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EFFECTS OF ENDOPHYTE INFECTION IN TALL FESCUE (*FESTUCA ARUNDINACEA*: POACEAE) ON COMMUNITY DIVERSITY

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Recent studies have suggested that the presence of endophytes in tall fescue can lead to decreased species richness in the associated plant community. To assess the generality of this hypothesis, a field study tested the effects of endophyte infection on a 3-yr-old successional field dominated by *Festuca arundinacea*. The potential importance of endophyte infection relative to other environmental factors was tested by including two additional treatments: the effects of soil fertility and mowing. Contrary to previous studies, a positive relationship was found between endophyte infection frequency and diversity ($N = 23$, $F = 5.23$, $R^2 = 0.19$, $P < 0.03$). A strong interaction was found between the mowing treatment and endophyte infection frequency in predicting diversity ($N = 22$, $F = 36.1$, $R^2 = 0.84$, $P < 0.0001$), where the maximum species richness was present in plots that were both mowed and highly endophyte infected. The relationship between endophytes and diversity varied through the successional continuum (the mowing treatments) but was generally positive. The soil in mowed plots was drier than in unmowed plots ($t = 2.1$, df $= 28$, $P < 0.05$). We suggest that heavy mowing decreases soil moisture levels enough to reduce the interspecific competitive ability of infected *F. arundinacea*, thereby promoting local diversity. Endophyte presence is important, but the previously reported negative relationship between endophyte infection and community diversity is probably overly simplistic in complex ecological settings.

Keywords: community diversity, cool season grasses, C_3, endophyte, *Festuca arundinacea*, tall fescue.

Introduction

An endophyte is a parasitic to mutualistic internal fungal resident of a host plant that is asymptomatic for some portion of the life cycle of the fungus (Carroll 1988; Clay 1988; Petrini 1996). Endophytic fungi in grasses occur throughout the aboveground parts and have more fungal biomass than endophytes of other plants. Many grass endophytes are maternally transmitted via the seed; these are an extremely host-specific and specialized subset of perennial, systemic endophytes (Clay 1988, 1992; Bills 1996; Petrini 1996). The majority of these belong to the asexual genus *Neotyphodium* (sexually Ascomycota; Clavicipitaceae) (White 1987; Glenn et al. 1996) and infect the Pooideae grasses that dominate temperate areas (Clay 1990*a*, 1997*b*). Thus, it is likely that *Neotyphodium* and the Pooideae are coevolutionarily linked (Clay 1990*b*).

Despite the knowledge of systemic fungal infections, the ecological significance of endophytes was not realized until the mid-1970s, when the connection between the presence of fungi in forage and various livestock poisonings was made (Bacon et al. 1977). Consequently, the majority of grass endophyte studies have dealt with the two widely planted forage grasses, *Festuca arundinacea* Shreber. (tall fescue) and *Lolium perenne* L. (perennial ryegrass) and their predominantly mutualistic endophytes (Morgan-Jones and Gams 1982; Siegel et al. 1987; Bacon and Siegel 1988; Siegel 1993; Clay 1998). Studies of *F. arundinacea* are of particular importance since, while native to Europe, it is widely planted in temperate regions of the world, providing a mosaic of endophyte-infected and endophyte-free populations (Gibson and Newman 2001; Spyreas et al. 2001).

Benefits to the fungi include nutrition, long-term protection, and improved dissemination (by seeds) (Siegel et al. 1987). Reported host benefits include increased resistance to both grazing and insect herbivory, decreased nematode predation, antimicrobial and antifungal properties, both heat and drought stress tolerance, and increased overall vigor (e.g., tiller production, biomass, height, seed crop, seed germination; Clay 1987, 1990*a*, 1997*b*; Cheplick et al. 1989; Petroski et al. 1990; Guo et al. 1992; Siegel 1993). Costs to the plant, though poorly understood, are thought to be insignificant (Hill 1994; Bacon and Hill 1996).

Previous Population and Community-Scale Studies

Greenhouse studies have indicated increased vegetative vigor in infected versus uninfected individuals of the same species, with and without herbivory (Clay 1997*b*). *Festuca arundinacea* has almost exclusively been the species studied although field observations suggest that increased vigor with endophytic infection also occurs in other grass species (Clay 1997*b*). The primary exception reported to this pattern may be a slight advantage to uninfected *F. arundinacea* and *Lolium perenne*

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reported under low-nutrient conditions (Arachevaleta et al. 1989; Cheplick et al. 1989).

