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Basic and Applied Ecology ■ (■■■■) ■■■-■■■

Basic and
Applied Ecology

www.elsevier.de/baae

Benefits of a fungal endophyte in *Elymus virginicus* decline under drought stress

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Received 17 July 2007; accepted 6 December 2007

Abstract

In most natural systems, it remains unclear whether microbial symbionts can help plants adapt to rapidly changing environments. Here, we tested the hypothesis that fungal endophyte symbiosis in a widespread, native grass species, *Elymus virginicus*, would promote host tolerance of drought. In a growth chamber experiment, we manipulated water availability to plants naturally infected with the endophyte *Epichloë elymi* and to plants that were experimentally disinfected. The drought treatment imposed a ~67% reduction in water, corresponding to the very high end of drought predictions for the Midwestern US, where *E. virginicus* is common. In contrast to expectations, *E. virginicus* benefited more from the endophyte under daily watering than under drought. Disinfected plants had 45% less aboveground biomass than naturally infected plants under daily watering and only 23% less under drought. Both the number of tillers produced and root biomass were reduced by disinfection, with a greater difference between endophyte treatments under daily watering than under drought. There were no effects of either the endophyte or drought on aphid colonization of the plants. To put these results into a broader geographic context, we surveyed endophyte infection frequencies across 43 populations, including wild populations, commercial stock and germplasm resources. Endophyte frequencies varied from 0% to 100%, and frequencies were much higher for wild populations than germplasm resources. Natural variation in endophyte frequencies combined with the heritable transmission of endophytes from plants to seeds provides opportunities for climate change to affect selection on the maintenance of endophyte symbiosis in *E. virginicus*.

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Zusammenfassung

Bei den meisten natürlichen Systemen ist weiterhin unklar, ob mikrobielle Symbionten Pflanzen helfen können sich an schnell wandelnde Umweltbedingungen anzupassen. Wir testeten hier die Hypothese, dass die Symbiose mit einem endophytischen Pilz bei einer weit verbreiteten, indigenen Grasart, *Elymus virginicus*, die Trockenheitstoleranz des Wirtes stärken sollte. In einem Zuchtkammer-Experiment manipulierten wir die Wasserverfügbarkeit für natürlich mit dem Endophyten *Epichloë elymi* infizierte und experimentell desinfizierte Pflanzen. Die Trockenvariante bedeutete eine Reduktion der Wasserverfügbarkeit um ca. 67%; dies entspricht dem oberen Ende der Vorhersagen aus Klimamodellen für den mittleren Westen der USA, wo *E. virginicus* verbreitet vorkommt. Im Gegensatz zu unseren Erwartungen profitierte *E. virginicus* bei täglicher Wässerung mehr vom Endophyten als bei Trockenheit. Desinfizierte Pflanzen hatten bei Bewässerung 45% weniger oberirdische Biomasse als natürlich infizierte und nur 23% weniger

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unter Trockenheit. Sowohl die Anzahl produzierter Schößlinge als auch die Wurzelbiomasse waren bei Desinfektion reduziert, wobei die Differenzen zwischen den Endophytenbehandlungen bei täglicher Bewässerung größer als bei Trockenheit waren. Weder Endophyt noch Trockenheit beeinflussten den Befall durch Blattläuse.

Um diese Ergebnisse in einen breiteren geographischen Kontext zu stellen, untersuchten wir die Infektionsraten von 43 Populationen, darunter wilde Populationen, kommerzielle Bestände und eingelagertes Keimmateriale. Die Infektionshäufigkeiten variierten zwischen 0% und 100%, und sie waren bei Wildpopulationen viel höher als im eingelagerten Material. Die natürliche Variation der Endophyteninfektionsrate verbunden mit der erblichen Weitergabe des Endophyten von der Pflanze zum Samen bietet Gelegenheiten zum Einwirken der Selektion auf den Erhalt der Symbiose. Unsere Ergebnisse legen nahe, dass die Zunahme der Trockenheit, die von aktuellen Klimamodellen vorhergesagt wird, selektiv auf eine Reduktion der natürlichen Endophytenhäufigkeit bei *E. virginicus* hinwirken könnte.

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Keywords: Climate change; *Epichloë elymi*; Symbiosis; Native grass; Forage; Restoration

Introduction

Climate models predict net losses of soil moisture and more variable precipitation events in many regions of North America and abroad (Houghton et al. 2001; IPCC 2007). Accumulating evidence demonstrates that many species have already responded ecologically to changes in climate, including an estimated 40% of wild species in one recent study (Parmesan & Yohe 2003). Largely sedentary organisms, like plants, may be among the most limited in ability to cope with rapid environmental change (Jump & Peñuelas 2005). Importantly, altered types and intensities of species interactions may contribute to the dynamic response of plants to a warmer environment (Parmesan 2006). For example, most plants form symbioses with other organisms, including mycorrhizal fungi, nitrogen-fixing bacteria, and fungal endophytes (Bacon & White 2000; Brundrett 2002). These symbionts can ameliorate nutrient and water limitation (Auge 2001; Smith & Read 1997) as well as protect against herbivore and pathogen attack (Barbosa, Krischik, & Jones 1991; Clay, Holah, & Rudgers 2005; Rudgers & Clay 2005). Furthermore, the relative costs and benefits of symbionts to host plants often vary with environmental conditions (Cheplick 2004; Johnson, Graham, & Smith 1997; Lehtonen, Helander, & Saikkonen 2005; Malinowski, Belesky, & Lewis 2005).

