

Intraspecific competition of endophyte infected vs uninfected plants of two woodland grass species

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Grass endophytes (Clavicipitaceae, Ascomycota) are generally considered to be mutualists which increase the host's fitness. Infected plants are often more persistent and competitive than uninfected plants, influencing population dynamics and plant community diversity. However, most of this empirical evidence is based on studies focusing on agronomically important grass species such as tall fescue or perennial ryegrass and their implications for livestock and man-made habitats. Recent studies indicate that endophyte-plant associations may be more variable, ranging from parasitic to mutualistic. In the present study, we investigated the influence of endophyte infection on two wild woodland grasses, which are naturally infected with distinct fungal endophytes: *Brachypodium sylvaticum* with *Epichloë sylvatica* and *Bromus benekenii* with *Epichloë bromicola*. An intraspecific competition experiment was conducted over two growing seasons in the greenhouse and in an experimental garden. At first harvest (after 12 weeks growing), endophyte infection had a significant negative effect on above ground dry matter yield (DMY) of *B. sylvaticum*, but a significant positive effect on DMY of *Br. benekenii* under competition. The same differential effects on DMY and on total seed number were also observed at final harvest (after 62 weeks growing). Results from *Br. benekenii* were consistent with our hypothesis of increased competitive abilities of infected plants in nature which could explain the high infection rate observed in natural populations. In contrast, this explanation does not hold true for *B. sylvaticum*, and other factors such as increased herbivore and pathogen resistance together with frequent horizontal transmission may be responsible for the very high incidence of this association in nature. Our results confirm previous predictions that beneficial effects of endophyte infection in wild grasses can vary for different grass species, even in comparable habitats.

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Endophytes are microorganisms that colonize internal tissues of plants for all or part of their life cycle without causing signs of tissue damage (Wennström 1994, Wilson 1995). A widespread and very common group of endophytes in certain grassland communities are systemic clavicipitaceous fungi infecting grasses in the subfamily Pooideae (White 1987, Clay and Leuchtmann 1989). Associations with these endophytes can be an important factor influencing seedling establishment and competitive interactions among plants (Clay 1987). Taxonomically they are referred either to the sexual reproducing genus *Epichloë* (Clavicipitaceae, Ascomy-

cota) or to the asexual counterpart *Neotyphodium* (formerly *Acremonium* (Glenn et al. 1996)). The asexual endophytes are asymptomatic and are maternally transmitted by growth into the ovules and seeds of infected plants (White et al. 1993). Sexually reproducing endophytes form cylindrical stromata on the surface of culms, preventing floral development and seed set of the plant (choke disease). Therefore, the fungus' mode of reproduction imposes different costs to the host grass. Asymptomatic associations are assumed to be highly mutualistic, while stromata-forming endophytes show clear aspects of parasites.

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Benefits for the plant may arise from fungal alkaloids, which protect the host against herbivores (Breen 1994, Bush et al. 1997). Other benefits of endophyte infection include enhanced growth and increased competitive abilities (Clay 1990, Clay et al. 1993, Malinowski et al. 1997a, Clay and Holah 1999). Moreover, endophytes may provide increased resistance to drought, pests and fungal pathogens (Kimmons et al. 1990, Schuster et al. 1995, Elbersen and West 1996, Yue et al. 2000); but see also Cheplick et al. (2000).

In the last decade, the ecological importance of this fungal group has been studied intensively in different grass-endophyte systems. However, most studies focused on agronomically important grass species of pastures or turf, such as perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and red fescue (*Festuca rubra*) (Siegel and Bush 1994, Cheplick 1998, Clay and Holah 1999, Matthews 2001); but see also Saikkonen (2000). By contrast, knowledge on the influence of fungal endophytes in associations with wild grasses is rather limited (Saikkonen et al. 1998). Recent investigations on grasses in natural populations and also with *L. perenne* indicated that consequences of endophyte infection for the fitness of the host can be variable or neutral (Schulthess and Faeth 1998, Saikkonen et al. 1999, Cheplick et al. 2000). Saikkonen et al. (1998) proposed a continuum of interactions, where costs and benefits of endophyte infection may vary spatially and temporally in natural populations, and thus selection and frequency of infected or uninfected hosts. Consequently, natural populations may be mosaics of unique endophyte-host plant genotypic combinations that are adapted to local biotic and abiotic environments.

