Mycologia, 88(5), 1996, pp. 809–815.

Some effects of water potential upon endophytic *Acremonium* spp. in culture

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Abstract: *Acremonium coenophialum, A. starrii,* and *A. typhinum* were grown on potato dextrose agar (PDA) amended with KCl (PDA + KCl) or sucrose (PDA + S) to attain a wide range of osmotic water potentials. Radial growth was greatest between −0.3 MPa (PDA without amendment) and −3.0 to −4.0 MPa, below which growth diminished to near zero at −8 MPa. *Acremonium* isolates grew well on corn meal agar (CMA + KCl), but antibiotic and halo production were not detected on these media. Halos consisting of an unknown opaque deposit formed within the media around colonies of some isolates in PDA and in certain PDA + KCl combinations. Inhibition zones were not detected on media adjusted to a water potential below about −3.5 to −4.2 MPa. Aerial hyphae were sparse on CMA + KCl at −5.47 MPa or lower, facilitating visual observation of sporulation. Sporulation by some isolates occurred over the entire range of water potentials. Isolates varied in radial growth, production of inhibition zones, sporulation, and in production of halos within the media and these responses appear to be characteristic of the isolate rather than the species. Growth of an unknown *Acremonium* species from *Stipa robusta* was restricted at −2.33 MPa on PDA amended with sucrose.

Key words: Antibiotic, *Festuca, Hordeum,* sporulation, *Stipa,* water relations

INTRODUCTION

Acremonium endophytes of grasses belong to the section *Albo-lanosa* (Morgan-Jones and Gams, 1982) of the genus *Acremonium.* All live intercellularly, thus they depend upon the living host for nutrients and water, and they are subjected to the water potentials of the apoplast. They persist in seed as dormant hyphae or as vegetative hyphae in perennial hosts. Hyphae must, therefore, adapt to a wide range of water potentials that exist within the living host from seedling to dormant seed. Water potentials within mesophytic grasses in the field are usually lower than the water potential of potato dextrose agar (−0.3 MPa, Sommers et al., 1970), and in dryland wheat at heading or shortly thereafter they range downward to at least −3.0 to −4.0 MPa (Cook and Duniway, 1981), and even to −6.0 MPa in maturing seed (Sung and Cook, 1981). In nature, wild grasses such as *Hordeum* and *Stipa* spp. are subjected to wide ranges of water potentials, and the endophytes, in order to survive, must adapt to these changes, down to dormant hyphae in dried seed, in which the water potential may drop to −50.0 MPa (Papendick and Campbell, 1975). We consider water potentials down to about −4.0 MPa pertinent to the active life of these fungi within their hosts.

Most mesophytic fungi grow fastest between about −0.6 and −3.0 MPa (Cook and Papendick, 1972), below which growth declines, usually linearly, at lower water potentials. Richardson et al. (1993) grew *A. coenophialum* on corn meal malt agar using glycerol to regulate water potential and found the optimum at about −0.4 MPa and no growth at −7.2 MPa.

Previous workers (Bacon, 1988; Christensen et al., 1991) cautioned that *Acremonium* isolates can change quickly in culture, especially in regard to production of secondary metabolites and that physiological studies are best made with fresh isolates. Secondary metabolites produced by *Acremonium* endophytes include a wide spectrum antifungal antibiotic(s) (White and Cole, 1985, 1986; Siegel and Latch, 1991), arabitol (Richardson et al., 1993), and a cloudy opaque deposit of some kind that forms a continuous halo about the colony (White and Cole, 1985; FIG. 8 in White et al., 1993). We studied the effect of water potential upon radial growth, inhibition zones, sporulation, and on production of an opaque halo-forming substance.

MATERIALS AND METHODS

The fungal isolates were grown on Difco potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) and corn meal agar (CMA), and 2% water agar made with deionized water. Water potential in the

Accepted for publication April 10, 1996.

TABLE I. Isolates of endophytic *Acremonium* spp.