What role will symbioses play in plant responses to climate change? Few studies in natural systems have directly examined this issue (but see Groppe et al. 1999; Meijer & Leuchtman 2000), with cultivated plants receiving somewhat more attention (Motha & Baier 2005). Furthermore, most studies have focused on the response of plant–microbe symbioses to increases in CO₂, rather than to predicted changes in climate (e.g., Groppe et al. 1999; Hartwig et al. 2002; Hunt, Rasmussen, Newton, Parsons, & Newman 2005; Marks & Lincoln 1996; Newman et al. 2003; Staddon, Gregersen, & Jakobsen 2004). However, a recent

example (Suttle, Thomsen, & Power 2007) highlights the potential importance of symbiosis. A field experiment in California grasslands showed that predicted increases in rainfall promoted a rapid increase then decline in nitrogen-fixing legumes. The authors hypothesized that elevated precipitation first benefited the symbiosis between legumes and bacteria, which in turn increased nitrogen in the system. The increased nitrogen is presumed to have escalated the invasion of grasses that ultimately out-competed the legumes. Although symbiotic nitrogen-fixing bacteria were not directly implicated, this work strongly suggested that symbiosis was a keystone mediator of plants' responses to climate change.

Here, we focus on symbiosis between grasses and systemic fungal endophytes to examine potential costs and benefits of symbiosis in light of a changing climate. Fungal endophytes in the genera *Epichloë* and *Neotyphodium* grow systemically in the aboveground tissues of many grass species (Schardl, Leuchtman, & Spiering 2004). In a few agronomically important grasses (notably *Lolium* spp.), endophytes have been shown to enhance tolerance of drought (reviewed by Clay & Schardl 2002; Malinowski et al. 2005). For example, under drought stress *Neotyphodium coenophialum* can alter the water potential of tall fescue grass (*Lolium arundinaceum*), increase sugar alcohols, promote more rapid closure of stomata, and enhance survival and growth relative to uninfected plants (reviewed by Malinowski et al. 2005). If drought amelioration is a common result of endophyte symbiosis, then increases in the severity and duration of summer drought in North America (Houghton et al. 2001; IPCC 2007) could drive the replacement of endophyte-free grasses by endophyte-infected hosts. In contrast, water limitation can increase the cost of supporting some endophytes (e.g., Cheplick 2004; Muller & Krauss 2005), potentially leading to reduced frequencies of symbiosis in response to increasing drought.

Despite the estimate that 20–30% of all grass species host systemic fungal endophytes (Leuchtmann 1992), little is known about the potential for drought amelioration in non-agronomic grass populations (Muller & Krauss 2005; Saikkonen, Lehtonen, Helander, Koricheva, & Faeth 2006). Broad geographic patterns suggest that endophytes may be important for drought tolerance. In France, the level of endophyte infection across 57 populations of native *Lolium* spp. was significantly positively correlated with evapotranspiration, suggesting that the endophyte may ameliorate drought stress (Lewis, Ravel, Naffaa, Astier, & Charmet 1997). Under strong drought stress, *Festuca arizonica* plants that were naturally endophyte-free had reduced aboveground biomass and relative growth compared to endophyte-infected plants (Morse, Day, & Faeth 2002; Morse, Faeth, & Day 2007). However, in another greenhouse experiment, benefits of the endophyte for *Festuca rubra* and *F. pratensis* did not increase under drought stress (Ahlholm, Helander, Lehtimäki, Wali, & Saikkonen 2002). In total, few studies exist to predict how wild plant–endophyte symbioses will respond to climate change.

Here, we tested the hypothesis that symbiosis with the endophyte *Epichloë elymi* would promote tolerance of drought by a widespread, native North American grass species, *Elymus virginicus*. Specifically, we addressed the following questions. (1) Does the endophyte improve plant performance? (2) Does drought reduce plant performance? (3) Does the endophyte ameliorate drought stress? We also provide data on the frequency of symbiosis across populations of *E. virginicus*, including material available for restoration. Answers to these questions are important to both basic and applied ecology: if endophytes promote drought tolerance, the frequency of symbiosis is expected to increase if summer drought becomes more severe. Similarly, if endophytes enhance drought tolerance, then choosing symbiotic plant material will be advantageous when planting grasses for restoration or forage.

Methods

Study organisms

E. virginicus (Virginia wild rye) is a widespread, cool season grass native to North America and distributed from Newfoundland to Alberta, south from Florida to Arizona (Gleason & Cronquist 1991). This grass has been recommended for forage and restoration planting in North America (Sanderson, Skinner, Kujawski, & van der Grinten 2004). The endophyte was first reported in *E. virginicus* in White (1987), and has been identified as *E. elymi* (Schardl & Leuchtmann 1999). The endophyte is transmitted vertically to seeds, and, less

commonly, produces stromata for sexual reproduction. Stromata production is costly to plants because tillers bearing stromata fail to reproduce. No plants formed stromata during our study.

Seed source for experiments

We obtained seeds from two commercial native plant seed distributors for use in the experiment: JFN: J. F. New, Indianapolis, IN, and PMN: Prairie Moon Nursery, Winona, MN, USA. PMN seeds were originally collected in southeastern Minnesota (exact location information is not available) and then propagated for two to three generations in a large production field and harvested by combine in 2004. JFN seeds were originally collected from a natural population in Rock Co. WI in 1995. Seeds were propagated for three generations in the field in Wisconsin (Agregcol Corp, Madison, WI). JFN seeds for the experiment were harvested by combining in 2003 from a 3.01 ha field planted at a density of ~37,070 plants per ha. Original seed stock was 100% infected in both populations (Appendix). We scored endophyte presence microscopically following Bacon and White (1994) (Fig. 1A). The stain detects the endophyte regardless of its viability; thus, we grew a subset of plants from seed, and confirmed 100% infection in both populations following Clark, White, and Patterson (1983) (Fig. 1B, Appendix).