In this study we focused on two wild woodland grasses, *Brachypodium sylvaticum* (Huds.) P.B. and *Bromus benekenii* (Lange) Trimen, infected by their host-specific endophytes. The high infection frequencies, reaching almost 100% in *B. sylvaticum*, suggest that the endophytes may be beneficial by enhancing the competitive ability of infected host plants and thus increasing the proportion of infected plants over time in natural populations. Enhanced competitive abilities of endophyte-infected grasses have been well documented for tall fescue (Hill et al. 1991), perennial ryegrass (Clay et al. 1993) and meadow fescue (*Festuca pratensis*) (Malinowski et al. 1997a). These effects may be related to changes induced by the endophyte, such as modifications in shoot and root growth (Latch et al. 1985, De Battista et al. 1990, Malinowski et al. 1997a, b), physiological responses to abiotic stress (West 1994, Malinowski et al. 1997a, b), production of anti-herbivore substances (Barker et al. 1983, Clay 1988, Siegel et al. 1990, Schardl and Philips 1997) and allelopathic compounds (Petroski et al. 1990). However, changes at the physiological level resulting from the symbiosis are not well understood, and mechanisms which directly influence grass behavior are still unknown.

The objective of our study was to examine the effect of endophyte infection on the competitive ability of two host grasses in intraspecific competition during the stage of plant establishment. We were interested, whether or not the two wild grasses, which occupy the same habitat but are infected by different endophyte species, are equally influenced by endophyte infection and whether results known from agricultural grasses are valid also for wild grasses.

Materials and methods

The study system

The clump-forming grass *Brachypodium sylvaticum* is a common element of beech forests in temperate Eurasia (Ellenberg 1996). Surveys in Switzerland and other European countries suggest that probably all plants of *B. sylvaticum* are infected with the strictly host-specific fungus *Epichloë sylvatica* Leuchtman & Schardl (Bucheli and Leuchtman 1996, Leuchtman and Schardl 1998). The association is characterized by a high rate of asymptomatic, seed-transmitted infections, and only rare sexual reproduction of the fungus, which appears to be genotypically controlled by the endophyte (Meijer and Leuchtman 2000, 2001). The broad distribution of *E. sylvatica* and the consistent infection of host plants suggest that infection should provide host plants with a substantial fitness advantage. Plants of *B. sylvaticum* associated with *E. sylvatica* have been analyzed for the presence of lolines, ergovalines, and ergot alkaloids but none of these compounds could be detected (Leuchtman et al. 2000); nevertheless, infected *B. sylvaticum* plants are resistant against the herbivore *Spodoptera frugiperda* (J. E. Smith) (Brem and Leuchtman 2001).

The predominant mode of reproduction of *E. sylvatica* is asexual. The hyphae grow into the seeds, and derived offspring are infected with the same fungal clone. The sexual stage, also known as "choke disease" involves the production of ascospores after fly-mediated cross-fertilization (Bultman et al. 1998). However, stromata are rarely seen at natural sites and are restricted to clusters within populations of the grass. Horizontal transmission by ascospores has been assumed to be rare, but recent studies have shown that it can be frequent (Brem and Leuchtman 1999, Meijer and Leuchtman 2000).

Bromus benekenii is a clump-forming grass with short rhizomes, and is common in clearings of European and west Asiatic deciduous and coniferous forests on rich soils (Hoffmann 1989, Ellenberg 1996). It often co-occurs and competes with the related species *Bromus ramosus* Huds. Because of better adaptation to water stress and light intensity, *Br. ramosus* seems to have an advantage in competition with *Br. benekenii* (Hoffmann

1989). Unlike *B. sylvaticum*, *Br. benekenii* plants do not form dense populations; they rather grow more scattered within large populations. A high percentage of *Br. benekenii* plants are infected by asexual strains of *Epichloë bromicola* Leuchtmann & Schardl (Leuchtmann 1992, Leuchtmann and Schardl 1998) in nature. No stromata have ever been observed on this host in natural populations, thus systemic infection of seeds in infected mother plants appears to be the only mechanism of endophyte transmission. Because of the considerable peramine content found in infected *Br. benekenii* plants, increased insect herbivore resistance of infected plants are expected (Leuchtmann et al. 2000).

