REPORT

Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities

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Abstract

Asexual, vertically transmitted endophytes are well known for increasing competitive abilities of agronomic grasses, but little is known about endophyte–host interactions in native grasses. We tested whether the asexual *Neotyphodium* endophyte enhances competitive abilities in a native grass, Arizona fescue, in a field experiment pairing naturally infected (E+) and uninfected (E−) plants, and in a greenhouse experiment pairing E+ and E− (experimentally removed) plants, under varying levels of soil water and nutrients. In the field experiment, E− plants had greater vegetative, but not reproductive, growth than E+ plants. In the greenhouse experiment, where plant genotype was strictly controlled, E− plants consistently outperformed their E+ counterparts in terms of root and shoot biomass. Thus, *Neotyphodium* infection decreases host fitness via reduced competitive properties, at least in the short term. These findings contrast starkly with most endophyte studies involving introduced, agronomic grasses where infection increases competitive abilities, and the interaction is viewed as highly mutualistic.

Keywords

Competition, drought, endophytic fungi, *Festuca arizonica*, mutualism, native grasses, *Neotyphodium*, parasitism, symbiosis.

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INTRODUCTION

Symptomless, systemic and asexual grass endophytes that are transmitted vertically from mother plant to offspring have traditionally been considered strong plant mutualists (e.g. Clay 1988, 1990; Wilkinson & Schardl 1997; Clay & Schardl 2002). Evolutionary theory predicts that strong mutualistic associations arise between host and microbial symbionts when symbionts lose sexuality and transmission is vertical, and thus host and microbe reproduction are closely tied (e.g. Law 1985; Ewald 1994; Frank 1994; Wilkinson & Schardl 1997). This prediction has been supported for agronomically important, but non-native grass cultivars (mainly, tall fescue, *Festuca arundinacea*, Kentucky-31 cultivar) introduced from Eurasia to North America, New Zealand, Australia and South America. *Neotyphodium*-infected agronomic grasses are generally more vigorous and competitive compared with their uninfected counterparts (e.g. Marks *et al*. 1991; Clay *et al*. 1993; Bacon & White 1994; Clay & Holah 1999), either as a result of increased fitness under stressful environmental conditions, such as drought (e.g. Bacon & White 1994) and low nutrients (Malinowski & Belesky 1999), increased resistance to invertebrate and vertebrate herbivores (e.g. Clay 1988;

Breen 1994) and seed predators (e.g. Knoch *et al*. 1993), or via allelopathy (e.g. Matthews & Clay 2001). Infection frequencies of agronomic grasses, such as tall fescue and ryegrass, often rapidly increase within a few years to near 100% because of increases in general competitive abilities (e.g. Clay 1998; Clay & Holah 1999).

Neotyphodium infection frequencies in natural grass populations are much more variable than domesticated grasses, ranging from 0 to 100%, even among populations of the same grass species (e.g. Saikkonen *et al*. 1998; Schulthess & Faeth 1998; Vinton *et al*. 2001). Indeed, infection frequency of perennial ryegrass (e.g. Lewis *et al*. 1997) and tall fescue (e.g. Clement *et al*. 2001) in native habitats is also much more variable than high infection frequencies typically found under cultivation (e.g. Clay 1998). This variability in infection frequency within and among native grass species suggests the costs and benefits of harbouring endophytes change among different environments (Faeth 2002; Faeth & Bultman 2002) as has been shown for some domesticated grasses (e.g. Clay *et al*. 1993; Cheplick *et al*. 2000) and with different plant and endophyte genotypes (e.g. Meijer & Leuchtmann 2001). In systemically infected grasses with high mycelial biomass, resources for harbouring endophytes may be limiting, and compete with host plant growth and

reproduction. Systemic endophytes like asexual *Neotyphodium* and sexual *Epichloë* also may produce several types of alkaloids, nitrogen-rich compounds, which confer resistance to herbivores, but may also compete with the host for available nutrients (Faeth 2002; Faeth & Bultman 2002). Some costs may be offset if systemic endophytes enhance water use efficiency (e.g. Bacon & White 1994) or increase uptake of soil nutrients (Malinowski & Belesky 1999). Nonetheless, the costs of infection may outweigh the benefits in certain hosts and environments (e.g. Brem & Leuchtmann 1999; Ahlholm *et al.* 2002), assuming that allocation of limited resources is negatively correlated among competing plant functions (e.g. Hamilton *et al.* 2001), and even asexual endophytes may interact parasitically with their hosts (Faeth & Sullivan 2003).