Elimination of the endophyte

We experimentally eliminated the endophyte using a standard heat treatment (6 d at 60 °C) in a convection drying oven. Prior to the experiment, we grew seedlings from heat-treated seeds to confirm experimental reductions in the endophyte. In this trial, heat treatment reduced the endophyte from 100% to 28.5% in JFN and to 17.6% in PMN. There were no significant differences in seed mass between heat-treated and control seeds. Mean mass of 10 seeds \pm S.E. was 0.0154 ± 0.0005 g for the control, and 0.0150 ± 0.0004 g for heat [ANOVA, $F_{1,32} = 0.3$, $P = 0.6$; $n = 10$ replicates of 10 seeds each; Power = 1.0 to detect a difference of 0.005 g (Hintz 2001)]. We also found no significant effect of the heat treatment on germination. We placed 6 PMN seeds from each treatment in each of nine replicate pots (54 seeds/treatment) filled with Metromix 200 (Sungro Horticulture, Bellevue, WA). Germination was recorded every 2 d for 4 wks. Heat treatment did not significantly affect the number of days to first seedling emergence (mean days \pm S.E., control = 8.22 ± 1.9 ; heat = 8.25 ± 1.2 ; ANOVA $F_{1,15} = 0.03$, $P = 0.9$; Power = 1.0 to detect a difference of 0.5 d) or the proportion of seeds germinating (mean % \pm S.E., control = $67 \pm 4\%$, heat = $59 \pm 8\%$, ANOVA

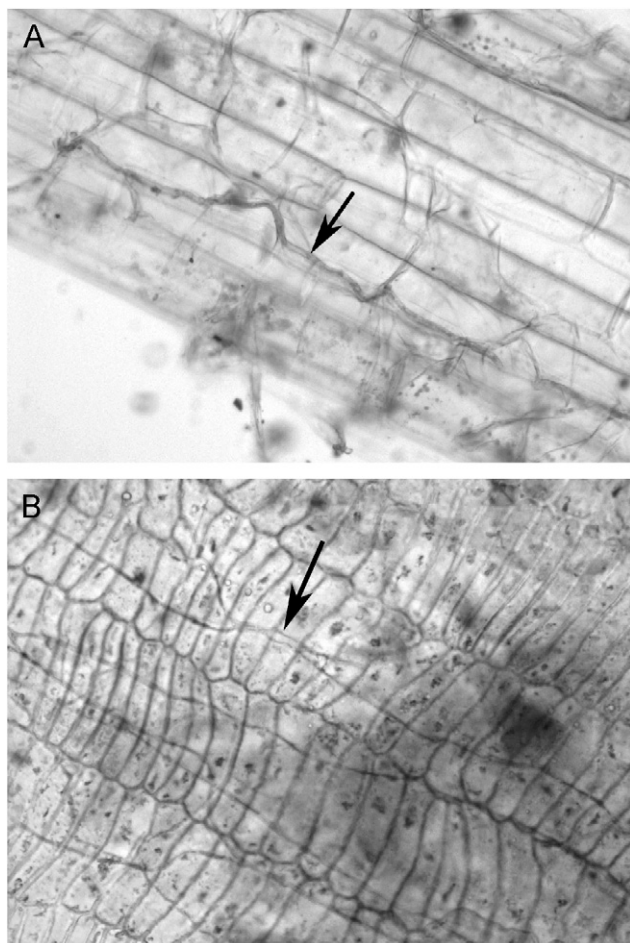


Fig. 1. (A) Photograph of hyphae of the *Epichloë* endophyte hyphae in leaf tissue of *Elymus virginicus* (S. F. Austin-400A) taken at $200\times$ magnification on a Leica DFC-480 digital camera attached to a Leica DM-2500 compound microscope (Leica Microsystems, Wetzlar, Germany). (B) Photograph of endophyte hyphae in seed coat tissue of *E. virginicus* (Bayles Road-C population) also taken at $200\times$ magnification. Endophyte hyphae were also found in the aleurone layer. Arrows indicate the endophyte.

$F_{1,15} = 1.1$, $P = 0.3$; Power = 0.19 for this test, and 0.75 to detect a difference of 50%).

Growth chamber experiment

Following heat treatment, seeds were grown in a greenhouse at ambient light ($15\text{--}28^\circ\text{C}$) for 8 wks. On 2 July 2006, we removed two clones from each plant. Each clone had, on average, 3.5 ± 1.8 tillers. By cloning, we aimed to ameliorate possible side effects of the heat treatment by creating initial plants of similar size for each treatment. Endophyte treatments did not significantly differ in the initial number of tillers per clone ($F_{1,46} = 1.1$, $P = 0.3$). Clones were planted individually into Miracle-Gro potting soil (0.21% N, 0.07% P,

0.14% K) (Miracle-Gro Lawn Products; Marysville, OH) in $10 \times 10 \times 9$ cm deep plastic pots. After transplant, clones were moved to an Enconair growth chamber (Model GC-20, Ecological Chambers, Incorporated; Winnipeg, Manitoba, Canada) and held at 22°C , with 14 h of light (363 PAR).

Successful endophyte elimination was confirmed by removing one leaf from every plant in early August 2006 and staining leaf sheath peels following Clark et al. (1983) (Fig. 1B). Not all plants survived cloning, and heat-treated seeds that retained the endophyte were not used. Thus, final sample sizes were: $E+ = 31$, $E- = 25$.