Competition experiments

Source of seeds and seedlings

Seeds of *B. sylvaticum* were collected in bulk in autumn 1997 from a natural population of the grass at "Risleten" (Sihlwald area, Kt. Zurich, Switzerland). Seeds of *Br. benekenii* were also collected in bulk from a natural population at "Zollikerberg" (Pfannenstiel area, Kt. Zurich). In our experiment we did not control for plant genotype, but experimental material is expected to have rather limited genetic variation due to the high rate of selfing of *B. sylvaticum* and the tendency for clonal growth of *Br. benekenii*. Seeds were surface-sterilized (Leuchtmann and Clay 1990) and subjected to a heat treatment at 37°C at 100% humidity for three weeks in the dark to kill the endophyte (Nott and Latch 1993). To break dormancy of the seeds, heat treated seeds were vernalized at 3°C for three weeks (*B. sylvaticum*) or six weeks (*Br. benekenii*) on water agar in sealed Petri dishes. For germination, dishes were transferred to an incubator with continuous light at 22°C. At emergence of the first leaf (7–10 d after germination), all seedlings were checked under the microscope at a magnification of 40× for emerging hyphae to verify that they were endophyte-free. Half of the endophyte-free seedlings of *B. sylvaticum* and *Br. benekenii* were then artificially inoculated with previously isolated and characterized fungal strains of the respective host plants. *B. sylvaticum* seedlings were inoculated with the fungal strain "A", the most common asexual isozyme genotype found in all natural populations of the host grass (Bucheli and Leuchtmann 1996, Meijer and Leuchtmann 1999). *Br. benekenii* seedlings were inoculated with the asexual fungal strain "DEB 9701", which was isolated from a host plant growing in the population of seed collection. While it was not possible to check the success of infection in young seedlings without destruction before the experiment began, we know from previous experiments with these hosts that the success rate is close to 100% using the inoculation technique described above. For consistency, endophyte-free seedlings were subjected to the

same inoculation treatment, but without fungal mycelium. After two weeks on agar, the seedlings were transferred to trays with standard potting substrate for another 5 weeks of growing.

Experimental design

Identical experimental designs were used for *B. sylvaticum* and *Br. benekenii* seedlings. Five seedlings of the same size (based on longest leaf) were transplanted into slightly conical plastic pots (diameter 11 cm, height 13.5 cm, 1000 cm³ volume), with one target plant in the middle and four surrounding competitor plants at equal distances from the target plant (average density 526 plants per m²). The potting substrate used was a commercial product (UFA®) with equal parts of peat, compost and natural fibers (68%) mixed with perlite (16%) and sand (16%). Each of the four possible intraspecific combinations (E^-/E^- ; E^-/E^+ ; E^+/E^- ; E^+/E^+) were replicated 20 times, resulting in three different treatments: a monoculture treatment, and two mixture treatments. One mixture treatment had an $E^+ : E^-$ ratio of 4:1 and the other treatment had a ratio of 1:4. In the first growing period, pots were randomly arranged in the greenhouse and rearranged every other week. They were watered daily and fertilized every week from the third week on with liquid fertilizer (20-20-15, N-P-K) dissolved in 100 ml water, resulting in an N supply of 10 mg per pot per week. In autumn 1998, after the first growing season in the greenhouse and after first harvest plants were transferred to an experimental garden with the pots. Arrangement was randomized within 10 rows (block), each consisting of eight pots, two of each combination. No rearrangement was done during the second growing season. Subsequently, they were watered as needed but left unfertilized. Once a year, one plant per pot was randomly selected and checked microscopically for endophyte infection (not destructive). Neither new infections nor losses of endophyte infection were detected.

Measurements and response variables

To ensure survival of the plants after first harvest in autumn 1998, plants were clipped 2 cm above ground level for measurement of dry matter yield (DMY), whereas plants were clipped on ground level at the end of the experiment in 1999 (final harvest). At first harvest in 1998, DMY (excluding root mass) and tiller number were recorded as response variables. Seed number was included as an additional response variable at final harvest in 1999.

Statistical analysis

Measurements of DMY and tiller number were normally distributed. Data of seed numbers were log transformed to fit a normal distribution (data not shown). Since we were interested in comparing the competitive abilities of infected and uninfected plants, a two-way

analysis of variance (ANOVA) was used for infection against treatment as main effects. At final harvest, a three-way ANOVA was performed with “block” as a random factor in the analysis. Because block was a random factor, the other terms were tested against the mean squares of the interaction with block (infection against infection \times block, and treatment \times infection against block \times infection \times treatment, and so on). Tukey’s HSD test was used to determine statistically significant differences between means of DMY and seed number (log transformed).

Dry matter yields of infected individuals and uninfected individuals grown in mixture plots in the ratio 4:1 and 1:4 can be compared to those in the respective monocultures and defined as relative yields (RY) of infected and uninfected individuals (De Wit 1960). RYT then describes the resource complementarity between infected and uninfected individuals in a binary mixture (Snaydon and Satorre 1989).