Fungal species	Culture designation	Host of origin
A. coenophialum Morgan-Jones & Gams	GI 307	*Festuca arundinacea*
A. starrii White & Morgan-Jones	269406, 269406A	*Hordeum bogdanii*
A. starrii White & Morgan-Jones	401386	*Hordeum brevisubulatum*
A. typhinum Morgan-Jones & Gams	314696, 440413	*Hordeum bogdanii*
A. typhinum Morgan-Jones & Gams	440420	*Hordeum brevisubulatum*
Acremonium sp.	4-3, 5-3, 7-5, 9-3, 10-2	*Stipa robusta*

media was adjusted by incorporating KCl or sucrose (S) prior to sterilization in accordance with tables of Robinson and Stokes (1959) or Harris (1981). Approximately 25 mL of medium was added per 85 mm-diam plastic Petri dish.

Isolates of *Acremonium* spp. from *Festuca arundinacea* Schreb, selection GI 307; *Hordeum bogdanii* Wilensky, PI 269406, PI 314696, and PI 440413; *H. brevisubulatum* (Trin.) Link, PI 401386 and PI 440420; and five isolates from *Stipa robusta* (Vasey) Scribner were studied (TABLE I). They have been deposited in the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852. PI (plant introduction) numbers refer to specific accessions of grasses maintained by the U.S. National Plant Germplasm System.

All cultures used as inoculum were maintained on PDA at 22 C. Cultures of *Acremonium* isolates were 30–60 d old at the time of use and inoculum (blocks 1–2 mm square) was obtained from the margins of the colonies. All dishes were sealed with Parafilm to minimize water loss. In studies with incubation periods of at least 20 d, dishes were incubated within the plastic sleeves in which they were shipped and the sleeves were closed with rubber bands.

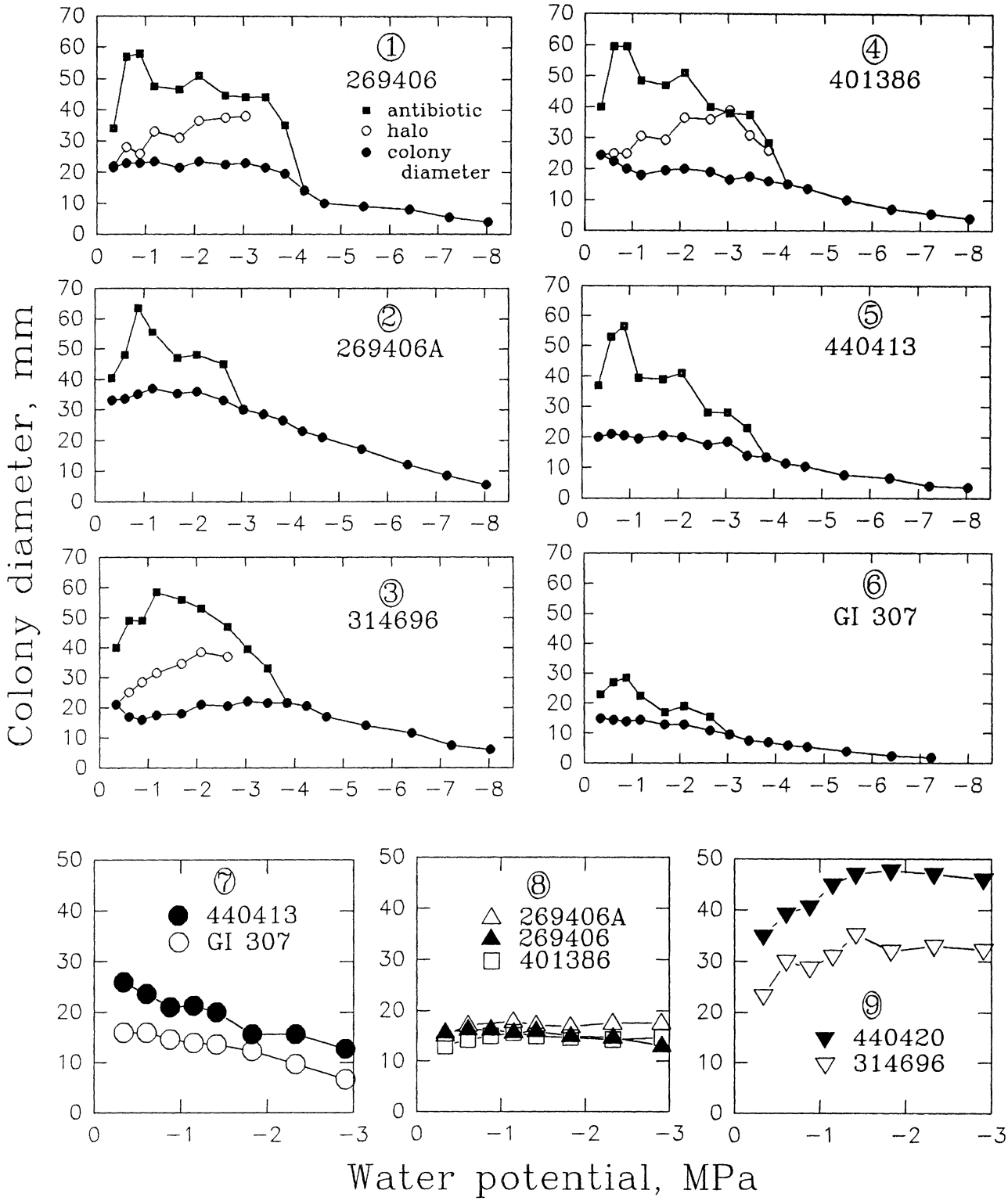
Cultures were bioassayed for inhibition zone production by spraying the surface of the agar with an aqueous suspension of yeast cells (*Rhodotorula rubra* (Demme) Lodder, ATCC 9540) using a DeVilbis hand atomizer when the *Acremonium* cultures were 20 or more d old. On favorable media the yeast lawn was adequate for measuring inhibition zones in 2–3 d, but longer incubation was needed on relatively unfavorable (dry) media. Growth rate studies had from 4–10 replicates; inhibition zone studies had two to four replicates. All growth-rate experiments were repeated. Significant departures from these general materials and methods will be presented with individual experiments. All data points in the graphs were analyzed for their standard deviation and only a few variations were greater than ± 1.5 mm per colony.