We allowed plants to recover from transplant for 4 wks, and began the drought on 5 August 2006. Paired clones were randomly assigned to locations within the chamber. Plants within each clone pair were assigned to drought treatments at random. Plants were either watered daily to saturation with 50 ml tap water (daily watering), or watered every third day with 50 ml (drought). The reduction in water (67%) falls at the extreme high end of drought trends predicted by climate models, particularly by the older Canadian model (Houghton et al. 2001, see also <http://www.usgcrp.gov/usgcrp/nacc/midwest.htm>). Predictions vary substantially among the most current climate models (IPCC 2007). At the extreme end of drought predictions, the MIROC3.2.medres model predicted up to 30% reductions in annual mean precipitation during the next century within the range of *E. virginicus*. At the opposite end, the GISS-ER model predicted up to 30% increases in annual precipitation. Averaged across the 21 current climate models, a maximum of 10% reduction in annual mean precipitation is predicted across the range of *E. virginicus*, with less precipitation toward the western range limit (Christensen et al. 2007). It remains unclear how increases in surface temperature will interact with predicted changes in precipitation to affect soil moisture levels (Christensen et al. 2007).

Response variables

We tracked changes in vegetative biomass by counting the number of tillers (independent stems) and measuring maximum plant height to the nearest 0.1 cm on 4 September, and 2 November 2006. From 9 to 11 December 2006, we harvested plants and washed roots (US Standard Sieve No. 18, 1 mm opening). Biomass was dried for 4 wks at 60°C in a convection oven then weighed to the nearest 0.001 g.

Aphids (*Rhopalosiphum maidis*) naturally colonized from outdoor populations. We counted aphids on 9 September 2006 using an 18×3 mm transparent grid comprised of 9 mm^2 squares. The grid was placed at three points (base, midpoint, and tip) along each of two randomly selected leaves per plant to determine the

average density of aphids per plant. We subsequently sprayed plants once with insecticidal soap (2% potassium salts of fatty acids; Safer, Inc. Lititz, PA), which suppressed aphids thereafter.

Data analysis

We evaluated host responses to the treatments with general linear models (SAS Institute 2004). Independent effects included the endophyte treatment, drought treatment, source population, clone (nested within source population \times endophyte), and all possible interactions. For biomass response variables, we used factorial ANOVA. For tiller number and maximum height, we used repeated measures MANOVA. To achieve normality of residuals and homogeneity of variances, we applied transformations: tiller number (square-root), maximum height (log), and root:shoot ratio (arcsin square-root). In each model, we additionally tested two *a priori* contrasts: (1) do E+ and E– differ under drought? and (2) do E+ and E– differ under daily watering?

Geographic variation in endophyte frequency

We surveyed the endophyte in 43 populations of *E. virginicus*. Locations included 12 wild populations (Indiana and Texas), 2 commercial sources, and 29 accessions from the USDA Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl), from which we examined all available North American accessions (Appendix). We stained for endophyte presence in seeds and/or leaves, using methods described above.

We were interested in examining whether endophyte frequencies correlated positively with precipitation gradients. However, because USAD-GRIN accessions strongly differed in infection from wild populations, we could not combine sources. Summer precipitation averages are higher for Indiana than Texas, by >3-fold in July, based on 30-year averages (1971–2000) (<http://www.ocs.orst.edu/prism/products/matrix.phtml?vartype=ppt&view=maps>). We used ANOVA to test for differences in endophyte frequency between Texas and Indiana populations, but sample sizes were very small (Indiana $n = 6$ populations; Texas $n = 6$).

Results

Does the endophyte improve plant performance?

Experimental elimination of the endophyte reduced several aspects of plant performance. Disinfected plants (E–) had 34% lower shoot biomass than naturally

infected (E+) plants (Fig. 2A, $F_{1,41} = 11.0$, $P = 0.002$) and produced 28% less root biomass (Fig. 2B, $F_{1,41} = 6.2$, $P = 0.017$). On average, tiller number declined 37% under disinfection in September and 30% in November relative to infected plants (Fig. 3A). Similarly, maximum plant height was 22% lower under disinfection in September and 16% in November relative to infected plants (Fig. 3B). There was no significant main effect of the endophyte on the root:shoot ratio (Fig. 2C, $F_{1,41} = 0.7$, $P = 0.4$). Root biomass was the only case where treatment effects depended on the source population (endophyte \times source population: $F_{1,41} = 5.6$, $P = 0.023$; all other response variables: $P > 0.3$). For roots, disinfection significantly reduced biomass for the Minnesota population (by ~50%, PMN, $P = 0.01$), but not for the Indiana population (JFN, $P = 0.9$) according to a post-hoc Tukey HSD test. Mean root biomass (g) \pm S.E. for PMN was E+ = 0.94 ± 0.09 and E– = 0.48 ± 0.10 ; for JFN, E+ = 0.59 ± 0.09 and E– = 0.57 ± 0.10 . Finally, the endophyte did not significantly alter aphid density per plant (mean aphids per $\text{mm}^2 \pm$ S.E., E+ = 0.071 ± 0.007 , E– = 0.066 ± 0.005 ; $F_{1,41} = 0.7$, $P = 0.4$).

Does drought reduce plant performance?

Experimental drought significantly reduced root biomass by 40% relative to daily watered plants (Fig. 2B, $F_{1,41} = 8.5$, $P = 0.006$). Drought reduced the root:shoot ratio in a similar magnitude (39% relative to daily watering; Fig. 2C, $F_{1,41} = 18.8$, $P < 0.0001$). In contrast, drought had no significant main effect on aboveground performance, including tiller number ($P > 0.4$), shoot mass ($P > 0.9$), and plant height ($P > 0.7$) (Figs. 2 and 3). Drought also did not affect aphid density (mean \pm S.E., drought (W–) = 0.072 ± 0.006 ; daily watering (W+) = 0.064 ± 0.006 ; $F_{1,41} = 1.2$, $P = 0.3$).