There have been several studies investigating both intraspecific and interspecific competition in *F. arundinacea* under controlled environmental conditions that have shown that infected plants are better competitors both with and without herbivory (Clay 1990*a*, 1997*a*; Hill et al. 1991; Marks et al. 1991; Clay et al. 1993; Clay and Holah 1999). This may explain why attempts to establish endophyte-free pastures from highly uninfected seed have at times been unsuccessful as a few seeds in the mix will contain live endophytes. Dramatic increases in infection frequencies within pastures often result (Bouton et al. 1993; Shelby and Dalrymple 1993), indicating high intraspecific competitiveness from endophyte-infected plants. However, infection frequency in wild fescues (including *F. arundinacea*) varies greatly within and among populations, suggesting that infected plants may not always outcompete uninfected plants (Spyreas et al. 2001). Furthermore, competitive ability may be highly variable under field environmental conditions, and competition studies have been conducted largely under greenhouse conditions.

There are a paucity of grass endophyte studies within natural communities (Clay and Leuchtmann 1989; Clay 1997*a*, 1997*b*). One study correlated infection frequencies with diversity. A field was disked, tilled, and planted with endophyte-free and endophyte-infected *F. arundinacea* of the commercial KY-31 variety (Clay and Holah 1999). After 4 yr, endophyte-infected fields showed less vascular-species diversity than endophyte-free fields, probably because fescue biomass increased with endophyte infection. The authors suggest that dominance by endophyte-infected grasses can reduce the diversity of the plant communities in which they occur. This idea has significant implications since endophyte-infected grasses (e.g., *F. arundinacea*, *L. perenne*, *Dactylus glomerata*, *Phleum pratense*, *Poa* spp.) are more abundant now than at any other time in history, and many planted fescues readily escape and establish themselves in the local flora (Clay 1997*a*; Spyreas et al. 2001).

Our study attempts to address the problem of relying on greenhouse experiments to ascertain the role of endophytes in grasslands. We examine the effects of endophyte infection on plant α diversity in a naturally established grassland. We hypothesized that endophyte-infected *F. arundinacea* would outcompete other plants in the community, leading to lessened community diversity compared with areas where uninfected *F. arundinacea* occurs. We also assessed the importance of abiotic factors on diversity through measuring several variables and through experimental manipulation using fertilizer and mowing treatments. Abiotic variables are hypothesized to be less important than the endophyte-infection frequency of *F. arundinacea* in controlling community diversity.

Material and Methods

Site Description and Sampling Methods

Our study was conducted at the Touch of Nature Environmental Center at Southern Illinois University at Carbondale (SIU-C) (lat. 37°37′15″N, long. 89°09′30″W). This area consists of fine, silty-mixed soils of the order Typic Fragiudalfs. The site was a successional old field containing infected and uninfected *Festuca arundinacea* grassland (Spyreas et al. 2001). This grassland lies on an upland ridge surrounded by dry-mesic forest with some of the most common species including *Panicum anceps* Michx., *Cardamine hirsuta* L., *Andropogon virginicus* L., *Sorghum halepense* (L.) Pers., *Tridens flavus* (L.) Hitchcock., *F. arundinacea*, *Vernonia missurica* Raf., *Setaria faberi* Hermm., and *Lespedeza cuneata* (Dum.-Cours.) G. Don., as well as the invasive shrubs *Elaeagnus umbellata* Thunb., *Rubus pensylvanicus* Poir., and *Rosa multiflora* Thund. The area studied was used as cropland until 1949 and then as a horse pasture until 1977. The field was plowed in spring 1981 and mowed annually until 1987, when it was abandoned. Our experiment was initiated in early spring 1996, when the field was disked (Gibson et al. 1999). Mowing and fertilizer treatments were initiated in late spring 1996. It is not known when or if *F. arundinacea* was sown into the field, but it was a dominant species before establishing the field experiment in 1996. It reestablished without reseeding to become a dominant species following establishment of the field experiment in late spring 1996.

A split-plot experimental design was established with 32 0.01-ha plots in eight blocks of four plots each. Half of the plots in each block were mowed and hand raked twice during the growing season, with half of these plots serving as controls (no mowing). The plots were randomly assigned to two fertilizer treatments, including annual fertilizer and control (no fertilizer). Each year, fertilized plots received 0.08 kg m^{-2} of diammonium phosphate (18-46-0), 0.06 kg m^{-2} of potassium (Potash K_2O, 0-0-60), and 0.07 kg m^{-2} of ammonium nitrate (33-0-0) in the spring and 0.07 kg m^{-2} of ammonium nitrate in the fall. The fertilizer was in granular form and broadcast evenly across each plot by hand. Soil tests revealed that added nutrients did not leach into adjacent control plots.