To check for sporulation on CMA and PDA at different water potentials, cultures were sampled for spores at 35 and at 80 d. Mycelial discs 1 cm diam were shaken for 30 s with a Vortex mixer in 10 mL of sterile deionized water in culture tubes 23 mm diam. The wide culture tubes provided free movement of the mycelial discs during shaking. One mL of undiluted spore suspension was spread over the surface of PDA in plastic Petri dishes and the free water was removed by evaporation in a laminar flow hood. After 5 d at 23 C all germlings between parallel lines drawn 3 mm apart in two perpendicular sets on the bottom of the Petri dish were counted using a dissecting microscope.

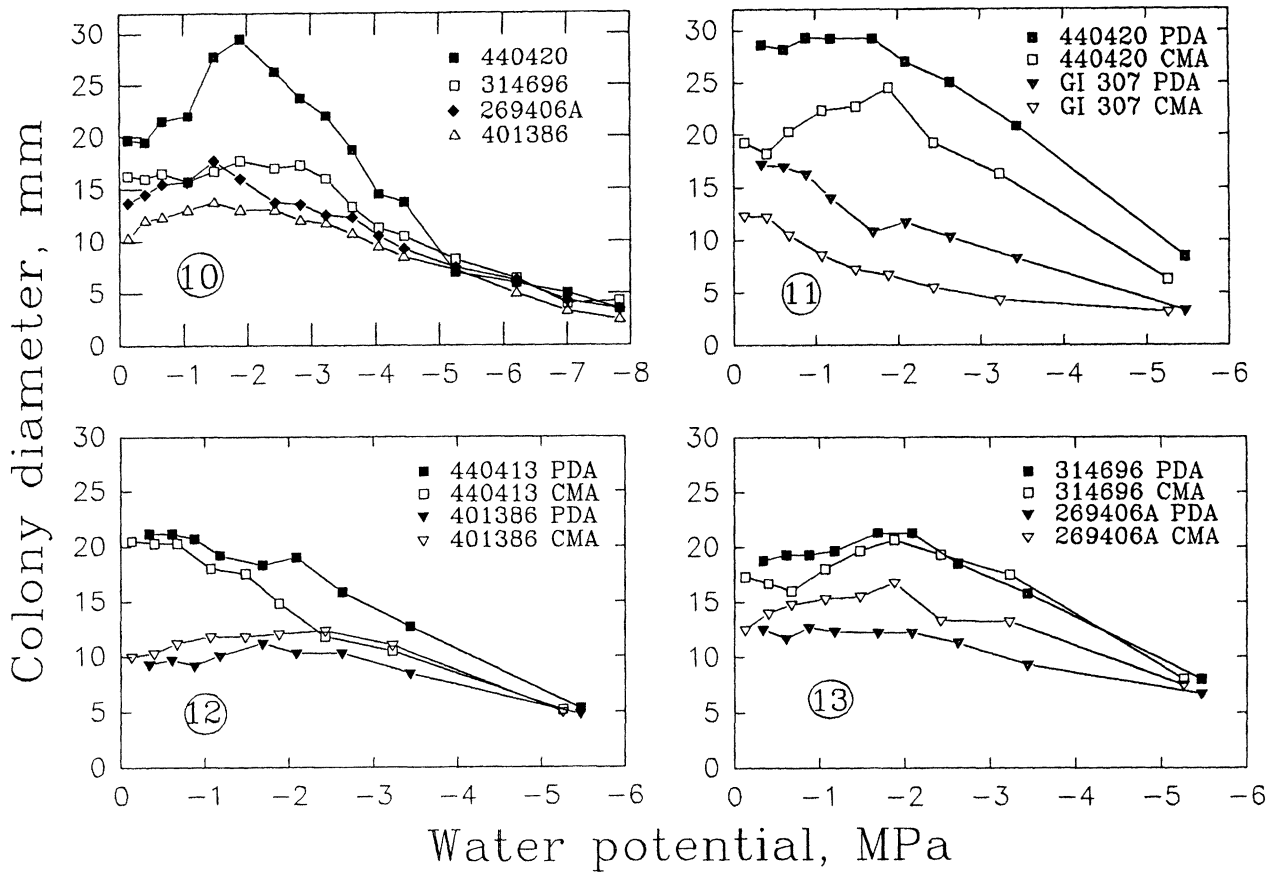
RESULTS

Colony diameters on PDA and KCl at 22.5 C in the dark after 33 d were relatively unresponsive over a wide range of osmotic water potentials, with no marked optimum in any isolate (FIGS. 1–6). The diam of inhibition zones increased when the water potential was reduced to about −1 MPa but below −3 to −4 MPa inhibition zones were undetected (FIGS. 1–6). Halos in unamended PDA (−0.3 MPa) were faint or undetected, but they increased in diameter when osmotic water potentials were lowered to about −2 to −3 MPa, below which they were undetected (FIGS. 1,3,4). There is little or no relationship between halo production and inhibition zones (FIGS. 1,3,4). The above experiment was repeated, except that incubation was 26 d in the dark at 25 C and isolate 440420 was added. Isolate 440420 produced little antibiotic and no measurable halos.