Does the endophyte ameliorate drought stress?

Contrary to our initial prediction, we found no effect of the endophyte in ameliorating drought stress. In fact, benefits of the endophyte were stronger under the daily watering treatment than under drought. At the end of the experiment, disinfected plants supported 45% less aboveground biomass than infected under daily watering and only 23% less under drought (Fig. 2A). Similarly, disinfected plants had 30% less root biomass than infected under daily watering and 24% less under drought (Fig. 2B). There was no significant interaction between the endophyte and drought for root:shoot ratio (Fig. 2C; $F_{1,41} = 0.8$, $P = 0.38$) or for aphid densities per plant ($F_{1,41} = 0.3$, $P = 0.6$). Statistical models explained substantial variation in the data (tiller number $r^2 = 0.16$,

shoot mass $r^2 = 0.25$, root mass $r^2 = 0.37$, root:shoot $r^2 = 0.45$).

The exceptions to the general pattern that the endophyte was more beneficial under daily watering than under drought were the early (September) estimates of plant performance. In September, benefits of the endophyte were significant in both drought and daily watered treatments, with 22% shorter height in disin-

fectured relative to infected plants (Fig. 3A) and 34% fewer tillers for disinfected plants (Fig. 3B). By November, disinfected plants still had 34% fewer tillers than endophyte-infected under daily watering, but had just 20% fewer under drought. Similarly, in November, disinfected plants were 26% shorter under daily watering but only 3% shorter under drought (Fig. 3A).

Geographic variation in endophyte frequency

E. virginicus populations exhibited considerable variation in natural frequencies of the endophyte, ranging from 0% to 100% (mean % infection = $6.8 \pm 3.0\%$ S.E., $n = 43$ populations, see Appendix). There were large differences in endophyte frequency between germplasm and wild material. Wild populations were on average $35.0 \pm 9.0\%$ infected ($n = 12$). By contrast, germplasm (USDA-GRIN) samples were $0.3 \pm 0.3\%$ infected, with just one of the 29 accessions showing infection. The two commercial stocks we sampled were 100% infected. There was no significant difference in the endophyte frequency of wild populations in Indiana versus Texas ($F_{1,10} = 0.5$; $P = 0.48$). Although the frequency of infection in Indiana was $1.5 \times$ higher than in Texas, sample sizes were very low, which limited statistical power (mean % infection \pm S.E., Indiana = $42.2 \pm 9.7\%$, $n = 6$ populations; Texas = $27.8 \pm 15.4\%$, $n = 6$; Power = 0.15 for this test and 0.35 to detect a difference of 25% in frequency).

Discussion

The elimination of endophyte symbiosis in *E. virginicus* reduced plant performance estimates by ~ 20 – 50% . Effects were robust across seed sources from two geographically distant locations (Minnesota and Indiana). Although several studies have documented the presence of endophytes in wild grasses (e.g., Clay & Leuchtman 1989; Clay & Schardl 2002; Leuchtman 1992; White 1987), experimental work has been rare in natural systems relative to managed and cultivated forage and turf grasses (Saikkonen et al. 2006). In our experiment, the magnitude of endophyte benefits observed was higher than magnitudes

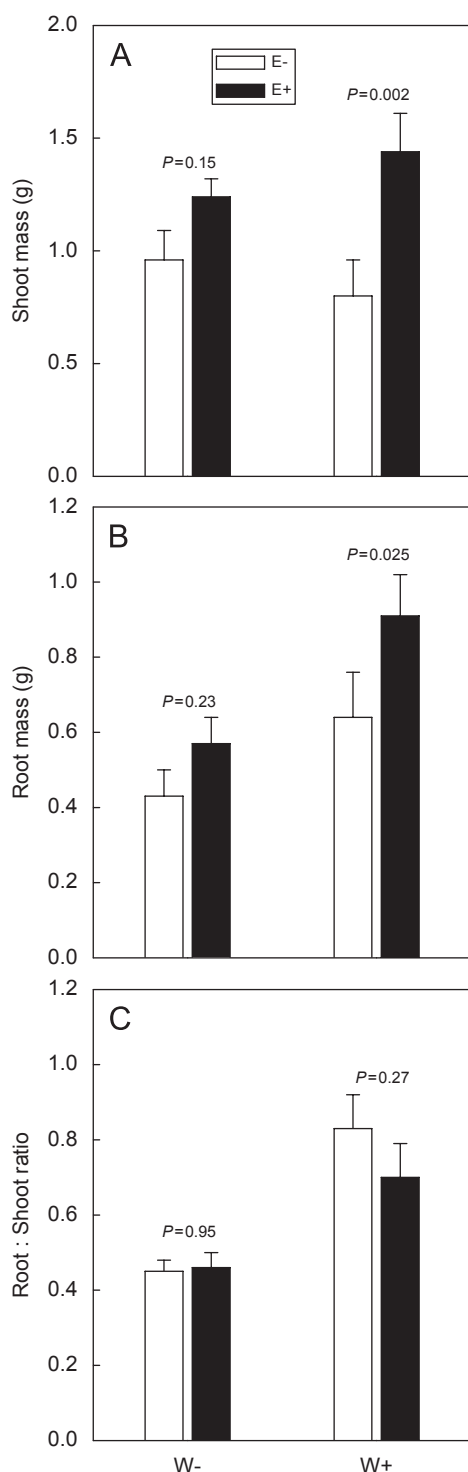


Fig. 2. Combined effects of the endophyte *Epichloë elymi* and experimental drought on the (A) shoot biomass, (B) root biomass, and (C) root:shoot ratio of *Elymus virginicus*. Bars indicate means \pm S.E. Filled bars are E+ plants cloned from plants naturally infected with *Epichloë elymi*. Open bars are E- plants cloned from experimentally disinfected plants. W- = experimental drought treatment. W+ = daily watering to saturation. For each response variable, P-values indicate significant differences for *a priori* contrasts (see text). Sample sizes were W-E+ = 12, W-E- = 13, W+E+ = 13, W+E- = 12.

