 30°C , respectively) (Figs. 2 and 4). Furthermore, between osmotic potentials of -2.0 and -5.0 MPa, *M. poae* grew best at 35°C . Alternatively, the growth of *G. incrustans* at 35°C continued to decline with decreasing osmotic potential (Fig. 3). In comparison, growth of all three fungi on MgCl_2 -amended medium was poor.

DISCUSSION

The solute used to regulate osmotic potential in liquid shake culture had a significant impact on fungal dry weight. Although the growth of all three fungi on minimal salts medium amended with KCl or PEG did not differ from growth on nonamended medium, dry weights increased 11 to 63% when grown on medium amended with MgCl_2 . In a similar study, Brownell and Schneider (3) found that osmoregulant affected the growth of *F. oxysporum* on a basal medium amended with either MgCl_2 or KCl. Others have hypothesized that the growth response of ectotrophic root-infecting fungi, such as *G. graminis* var. *avenae*, *G. graminis* var. *graminis*, and *G. graminis* var. *tritici* (4,25), and unrelated fungi, including *F. culmorum* (6) and *Phytophthora* spp. (22), to medium osmotically adjusted with salts is a function of water potential (4,6) or osmotic potential (22,25) rather than a function of the specific ion utilized as an osmoregulant. These conclusions, however, were based on studies that used only KCl, NaCl, sucrose, or a mixture of KCl, NaCl, and Na_2SO_4 to adjust osmotic potential. The current study provides evidence that osmoregulant should be considered when evaluating the impact of osmotic potential on fungal growth.

The growth response of *M. poae* and *L. korrae* on nonamended minimal salts medium was similar when evaluated in liquid shake culture and on solid-agar medium.

Table 4. Average growth rates^y of *Magnaporthe poae*, *Gaeumannomyces incrustans*, and *Leptosphaeria korrae* on nonamended minimal salts medium (-0.12 MPa) in liquid shake culture at 25°C and on nonamended minimal salts medium (-0.12 MPa) at temperatures optimum for growth

Fungal species	Liquid culture (mg/day) ^z	Minimal salts medium	
		Optimum temperature ($^{\circ}\text{C}$)	Radial growth (mm/day) ^z
<i>M. poae</i>	0.98 a	30	7.00 a
<i>G. incrustans</i>	0.56 b	30	5.74 b
<i>L. korrae</i>	0.52 b	25	2.24 c

^y Fungal dry weight (milligrams) in liquid shake culture was measured at 8 (*M. poae* and *G. incrustans*) or 10 (*L. korrae*) days after inoculation. Colony radius on minimal salts medium was measured every 48 h from 2 to 6 (*M. poae* and *G. incrustans*) or 2 to 14 (*L. korrae*) days after inoculation.

^z Means within a column followed by the same letter are not significantly different ($P < 0.05$, least squares means). Each mean represents the average of two isolates of each fungal species for two experiments.

Fig. 1. Mean colony diameter ($\pm$ standard error) for two isolates each of (A) *Magnaporthe poae*, (B) *Gaeumannomyces incrustans*, and (C) *Leptosphaeria korrae* grown for 6 (*G. incrustans* and *M. poae*) or 14 (*L. korrae*) days on nonamended minimal salts agar medium (-0.12 MPa) at four temperatures. Each data point represents the mean of two experiments for each isolate.

Even though liquid culture experiments were performed at a temperature that was sub-optimal (25°C) for growth of *M. poae*, the average growth rate of this fungus was greater than the average growth rate of *L. korrae*. On the minimal salts solid-agar medium (-0.12 MPa), the average growth rate of *M. poae* and *G. incrustans* at the optimal temperature of 30°C was 7 and 5.7 mm/day, respectively. These rates are 20 and 27% less than those reported by Landschoot and Jackson (16) (8.8 and 7.8 mm/day for *M. poae* and *G. incrustans*, respectively) who used a more nutrient-rich growth medium containing half-strength PDA (-0.3 MPa) at 30°C. The average growth rate of *G. incrustans* was greater than the average growth rate of *L. korrae* on solid minimal salts medium (-0.12 MPa) but was similar when evaluated in liquid shake culture.