The isolates formed three groups when grown on PDA + S at 25 C for 30 d at −03. to −2.9 MPa (FIGS. 7–9). Isolates 440413 and GI 307 grew more slowly even with moderate reductions in water potential (FIG. 7), isolates 269406, 269406A, and 401386 responded little to decreased water potential over this range (FIG. 8), and the growth of 314696 and 440420 increased with reduced water potential (FIG. 9). Using spore measurements isolates 440413 (FIG. 7), 314696 and 440420 (FIG. 9) are *A. typhinum* but



FIGS. 1–9. Influence of water potential upon colony diam, diam of inhibition zones, and of halos produced on potato dextrose agar. 1–6. Water potential adjusted with KCl, incubation in the dark at 22.5 C for 33 d. 7–9. Water potential adjusted with sucrose, inhibition in the dark at 25 C for 21 d.



FIGS. 10–13. Influence of water potential upon colony diameters of *Acremonium* isolates. 10. Incubated on corn meal agar + KCl in the dark at 25 C for 21 d. 11–13. Incubated on corn meal agar and potato dextrose agar on a laboratory bench for 26 d at 23 C.

they responded differently to reduced water potential on PDA + S. All inhibition zones were smaller on PDA + S than on PDA + KCl, and no halos were observed on PDA + S, causing us to use PDA + KCl extensively.

