Asexual Endophytes in a Native Grass: Tradeoffs in Mortality, Growth, Reproduction, and Alkaloid Production

By: Stanley H. Faeth, Cinnamon J. Hayes, Dale R. Gardner

Abstract:

Neotyphodium endophytes are asexual, seed-borne fungal symbionts that are thought to interact mutualistically with their grass hosts. Benefits include increased growth, reproduction, and resistance to herbivores via endophytic alkaloids. Although these benefits are well established in infected introduced, agronomic grasses, little is known about the cost and benefits of endophyte infection in native grass populations. These populations exist as mosaics of uninfected and infected plants, with the latter often comprised of plants that vary widely in alkaloid content. We tested the costs and benefits of endophyte infections with varying alkaloids in the native grass *Achnatherum robustum* (sleepygrass). We conducted a 4-year field experiment, where herbivory and water availability were controlled and survival, growth, and reproduction of three maternal plant genotypes [uninfected plants (E−), infected plants with high levels of ergot alkaloids (E+A+), and infected plants with no alkaloids (E+A−)] were monitored over three growing seasons. Generally, E+A+ plants had reduced growth over the three growing seasons and lower seed production than E− or E+A− plants, suggesting a cost of alkaloid production. The reduction in vegetative biomass in E+A+ plants was most pronounced under supplemented water, contrary to the prediction that additional resources would offset the cost of alkaloid production. Also, E+A+ plants showed no advantage in growth, seed production, or reproductive effort under full herbivory relative to E− or E+A− grasses, contrary to the predictions of the defensive mutualism hypothesis. However, E+A+ plants had higher overwintering survival than E+A− plants in early plant ontogeny, suggesting that alkaloids associated with infection may protect against below ground herbivory or harsh winter conditions. Our results suggest that the mosaic of E−, E+A+, and E+A− plants observed in nature may result from varying biotic and abiotic selective factors that maintain the presence of uninfected plants and infected plants that vary in alkaloid production.

Article:

Introduction

Fungal endophytes are abundant and diverse microbial symbionts that inhabit the above-ground parts of plants (e.g., [2]). In cool season pooid grasses, fungal endophytes in the genus *Neotyphodium* are specialized symbionts that live intercellularly, systemically, and asymptomatically within grasses tissues and are strictly vertically transmitted (hyphae grow into developing seeds) [4, 32, 36]. *Neotyphodium* endophytes are closely related and derived from their *Epichloë* sexual ancestors, which can be transmitted vertically or horizontally, depending on strain and environmental conditions (e.g., [36]). Traditionally, asexual endophytes are viewed as interacting mutualistically with their host grasses (e.g., [3, 6]), although more recent evidence indicates the interaction ranges from antagonistic to mutualistic, especially in native grasses (e.g., [2, 9]).

Neotyphodium infections may provide a suite of benefits including increased growth, reproduction and competitive abilities, resistance to abiotic stresses, and enhanced nutrient uptake (e.g., [10, 28]). However, the most renowned and oft-cited benefit of infection is increased resistance to herbivores via the production of fungal alkaloids (e.g., [3, 6]). *Neotyphodium* may produce four different general types of alkaloids, each with varying biological activity against invertebrate and vertebrate herbivores ([24, 36]). Thus, fungal endophytes have been termed "acquired defenses" [1] and "defensive mutualists" [3] of grasses which often lack their own chemical defenses against herbivores. Increased resistance via endophyte alkaloids has been demonstrated in laboratory bioassays and field tests involving mostly introduced agronomic grass cultivars with mostly agricultural insect pests (e.g., [8, 34]). Infection frequencies in introduced, agronomic grasses such as tall fescue (*Lolium arundinaceum*) and perennial ryegrass (*Lolium perenne*) typically increase over time and are often near 100%, purportedly because of increased fitness under intense vertebrate or invertebrate herbivore pressure [5, 7] or because of other benefits associated with endophytes. Studies involving native grasses are relatively scarce and short in duration, and results range from increased (e.g., [22]) to decreased herbivore resistance (e.g., [33, 40]). Long-term experimental tests of the defensive mutualism hypothesis in *Neotyphodium*-infected native grasses that are embedded in natural communities where selective pressures from native herbivores and abiotic factors are controlled are rare.