The competitive ability of infected against uninfected plants in a binary mixture can be described by the aggressivity index (A), as used by McGilchrist and Trenbath (1971) and adjusted for unequal proportions of individuals by Gleeson and McGilchrist (1980). Infected and uninfected plants have the same competitive ability if the A value is zero. An A value greater than zero indicates greater competitive ability of infected plants compared to uninfected plants. An A value less than zero indicates the opposite. The differences of an actual RYT value (pretest value) from 1.0 (post test value) and an actual A value from zero were analyzed with the paired comparisons t -test at $p = 0.05$. Statistical tests were performed with the statistical packages JMP 3.2.2 and SPSS 10.0.7a for Macintosh.

Results

Brachypodium sylvaticum

Uninfected target plants had significantly more DMY ($F = 15.3$, $p < 0.001$) than infected target plants in 1998, independent of the infection status of the competitor plants (Fig. 1a). DMY of infected and uninfected competitor plants were not significantly different ($F = 0.69$, $p = 0.41$), ranging from 0.70 g to 0.84 g. Although no significant differences of target plant dry weight were recorded in 1999 ($F = 1.21$, $p = 0.28$), there was still a trend for higher DMY in uninfected target plants in competition with uninfected competitor plants (Fig. 1a). As in 1998, values of DMY of competitor plants were not significantly different ($F = 2.79$, $p = 0.10$), ranging from 0.81 g to 1.01 g. After 62 weeks, mean numbers of seeds produced by uninfected target plants were higher than

for uninfected target plants, but differences were not statistically significant (Fig. 2a).

In the two-way ANOVA of DMY in 1998, infection, treatment and the interaction were all significant indicating that infected and uninfected plants were performing differently under the three different treatments (Table 1). Only infection status significantly influenced tiller number in 1998 (Table 1). In the ANOVAs of DMY, tiller number and seed number in 1999, main factors and their interactions were not significant anymore (Table 1), except for the factor Block regarding the tiller number. There is a substantial increase in the number of tillers of uninfected target plants produced after the first harvest compared to infected target plants. However, tiller number of uninfected and infected competitor plants was almost equal before and after the first harvest (Fig. 3a, b).

Contrary to our expectations, the aggressivity indices (A), based on dry matter yield were consistently lower for infected plants of *B. sylvaticum* compared to uninfected plants in both years of the experiment. In 1998, relative yield totals (RYT) of infected competitor plants were significantly larger than unity (Table 2).

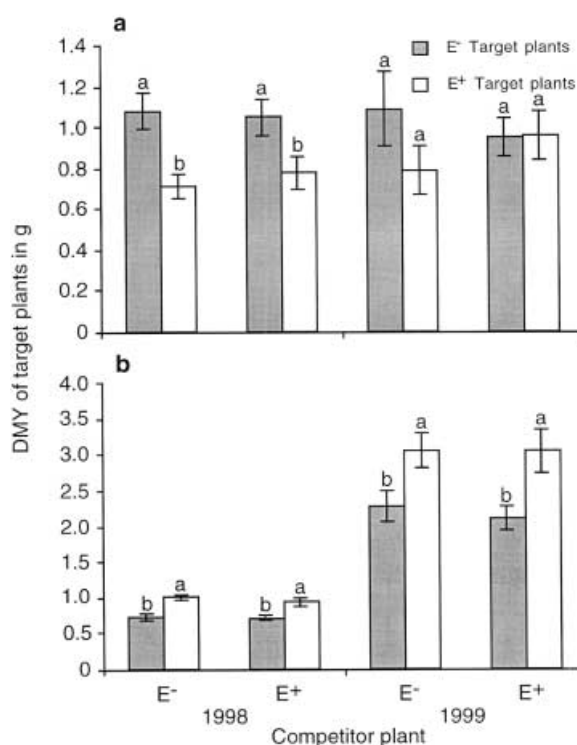


Fig. 1. Above-ground dry mass (DMY) of (a) *B. sylvaticum* and (b) *Br. benekenii* target plants in 1998 and 1999 in intraspecific competition. Different letters between treatments indicate significant differences among treatment means (Tukey HSD Test, $P < 0.05$). Error bars represent SEs.