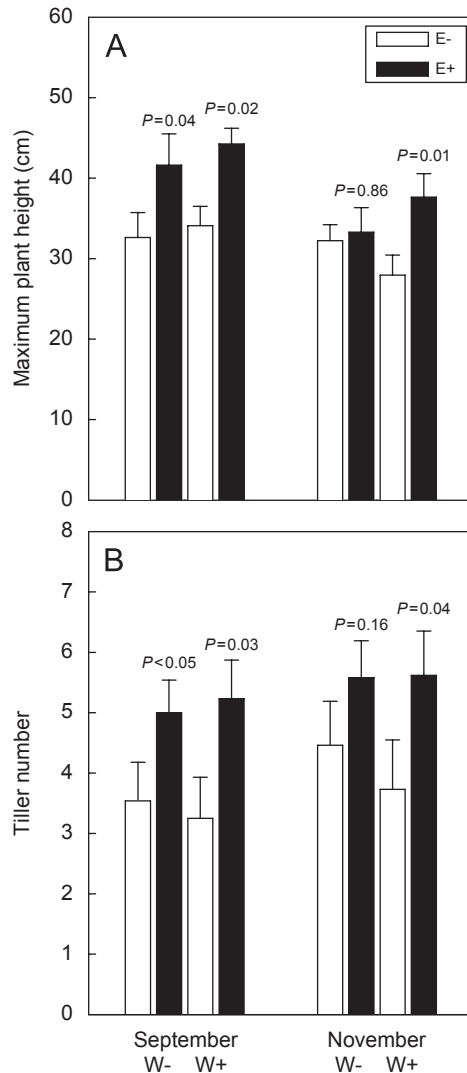


Fig. 3. Combined effects of the endophyte *Epichloë elymi* and experimental drought on (A) maximum plant height (cm) and (B) the number of tillers of *Elymus virginicus*. Bars indicate means \pm S.E. for each census. Filled bars are E+ plants cloned from plants naturally infected with *Epichloë elymi*. Open bars are E- plants cloned from experimentally disinfected plants. W- = experimental drought treatment. W+ = daily watering to saturation. *P*-values indicate significant differences for *a priori* contrasts (see text). Repeated measures MANOVA. Tiller number: endophyte $F_{1,41} = 8.8$, $P = 0.005$; time $F_{2,40} = 4.0$, $P < 0.05$; for all other factors, $P > 0.2$. Plant height: endophyte $F_{1,41} = 8.2$, $P = 0.007$; time $F_{2,40} = 23.5$, $P < 0.0001$; time-source population $F_{2,40} = 5.2$, $P = 0.028$; for all other factors, $P > 0.08$. Sample sizes were W-E+ = 12, W-E- = 13, W+E+ = 13, W+E- = 12.

reported for non-cultivated grasses in a recent review (Saikkonen et al. 2006). Given the benefits of the endophyte documented in our study and the substantial variation among populations in endophyte frequency, we suggest that when considering *E. virginicus* material for experiments, restoration, or forage planting, it will be useful to consider its endophyte status.

Contrary to our initial prediction, we were unable to document significant effects of the endophyte in ameliorating drought stress. In fact, endophyte benefits were generally stronger under daily watering than under drought, suggesting that drought stress may be experienced by the endophyte as well as the host plant. For some responses (e.g., plant height and tiller number after 1 month of growth), there were trends toward greater performance of endophyte-infected plants than endophyte-free under drought; increased sample sizes could possibly detect some benefits of the endophyte under drought as well as daily watering.

How does the endophyte benefit its host? In our experiment, aphids were the only herbivores present, and these were not significantly reduced by the endophyte. So, it is unlikely the observed differences were due to herbivore deterrence, which is commonly documented for other endophyte hosts and is caused by alkaloid production by the endophyte (Clay 1996; Clay & Schardl 2002; Siegel et al. 1990). The lack of an effect on aphids in our study does not rule out the possibility that herbivore deterrence is a primary benefit under field conditions. Some aphids appear relatively insensitive to the presence of endophytes in other systems (Siegel et al. 1990); thus, other herbivores may be more likely to be deterred. Additional benefits of *E. elymi* may include increased nutrient uptake (Lyons, Evans, & Bacon 1990; Malinowski, Alloush, & Belesky 2000) or enhanced photosynthesis (Marks & Clay 1996; Newman et al. 2003), both of which have been documented in tall fescue grass. Similar benefits could occur for *E. virginicus*, and the 50% reduction in root biomass that we observed for disinfected Minnesota (PMN) plants is consistent with an endophyte benefit via increased nutrition. Further experiments will be needed to uncover the precise mechanisms through which endophytes alter host performance in *E. virginicus*.