To our knowledge, relative competitive abilities of native grasses infected with the asexual *Neotyphodium* endophyte have not been experimentally tested. We tested the relative competitive abilities of E+ and E− Arizona fescue (*Festuca arizonica* Vasey) over varying soil moisture and nutrient environments. In the field experiment, we tested naturally infected and uninfected plants grown under varying soil moisture and nutrient conditions. In the greenhouse experiment, we tested clones of four E+ maternal plants grown, under varying soil moisture and nutrients, in competition with their E− counterparts, from which the endophyte was experimentally removed. Based upon evolutionary theories of host–microbe interactions (e.g. Law 1985; Ewald 1994; Schardl & Clay 1997) and empirical studies of asexual, systemic endophytes in agronomic grasses (Clay 1988, 1990; Cheplick *et al.* 1989; Marks *et al.* 1991), we predicted that E+ Arizona fescue should generally perform better than E− grasses. Because Arizona fescue grows in habitats characterized by seasonal and yearly droughts and low soil nutrients (Schulthess & Faeth 1998), we predicted that infection by *Neotyphodium* should be most advantageous when water availability is low, but less so when water is supplemented. Predictions based upon nutrient availability are less clear. If infection and associated fungal alkaloids compete with other plant functions for limiting nutrients, such as nitrogen, then infection may be more advantageous in environments with high soil nutrients (e.g. Cheplick *et al.* 1989; Marks *et al.* 1991) Alternatively, if systemic endophytes enhance nutrient uptake by the host (e.g. Malinowski & Belesky 1999), then infected plants may outcompete uninfected plants when nutrients are limited.

MATERIALS AND METHODS

The host plant

Arizona fescue [*F. arizonica* Vasey (Pooideae)] is a dominant and native perennial bunchgrass in open Ponderosa pine woodlands in semi-arid plains at elevations between 2300 and 3200 m in Arizona, Nevada, New Mexico and Colorado in the USA and in northern Mexico. Sixty to 100% of individuals in populations of Arizona fescue are infected by a fungal endophyte *Neotyphodium starrii*, depending on the population (Schulthess & Faeth 1998). Arizona fescue reproduces by seed and plants begin reproducing within about 1 year in the field (Saikkonen *et al.* 1999). The climate in Arizona fescue habitats is characterized by two rainy seasons in winter and summer, typically separated by periods of very low precipitation, high evaporation and seasonal droughts, especially in May and June of each year.

The endophyte

Neotyphodium (Ascomycota: Clavicipitaceae) is a systemic endophyte of cool season grasses (Pooideae subfamily). Hyphae grow in intercellular spaces in mostly leaf sheaths, culms and seeds. In Arizona fescue, the endophyte remains near meristematic tissue when above-ground tissues senesce in the winter or are combusted during frequent fires, and the grasses remain infected throughout their life span (Faeth *et al.* 2002b). *Neotyphodium* (formerly *Acremonium*) is considered the exclusively asexual form of *Epichloë*: *Neotyphodium* is always transmitted vertically (type III infection, Schardl & Clay 1997 but see White *et al.* 1996), while strains of *Epichloë* can either be transmitted vertically via seeds or horizontally via spores in stromata (type II infection). *Neotyphodium* is evolutionarily derived from the sexual and horizontally transmitted form, *Epichloë*, that has lost sexuality (Clay 1990). *Epichloë* has never been observed in more than 10 years of observations of hundreds of populations of Arizona fescue

At least two species of *Neotyphodium* have been described from Arizona fescue (An *et al.* 1992; White *et al.* 1993), although the taxonomy is still unsettled. Based upon spore and conidia morphology and microsatellite DNA (Sullivan & Faeth 2004), *Neotyphodium* from experimental plants most closely resembles *N. starrii* and we use this taxonomic convention here. Like many native grasses, *Neotyphodium* infections in Arizona fescue produce only the alkaloid peramine, and levels are generally low and highly variable, ranging from 0 to 3.0 p.p.m. (Faeth *et al.* 2002a).

Field experiment

We used half-sib seeds from naturally infected and uninfected field plants. We collected seeds from 50 randomly selected maternal plants from within a large population of Arizona fescue at Buck Springs, a drainage meadow on Mogollon Rim, Arizona (elevation 2500 m) in September 1996. Seeds from each mother plant were stained and screened to detect endophyte infection status (Saha *et al.*

1988). From the original collection of 50 plants, we used seeds from eight infected (E+) and seven uninfected (E−) plants with sufficient seeds for the experiment. We counted the number of seeds from each plant. Seeds were then cold-treated at 4 °C for 3 months prior to the experiment. The seeds were then germinated on moist filter paper on Petri dishes and the percent germination was determined for seeds from each mother plant. We then transferred the seedlings to pots with equal amounts of sand, peat and potting soil and counted the seedling survival of the progeny of each mother plant (half-sib families) after 5 weeks.

At the end of January 1997, we planted the seedlings, two per 1 L pots, in natural soil collected from the Arboretum at Flagstaff, Arizona. In each pot, we planted either two infected (E+E+), two uninfected (E−E−), or one infected and one uninfected (E+E−) plant. The pots from each group were assigned randomly to 10 blocks of 12 pots, with 10 replicates of each treatment. The plants were grown in the greenhouse for an additional 4 months, and all were provided with equal and liberal amounts of water for growth, but no nutrients. Some plants in the pairs did not survive and these pairs were excluded from the experiment.