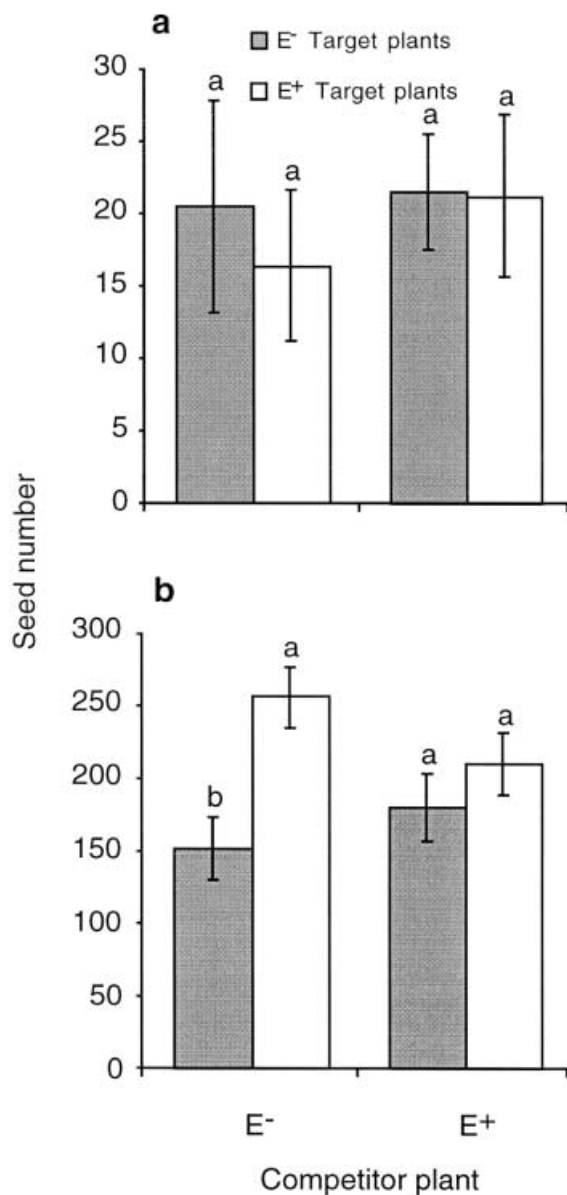


Fig. 2. Seed number of (a) *B. sylvaticum* and (b) *Br. benekenii* target plants in 1999 in intraspecific competition. Different letters between treatments indicate significant differences among treatment means (Tukey HSD Test, $p < 0.05$). Error bars represent SEs.

Bromus benekenii

Results of the intraspecific competition experiment with *Br. benekenii* plants were diametrically opposite to the results obtained with *B. sylvaticum* plants. Means of DMY of infected target plants were significantly higher (Tukey HSD, $p < 0.05$) compared to uninfected target plants at first and at final harvest in 1999, independent of competitor plants (Fig. 1b). There was also a significant increase in DMY in both years for infected competitor plants (0.75 g or 2.55 g, respectively) grown in

competition with uninfected target plants (0.69 g or 1.91 g, respectively), compared to uninfected competitor plants (Tukey HSD, $p < 0.05$). Seed number of infected target plants was significantly higher than seed number of uninfected target plants (Tukey HSD, $p < 0.05$) when in competition with uninfected competitor plants (Fig. 2b).

In the ANOVAs of DMY and tiller number in 1998, and of DMY, tiller number and seed number in 1999, infection was the only main effect significant in both growing periods, whereas the treatment was only in 1998 a significant main effect (Table 3). The interaction treatment \times infection was also significant in both growing periods for all three response variables (Table 3). During the whole experiment, infected target plants had higher tiller numbers than uninfected target plants, independent of infection status of competitor plants. Tiller numbers of infected and uninfected competitor plants were similar at first and at final harvest, except for infected competitors competing with an uninfected target plant, which had fewer tillers.

Aggressivity was very different for competitor and target plants with indices A significantly smaller than unity for infected competitor plants, but significantly greater than zero for infected target plants. This effect could be observed also in the second year. RYT of infected competitor plants was significantly lower than unity in both years (Table 2).

Discussion

Influence of endophyte infection

The results of this study indicate that endophyte infection can have a significant influence on dry matter yield, tillering and seed number of wild grasses under intraspecific competition. Further, the experiments suggest that endophyte-plant interactions in different wild grasses, which occupy comparable habitats, may not be uniform and predictable. This was most obvious at the end of the first growing season of young plants in the greenhouse. Indicated by different DMY, endophyte infection influenced competitive abilities of *B. sylvaticum* and *Br. benekenii* significantly in opposite directions. Infected plants of *Br. benekenii* were stronger competitors than uninfected plants, whereas plants of *B. sylvaticum* were the better competitors when not infected with the endophyte. Overall, endophyte infection stimulated plant growth and seed number of *Br. benekenii* in both years while growth of infected *B. sylvaticum* plants was significantly reduced in the first growing period.