In the current study, the relationship between fungal growth and osmotic potential on solid mineral salts medium was linear or curvilinear. At most of the temperatures tested, mean colony diameter was greatest at the highest amended osmotic potential (-0.5 MPa) and declined with decreasing osmotic potential. The relationship between growth and osmotic potential depended on both osmoregulant and temperature. As osmotic potential decreased (from -1.0 to -5.0 MPa), $MgCl_2$ was more inhibitory to the growth of *M. poae* and *G. incrustans* than KCl at varying temperatures. In a similar study, Brownell and Schneider (3) reported that the growth of *F. oxysporum* was inhibited at osmotic potentials less than -2.0 MPa on solid-agar medium amended with $MgCl_2$ compared to solid medium amended with KCl. At relatively low os-

motric potentials, KCl permits fungal growth to continue because potassium ions are absorbed easily by fungal cells and may function as a compatible cytoplasmic osmoticum with low toxicity (8). Because growth was inhibited on medium amended with $MgCl_2$ at osmotic potentials less than -2.0 MPa, Brownell and Schneider (3) theorized that $MgCl_2$ is either not absorbed by hyphal cells or accumulates to toxic concentrations at low osmotic potentials. In the current study, the inhibitory effect of $MgCl_2$ was even more pronounced for *M. poae* and *G. incrustans* when they were maintained at a temperature that was least optimal (35°C) for growth.

When KCl was used as the osmoregulant and temperatures were optimal or nearly optimal (25 to 30°C) for growth of *M. poae*, colony diameter was greatest at the

Fig. 2. Impact of osmotic potential on fungal growth of *Magnaporthe poae* (isolates ATCC 64411 and 64412) grown for 6 days at 20, 25, 30, and 35°C on minimal salts agar medium amended with $MgCl_2$ or KCl. Each data point represents the mean of two experiments, and all regression relationships are significant at $P < 0.01$.

highest osmotic potential (−0.5 MPa) and was reduced approximately 50% at an osmotic potential of −2.5 MPa. Using the same basal salts medium at 25 and 30°C, Kackley et al. (9) determined that maximal growth of their *M. poae* isolates occurred between −0.12 and −0.35 MPa and was reduced 50% at osmotic potentials of −1.03 and −1.5 MPa. It is not unusual for isolates within a species to vary in tolerance to extremes in temperature and osmotic potential (21). Therefore, this disparity could be attributed simply to physiological differences among isolates of *M. poae*.

At temperatures that are supraoptimal for growth of *M. poae* and *L. korrae* (35 and 30°C, respectively), maximal growth of both species on medium amended with KCl was observed at −2.5 to −3.0 MPa (Figs. 2 through 4). The relationship between growth

and osmotic potential was curvilinear and represented a significant shift in the osmotic optima for these two pathogens. A similar shift was not observed for *G. incrustans* at 35°C. These results are consistent with previous studies that investigated the impact of temperature and osmotic potential on the growth of ectotrophic fungi (9,25); however, this is the first report of such a phenomenon for *L. korrae*.

Cook and Christen (4) working with *G. graminis* var. *tritici* and two species of *Fusarium* demonstrated that progressively drier conditions (i.e., lower water potentials) were required for maximal growth of these pathogens when the incubation temperature was raised from 10 to 35°C. This response was presumed to be an adaptive mechanism by these fungi in response to high temperature and low moisture conditions, which often

occur during summer, when soil surface temperatures may exceed 35°C. Wong (25) observed a similar adaptation for *G. graminis* var. *avenae*, *G. graminis* var. *graminis*, and *G. graminis* var. *tritici*. The shift in maximal growth of *M. poae* and *L. korrae* as substrates become drier may enhance the survival of these fungi when temperatures approach their upper limit for growth.