In May 1997, the plants were transplanted to a field plot at the Arboretum of Flagstaff, Arizona. Each plant in the pairs was approximately the same size, consisting of eight to 10 tillers each. The Arboretum is located in a Ponderosa pine – perennial bunchgrass habitat, and Arizona fescue is the dominant grass. The plot was an area in which Arizona fescue was naturally growing. The Arboretum is surrounded by a 4 m fence, which excludes large grazing vertebrates (native elk and deer and introduced cattle) but not invertebrates or small vertebrates. Thus, above- and below-ground herbivory were random factors, and any differences between E+ and E− plants should be reflected in overall estimates of growth and reproduction.

Before planting, all plants in the plot were removed and the soil within the plot was cultivated with hand tools. Individual pairs of plants were randomly assigned a position within the plot, removed from the pots, and planted (in original pairs and in the same proximity to each as when growing in the pots) and labelled with flagging and a metal stake with an aluminium tag. Pairs of plants were planted 1 m apart from other pairs to prevent competition between pairs. In nature, adult Arizona fescue plants typically grow widely spaced (c. 1 m) from each other (Schulthess & Faeth 1998). Thus, planting in close proximity (< 10 cm) greatly increased the likelihood of competition as plants grew. In the first year, we removed any plants other than the experimental plants weekly. In the second year, the entire plot was covered with Dalen® (Dalen Products Inc., Knoxville, TN, USA) weed barrier and then covered with commercial Ponderosa pine bark chips. The porous weed barrier allows water and nutrients to penetrate, but prevents unwanted growth from non-experimental plants. At each experimental pair, a 0.25 m diameter hole was cut into the barrier to allow for above-ground expansion of the experimental plants. Any non-experimental plants growing in the holes were removed every 2 weeks by hand.

The plant pairs were subjected to treatments consisting of two factors, water and nutrients. The high nutrient treatment consisted of 0.5 L of 2.6 g L^{-1} of 15N–30P–15K nutrient solution every 2 weeks, while the low nutrient plants were grown in the natural soil. Soil at the Arboretum, and in Arizona, generally is very low in nutrients (nitrogen content of soil at the Arboretum c. 0.20 mg 100 g^{-1}, Schulthess & Faeth 1998). Low water plants received only ambient precipitation while the supplemented plants were watered (4 L) two times per week during the two growing seasons. These treatments began after the plants were transplanted to the field of Arboretum at Flagstaff in May 1997. Some plant pairs were excluded from analyses because one or both of the plants had died during the experiment.

In May 1998 (the beginning of the second growing season), we measured the bunch diameter and height of each plant. Because Arizona fescue is a bunchgrass and grows approximately in the shape of a cylinder, volume of each plant was estimated based upon the formula for volume of a cylinder ($V = \pi r^2 h$). Plant volume is a reliable estimator of above-ground biomass (Faeth & Sullivan 2003). In September 1998, after two growing seasons, we extracted all living plants, returned them to the laboratory and counted the number of panicles (culms with flowering spikelets) for each plant in each pair. We also measured the number of tillers of each plant; however, because these results are qualitatively similar to volume and above-ground dry mass, we exclude analyses here. We determined dry mass of the above-ground parts after oven drying at 65 °C for 2 days. Because roots of most plant pairs were inextricably tangled, we could not distinguish roots of one half of the pair from the other, and did not use root biomass as dependent variable in the analyses. At the end of the experiment, we reconfirmed the endophyte infection status of each plant by screening at least eight seeds from each experimental plant (Saha et al. 1988).

Statistical analyses – field experiment

We first tested for differences in seed production, germination success and seedling survival in the greenhouse between the eight E+ and seven E− maternal plants, using separate t-tests for each variable. We used analysis of variance (ANOVA) to test for a block effect (original position of potted plants in the greenhouse before transplanting to the field). Because block had no effect on above-ground dry mass ($F = 0.85$, d.f. $= 9,180$, $P = 0.57$), number of panicles ($F = 1.13$, d.f. $= 9,180$, $P = 0.34$), or plant volume

($F = 1.55$, d.f. $= 8,180$, $P = 0.24$), we excluded block from subsequent analyses.

We then used ANOVA to test for effects of partner, infection, and treatments on bunchgrass above-ground dry mass, panicles (culms with flowering spikelets), and volume using the GLM procedure of the SYSTAT Version 10.0 statistical software (SYSTAT 2000). Our focal question was: did E+ and E− plants perform differently depending on whether they were growing with the same (E+E+ and E−E−) or different (E+E−) partner? Thus, E+ and E− plants growing in pure combinations were considered as controls, respectively, for those growing in the mixed combination (E+E−), because we are interested in relative competitive abilities of E+ and E− plants. Comparison of performance of just E+E− pairs without reference to pure combinations of each infection status could be misleading if either E+ and E− plants generally performed worse or better than the other, regardless of the infection status of the competing partner. Because of the large number of possible combinations of eight E+ and seven E− maternal genotypes, we could not replicate all possible combinations in all four treatments, and some pairs were excluded due to death of one individual of the pair in the greenhouse or in the field. Therefore, maternal plant genotype was used as a nested factor within partner type in the analyses.