Four isolates were studied on corn meal agar modified with KCl to vary from −0.15 to −7.8 MPa (FIG. 10) and some were grown on both PDA and CMA in direct comparison (FIGS. 11–13). The experiment with only CMA was incubated at 25 C in the dark for 21 d; the CMA plus PDA trial was incubated 26 d. Isolate 440420 (FIGS. 10, 11) grew faster on CMA + KCl at near −2 MPa than on unamended CMA, and isolate 440420 was the only isolate whose response to CMA and PDA differed markedly (FIG. 11). The other isolates responded to varying water potential relatively similarly on both basal media. Inhibition zones and halos were produced only when PDA was the basal medium.

In estimating sporulation we found that high concentrations of KCl reduced the gel-forming ability of agar, making it soft and watery. In general, sporulation per unit of colony surface was higher on PDA than on CMA and spores were produced over the entire range of water potentials (TABLE II). These data are unreplicated, so only general observations are justified.

Of all the isolates studied, those from *S. robusta* were least able to tolerate sucrose as the osmoticum (FIGS. 14–17). All isolates produced clear inhibition zones on PDA + S at −0.9 and −1.4 MPa but on PDA + KCl inhibition zones were sporadic. Halos were not observed on unamended PDA, but they were produced by isolates 4–3 and 5–3 on PDA + KCl (FIGS. 14, 15), with the density of the halos, as visually estimated, diminishing as their diameters increased.

DISCUSSION

We expected the response to water potential by isolates within a species to be quite similar (Griffin, 1972, p. 97), but the response was more a characteristic of the isolate than of the species. Latch

TABLE II. Germlings, 10^3/ cm^2 of 35- and 80-d-old *Acremonium* cultures on corn meal agar (CMA) and potato dextrose agar (PDA) adjusted to differing water potential with KCl

Water potential, MPa	Isolates on CMA					
	269406		401386		440413	
	Incubation time (d)					
	35	80	35	80	35	80
−0.1	37	27	26	3	1	0
−0.4	41	18	18	4	3	2
−0.7	41	27	23	16	2	4
−1.1	35	24	27	11	4	3
−1.5	45	49	30	24	3	5
−1.9	45	23	26	17	5	6
−2.4	44	44	22	13	8	10
−3.2	49	41	19	23	6	11
−5.3	143	70	18	77	9	9
	Isolates on pDA					
−0.3	100	24	49	73	17	11
−0.6	76	0	51	42	12	5
−0.9	75	44	36	46	20	15
−1.2	75	27	29	37	37	15
−1.7	67	26	47	33	36	23
−2.1	64	28	65	36	41	25
−2.6	76	25	44	31	49	18
−3.5	121	31	52	55	63	49
−5.5	198	61	124	+[a]	76	+[a]

+[a] = spores too numerous to count.