Natural populations of grasses usually exist of mosaics of uninfected (E−) and infected grasses [8] with infection frequencies that are highly variable and often less than 100% (see Table 5.5 in [2]). Furthermore, recent evidence indicates that unlike most agronomic or invading E+ agronomic grasses that are infected by a single *Neotyphodium* haplotype, natural populations are usually comprised of plants harboring multiple haplotypes [2]. These multiple haplotypes differ greatly in endophyte-mediated changes in host phenotypes, including alkaloid types and levels, such that variation among host traits with different endophyte haplotypes may be greater than

that between E− and E+ plants [*9, 11, 15, 26, 27*]. For example, *Achnatherum robustum* or sleepygrass is often infected with *Neotyphodium* and well known for its toxic and narcotizing effects on vertebrates due to extremely high levels of ergot alkaloids [*20, 29*]. Yet, a more recent survey showed that whereas infection frequencies are high throughout its range, the high levels of alkaloid are found in only a small subset of the population [*15*]. Many infected plants produce either very low or no alkaloids at all, at least proximally due to different *Neotyphodium* haplotypes [*15*]. However, ultimately, the persistence of E− and E+ grasses with varying alkaloid levels may be caused by biotic and abiotic selective pressures that vary over time and space and thus change the cost and benefits of harboring different endophyte haplotypes (e.g., [*8*]). For example, if endophytic alkaloids protect against herbivory but are costly to produce, then one would expect infected plants with high alkaloids to be more frequent in environments with high herbivore pressure and abundant resources than uninfected plants or infected grasses that produce few or no alkaloids [*8*]. These varying costs and benefits of endophyte infection in populations of wild grasses are poorly understood [*2*], yet these tradeoffs likely underlie the patterns of distribution and frequency of uninfected and infected wild grasses in nature.

We measured the relative growth and reproduction of offspring of three maternal lines of *A. robustum* (sleepygrass), an uninfected line, an infected line that produced no alkaloids, and an infected line that produced high levels of ergot alkaloids, under varying soil moisture and herbivory levels over three growing seasons to ascertain the relative costs and benefits of different endophyte infections. Based upon the defensive mutualism hypothesis, we predicted that infected grasses that produced high levels of alkaloids (E+A+) would grow and reproduce better than uninfected (E−) or infected grasses with that did not produce alkaloids (E+A−) when herbivores were present. Furthermore, if alkaloids are costly to produce, then E+A+ should grow and reproduce better when additional resources are provided.

Methods

Study System

A. robustum (Vasey) Barkworth [= *Stipa robusta* (Vasey) Scribn. = *Stipa vaseyi* Scribn.] (Pooideae: Tribe Stipeae) is a cool season, perennial native bunchgrass found at high elevations throughout the western and southwestern USA in semiarid pine grasslands [*20*]. Sleepygrass is an obligate outcrossing species and reproduces only by seed [*41*]. The common names of *A. robustum* are robust needlegrass and sleepygrass. The latter name is derived from its long known toxic and narcotizing properties to livestock (e.g., [*25*]). It was discovered much later that this toxicity was caused by infection of the endophyte *Neotyphodium* [*21*] which produced extraordinarily high levels of ergot alkaloids, notably ergonovine, isolysergic acid amides, and ergonovinine [*15*].

Sleepygrass in natural populations are often infected by *Neotyphodium*, an asexual endophyte that is vertically transmitted by hyphae growing in culms and eventually into seeds [15]. However, infection levels are highly variable among populations in New Mexico and Colorado, USA, ranging from 50–100% infectivity [15]. *Neotyphodium* in sleepygrass has not yet been identified to species level, with morphological traits intermediate between *Neotyphodium starrii* and *Neotyphodium chisosum* [21], and one hybrid isolate genetically related to the sexual forms *Epichloë festucae* and *Epichloë elmyi* [26]. However, like other native grasses (e.g., [39]), different *Neotyphodium* haplotypes and perhaps species exist within and among populations. In sleepygrass, there are at least two haplotypes—a nonhybrid form that produces high levels of alkaloids and a hybrid form that produces no alkaloids [15]. Hybrids (heteroploids) are common in*Neotyphodium* and arise from parasexual fusion of a co-occurring *Epichloë* with *Neotyphodium* or two *Epichloë* (e.g., [35]).