Assumptions of ANOVA were tested. Variances of the dependent variables, number panicles and dry mass were normalized by square root transformation. A total of 190 plants in 95 pairs were used in the statistical analyses.

Greenhouse experiment

To test the effect of endophyte infection on competitive abilities, we planted E+ and E− seeds collected from four infected maternal plants at one density (four plants per pot). In this experiment, plant maternal genotype was strictly controlled because we used E− seeds from clones of the maternal plants from whence the endophyte infection was experimentally removed. In brief, *Neotyphodium* infections were removed from clones of four infected maternal plants (termed A, B, C, D) via hydroponic treatment with low levels of fungicide. These E− clones were then potted, split multiple times as replicates, and then grown in the field for 4 years. Other clones from the four maternal plant genotypes were treated similarly but without fungicide, and thus remain infected (E+). Finally, clones of one plant genotype (A) were treated with fungicide, but remain infected (fungicide treatment removes the endophyte in *c*. 50% of clones). These plants were used as controls (E+F) to test for any extraneous effects of the fungicide, of which there were none (Faeth & Sullivan 2003). Seeds were then collected from E− and

E+ plants in September 2001. Seeds were cold-treated for 30 days at 5 °C and then stored at room temperature. Details of endophyte removal are in Faeth & Sullivan (2003).

E+ and E− seeds from the four maternal plants were singly planted in cone containers (50 : 50 mixture of native and potting soil) in September 2002. After germination and formation of two tillers, seedlings were transplanted into 4 L pots (50 : 50 mixture of native and potting soil) in the following arrangement: 2 E+ and 2E− seedlings from each maternal genotype were planted on 12 and 13 December 2002. E+ and E− seedlings were planted in opposite corners of a square, in an X-shaped design, arranged equidistant from the edge of the pot, and each plant was marked with a colour-coded toothpick. Pots of each maternal genotype were replicated 40 times. All pots were watered liberally until 3 February 2003. On that date, 10 pots from each genotype were randomly assigned to one of four blocks on greenhouse benches and randomly assigned one of four treatments: (1) low water, low nutrients – 125 ml water per pot twice per week, no supplemented nutrients; (2) high water, no nutrients – 125 ml water per pot four times per week, no supplemented nutrients; (3) low water, high nutrients – 125 ml fertilizer solution (2.6 gm L^{-1} of 15N–30P–15K fertilizer) once per week; and (4) high water, high nutrients – 125 ml fertilizer solution four times per week. The amount of water in the low and high water treatments was based upon previous experiments and maintained *c*. 20 and 80% soil moisture content, respectively. During the course of the experiment, absolute water levels were adjusted to maintain soil moisture content (as measured by a soil moisture probe), but low water treatments always received half of water for the high water treatments. Adjustment of absolute water levels was necessary because of seasonal growth (Arizona fescue grows continuously in the greenhouse but rate of growth slows during lower light levels in winter months). The four blocks of pots were rotated sequentially in position on the greenhouse benches every 2 weeks to minimize within-greenhouse variation in lighting and temperature. The greenhouse was maintained at 20 °C during the day and 12 °C at night. A few plants died during the experiment and these pots were excluded from analyses. Infection status was confirmed by a modified tissue print immunoblot (Schulthess & Faeth 1998).

Treatments and rotation continued until 3 July 2003; plants appeared to reach maximum growth in mid-June 2003. All plants were then harvested. Roots and above-ground shoots were separated from each plant, dried at 65 °C, and then weighed. Mean values of root, shoot and total dry mass (root plus shoot) of each pair of E+ and E− plants in each pot (the experimental unit) were used as dependent variables in statistical analyses.

Statistical analyses – greenhouse experiment

To test the competitive abilities of E+ plants of the same maternal plant genotype with and without the endophyte, we used ANOVA [GLM procedure of the SYSTAT Version 10.0 statistical software (SYSTAT 2000)], with root, shoot and total dry biomass as dependent variables, and plant genotype, infection status, and treatments, and their interactions, as independent variables. Assumptions of ANOVA were tested. Variances of the dependent variables were normalized by log transformations.