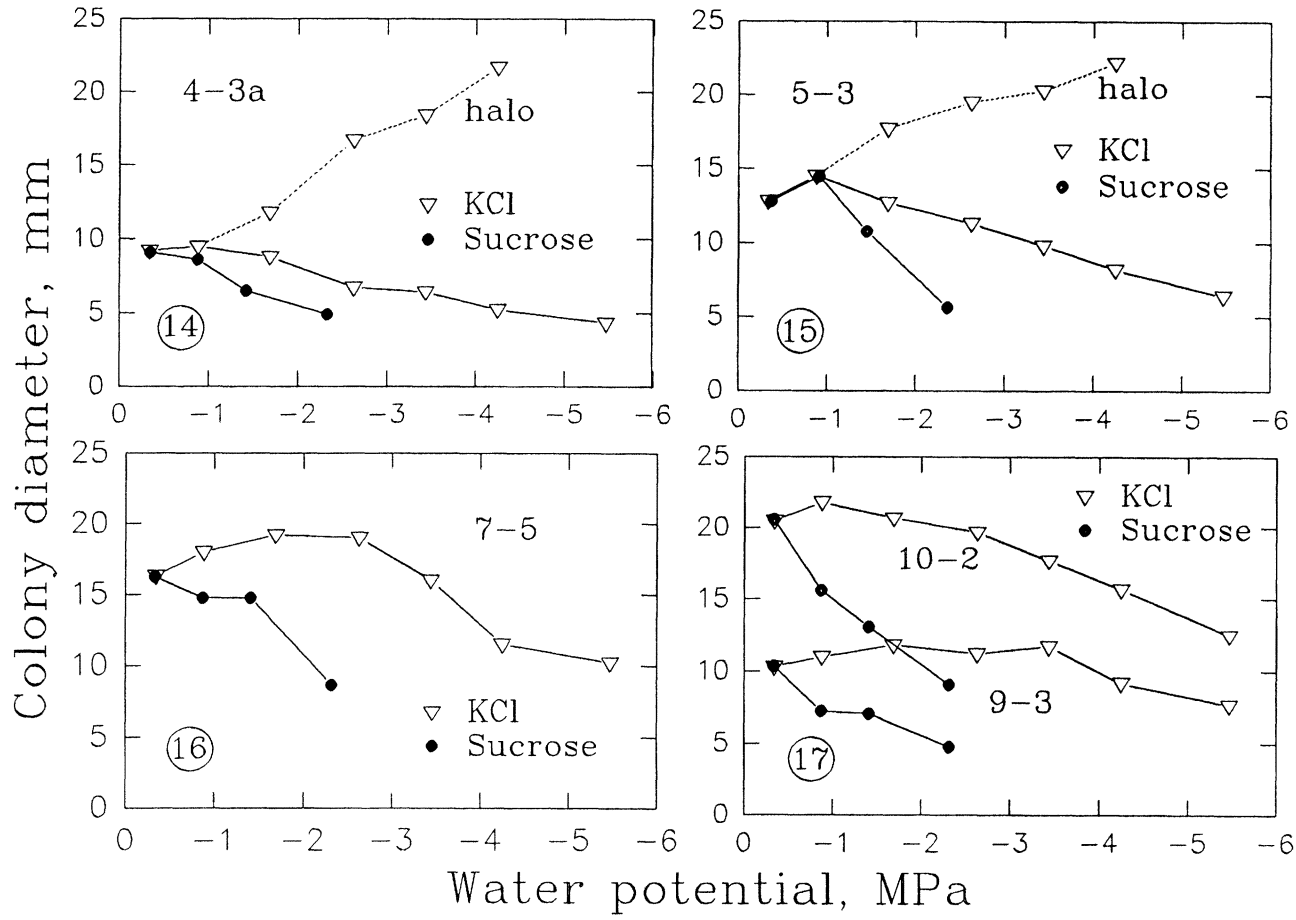
(1993), after reviewing the research on endophytes, concluded that isolates within a species vary greatly in their metabolism. Our observations on mycelial growth, inhibition zone, and halo production support his conclusion. Richardson et al. (1993) and we used different osmotica (glycerol, KCl, sucrose) to study the water relations of *A. coenophialum,* so the results cannot be directly compared, but it is doubtful that, even if the isolates of these studies had been tested under the same conditions, they would have responded similarly. White and Morgan-Jones (1987) found differences in the response of isolates within a species to temperature, another fundamental character.

Bacon and Siegel (1988) found that hot, dry weather reduced the percent of infected seed produced by *F. arundinacea.* We made no temperature × water interaction studies but the ability of all isolates to make significant growth down to −4 MPa water potential suggests that it is unlikely that water stress would inhibit the endophyte as much as the host, more likely that the fungi would continue development under conditions too dry to support vegetative growth of a grass.

Production (or diffusion) of antibiotic and halo substance are more sensitive to water stress than hyphal growth or sporulation. Production of halos and inhibition zones ceased when water potential fell below about −3.5 MPa, well before hyphal growth of any isolate was greatly reduced by water stress. In contrast, *Cephalosporium gramineum* Nisikado and Ikata produced antibiotic over its entire growth range (Bruehl et al., 1972). Bacon et al. (1979) observed that a *Balansia* isolate produced more alkaloid when 100 g/L of sorbitol or mannitol were added to the medium, even though the fungus did not use either as a nutrient. Alkaloid production may respond to water stress as did antibiotic and halo substance. Belesky et al. (1989) and Barker et al. (1993) found that water stress increased alkaloid production within some endophyte-host associations.