Endophyte-infection sampling was conducted in April 1999. Within each of the 32 plots sampled, up to 20 tillers (when present) of *F. arundinacea* were collected randomly for testing for endophytic infection. One tiller per *F. arundinacea* clump was sampled, except where plots had less than 20 clumps and then more than one sample per clump had to be taken to obtain 20 for the plot. Sample size was less than 32 in some analyses using endophyte-infection frequency because only 24 plots contained *F. arundinacea* tillers large enough to be sampled and identified in the spring. Percentage canopy cover of all vascular plants was estimated visually using a modified Daubenmire scale (0%–1%, 1%–5%, 6%–25%, 26%–50%, 51%–75%, 76%–95%, 96%–100% cover classes) (Abrams and Hulbert 1987). The spring flora was sampled in the first week of April 1999. All plots were resampled in the first week of September to detect species that were unidentifiable (including *F. arundinacea*) in the spring. On October 15, three measurements of both soil pH and moisture were taken in each plot using a Kelway soil tester (Kelway Instruments, Wyckoff, N.J.). The average of the three measurements was used in analyses.

Endophyte Staining

We used a quick-staining technique for detecting endophytic infection in *F. arundinacea* (Clark et al. 1983). Tissue samples

Table 1

Spearman's Rank Correlations (*R* Values) for All Variables Correlated with Species Richness, Endophyte Infection (EI), Fescue Cover (FC), Mowing (MO), and Fertilizer (FZ) at the Touch of Nature Field Site

	MO	FZ	PH	WA	FC	EI	N1	N2
Species richness	0.86***	−0.06	0.34	−0.39*	0.13	0.36†	0.84***	0.76***
Endophyte infection	0.46*	−0.07	0.06	−0.21	−0.13	…	0.3†	0.3†
Fescue cover	0.16	0.43*	−0.04	0.03	…	…	0.01	−0.05
Mowing	…	0.0	0.26	−0.29	…	…	0.78***	0.71***
Fertilizer	…	…	0.52*	0.56**	0.43*	…	−0.08	−0.04

Note. Abbreviations are as follows: WA = soil moisture; PH = soil pH; N1, N2 = Hill's diversity index; $n = 32$.
† $P < 0.1$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

were taken from leaf sheath bases because these contain the highest density of endophyte mycelium (Morgan-Jones and Gams 1982). Plant tissues with visible epiphytic fungal infection that consisted of germinated surface spores, extensive colonization of netted, obviously saprobic fungal hypha, or senescent leaf tissue were discarded. Staining methods are described further in Spyreas (2000).

Explanation of Measured Variables

The mowing (MO) and fertilizer (FZ) treatments were recorded as binomial variables. Endophyte-infection frequency (EI) is the percentage of the 20 tillers of *F. arundinacea* sampled per plot in which endophyte infection was noted. Local diversity was estimated according to three measures of diversity belonging to Hill's family of numbers (Ludwig and Reynolds 1988), where SN is species richness, N1 incorporates the Shannon index to estimate the number of abundant species, and N2 incorporates the Simpson index to estimate the number of very abundant species. Abundant and very abundant can be thought of as those species that are very common and dominant in a given area, so much so that they preclude the possibility of a diverse community. High values of N1 indicate that there were few abundant species with low overall evenness. High values of N2 indicate that there were few very abundant species, with even lower evenness. Diversity measures were calculated using midpoints of the cover classes and the number of species.

Data Analysis

The following general model was tested:

$$\begin{aligned}\text{local diversity} = {} & a + b\,(\%\ \text{endophyte infection}) \\ & + c\,(\text{soil pH}) + d\,(\text{soil moisture}) \\ & + e\,(\text{fertilizer treatment}) \\ & + f\,(\text{mowing treatment}).\end{aligned}$$

It was expected that local diversity would be less correlated with environmental variables or treatments than it would be with endophyte infection. The values for endophyte infection were found to contain two outliers that were omitted in the analyses. The percentage variables, endophyte infection, and soil moisture were nonnormal. Only endophyte-infection frequency could be normalized with an arcsine transformation (Kolmogorov-Smirnoff test; $P = 0.226$) (Sigma Stat 3.0; Jandel Corporation 1995). All variables had equal variances (Levene median test; $\alpha > 0.05$) (Sigma Stat 3.0; Jandel Corporation 1995). Residual values in all analyses were normal and had equal variances (Sigma Stat 3.0; Jandel Corporation 1995).

Initial analysis (Spearman's rank correlation) was performed on the raw, untransformed data to determine whether multicollinearity existed among variables (SAS Institute 1990). Selected variables were then analyzed (transformed and without outliers) using linear regression analysis on continuous variables, one-way ANOVA on discrete variables, and multiple regression analysis to generate predictor equations (SAS Institute 1990). For all tests in the study, the significance level was set at $P < 0.05$.

Results

Spearman's Rank Correlation Test for Multicollinearity

Species richness was not significantly correlated with endophyte infection, but the data suggest a weakly positive correlation. Species richness was negatively correlated with soil moisture and positively correlated with mowing. Mowing was also positively correlated with the two other diversity values (N1, N2). Endophyte infection frequency was positively correlated with mowing, and fertilizer was positively correlated with soil pH, soil moisture, and cover of *Festuca arundinacea* (table 1).