Growth responses recorded for *M. poae*, *G. incrustans*, and *L. korrae* in the current study appear to match their ecological adaptation in nature. *L. korrae* is capable of growth at higher temperatures and lower water potentials than its Kentucky bluegrass host (21). This supports the observation that necrotic ring spot occurs in the field over a wide range of environmental conditions and that foliar symptoms often

Fig. 3. Impact of osmotic potential on fungal growth of *Gaeumannomyces incrustans* (isolates ATCC 64416 and 64417) grown for 6 days at 20, 25, 30, and 35°C on minimal salts agar medium amended with $MgCl_2$ or KCl. Each data point represents the mean of two experiments, and all regression relationships are significant at $P < 0.01$.

are intensified by drought (21,26). For example, the growth of *L. korrae* in vitro on medium amended with KCl was not reduced by 50% until the osmotic potential approached -3.0 and -3.5 MPa at 20 and 25°C, respectively.

Summer patch, caused by *M. poae*, is most severe in the field during hot (30 to 35°C), wet weather or when high temperatures are followed by periods of heavy rainfall (20). In vitro *M. poae* grew best at 30°C on nonamended and KCl-amended minimal salts agar medium. Moreover, at this temperature on basal medium amended with either KCl or $MgCl_2$, optimum colony diameter was observed at -0.5 MPa and decreased with a decrease in osmotic potential. Because the growth of Kentucky bluegrass roots is limited by temperatures between 20 and 25°C and essentially stops

at temperatures higher than 26°C (1), high soil temperatures could provide *M. poae* with a competitive advantage over this host (21). Because *M. poae* and *L. korrae* have the ability to grow at low water potentials (less than -2.0 MPa) and supraoptimal temperatures (35 and 30°C, respectively), this advantage may be enhanced further when turf is subjected to moderate drought stress. This is supported by the observation that *M. poae* grew best between -2.5 and -3.0 MPa at 35°C on medium amended with KCl. In cooler (less than 20°C), moist soils, however, the host often is favored, and summer patch development is often minimal or nonexistent (21). Between 20 and 25°C, however, symptoms of this disease usually are more severe on turf subjected to moderate drought stress (-0.4 MPa) (10, 11,18).

During periods of drought stress, plants are capable of drawing water from deep in the soil profile, whereas ectotrophic pathogens often are exposed to restrictive water potentials near the soil surface, where evapotranspiration is high (5). As a result, these fungi must be able to adjust to a drier soil environment. Griffin (7) suggested that although soil water potential may not directly affect the population of a pathogen, it may adversely affect the host's ability to prevent infection. Therefore, as the environment becomes unfavorable for growth of the host, various physiological processes, such as disease resistance, may be impaired (5). This could significantly reduce the degree of host resistance and, thus, enhance disease development.

Although *G. incrustans* has been isolated from the roots of plants exhibiting

Fig. 4. Impact of osmotic potential on fungal growth of *Leptosphaeria korrae* (isolates PjL1 and PjL2) grown for 14 days at 15, 20, 25, and 30°C on minimal salts agar medium amended with $MgCl_2$ or KCl. Each data point represents the mean of two experiments, and all regression relationships are significant at $P < 0.01$.

foliar symptoms of summer patch (16), a proven association between *G. incrustans* and this disease has not been established. Pathogenicity has been confirmed in the growth chamber with *Poa annua* (16) and in growth-chamber and field trails with selected species and cultivars of fine fescues (12); however, *G. incrustans* does not appear to be as pathogenic to turf as *M. poae* (12,13).

It is apparent that temperature plays an important role in the growth of fungi. Most of the initial work describing temperatures optimal for the growth of ectotrophic root-infecting fungi (16,21) was conducted on nonamended nutrient agar at an osmotic potential of approximately -0.3 MPa. White et al. (24), assessing the relationship between leaf water potential and soil moisture for three tall fescue selections, found that the water potential of leaf tissue ranged from -0.6 to -3.0 MPa depending on the degree of soil moisture stress. Papendick and Cook (17) found a similar range of water potential (-2.0 to -3.5 MPa) within the leaves and roots of mature wheat tissue. Although the temperature optima reported for the growth of ectotrophic fungi on nonamended media at high osmotic potentials adds to our general understanding of these fungi, it may not accurately reflect their growth over the full range of water potentials encountered during the host-pathogen process (4,25). Therefore, to adequately evaluate the impact of temperature and osmotic potential on the growth of ectotrophic pathogens of turf, studies must continue to investigate these factors, both independently and interactively. Moreover, it is apparent that the osmoregulant must be considered when evaluating the effect of osmotic potential on fungal growth.

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