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The Effect of Water Potential upon Radial Growth of Epichloë and Claviceps purpurea Isolates in Culture Author(s): W. J. Kaiser and G. W. Bruehl Source: *Mycologia*, Vol. 88, No. 5 (Sep. - Oct., 1996), pp. 816-818 Published by: <u>Mycological Society of America</u> Stable URL: <u>http://www.jstor.org/stable/3760977</u> Accessed: 18/10/2010 08:58

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The effect of water potential upon radial growth of *Epichloë* and *Claviceps purpurea* isolates in culture

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Abstract: Claviceps purpurea and single isolates of Epichloë amaryllans and E. baconii grew well between -0.3 and -3.0 MPa water potential, below which growth declined with declining water potential. The isolates of E. clarkii and of E. typhina were less tolerant to reduced water potential. Antibiotic was weakly produced by some Epichloë isolates but not by C. purpurea.

Key Words: Antibiotic, Clavicipitaceae, water relations

Endophytic Acremonium Link isolates varied in response to reduced water potential (Richardson et al., 1993; Bruehl and Kaiser, 1996), but all were sufficiently tolerant to make it improbable that drought would prevent an endophyte from maintaining its essential relationships within the growing host (Bruehl and Kaiser, 1996). The production of antibiotic(s) (White and Cole, 1985, 1986; Christensen et al., 1991; Siegel and Latch, 1991) and an unknown opaque substance that forms haloes within the media around growing colonies (White and Cole 1985) is strongly influenced by water potential (Bruehl and Kaiser, 1996). This paper reports the results of studies on Epichloë spp. and Claviceps purpurea (Fr.) Tul., as well as determining if similar response patterns exist among three groups of fungi (Acremonium endophytes, Epichloë spp., and C. purpurea).

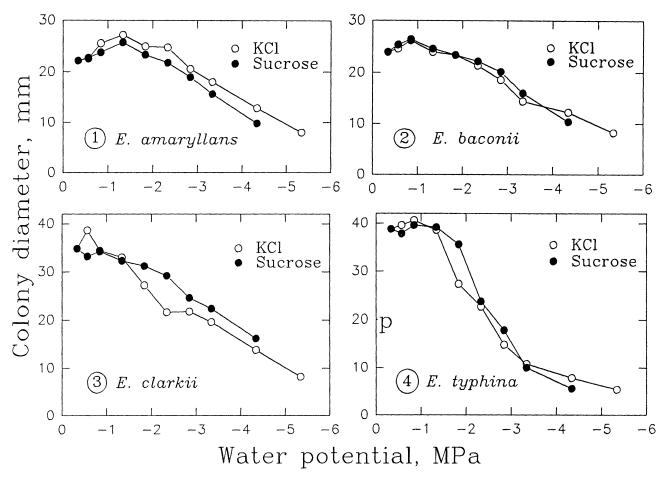
Single isolates of *Epichloë* spp. (*E. amaryllans* White, isolate A3 from *Agrostis hiemalis* (Walt.) B.S.P.; *E. baconii* White, isolate As 6 from *Agrostis capillaris* L.; *E. clarkii* White, isolate Holcus 1 from *Holcus lanatus* L.; and *E. typhina* (Pers.:Fr.) Tul isolate Dg5 from *Dactylis glomerata* L.), all provided by J.F. White, Jr., were studied along with four isolates of *C. purpurea* (one each from *Festuca spectabilis* Jan

ex Bertoloni, PI 380862; Hordeum vulgare L., CI 11012; Lolium rigidum Gaudin, PI 239781; and Secale cereale L., PI 534952). PI are plant introduction numbers and CI are cereal inventory numbers of the U.S. National Plant Germplasm System. The water potential of Difco potato dextrose agar was modified with KCl or sucrose (S) according to Harris (1981). Inoculum from the leading edge of cultures maintained on PDA was placed at the center of 85 mm-diam plastic Petri dishes containing 25 mL of medium, six replicates per treatment. After inoculation the dishes were sealed with Parafilm to reduce water loss and incubated on a laboratory bench at 22 C for 13 d. Assays for growth, inhibiton zone production, and halo formation were as described by Bruehl and Kaiser (1996). After colony measurements and observation for haloes, inhibition zones were determined by spraying the cultures with an aqueous suspension of Rhodotorula rubra (Demmi) Loder, ATCC 9540.