Field Experiment

To test the cost and benefits of endophyte infection and corresponding alkaloid production, we designed a long-term field experiment where herbivory and soil moisture were controlled and plant growth and reproduction were monitored. We first collected seeds from sleepygrass plants whose infection status and alkaloid levels were previously known [15] from wild grass populations near Cloudcroft, NM, USA in Fall, 2003. We chose seeds from three maternal plants for a field experiment that spanned the variation in infection and alkaloid levels—uninfected seeds (E−), infected seeds from a maternal plant that produced no alkaloids (E+A−), and infected seeds from a maternal plant that produced high levels of ergot alkaloids (E+A+). The plants were from a population near Cloudcroft, NM, USA and part of a larger study of the variation in alkaloid content within and among sleepygrass populations and the consequences for associated arthropod communities [19]. The alkaloid content of the original E+A+ plant was 39.8-ppm ergot alkaloids, a relatively high concentration of alkaloids for *Neotyphodium*-infected plants [15, 24] and one that is well above levels known to negatively affect invertebrate and vertebrate herbivores (e.g., [20, 37, 38]). There were no detectable alkaloids in the original E+A− maternal plant.

Seeds of the three maternal plants were germinated and planted in mixture of potting soil and native soil and grown in individual pots in the greenhouse in Fall 2004. A field plot at The Arboretum of Flagstaff, Flagstaff, AZ, USA was prepared by disking in May 2003 to remove existing vegetation. The original plot was in a natural and previously undisturbed semiarid, Ponderosa pine-grassland habitat which harbored native plant species, mostly grasses. The entire plot was covered with a weed barrier (Dalen®) that prevents growth of unwanted plants but is pervious to water and nutrients. The weed barrier was then covered in a layer of pine bark chips to ameliorate any temperature changes caused by the weed barrier. To prevent disturbance from large vertebrates (elk) which trample and pull up new plants, the plot was surrounded with large mesh fencing that allowed free access by small vertebrates.

In May 2004, 44 E+A− and 44 E+A+ seedlings, selected for similar starting size, were randomly assigned a position in the plot and planted 2 m apart into holes cut into the weed barrier. Because of insufficient numbers surviving in the greenhouse in 2004, E− plants were not planted until May 2005, when 44 E− grasses were planted in randomly assigned positions in the plot. However, these E− plants were matched in size to the E+A− and E+A+ plants currently growing in the plot. The herbivory and water treatments were randomly assigned and initiated in 20 August 2005, with each plant type and water and herbivory treatment replicated 11 × (total plants = 132). The two water treatments were ambient water (normal precipitation) and supplemented water (drip irrigation, 8 l per day per plant). This amount of supplemented water is sufficient to increase soil moisture about 25% above ambient [9]. Herbivory treatments included two levels, ambient herbivory (from arthropods and small mammals) to greatly reduced herbivory (exclusion of arthropods and small mammals). Reduced herbivory treated plants were sprayed every 2 weeks with esfenvalerate, a synthetic pyrethroid, which has minimal effects on plant functions [31]. Treatment with this insecticide at these intervals reduces arthropod abundances more than 10-fold [13]. Reduced herbivory plants were enclosed in hardware cloth (1 cm mesh) to exclude small mammals. The 1-m-high cylindrical cages were open on top to allow unrestricted plant growth and ambient sunlight.

Plant Overwintering Survival

We monitored overwintering survival of plants after each growing season by observing living and dead plants in May 2005, 2006, and 2007. Because E− plants were not planted until the May 2005, the survival of only E+A− and E+A+ plants were monitored in May 2005.

Vegetative and Reproductive Biomass

We estimated the volume of all plants by measuring diameter and height of each plant in May 2005 (the beginning of the growing season) and then calculating the volume of a cylinder. As a bunchgrass, sleepygrass approximates the shape of a cylinder. This method of estimating plant size has been used previously and verified [11]. On 16 September 2005 (the end of the growing season), we remeasured all plants and determined the change in volume. At the end of the growing season in 2006 and 2007, we determined above vegetative biomass by hand cutting all above-ground biomass and then oven drying and weighing biomass from each plant individually. In 2007 (the first year plants produced flowering culms and seeds), we determined reproductive biomass by hand cutting all culms (flowering stems) and panicles (inflorescences) at the time of seed maturity in September before the seeds shattered and were then dried. After drying, seeds were removed by hand from each plant and separated from culms and panicles and weighed separately for each plant.

Alkaloid Determination

The concentration of alkaloids in infected plants is known to vary based largely upon endophyte haplotype but also upon plant genotype and environmental factors [2, 8]. To ensure that alkaloid

concentrations in offspring plants remained similar to their maternal lines, we measured the concentration of alkaloids (total alkaloids and the ergonovine, isolysergic acid amides, and ergonovinine fractions) from leaf tissue samples from a random sample of 12 experimental plants from each maternal line plant in 2006. Alkaloid analyses were via previous published methods [15].