The production of a wide spectrum antifungal antibiotic by some isolates (Christensen et al., 1991; Siegel and Latch, 1991; White and Cole, 1985, 1986) led to speculation that antibiotic production within the host might increase or induce resistance to some pathogens and contribute to host survival. Gwinn and Gavin (1992) found that *A. coenophialum* in *F. arundinacea* seedlings offered significant protection against *Rhizoctonia zeae* Voorhees but Burpee and Bouton (1993) reported no protection of seedlings against *R. solani* Kühn. Welty et al. (1993) reported no protection in *F. arundinacea* against infection by *Puccinia graminis* (Pers.; Pers.) subsp. *graminicola* Z. Urban. Fungus concentration in the host is usually greatest near the base of the plant, in leaf sheaths (Musgrave, 1984), and within seed near the aleurone layer. Some *Acremonium* isolates produced small inhibition zones and some produced none. It seems unlikely that antibiotic produced by these fungi plays a significant direct role in plant protection. Five alkaloids studied by Siegel and Latch (1991) did not inhibit their test fungi.

Latch and Christensen (1985) described a host inoculation technique that involved incubating wounded, inoculated seedlings aseptically on the surface of agar. It is possible that their method could be made more effective if the seedlings were transferred to a medium with reduced water potential immediately after inoculation and held there for a week or so. Lindstrom et al. (1976) found that elongation of wheat seedlings was greatly reduced below −1 MPa. Theoretically, the seedlings could be held static and the fungus not hindered or even stimulated by incubating the inoculated seedlings at −2



FIGS. 14–17. Some responses of four *Acremonium* isolates from *Stipa robusta* to osmotic water potentials on potato dextrose agar adjusted with KCl or sucrose. Incubation in the dark at 23 C for 30 d. Colony diameters connected by solid lines, halos by dotted lines. Isolates 4–3a, 5–3, 7–5, 9–3, and 10–2 identified within the figures.

MPa for some time after inoculation, followed by transferring them to a growth medium.

ACKNOWLEDGMENTS

We thank T.D. Murray for the yeast culture, and B.C. Hellier for the graphs.

LITERATURE CITED

Bacon, C. W. 1988. Procedure for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. *Appl. Environ. Microbiol.* 54: 2615–2618.

———, and M. R. Siegel. 1988. Endophyte parasitism of tall fescue. *J. Prod. Agric.* 1: 45–55.

———, J. K. Porter, and J. D. Robbins. 1979. Laboratory production of ergot alkaloids by species of *Balansia*. *J. Gen. Microbiol.* 113: 119–126.

Barker, D. J., E. Davies, G. A. Lane, G. C. M. Latch, H. M. Nott, and B. A. Tapper. 1993. Effect of water deficit on alkaloid concentrations in perennial ryegrass endophyte associations. Pp. 67–71. *In: Proc. 2nd international symposium on Acremonium/grass interactions.* Eds., D. E. Hume, G. C. M. Latch, and H. S. Eaton. Ag Research, Grasslands Research Centre, Palmerston North, New Zealand.

Belesky, D. P., W. C. Stringer, and R. D. Plattner. 1989. Influence of endophyte and water regime upon tall fescue accessions. II. Pyrolizidine and ergopeptine alkaloids. *Ann. Bot.* 64: 343–349.

Bruehl, G. W., B. Cunfer, and M. Toivianen. 1972. Influence of water potential on growth, antibiotic production, and survival of *Cephalosporium gramineum*. *Can. J. Plant Sci.* 52: 417–423.

Burpee, L. L., and J. H. Bouton. 1993. Effect of eradication of the endophyte *Acremonium coenophialum* on epidemics of Rhizoctonia blight in tall fescue. *Plant Dis.* 77: 157–159.

Christensen, M. J., G. C. M. Latch, and B. A. Tapper. 1991. Variation within isolates of *Acremonium* endophytes from perennial rye-grasses. *Mycol. Res.* 95: 918–923.