This work contributes to a growing body of literature demonstrating that fungal symbioses can increase plant performance and alleviate biotic and abiotic stress. Both evolutionary adaptation and phenotypic plasticity in plants may be strongly influenced by the presence of cryptic symbionts, such as endophytes (Bacon & White 2000; Clay & Schardl 2002; Schardl et al. 2004). Symbiotic microorganisms may also alter plant competitive hierarchies (Clay et al. 2005; Rudgers, Koslow, & Clay 2004; Rudgers, Mattingly, & Koslow 2005), influence higher trophic levels (Bultman & Bell 2003; Omacini, Chaneton, Ghersa, & Muller 2001; Rudgers & Clay 2005, 2007) and even change the trajectory of plant succession (Rudgers, Holah, Orr, & Clay 2007). The function of endosymbionts in ecological communities, particularly natural systems, deserves greater attention. Here, our results showed up to 50% declines in the performance of *E. virginicus* when the endophytic symbiont was eliminated. Benefits were weaker when

plants were subjected to drought stress. Because endophyte frequencies vary naturally among populations (Appendix), benefits of the endophyte depend on drought, and endophytes are inherited by seeds, we predicted that climate change will have the potential to influence selection on the *Epichloë–Elymus* symbiosis.

Acknowledgments

Many thanks to Megan Rúa, Michelle Afkhami, P.J. Pulliam, Sonia Kannadan, Reem Sabouni, Anika Vinze, Kate Boles, and Sami Hammer for assistance. Ken Whitney and Michelle Afkhami provided thoughtful comments on the manuscript. Thanks to Keith Clay and the Indiana University Research and Teaching Preserve, Ron Thill and the Stephen F. Austin Experimental Forest, and the National Forests and Grasslands of Texas for permission to collect samples. This work was funded by the National Science Foundation DEB 054278 and the Godwin Assistant Professorship to J.A.R.

Appendix. Supplementary materials

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.baae.2007.12.004](https://doi.org/10.1016/j.baae.2007.12.004).

References

- Ahlholm, J. U., Helander, M., Lehtimäki, S., Wali, P., & Saikkonen, K. (2002). Vertically transmitted fungal endophytes: Different responses of host-parasite systems to environmental conditions. *Oikos*, *99*, 173–183.
- Auge, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, *11*, 3–42.
- Bacon, C. W., & White, J. F. (Eds.). (2000). *Microbial endophytes*. New York: Marcel Dekker, Inc.
- Bacon, C. W., & White, J. F., Jr. (1994). Stains, media, and procedures for analyzing endophytes. In C. W. Bacon, & J. F. White Jr. (Eds.), *Biotechnology of endophytic fungi of grasses* (pp. 47–56). Boca Raton, FL, USA: CRC Press.
- Barbosa, P., Krischik, V. A., & Jones, C. G. (1991). *Microbial mediation of plant–herbivore interactions*. New York: Wiley.
- Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, *154*, 275–304.
- Bultman, T. L., & Bell, G. D. (2003). Interaction between fungal endophytes and environmental stressors influences plant resistance to insects. *Oikos*, *103*, 182–190.
- Cheplick, G. P. (2004). Recovery from drought stress in *Lolium perenne* (Poaceae): Are fungal endophytes detrimental. *American Journal of Botany*, *91*, 1960–1968.
- Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., et al. (2007). 2007: Regional climate projections. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, H. L. Miller (Eds.), *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge, UK and New York, NY: Cambridge University Press.
- Clark, E. M., White, J. F., & Patterson, R. M. (1983). Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of *in vitro* culture of the fungus. *Journal of Microbial Methods*, *1*, 149–155.
- Clay, K. (1996). Interactions among fungal endophytes, grasses and herbivores. *Researches on Population Ecology*, *38*, 191–201.
- Clay, K., Holah, J., & Rudgers, J. A. (2005). Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proceedings of the National Academy of Sciences USA*, *102*, 12465–12470.
- Clay, K., & Leuchtman, A. (1989). Infection of woodland grasses by fungal endophytes. *Mycologia*, *81*, 805–811.
- Clay, K., & Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist*, *160*, S99–S127.
- Gleason, H. A., & Cronquist, A. (1991). *Manual of vascular plants of Northeastern United States and adjacent Canada* (2nd ed.). Bronx, New York: The New York Botanical Garden.
- Groppe, K., Steinger, T., Sanders, I., Schmid, B., Wiemken, A., & Boller, T. (1999). Interaction between the endophytic fungus *Epichloë bromicola* and the grass *Bromus erectus*: Effects of endophyte infection, fungal concentration and environment on grass growth and flowering. *Molecular Ecology*, *8*, 1827–1835.
- Hartwig, U. A., Wittmann, P., Raun, R. B., Hartwig-Raz, B., Jansa, J., Mozafar, A., et al. (2002). Arbuscular mycorrhiza infection enhances the growth response of *Lolium perenne* to elevated atmospheric pCO₂. *Journal of Experimental Botany*, *53*, 1207–1213.
- Hintz, J. (2001). NCSS and PASS Number Cruncher Statistical Systems. Kaysville, UT.
- Houghton, J., Ding, Y., Griggs, D., Noguer, M., van der Linden, P., Dai, X., et al. (2001). *Climate change 2001: The scientific basis. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge, UK: Cambridge University Press.
- Hunt, M. G., Rasmussen, S., Newton, P. C. D., Parsons, A. J., & Newman, J. A. (2005). Near-term impacts of elevated CO₂, nitrogen and fungal endophyte-infection on *Lolium perenne* L. growth, chemical composition and alkaloid production. *Plant, Cell, and Environment*, *28*, 1345–1354.
- IPCC. (2007). *Climate change 2007: The physical science basis. Summary for policy makers* (18pp.). Intergovernmental Panel on Climate Change, Geneva, Switzerland.
- Johnson, N. C., Graham, J. H., & Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, *135*, 575–586.
- Jump, A. S., & Peñuelas, J. (2005). Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*, 1010–1020.
- Lehtonen, P., Helander, M., & Saikkonen, K. (2005). Are endophyte-mediated effects on herbivores conditional on soil nutrients? *Oecologia*, *142*, 38–45.