ANOVA and Simple Regression

Variables of interest to our hypothesis were analyzed using one-way ANOVA and simple linear regression. In all results, summaries of the untransformed data are graphed while results using transformed variables are presented in the text. All diversity measures (SN, N1, N2) were positively related to endophyte infection (fig. 1; species richness: $F = 5.23$, df = 23, $R^2 = 0.19$, $P < 0.03$). Species richness and endophyte infection were both higher in mowed versus unmowed plots (fig. 2*A*; ANOVA: SN, $F = 9.4$, df = 1,28, $P < 0.0001$) (fig. 2*B*; EI, $F = 2.6$, df = 1,22, $P < 0.02$). Soil moisture was lower in mowed plots (fig. 2*C*; ANOVA: $F = 2.1$, df = 1,28, $P < 0.05$). A linear regression using endophyte infection as the

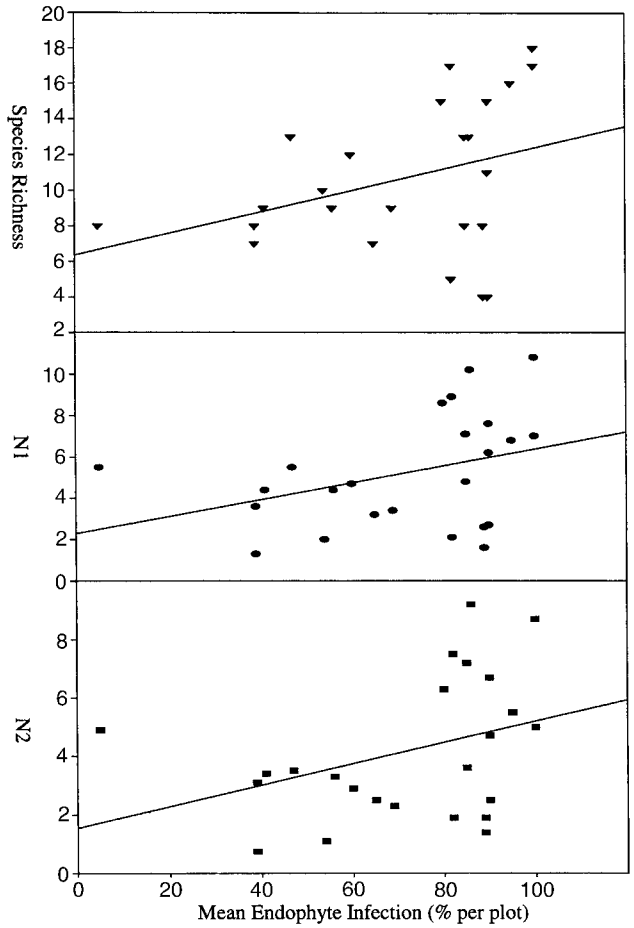

Fig. 1 Species diversity plot^{-1} by endophyte infection frequency plot^{-1} at the Touch of Nature field site. Arcsine values were used in calculations; raw data are plotted in this figure. Species richness = 6.339 + (0.060 × endophyte infection) (N = 24, R^2 = 0.19, P < 0.03); N1 = 2.276 + (0.041 × endophyte infection) (N = 24, R^2 = 0.19, P < 0.03); N2 = 1.549 + (0.036 × endophyte infection) (N = 24, R^2 = 0.19, P < 0.03).

dependent variable and soil moisture as the independent variable showed a significant negative relationship (fig. 3; F = 5.99, df = 23, R^2 = 0.21, P < 0.02).

Multiple Linear Regression

Multiple regression was performed on the three diversity measurements (SN, N1, and N2) and their significant predictor variables (table 2). Species richness was the primary dependent variable used as it had the highest correlation with the measured independent variables. Species richness was found to be best predicted by the single independent variable mowing (table 2; R^2 = 0.758, P < 0.0001). There were no other variables that significantly predicted species richness for the plots. When we deliberately added endophyte infection to the model to assess its predictive value, it increased the R^2 by only 0.002 and was not a significant individual predictor (table 2, model 1).