The isolates of Epichloë clarkii and E. typhina grew more rapidly at high water potentials than those of E. amaryllans and E. baconii and all tolerated KCl and sucrose (FIGS. 1-4). Growth of our single isolate of E. clarkii decreased gradually with reduced water potential (FIG. 3), growth of E. typhina declined rapidly between about -1.5 and -3.0 MPa (FIG. 4), and growth of E. amaryllans and E. baconii was maintained well to -3.0 MPa (FIGS. 1, 2). Inhibition zones were produced on KCl-amended media by E. amaryllans at -0.57 and -0.85 MPa, by E. clarkii at -0.85 and -1.34 MPa, by E. typhina at -0.57 to -1.34 MPa, but not by E. baconii. Inhibition zones were small and haloes were absent. The above observations are of single isolates of the Epichloë spp. and isolates may differ within the species, so we do not know whether the results are typical of the species.

The responses of four isolates of *C. purpurea* to water potential (FIGS. 5–8) were less diverse, with growth accelerating from -0.3 to about -1.3 MPa, below which growth declined. The isolates from *F. spectabilis* (FIG. 5) and *S. cereale* (FIG. 6) tolerated lower water potential than the other two. The *L. rigidum* isolate (FIG. 8) preferred KCl over sucrose as the osmoticum. Neither antibiotic nor halo production was observed around any isolate.

Accepted for publication April 10, 1996.



FIGS. 1-4. Colony diam of *Epichloë* isolates after 13 d on potato dextrose agar modified with KCl or sucrose to attain different water potentials.

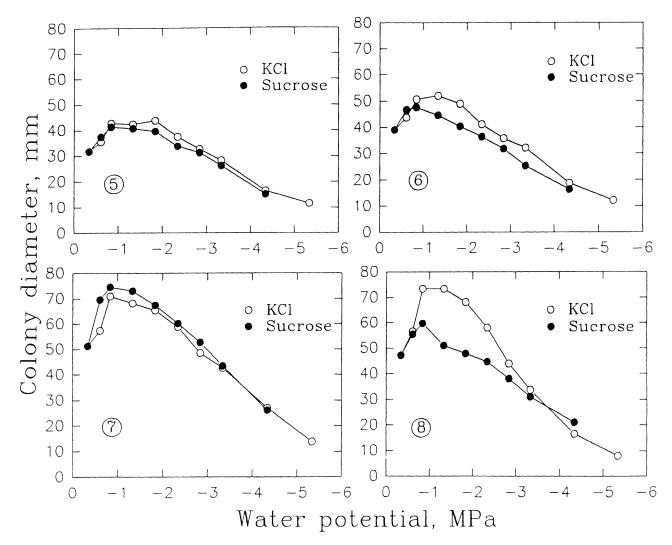
Growth responses to decreasing water potential were less diverse among C. purpurea isolates than among Epichloë isolates, and the types of responses of the latter were similar to those found among Acremonium endophytes (Bruehl and Kaiser, 1996). We found no isolate of C. purpurea whose growth decline with declining water potential was as strictly linear as the isolate of E. clarkii (FIG. 3) in this study or as isolate GI 307 (A. coenophialum Morgan-Jones & Gams (FIGS. 6,7,11), and isolate 440413 (A. typhinum Morgan-Jones & Gams (FIGS. 5,7,12) of our earlier study. Inhibition zone production was commonly stronger among Acremonium isolates than among Epichloë isolates, as was halo production. Inhibition zones and haloes were both lacking around colonies of C. purpurea.

Two very different solutes, KCl and sucrose, were used because similar responses to both should indicate that even in high concentrations they are not toxic to the test fungi and that the resulting curves represent response to water, not to a particular solute. With the exception of the *C. purpurea* isolate from L. rigidum (FIG. 8), and possibly the S. cereale isolate (FIG. 6), the growth curves were remarkably similar, indicating that both chemicals are suitable for water studies with these fungi. The standard deviations of the data points where growth on the two media differed (-1 to -3 MPa, FIGS. 6 and 8) do not overlap, so the differences are statistically valid. Standard deviations for the data points for colony diameters of *Epichloë* isolates and for *Claviceps purpurea* averaged ± 1.3 mm, most of them falling within or very close to the edges of the symbols, so they are omitted from the graphs.

We hoped that studies of water potential would reveal relationships among *Claviceps purpurea, Epichloë* species, and endophytic *Acremonium* species (Bruehl and Kaiser, 1996), but the responses among isolates of the same species varied so greatly this objective was not attained.

ACKNOWLEDGMENTS

We thank J. F. White, Jr., Department of Plant Pathology, Auburn University, Montgomery, Alabama for the *Epichloë*



FIGS. 5-8. Colony diam of *Claviceps purpurea* isolates after 13 d on potato dextrose agar modified with KCl or sucrose to attain different water potentials. 5. Isolate from *Festuca spectabilis*, 6. Isolate from *Secale cereale*, 7. Isolate from *Hordeum vulgare*, 8. Isolate from *Lolium rigidum*.

isolates and H.E. Bockelman, USDA, ARS, Aberdeen, Idaho for the sclerotia of *C. purpurea*.

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