Cook, R. J., and J. M. Duniway. 1981. Water relations in the life-cycles of soilborne plant pathogens. Pp. 119–139. *In: Water potential relations in soil microbiology.* Eds., J. F. Parr, W. R. Gardner, and L. F. Elliott. *Soil Science Soc. Am., Spec. Publ. No. 9.* Soil Science Soc. Am., Madison, Wisconsin.

———, and R. I. Papendick. 1972. Influences of water potential of soils and plants on root disease. *Annu. Rev. Phytopathol.* 10: 349–374.

Griffin, D. M. 1972. *Ecology of soil fungi.* Syracuse University Press, Syracuse, New York, 193 pp.

Gwinn, K. O., and A. M. Gavin. 1992. Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. *Plant Dis.* 76: 911–914.

Harris, R. F. 1981. Effect of water potential upon microbial growth and activity. Pp. 23–84. *In: Water potential relations in soil microbiology.* Eds., J. F. Parr, W. R. Gardner, and L. F. Elliott. *Soil Science Soc. Am., Spec. Publ. No. 9.* Soil Science Soc. Am., Madison, Wisconsin. 151 pp.

Latch, G. C. M. 1993. Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. *Agric. Ecosystems Environ.* 44: 143–156.

———, and M. J. Christensen. 1985. Artificial infection of grasses with endophytes. *Ann. Appl. Biol.* 107: 17–24.

Lindstrom, M. J., R. I. Papendick, and F. E. Koehler. 1976. A model to predict winter wheat emergence as affected by soil temperature, water potential, and depth of planting. *Agron. J.* 68: 137–141.

Morgan-Jones, G., and W. Gams. 1982. Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina,* new taxa in one of two sections of *Acremonium. Mycotaxon* 15: 311–318.

Musgrave, D. R. 1984. Detection of an endophyte fungus of *Lolium perenne* using enzyme-linked immunosorbent assay (ELISA). *New Zealand J. Agric. Res.* 27: 283–288.

Papendick, R. I., and G. S. Campbell. 1975. Water potential in the rhizosphere and plant and methods of measurement and experimental control. Pp. 39–49. *In: Biology and control of soil-borne plant pathogens.* Ed., G. W. Bruehl. American Phytopathological Society, St. Paul, Minnesota.

Richardson, M. D., C. W. Bacon, N. S. Hill, and D. M. Hinton. 1993. Growth and water relations of *Acremonium coenophialum.* Pp. 181–184. *In: Proc. 2nd international symposium on Acremonium/grass interactions.* Eds., D. E. Hume, G. C. M. Latch, and H. S. Eaton. Ag Research, Grasslands Research Centre, Palmerston North, New Zealand.

Robinson, R. A., and R. H. Stokes. 1959. *Electrolyte solutions.* 2nd edition. Butterworth, England. 571 pp.

Siegel, M. R., and G. C. M. Latch. 1991. Expression of antifungal activity in agar culture by isolates of grass endophytes. *Mycologica* 83: 529–537.

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.

Sung J-M., and R. J. Cook. 1981. Effect of water potential on reproduction and spore germination by *Fusarium roseum* 'Graminearum', 'Culmorum', and 'Avenaceum'. *Phytopathology* 71: 499–504.

Welty, R. E., R. E. Barker, and M. D. Azevedo. 1993. Response of field-grown tall fescue infected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola. Plant Dis.* 77: 574–575.

White, J. F., Jr., and G. T. Cole. 1985. Endophyte-host associations in forage grasses. III. In vitro inhibition of fungi by *Acremonium coenophialum. Mycologia* 77: 487–489.

———, and ———. 1986. Endophyte-host associations in forage grasses. IV. The endophyte of *Festuca versuta. Mycologia* 78: 102–107

———, and G. Morgan-Jones. 1987. Endophyte-host associations in forage grasses. X. Cultural studies on some species of *Acremonium* Sect. *Albo-lanosa,* including a new species *A. starrii. Mycotaxon* 30: 87–95.

———, ———, and A. C. Morrow. 1993. Taxonomy of life cycle, reproduction and detection of *Acremonium* endophytes. *Agric. Ecosystems Environ.* 44: 13–37.