In unmowed plots, species richness decreased with infection

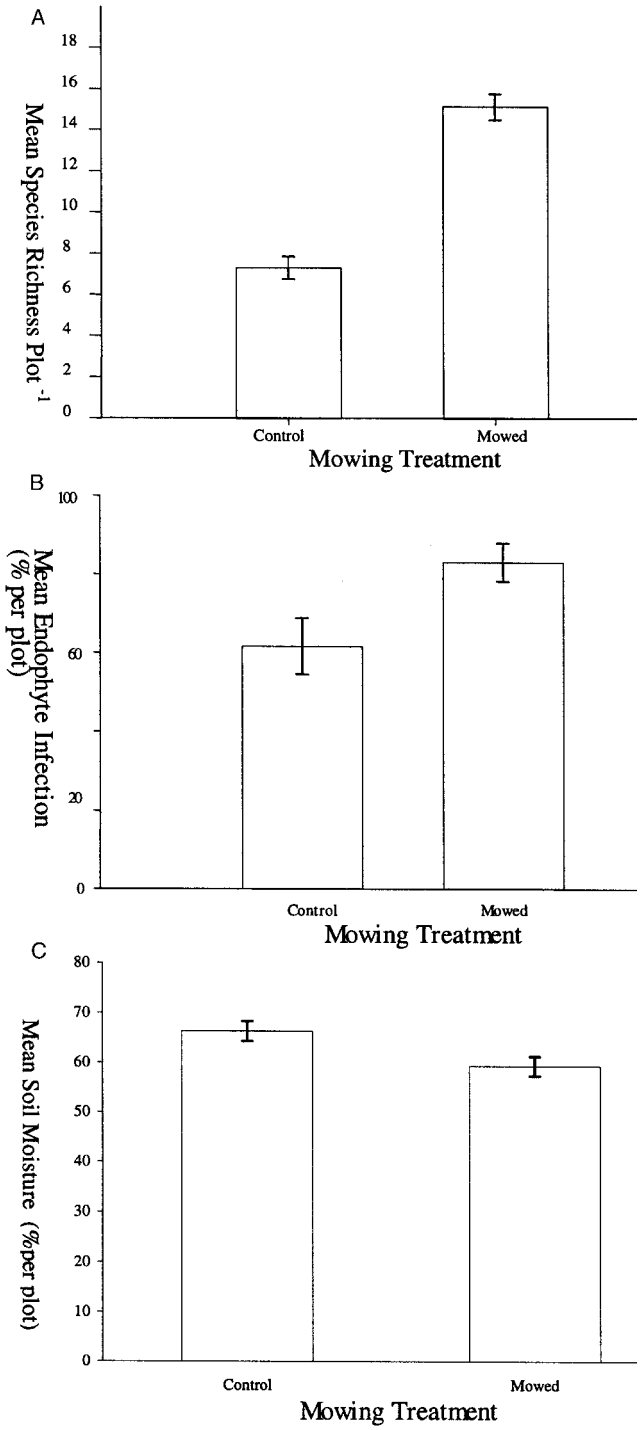

Fig. 2 *A*, Species richness (mean ± 1 SE) plot^{-1} by mowing treatment at the Touch of Nature field site (F = 9.4, df = 1,28, P < 0.0001). *B*, Endophyte infection frequency (mean ± 1 SE) plot^{-1} by mowing treatment at the Touch of Nature field site. Arcsine values were used in calculations; raw data were plotted (F = 2.6, df = 1,22, P < 0.02). *C*, Soil moisture (mean ± 1 SE) by mowing treatment at the Touch of Nature field site (F = 2.1, df = 1,28, P < 0.05).

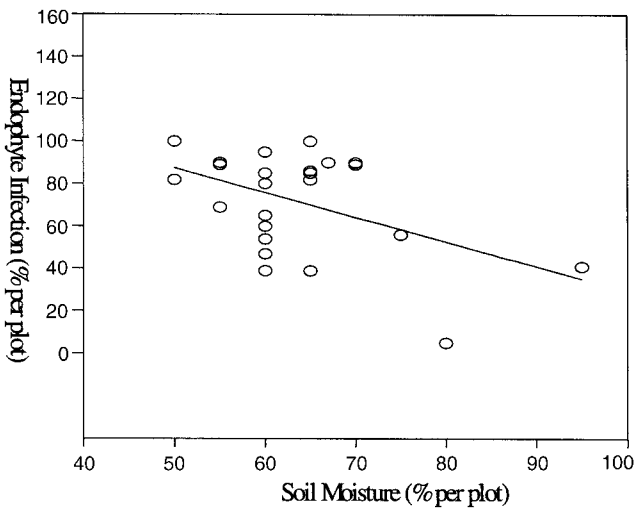

Fig. 3 Endophyte infection frequency plot^{-1} by soil moisture plot^{-1} at the Touch of Nature field site. Arcsine values were used in calculations; raw data were plotted. Endophyte infection = 145.381 − (1.160 × soil water) (F = 4.928, N = 24, adj. R^2 = 0.15, $P < 0.04$).

frequency, but in mowed plots, species richness increased with increasing infection frequency (fig. 4; table 2, mowing × endophyte interaction: $P < 0.003$). The interactive term between mowing and endophyte infection was added to the multiple regression model and was found to be a significant variable (table 2; $P < 0.0001$). When the variable accounting for the interaction was included in the regression analysis, the simple predictor variables (main effects) become nonsignificant (table 2, model 2).

Unless indicated, the results for the two diversity indices (N1, N2) mirrored those for species richness. The best variables at predicting N1 and N2 were mowing and *F. arundinacea* cover, though only mowing was a significant predictor (table 2). β values in our regression model show that mowing was associated with increased N2 levels and that *F. arundinacea* cover was associated with lower N2 (diversity). N2 was the only diversity value negatively correlated with *F. arundinacea* abundance. This finding is not surprising because *F. arundinacea* is a dominant species at the site, so those plots with a major dominant absent likely will be more diverse.