Nutrients are an important factor influencing endophyte-host associations and could have been the reason for different behavior before and after the first cut (Cheplick et al. 1989). In the first growing period,

fertilization was relatively intense, resulting in a high level of nutrients available to the plants. This probably conforms to conditions in nature, where both grass species occur mainly on fertile, calcareous soils (Grime et al. 1988). In the experimental garden plot, plants were no longer fertilized, and towards the end of the second growing season plants suffered from nutrient depletion, and competition for resources became more intense. There are several studies with different host grasses that suggest that endophyte infection is most beneficial under high nutrient conditions (Arachevaleta et al. 1989, Cheplick et al. 1989, Cheplick 1998, Schulthess and Faeth 1998). It has been argued that reduced growth of infected plants under low nutrient treatments might be the result of competition between plant and fungus for limited supply of nutrients. This hypothesis is not supported by our results, since infected *B. sylvaticum* plants were clearly weaker competitors than uninfected plants in the first growing period, where nutrient availability was high. In *Br. benekenii* plants, competitive abilities appeared to be unaffected by nutrient conditions. After regrowth of the plants, the influence of endophyte infection on competitive ability decreased in *B. sylvaticum*.

Plant genotypes may respond differently to endophyte infection or may modify expression of fungal traits. For example, in some agricultural grasses it has been shown that alkaloid production is independently regulated by plant and endophyte genotypes (Roylance et al. 1994, Wilkinson et al. 2000), or that drought tolerance varied among plant genotypes infected by the same or different endophytes (Hill et al. 1996, Cheplick et al. 2000). While we used only one selected endophyte genotype of each species involved, our experimental

design did not control for the host genotype. However, genetic variation of experimental plants is assumed to have been very low, because seeds were collected from few plants at a single site. Moreover, *B. sylvaticum* is known to be selfing at very high rate (Schippmann 1991) and populations of the rhizome-forming *Br. benekenii* have a tendency for clonal growth. The consistently strong effects of endophyte infection on growth parameters in both years (at least for *Br. benekenii*) suggest that there was little plant genotypic variation.

Brachypodium sylvaticum

The observed negative effect of endophyte infection on competitive abilities of young *B. sylvaticum* seedlings could be relevant for seedling establishment in natural populations, where seedlings of the same or different species compete with each other in the struggle for light, space and nutrients. Infected seedlings of *B. sylvaticum* may therefore be at disadvantage compared to uninfected seedlings in the important stage of establishment. Later in development, differences in competitive abilities of infected and uninfected plants were less pronounced with regard to DMY and seed set. The drawback of infection may therefore decrease after successful establishment of plants in a population. These results are unexpected, given that typically all plants of *B. sylvaticum* are endophyte-infected in nature (Bucheli and Leuchtman 1996, Meijer and Leuchtman 1999). Hence, other factors must be responsible for the obvious success of infected plants, or alternatively, mechanisms operate that purge uninfected plants from the population.

Table 1. ANOVA for above-ground dry mass (DMY), tiller number (1998 and 1999) and log transformed seed number (1999 only) for *Brachypodium sylvaticum*, growing in intraspecific competition.

Source	df	DMY			Tiller number			Seed number		
		MS	F	p	MS	F	p	MS	F	p
1998										
Infection ²	1	0.86	8.99	0.003	76.25	7.39	0.007			
Treatment ³	2	0.67	7.00	0.001	7.88	0.76	0.467			
Infection × Treatment	2	0.30	3.14	0.044	23.64	2.29	0.103		n.a. ¹	
Error	394	0.09			10.32					
1999										
Infection	1	0.06	0.35	0.569	0.05	0.01	0.936	0.02	0.45	0.517
Treatment	2	0.12	1.54	0.242	14.20	0.44	0.651	0.23	1.50	0.248
Block ⁴	9	0.32	3.10	0.259	132.20	7.14	0.024	0.32	3.26	0.088
Treatment × Infection	2	0.13	0.86	0.442	38.60	1.98	0.167	0.16	1.84	0.186
Infection × Block	9	0.18	1.14	0.380	6.82	0.34	0.953	0.04	0.42	0.129
Block × Treatment	18	0.07	0.49	0.930	32.26	1.66	0.147	0.15	1.72	0.909
B × I × T	18	0.15	0.71	0.795	19.47	0.68	0.833	0.09	0.73	0.771
Error	340	0.22			28.72			0.12		