RESULTS

Field experiment

Seed production and germination success did not differ between the E+ and E− mother plants (mean seed production per maternal plant: E−, $n = 7$, $\bar{x} = 59.0 \pm 18.90$; E+, $n = 8$, $\bar{x} = 75.88 \pm 11.11\%$, $t = -0.77$, d.f. = 13, $P = 0.46$; percent germination: E−, $n = 7$, $\bar{x} = 42.72 \pm 8.14\%$; E+, $n = 8$, $\bar{x} = 49.88 \pm 9.34\%$, $t = -0.58$, d.f. = 13, $P = 0.57$). Likewise, initial survival of seedlings in the greenhouse was not different for E− and E+ plants (seedling survival: E−, $\bar{x} = 78.14 \pm 7.95\%$; E+, $\bar{x} = 68.05 \pm 7.43\%$, $t = 0.93$, d.f. = 13, $P = 0.37$).

E− plants generally performed better than, or equal to, E+ plants (Table 1, Fig. 1). Above-ground dry mass and plant volume of E− plants was greater than E+ plants (Fig. 1a,c), and although not significantly different, mean number of panicles, an estimate of reproductive output, was also greater for E− plants (Fig. 1b). Growth and reproduction did not

depend on the whether the infection status of the partner was the same (E+E+ and E−E−) or different (E+E−) (no partner effect, Table 1) and did not vary with maternal plant genotype (Table 1). As expected, the treatments affected overall growth and reproduction (Table 1, significant treatment effects). Generally, above-ground biomass, number of panicles and plant volume were greatest in the supplemented water and nutrient, and lowest in the ambient water and treatment treatments, but supplemented water alone did not significantly increase these parameters relative to ambient conditions (Tukey HSD test of multiple comparison of treatment effects, $P < 0.05$, data not shown). Infection interacted with treatment to affect plant volume (Table 1, infection × treatment effect). E− plants had greater volume than E+ plants in the ambient water and nutrient treatment and the supplemented water treatment (Fig. 2). There were no other significant two- or three-way interactions (Table 1).

Greenhouse experiment

When endophytes are removed from their maternal hosts, competitive abilities increased, rather than decreased, as predicted. E+ plants had reduced root (Fig. 3a), shoot (Fig. 3b) and total dry biomass (Fig. 3c) relative to their E− counterparts (Table 2). The treatments altered root, shoot and total biomass (Table 2). Treatments with increased water, either alone or with added nutrients, increased root growth relative to the low water treatments (Tukey HSD test for multiple comparisons, $P < 0.05$, data not shown). Increased water or nutrients also significantly increased shoot biomass, but the high water and nutrient treatment had significantly greater shoot biomass than high water or

Source of variation	d.f.	Above-ground dry mass		Number of panicles		Plant volume	
		F	*P*	*F*	*P*	*F*	*P*
Infection	1	**3.39**	**0.07**	2.17	0.24	**7.09**	**0.008**
Partner	1	0.83	0.36	0.17	0.68	1.38	0.24
Treatment	3	**2.66**	**0.05**	7.25	<0.0001	6.42	<0.0001
Partner (maternal genotype)	16	0.86	0.62	0.86	0.61	0.92	0.62
Infection × partner	1	1.92	0.17	2.37	0.13	1.78	0.18
Infection × treatment	3	1.37	0.25	0.95	0.42	**2.87**	**0.04**
Partner × treatment	3	0.04	0.99	0.35	0.79	0.58	0.63
Infection × partner × treatment	9	0.76	0.52	1.12	0.34	0.59	0.55
Error	158,180						

Table 1 Summary of the analyses of variance of the effect of infection, partner (the same or different infection status), treatments, and maternal plant genotype on above-ground dry mass, number of panicles, and plant volume in the field experiment

Dependent variables were square root transformed to normalize variances. Error d.f. for dry mass and panicles (158) differ from volume (180) because not all plants were recovered for extraction. Significant ($P < 0.05$) or marginally significant ($0.10 < P < 0.05$) effects are in bold.
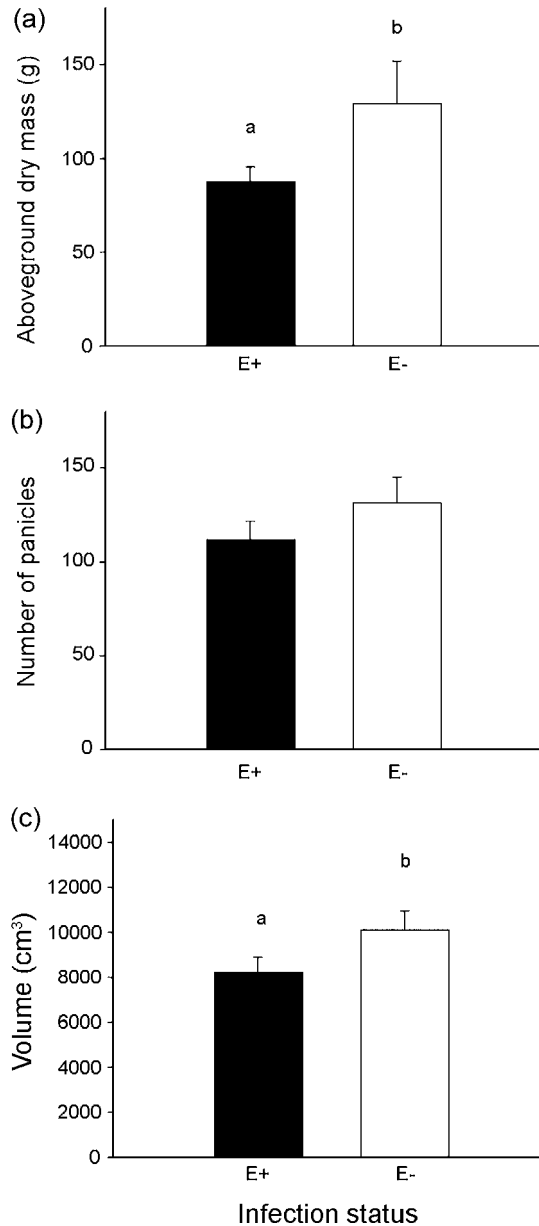
Figure 1 Effect of infection status (E− or E+) on mean (±SE) of (a) above-ground dry mass, (b) number of panicles and (c) plant volume. Data were square root transformed in statistical analyses to normalize variances; data presented are untransformed. Different letters above bars indicate significant (*P* < 0.05) or marginally significant (0.10 < *P* < 0.05) differences.