Discussion

In our study, endophyte infection was positively related to three separate measures of diversity (fig. 1). A previous study using *Festuca arundinacea* seeded into a newly disked field showed that endophyte-infected fields had much lower diversity compared to endophyte-free fields after 4 yr (Clay and Holah 1999). Our study used a previously established grass growing within a diverse matrix of co-associates, so it likely presents a more typical ecological picture of *F. arundinacea* community dynamics.

Mowing

Mowing and other disturbances increase diversity in grasslands as predicted by the intermediate disturbance hypothesis (Armesto and Pickett 1985; Wilson 1994; Collins et al. 1995). Not surprisingly, in this study, the lowest levels of diversity were found in unmowed plots because they were becoming dominated by dense, woody brush (*Eleagnus umbellata*, *Rubus* spp.) (fig. 2*A*).

Our study found a positive relationship between endophyte-infection frequency and mowing (table 1; fig. 2*B*). In contrast, Shelby and Dalyrymple (1993) found that uninfected, annually mown plots of *F. arundinacea* remained endophyte free after 4 yr, while unmowed plots increased to 8% infection. It is likely that drought was not a limiting factor for *F. arundinacea* in their study because they were not mown as intensively. Increased infection without mowing (Shelby and Dalrymple 1993) is likely explained by the dispersal and establishment of new, infected seed into their unmowed plots. In their study, mown *F. arundinacea* had higher density and ground coverage after 4 yr compared to unmown. These were cattle-grazed areas and because infected *F. arundinacea* seed can survive the digestive tract of livestock, animals can be a significant source of infected seed (Shelby and Schmidt 1991). Therefore, the mowed areas were likely too dense to allow new, infected seed recruitment. Van Santen et al. (1991) concluded that because mown plots are denser than unmown plots, endophyte-infected seedlings arising from the seed of neighboring fields do not become established.

Drought Tolerance of Tall Fescue and Diversity

The most significant finding of our study was that the pattern of species richness in unmowed versus mowed plots varied with infection frequency; i.e., there was an interactive effect. Species richness decreased with infection frequency in unmowed plots, but in mowed plots, species richness increased with increasing infection frequency (fig. 4). We expected richness to increase with disturbance (Armesto and Pickett 1985), but we did not expect endophyte-infected fescue to be more prominent in mowed areas. We suggest that this interaction is partially attributable to soil moisture/endophyte relationships. Mowed plots were cut almost to the soil surface after mowing and had increased solar radiation throughout the year (D. J. Gibson and B. A. Middleton, unpublished data). Unmowed plots had tall and often dense vegetation shading the fine, silty soil. The more drought-prone mown plots likely had high frequencies of infection because infected *F. arundinacea* has increased survivorship in droughts (West 1994). Thus, mowed *F. arundinacea* likely suffered drought stress, and those individuals that were infected were more likely to survive under summer stress.

In southern Illinois, *F. arundinacea* is commonly the dominant competitor when established in areas with ample moisture. Indeed, adjacent plots not used in this study that were only mowed once annually often contained *F. arundinacea* in over 100% of their area (D. J. Gibson and B. A. Middleton, unpublished data). Therefore, increased diversity in mown plots may be partially explained by a decreased interspecific competitive ability of *F. arundinacea* in drier areas. This may explain, in part, why soil moisture was negatively correlated

with diversity (table 1) and why diversity decreased with *F. arundinacea* dominance (table 2, N2). Competitive ability of early successional plants can be very sensitive to varying levels of water availability (Pickett and Bazzaz 1978; Bazzaz 1979).

Intraspecific advantage under drought stress has been shown for some endophyte-infected species (Clay 1997*b*). Endophyte-infected *F. arundinacea* has been found to have increased survivorship and growth under drought conditions (West 1994 and references within). *Festuca arundinacea* can be intolerant of moderate summer water deficits in areas such as the southeastern United States (West 1994). Infected *F. arundinacea* shows rapid stomatal closure under air-drying conditions and drought (Elmi et al. 1990; Buck et al. 1997), leading to decreased overall transpiration (Elmi et al. 1990). Earlier and greater leaf rolling and better long-term survival under water stress are related to endophyte-infected versus uninfected *F. arundinacea* (Arachevaleta et al. 1989). At high leaf temperatures, photosynthetic rates of up to 25% higher are found in infected plants (Marks and Clay 1996). Under water stress, infection-affected stomatal activity leads to reduced water loss from transpiration (Malinowski et al. 1997) as well as maintained turgor pressure (Bacon and Hill 1996). In support of this argument, we found that endophyte infection was negatively correlated with soil moisture (fig. 3), and mowed plots were significantly lower in soil-moisture levels (fig. 2*C*).