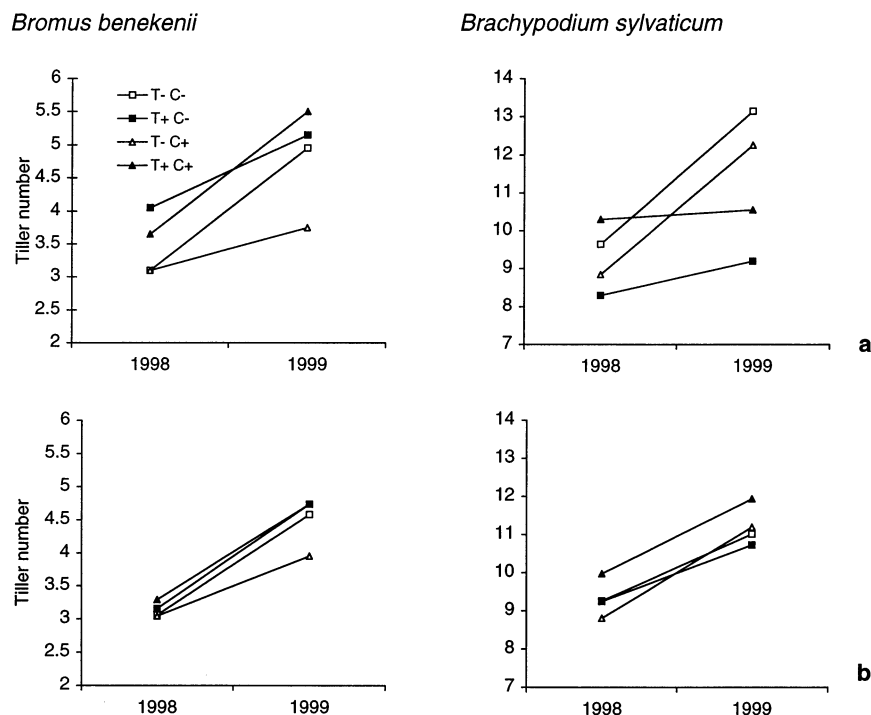
¹ n.a. not available, seed number lacking.

² Infection (infection status, E⁺ or E⁻).

³ Treatment (monoculture and mixtures with different ratio of infected and uninfected plants (4:1 and 1:4, respectively)).

⁴ Block (arrangement of pots in 10 rows, in 1999 only).

Fig. 3. Average tiller number of uninfected and infected plants of the two woodland grasses *Brachypodium sylvaticum* and *Bromus benekenii* in intraspecific competition at harvest of first and second growing period (1998 and 1999). (a) Tiller number of infected (T+) and uninfected (T-) target plants and (b) tiller number of infected (C+) and uninfected (C-) competitor plants.



Selective advantages for infected hosts could arise from increased resistance to plant pathogens or other pests (Burpee and Bouton 1993). Recent studies suggest that fungal inhibitors produced by endophytes may reduce colonization of infected hosts by potential fungal competitors (Yue et al. 2000). Moreover, there is evidence that infected grasses develop increased resistance against nematodes, resulting in enhanced plant growth (Kimmons et al. 1990, Schuster et al. 1995). Other benefits relate to the antiherbivore properties of fungal alkaloids produced in infected plants. In laboratory experiments, infected *B. sylvaticum* showed increased herbivore resistance against *Spodoptera frugiperda*, presumably mediated by unknown toxic compounds produced by the fungus (Brem and Leuchtmann, 2001). Thus, infected seedlings could be more persistent than uninfected seedlings and suffer less damage due to herbivores. Herbivore damages of uninfected plants in nature could compensate for competitive advantages observed in a stress-free environment (e.g. in the greenhouse) where herbivore pressure is negligible (Clay et al. 1993).

An alternative explanation for the lack of uninfected plants in natural populations could be the high rate of contagious spread of *E. sylvatica*. Horizontal transmission by ascospores is frequent and infection may take place not only through the inflorescence, but also through vegetative tissues (Brem and Leuchtmann 1999). In addition, transmission of asexual strains via epiphytically produced conidia cannot be ruled out (Moy et al. 2000). It is therefore conceivable that

seedlings or adult plants without endophyte infection will become infected by ascospores or conidia early during their life span. Once a population has reached a certain infection level, the occurrence of uninfected seedlings may be extremely rare. First, because of the very efficient vertical transmission of the endophyte which prevents production of uninfected seeds in accountable quantities and second, because infected seeds appear to have a higher germination rate than uninfected seeds (D. Brem unpubl.). Moreover, seedling recruitment in natural populations appears to be very low. Between 1 and 6 seedlings per m² became established after one year, independent of endophyte infection. In established populations of *B. sylvaticum* vegetative growth may also be important. *B. sylvaticum* forms long-living clumps with up to 200 tillers in often dense populations. These clumps are capable of vegetative reproduction through splitting into two or more separate plants during their long life span.