high nutrients alone (Tukey HSD test, *P* < 0.001, data not shown). Plant genotype influenced root but not shoot biomass (Table 2). Genotype D had reduced root mass relative to Genotype C, but not to the other genotypes (Tukey HSD test, *P* < 0.05). Shoot and root biomass were not affected by any two- or three-way interactions between infection status, plant genotype and treatment (Table 2).
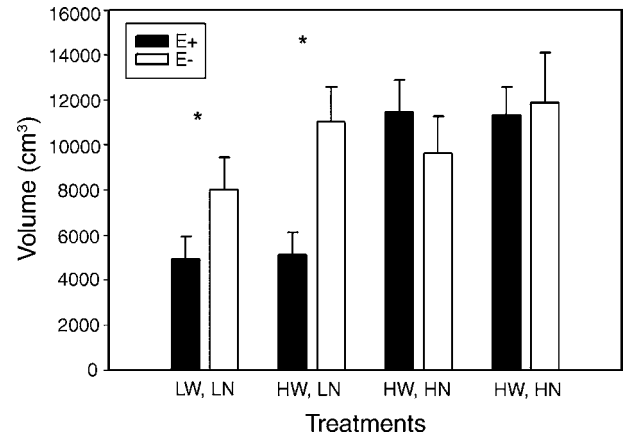

Figure 2 Interaction of infection status (E+ and E−) with treatments. Treatments were LW,LN = ambient water, nutrients, HW,LN = supplemented water, ambient nutrients, LW,HN = ambient water, supplemented nutrients, and HW,HN = supple-upplemented water and nutrients. Asterisks above bars indicate significant differences between E+ and E− plants for each treatment (*P* < 0.05, Bonferroni adjustment to α for multiple comparisons).

DISCUSSION

Our results do not support the hypothesis that infection by the asexual, *Neotyphodium* endophyte in Arizona fescue confers a competitive advantage over uninfected plants. Contrary to the prediction that E+ plants should generally be favoured in competition, E+ plants were either equivalent, or worse in competitive abilities in terms of growth and reproduction to E− plants in the field experiment. In the greenhouse experiment, E− consistently outperformed E+ plants in terms of root and shoot biomass. These findings conflict with evolutionary theory that predicts vertically transmitted symbionts should be strong mutualists (e.g. Law 1985; Ewald 1994), as well as empirical evidence from *Neotyphodium*-infected domesticated grasses (e.g. Schardl & Clay 1997; Wilkinson & Schardl 1997; Clay & Schardl 2002). Our results, however, should be interpreted cautiously because we used only two different densities and arrangements of plants; other designs may have yielded different outcomes (e.g. Reynolds 1999). Nevertheless, our findings are consistent in both the field and greenhouse experiments, and with other long-term studies of E+ Arizona fescue, where E− plants performed better than E+ plants when grown under no competition (Faeth & Sullivan 2003).