Endpophyte-related drought tolerance is coupled with intraspecific competitiveness in that maintenance of dense stands interferes with establishment and reproductive efficiency of endophyte-free plants (Clay 1991; Hill et al. 1991; West 1994). West (1994) claims that under field conditions, greater growth and persistence of infected plants probably results more from both enhanced competitiveness and resistance to drought and herbivory than from direct stimulation of growth. We assume similar levels of herbivory on fescue in mowed and unmowed plots, thus leaving drought stress as the factor likely affecting the grass/endophyte relationship. Interestingly, it has been shown that drought stress in infected *F. arundinacea* greatly increased endophyte-synthesized ergopeptine and pyrrolizidine concentrations (Belesky et al. 1989; Arachevaleta et al. 1992), suggesting an interaction between drought and the production of secondary compounds. Mechanisms for these changes in physiology leading to increased drought/heat tolerance and enhanced growth are not well understood. Influences might include the production of osmotically active substances, hormones, and alkaloids by the endophytes (Malinowski et al. 1997). Endophytes may produce or cause the plant to produce auxin-like plant growth regulators and/or inhibitors, or they may alter hormone metabolism. Auxin-like compounds have been isolated from cultures of *Balansia epichloë* (Siegel et al. 1987).

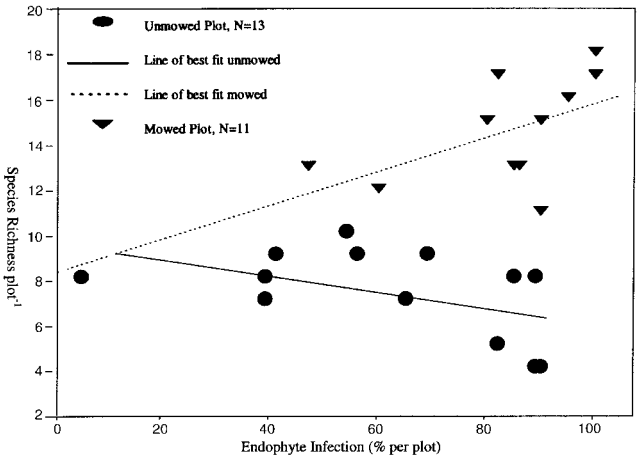

Fig. 4 Interaction of mowing and endophyte infection frequency plot^{-1} for species richness data at the Touch of Nature field site ($N = 24$, $R^2 = 0.84$, $F = 36.1$, $P < 0.0001$).

Table 2

Multiple Regression Analysis of Those Variables (Mowing, Fescue *Festuca arundinacea* Cover, and Endophyte Infection) That Best Predict Diversity (N1, N2, and Species Richness) at the Touch of Nature Field Site

	Partial R^2	Model R^2	Partial probability > F	Model probability > F
Diversity index N1:				
Mowing regime	0.5580	0.5580	0.0001	0.0001
Fescue cover	0.0510	0.6090	0.1120	0.0001
Diversity index N2:				
Mowing regime	0.4000	0.4000	0.0001	0.0009
Fescue cover	0.1080	0.5080	0.0440	0.0006
Species richness (model 1):				
Mowing regime	0.7580	0.7580	0.0001	0.0001
Endophyte infection	0.0020	0.7600	0.6610	0.0001
Species richness (model 2):				
Endophyte infection × mowing	0.8400	0.8400	0.0030	0.0001
Mowing regime	0.0010	0.8410	0.7030	0.0001
Endophyte infection	0.0000	0.8410	1.0000	0.0001

Note. Hill's diversity index N1 = 4.4 + 4.05(mowing) + −0.021(fescue cover) ($N = 24$, df model = 2, df = 23). Hill's diversity index N2 = 4.4 + 4.05(mowing) + −0.021(fescue cover) ($N = 24$, df model = 2, df = 23). Species richness model 1 (using simple variables) = 7.95 + 7.36(mowing regime) + −0.009(endophyte infection) ($N = 24$, df model = 2, df = 23). Species richness model 2 (using an interaction variable): interaction variables cannot be used in predictor equations ($N = 24$, df model = 3, df = 23).

Further Examination of the Obligate Mutualism

We may assume that the diversity of this old field is not driven by one dominant grass but by many interacting forces. Though we suggest some beneficial effects from endophytes in our study (drought tolerance), an obligate mutualism has not been shown. The level to which mutualistic endophytes are beneficial to grasses in natural systems has been questioned for a number of reasons. Saikkonen et al. (1998) argue that unlike the highly controlled agricultural studies conducted thus far, endophyte-plant-herbivore interactions should be more variable in natural systems. Other supporting arguments include the following:

1. Almost all research thus far has been on introduced species or cultivars. These plants and their endophytes have limited genotypic diversity because of selective breeding (Saikkonen et al. 1998) and may not behave like their wild progenitors in an ecological context. Indeed, *Neotyphodium coenophialum* from KY-31 *F. arundinacea* sampled in the United States was shown to be genetically depauperate compared to two native fescues and all other *F. arundinacea* (Leuchtmann and Clay 1990). We have also found variable infection frequencies among native populations of *F. arundinacea* in England, further supporting the nonobligate nature of the mutualism (Spyreas et al. 2001).