Bromus benekenii

Contrasting with the results of *B. sylvaticum* are the findings in seedlings of *Br. benekenii*. In both years of the experiment, infected seedlings and later young plants were more competitive than their uninfected competitors. However, similar to *B. sylvaticum*, the influence of endophyte infection was weaker in the second growing period, whereas seed number was considerably higher for infected *Br. benekenii* plants com-

Table 2. Relative yield totals (RYT) and aggressivity indices (*A*) calculated for infected *Brachypodium sylvaticum* and *Bromus benekenii* in the intraspecific competition experiment, in 1998 and 1999. RYT values significantly different from 1, or *A* values significantly different from 0 are indicated by * (*t*-test, $p < 0.05$).

	1998		1999	
	<i>A</i>	RYT	<i>A</i>	RYT
<i>Brachypodium sylvaticum</i>				
Target	−0.041	1.011	−0.043	0.966
Competitor	−0.009	1.128*	−0.088	0.970
<i>Bromus benekenii</i>				
Target	0.080*	1.034	0.106*	0.942
Competitor	−0.081*	0.899*	−0.196*	0.797*

Table 3. ANOVA for above-ground dry mass (DMY), tiller number (1998 and 1999) and log transformed seed number (1999 only) for *Bromus benekenii*, growing in intraspecific competition.

Source		df	DMY			Tiller number			Seed number		
			MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>	MS	<i>F</i>	<i>p</i>
1998											
Infection ²		1	0.90	26.84	<0.0001	5.29	8.75	0.003			
Treatment ³		2	0.39	11.61	<0.0001	1.58	2.62	0.074			
Infection × Treatment		2	0.53	15.73	<0.0001	3.13	5.19	0.006			
Error		393	0.03			0.60				n.a. ¹	
1999											
Infection		1	12.74	8.58	0.017	15.21	3.90	0.080	0.22	3.91	0.080
Treatment		2	2.10	2.30	0.129	3.21	0.50	0.615	0.15	2.45	0.115
Block ⁴		9	0.78	0.41	0.904	4.28	0.58	0.790	0.09	1.19	0.408
Treatment × Infection		2	3.34	6.82	0.006	14.51	5.14	0.017	0.33	7.49	0.004
Infection × Block		9	1.48	2.96	0.020	3.90	1.37	0.267	0.06	1.29	0.303
Block × Treatment		18	0.90	1.83	0.104	6.43	2.28	0.045	0.06	1.37	0.254
B × I × T		18	0.49	0.64	0.862	2.83	0.77	0.737	0.04	0.89	0.589
Error		337	0.77			3.67			0.04		

¹ n.a. not available, seed number lacking.

² Infection (infection status, E⁺ or E[−]).

³ Treatment (monoculture and mixtures with different ratio of infected and uninfected plants (4:1 and 1:4, respectively)).

⁴ Block (arrangement of pots in 10 rows, in 1999 only).

pared to uninfected plants. This is consistent with the high infection rate observed in nature, where usually over 70% of the plants are infected (Leuchtmann 1996).

Demography of *Br. benekenii* populations may be different from that of *B. sylvaticum* populations, because plants do not grow in dense populations, but form small, perennial clumps in scattered populations. The fact that *Br. benekenii* is able to colonize extreme and disturbed areas (Grime et al. 1988) would suggest that *Br. benekenii* is more dependent on seed dispersion and successful establishment of seedlings than *B. sylvaticum*. It is interesting to note that aggressivity of *Br. benekenii* varied with different mixture treatments (Table 2). Aggressivity of infected plants was significantly increased in mixtures with the low number of infected plants (1:4), whereas this could not be observed in mixtures with the high number of infected plants (4:1). Higher competitiveness of infected seedlings under intraspecific competition and higher seed set of adult plants should increase the number of infected plants over time.

Conclusions

The intraspecific competition experiment showed that endophyte infection had significant effects on both grass species. However, results for the two associations were diametrically opposite, unlike in agronomic grass cultivars (e.g. *Festuca arundinacea* and *Lolium perenne*) where endophyte infection almost always increases fitness and competitive abilities of the host. This study is among the first to provide empirical data on wild grass/endophyte associations and confirms the predictions made by Saikkonen et al. (1998) about variable influences of endophyte infection in natural grass populations. A holistic view considering the influences of many factors is essential to understand the association of wild grasses with their endophytes. Different factors are interacting and the outcome may be a combination of beneficial and less beneficial effects.

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