Maternal plant genotype did not influence growth and reproduction in the field experiment, but did affect root biomass in the greenhouse experiment where plant genotype was strictly controlled. However, increase in competitive abilities associated with removal of the endophyte from host
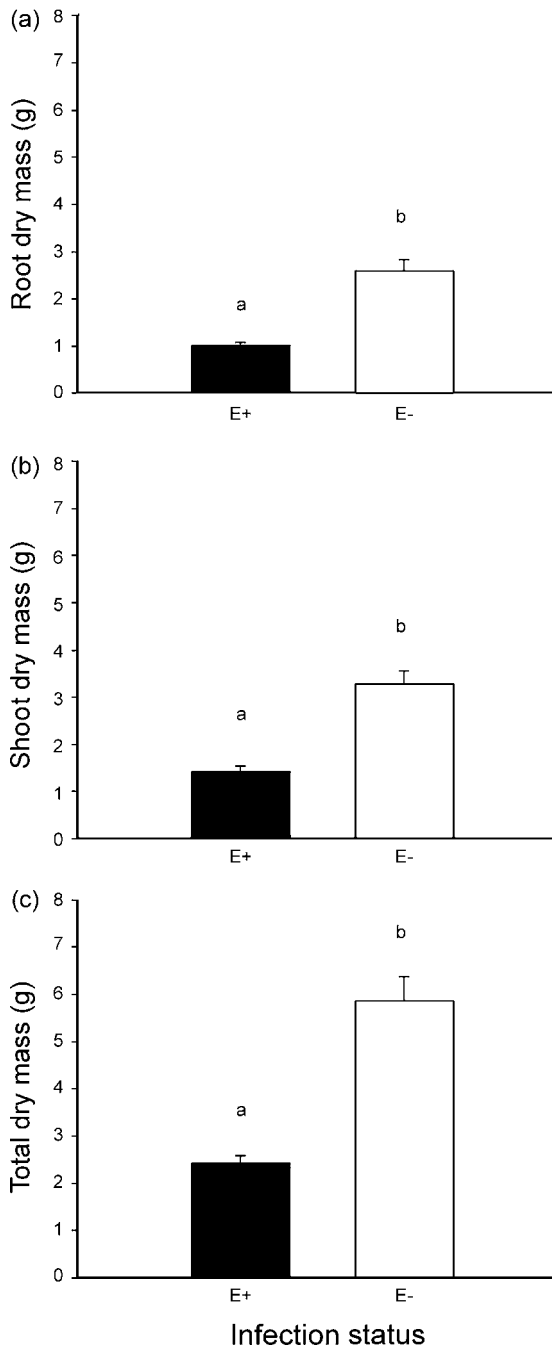
Figure 3 Effect of infection status (E− or E+) on mean (±SE) of (a) root dry mass, (b) shoot dry mass and (c) total dry mass. Data were square root transformed in statistical analyses to normalize variances. Different letters above bars indicate significant differences ($P < 0.0001$).

grasses was consistent across all plant genotypes (no genotype × infection interaction, Table 2). Both plant and endophyte genotype may influence competitive abilities and other properties, such as alkaloid content (e.g. Meijer &

Leuchtmann 2001). However, in Arizona fescue, plant genotype appears to override differences in endophyte genotypes (Faeth *et al.* 2002a). We could not measure root biomass in the field experiment, and although maternal genotype had no influence on above-ground growth, it may have also influenced root growth in this experiment.

The reduced competitive abilities of E+ plants relative to E− plants were also consistent across all of the treatments for the greenhouse experiment (Table 2, no treatment × infection interaction). However, in the field experiment, E+ showed reduced competitive abilities for both the low and supplemented water treatments, at least for plant biomass and volume (Fig. 2). These results are surprising because previous studies of *Neotyphodium*-infected agronomic grasses, especially tall fescue (*F. arundinaceae*) cultivars, show that infection typically increases competitive abilities under water stress (e.g. Clay *et al.* 1993; Bacon & White 1994; Elbersen & West 1996). However, increased resistance to drought is not universal even in E+ domesticated grasses. For example, *Neotyphodium*-infected perennial ryegrass (*Lolium perenne*), which is typically found in more mesic environments, is not more resistant to drought than E− plants (e.g. Cheplick *et al.* 2000). Because Arizona fescue grows in semi-arid habitats, where seasonal droughts are normal, and yearly droughts are highly probable within the perennial lifespan (>10 years) of the host grass (Schulthess & Faeth 1998), we expected that *Neotyphodium* infections should confer competitive advantages under low water availability. For example, *Neotyphodium* apparently enhances drought resistance in some native Morrocan fescues, growing in similar semi-arid habitats (e.g. Buck *et al.* 1994).

One explanation for lack of endophyte-enhanced competitive abilities at low soil moisture is that plants in the field experiment in the low (ambient) water treatment did not experience sufficient reductions in soil moisture. Generally, plants appeared more nutrient limited than water limited in this experiment, although we could not measure root biomass, the growth parameter affected by water availability in the greenhouse experiment. We relied upon ambient rainfall to simulate drought conditions for plants in the non-supplemented treatments, and installation of the weed barrier, although porous, may have reduced evaporation from the soil in the second growing season. Precipitation over the 2-year experiment ($\bar{x} = 54.6$) was very close to the 50-year average rainfall ($\bar{x} = 55.7$ mm), although the second growing season, when most growth occurred, was wetter than average (69 mm). However, in the greenhouse experiment, where soil moisture could be more closely controlled, and overall growth in high water treatments was greater than in the low water treatments, plants without their endophytes outperformed their E+ counterparts. Thus, we find no compelling evidence that infection confers