2. A second point concerns the extent to which competitive superiority can be attributed to herbivory-deterring alkaloids in the grass. It is known that antipathogenic and antiherbivory properties are conferred by a diverse array of fungally derived alkaloids that become present in plant tissue (Bush et al. 1993; Porter 1994). Many consider it to be logical that this relationship would evolve considering that within the plant kingdom, the Poaceae are one of only a few groups lacking the ability to produce secondary metabolites (such as alkaloids) on their own (Bacon and Hill 1996).

However, alkaloid production is under the influence of both the environment and host/fungal genomes (Arachevaleta et al. 1989; Leuchtmann 1992; Bacon 1993; Agee and Hill 1994; Roylance et al. 1994). Thus, constraints upon biosynthesis offer a wide spectrum of biological activity (Siegel and Bush 1997) and may not simply be a question of infected populations incurring less herbivory. The number of alkaloid types and concentrations produced by cultivar grasses may be atypically high. This may be related to either artificial selection or the atypically high-nutrient conditions of agricultural and turfgrass systems (Cheplick et al. 1989; Christensen et al. 1998; Saikkonen et al. 1998). Additionally, the frequency of infected plants may be unrelated to alkaloid levels, as these are highly variable among wild, infected plants, even within populations (Saikkonen et al. 1998). Unlike cultivars of *F. arundinacea*, which contain high levels of two or three types of alkaloids, seven other species of *Festuca* tested for alkaloids produce none or only one type of alkaloid (Siegel and Bush 1997). However, the relationship between environmental variables, such as soil fertility and endophytes, remains ambiguous, as a recent study on *F. arundinacea* found no effects on the abundance of *Neotyphodium hyphae* under three fertilization treatments (Hickam 1998). Similarly, field studies, including ours, show no endophyte-by-soil-fertility relationship (Schulthess and Faeth 1998) (table 1).

Wild *Festuca arizonica* and its endophyte *Neotyphodium starii* produce only one alkaloid, which occurs at very low levels and is typical of most native-grass populations (Siegel and Bush 1996). Studies on this grass in its native range of *Pinus ponderosa*/bunchgrass communities in the western United States indicate that *Neotyphodium* infection is actually lower in cattle-, elk-, and deer-grazed areas, compared to areas ungrazed for 20 yr (Schulthess and Faeth 1998), contradicting the idea that infection deters herbivory and increases grass survivorship. Most studies of endophyte-grass-herbivore interactions have indicated deterred herbivory with infection and have used either introduced livestock or nonnative, generalist invertebrate pests. Specialist herbivores may be more resistant to allelochemicals (Saikkonen et al. 1998), as most herbivore invertebrate species are specialists with few hosts (Crawley 1983).

3. Though endophyte infection is assumed to deter herbivory, herbivory on grasses may have weak effects on overall plant fitness and, therefore, population dynamics. Perennial grasses can be adapted to tolerate high and consistent levels of herbivory because of their basal meristems (Crawley 1983; Marquis 1992). Our study site has high levels of resident herbivores, including deer and rabbits (G. Spyreas, personal observation), as no hunting is allowed. Whether herbivory on *F. arundinacea* was detrimental to the vigor of the plant, as is commonly assumed, is not known. However, we found no indication that this was affecting competitiveness of infected versus uninfected *F. arundinacea* differentially.

Ecology of F. arundinacea *and Cool Season Grasses*

Study and speculation concerning host grass success has overwhelmingly emphasized the importance of herbivore deterrence by endophytes. The idea of a "super" grass, unencumbered by herbivory, outcompeting all other highly palatable neighbors, is an idea we believe to be too often coupled with endophytes. Though highly endophyte-infected cultivars of tall fescue often act as a long-lasting pest species when established, we found no evidence to indicate that endophyte-infected tall fescue decreases local diversity any more than its uninfected neighbors. Our study indirectly shows that herbivory is less important in explaining this potential mutualism than previously thought and that endophyte-induced drought tolerance may be more crucial. This and other studies show a narrow ecological range where endophytes are markedly mutualistic (Cheplick et al. 1989; West 1994; Marks and Clay 1996). For example, it is known that C_3 grasses are less successful than C_4 in more xeric conditions (Barnes et al. 1983; Archer 1984). Our research is consistent with the idea that under drought-stressed conditions, in the absence of an altered photosynthetic pathway, i.e., C_4, CAM, some endophytes can help cool season C_3 grasses better survive and compete.

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