- Leuchtman, A. (1992). Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins*, *1*, 150–162.
- Lewis, G. C., Ravel, C., Naffaa, W., Astier, C., & Charmet, G. (1997). Occurrence of *Acremonium* endophytes in wild populations of *Lolium* spp. in European countries and a relationship between level of infection and climate in France. *Annals of Applied Biology*, *130*, 227–238.
- Lyons, P. C., Evans, J. J., & Bacon, C. W. (1990). Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. *Plant Physiology*, *92*, 726–732.
- Malinowski, D. P., Alloush, G. A., & Belesky, D. P. (2000). Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. *Plant and Soil*, *227*, 115–126.
- Malinowski, D. P., Belesky, D. P., & Lewis, G. C. (2005). Abiotic stresses in endophytic grasses. In C. A. Roberts, C. P. West, & D. E. Spiers (Eds.), *Neotyphodium in cool-season grasses* (pp. 187–199). Ames, IA: Blackwell Publishing.
- Marks, S., & Clay, K. (1996). Physiological responses of *Festuca arundinacea* to fungal endophyte infection. *New Phytologist*, *133*, 727–733.
- Marks, S., & Lincoln, D. E. (1996). Antiherbivore defense mutualism under elevated carbon dioxide levels: A fungal endophyte and grass. *Environmental Entomology*, *25*, 618–623.
- Meijer, G., & Leuchtman, A. (2000). The effects of genetic and environmental factors on disease expression (stroma formation) and plant growth in *Brachypodium sylvaticum* infected by *Epichloë sylvatica*. *Oikos*, *91*, 446–458.
- Morse, L. J., Day, T. A., & Faeth, S. H. (2002). Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. *Environmental and Experimental Botany*, *48*, 257–268.
- Morse, L. J., Faeth, S. H., & Day, T. A. (2007). *Neotyphodium* interactions with a wild grass are driven mainly by endophyte haplotype. *Functional Ecology*, *21*, 813–822.
- Motha, R. P., & Baier, W. (2005). Impacts of present and future climate change and climate variability on agriculture in the temperate regions: North America. *Climate Change*, *70*, 137–164.
- Muller, C. B., & Krauss, J. (2005). Symbiosis between grasses and asexual fungal endophytes. *Current Opinion in Plant Biology*, *8*, 450–456.
- Newman, J. A., Abner, M. L., Dado, R. G., Gibson, D. J., Brookings, A., & Parsons, A. J. (2003). Effects of elevated CO₂, nitrogen and fungal endophyte-infection on tall fescue: Growth, photosynthesis, chemical composition and digestibility. *Global Change Biology*, *9*, 425–437.
- Omacini, M., Chaneton, E. J., Ghersa, C. M., & Muller, C. B. (2001). Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature*, *409*, 78–81.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics*, *37*, 637–669.
- Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*, 37–42.
- Rudgers, J. A., & Clay, K. (2005). Fungal endophytes in terrestrial communities and ecosystems. In E. J. Dighton, P. Oudemans, & J. F. J. White (Eds.), *The fungal community* (pp. 423–442). New York: M. Dekker.
- Rudgers, J. A., & Clay, K. (2007). Endophyte symbiosis with tall fescue: How strong are the impacts on communities and ecosystems? *Fungal Biology Reviews*, *21*, 107–124.
- Rudgers, J. A., Holah, J., Orr, S. P., & Clay, K. (2007). Forest succession suppressed by an introduced plant–fungal symbiosis. *Ecology*, *88*, 18–25.
- Rudgers, J. A., Koslow, J. M., & Clay, K. (2004). Endophytic fungi alter relationships between diversity and ecosystem properties. *Ecology Letters*, *7*, 42–51.
- Rudgers, J. A., Mattingly, W. B., & Koslow, J. M. (2005). Mutualistic fungus promotes plant invasion into diverse communities. *Oecologia*, *144*, 463–471.
- Saikkonen, K., Lehtonen, P., Helander, M., Koricheva, J., & Faeth, S. H. (2006). Model systems in ecology: Dissecting the endophyte-grass literature. *Trends in Plant Science*, *11*, 428–433.
- Sanderson, M. A., Skinner, R. H., Kujawski, J., & van der Grinten, M. (2004). Virginia wildrye evaluated as a potential native cool-season forage in the Northeast USA. *Crop Science*, *44*, 1379–1384.
- SAS Institute, Inc. (2004). *SAS version 9.1.3*. Cary, North Carolina, USA: SAS Institute.
- Schardl, C. L., & Leuchtman, A. (1999). Three new species of *Epichloë* symbiotic with North American grasses. *Mycologia*, *91*, 95–107.
- Schardl, C. L., Leuchtman, A., & Spiering, M. J. (2004). Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology*, *55*, 315–340.
- Siegel, M. R., Latch, G. C. M., Bush, L. P., Fannin, F. F., Rowan, D. D., Tapper, B. A., et al. (1990). Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. *Journal of Chemical Ecology*, *16*, 3301–3316.
- Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* (2nd ed.). San Diego: Academic Press.
- Staddon, P. L., Gregersen, R., & Jakobsen, I. (2004). The response of two *Glomus* mycorrhizal fungi and a fine endophyte to elevated atmospheric CO₂, soil warming and drought. *Global Change Biology*, *10*, 1909–1921.
- Suttle, K. B., Thomsen, M. A., & Power, M. E. (2007). Species interactions reverse grassland responses to changing climate. *Science*, *315*, 640–642.
- White, J. F., Jr. (1987). Widespread distribution of endophytes in the Poaceae. *Plant Disease*, *71*, 340–342.

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