Table 2 Summary of the analyses of variance of the effect of infection, genotype, and treatments on dry mass of roots, shoots and total biomass in the greenhouse experiment

Source of variation	d.f.	Dry mass roots		Dry mass shoots		Total dry mass	
		F	*P*	*F*	*P*	*F*	*P*
Infection	1	**26.22**	**<0.0001**	**35.50**	**<0.0001**	**34.03**	**<0.0001**
Genotype	3	**3.28**	**0.02**	1.40	0.24	2.02	0.11
Treatment	3	**38.36**	**<0.0001**	**91.09**	**<0.0001**	**67.97**	**<0.0001**
Infection × genotype	3	0.13	0.94	0.72	0.54	0.28	0.84
Infection × treatment	3	0.26	0.86	0.21	0.89	0.29	0.84
Genotype × treatment	3	0.94	0.49	0.65	0.75	0.60	0.80
Infection × genotype × treatment	9	0.38	0.94	0.34	0.96	0.36	0.95
Error	286						

Dependent variables were log transformed to normalize variances. Significant ($P < 0.05$) effects are in bold.

competitive advantages for Arizona fescue hosts in any of the soil moisture and nutrient environments that we tested.

Another purported mechanism increasing the competitive abilities of *Neotyphodium*-infected grasses, at least in agronomic tall fescue, is enhanced nutrient uptake in nutrient-poor soils, presumably by altering fine root structure and changing chemical environments near root zones (e.g. Malinowski & Belesky 1999). Previous experiments with agronomic grasses (Cheplick *et al.* 1989) indicate that E+ grasses perform better than E− at low soil nutrients, but not enriched ones. However, in our field experiment, E− plants performed better than E+ plants in the ambient (low) nutrient treatments, suggesting that the endophyte and host compete for limited nutrients. Similarly, in non-competitive experiments, E− *Festuca pratensis*, a native grass, performed better than E+ plants in low nutrient conditions (Ahlholm *et al.* 2002).

Maintenance of high infection levels in Arizona fescue

Our general finding that E+ Arizona fescue competes no better, and usually worse, in competition with E− grasses, poses a quandary: how then are the observed high infection levels in natural Arizona fescue populations (Schulthess & Faeth 1998) and other native grasses (e.g. Vinton *et al.* 2001; Faeth & Bultman 2002; Faeth 2002), maintained? *Neotyphodium* is asexual and strictly seed-borne (Clay 1990; Clay & Schardl 2002, but see White *et al.* 1996). Adult plants cannot gain infections, but infections may be lost, either because of imperfect transmission (*sensu* Ravel *et al.* 1997, hyphae fail to grow from culms into seeds) or loss of viability of hyphae in seeds and seedlings (Siegel *et al.* 1984). Therefore, unless *Neotyphodium* confers fitness benefits to the host, infection frequency should decline either because of random loss of the endophyte or selection against hosts with parasitic endophytes (e.g. Wilkinson & Schardl 1997).

The typically high infection frequencies in Arizona fescue may be attributable to some other benefit associated with *Neotyphodium*. Other benefits that indirectly increase competitive abilities and known from infected agronomic grasses include increased resistance to herbivores (e.g. Clay 1990; Breen 1994), seed predators (Knoch *et al.* 1993), and plant pathogens (e.g. Gwinn & Gavin 1992), increased germination rates (e.g. Clay 1987; Clay *et al.* 1993), and allelopathic effects (e.g. Matthews & Clay 2001). However, these purported benefits, as well as others (e.g. resistance to fire) are not found in infected Arizona fescue (e.g. Saikkonen *et al.* 1999; Tibbets & Faeth 1999; 2002b; Hamilton 2002; Faeth & Sullivan 2003; Neil *et al.* 2003). Furthermore, our field experiment, where plants were subjected to background levels of herbivory (although large vertebrates were excluded), root feeding, pathogens, and allelopathic effects, suggests that these other mechanisms associated with endophytes do not indirectly increase growth and reproduction for Arizona fescue.

One explanation for persistence of high infection levels is that asexual endophyte infections are infrequently mutualistic, but the positive effects occur at critical times, such as periods of rapid population decline such as during severe and prolonged droughts (Faeth 2002; Morse *et al.* 2002). For example, the benefits of VA mycorrhizal symbionts of xeric grasses are manifested mainly during severe droughts (Allen & Allen 1986). Our experiments, although relatively long term in nature, may have failed to capture long-term selective pressures associated with a long-lived host plant and its symbiont. Nevertheless, the question of how relatively high frequencies of *Neotyphodium* infection are maintained in natural grass populations remains unanswered. For Arizona fescue in typical semi-arid habitats, the answer does not appear to reside in the conventional tenet that asexual *Neotyphodium* provides advantages via increased general competitive abilities, at least under the environmental conditions and time frames tested here. To the contrary,

Neotyphodium infections in Arizona fescue appear to act parasitically by reducing competitive abilities.

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