Statistical Analyses—Plant Survival, Growth, and Reproduction

Because sleepygrass is an obligate outcrossing species, the seeds from each maternal line were half-sibs. Thus, in our statistical analyses, we are generally comparing the variation in survival, growth, and reproduction within these half-sib groups to the variation among groups.

We used G tests to compare overwintering survival of the field experimental plants in 2004, 2005, and 2006. In the winter of 2004 and before treatments began in the summer of 2005, only E+A− and E+A+ plants were available for comparison. In 2006 and 2007, all three types of plants were compared, as well as differences in survival between the herbivory and water treatments.

We used analysis of variance (ANOVA; Systat Ver. 10.1) to test for differences in the change in plant volume in the first growing season (May–September 2005). In the 2006 and 2007 seasons, we used analysis of covariance (ANCOVA; with plant volume or dry biomass at the end of the previous growing season as a covariate) to test for the effects of endophyte infection and type, herbivory, and water treatments and their interactions on aboveground vegetative dry biomass. In 2007, we used similar ANCOVAS to also test for differences in reproductive (culms, panicles, and seeds) dry biomass and seed to vegetative dry biomass ratios (arcsine square root transformed) with plant size at the end of the 2006 growing season used as a covariate. Seed to vegetative biomass ratio is an estimate of reproductive effort [30]. Because biomass of culms and panicles was positively and highly correlated with seed biomass, only the results from seed biomass are reported here. All assumptions of normality and heterogeneity were tested and met. Although most plants were repeatedly sampled over the growing seasons, we did not use repeated measures ANOVA because E− plants were not planted at the same time as E+A+ and E+A− plants and a few plants were replaced when they died during the first year of the experiment.

Results

Alkaloids

The original E+A+ maternal plant had 39.8-ppm total alkaloids, comprised mostly of ergonovine and lesser amounts of lysergic acid and isolysergic acid amides and ergonovinine alkaloids. The E+A− and E− maternal plants had no detectable alkaloids. E+A+ offspring plants used in the experiment had similar total ergot alkaloid levels (40.18 ± 8.97 ppm), whereas offspring plants from the E+A− and E− maternal plants had no detectable alkaloids.

Plant Overwintering Mortality

From September 2004 to the beginning of the growing season in May 2005, more E+A− plants (32.8%) died than E+A+ plants (15.2%; $G = 5.73$, $df = 1$, $P = 0.02$). After treatments began in 2005, there was no significant difference in mortality among E−, E+A−, and E+A+ plants in over the next two winters ($P > 0.10$). However, mortality did vary by herbivory treatments in the winter of 2005 to 2006 but not 2006 to 2007, with significant more mortality in the ambient herbivory treatments than the reduced herbivory treatments ($G = 6.45$, $df = 1$, $P = 0.01$). Mortality did not vary by soil moisture treatments either in the winter of 2005 to 2006 or 2006 to 2007.

Change in Volume, Vegetative Biomass, and Reproductive Biomass

In 2005, growth rate, as measured by change in plant volume, did not vary significantly among the three plant types (Table 1) although E+A+ plants tended to have reduced growth rates relative to E− and E+A− plants (Fig. 1a). However, in 2006, E+A+ had less vegetative biomass than E− and E+A− plants (Table 1; Fig. 1b). In 2007, E+A+ and E− plants showed less vegetative biomass than E+A− plants (Table 1; Fig. 1c). In 2007, E+A+ plants had significantly less seed biomass than E− but not E+A− plants (Table 1; Fig. 2a). E+A+ showed reduced reproductive effort, as measured by the ratio of seed to vegetative dry biomass than E− and E+A− plants (Table 1; Fig. 2b).

Table 1 Summary of P values and degrees of freedom from ANOVA (2005) and ANCOVA (2006 and 2007, with plant size from the end of the previous growing season as a covariate) on infection type (E−, E+A−, E+A+) and soil moisture and herbivory treatments on percent change in above-ground volume (2005) and above-ground vegetative dry biomass (2006 and 2007) and seed dry biomass and reproductive effort (2007, ratio of seed biomass to vegetative dry biomass, arcsine square root transformed) and the two- and three-way interactions

	2005		2006		2007			
	df	% volume change	*df*	Vegetative mass	*df*	Vegetative mass	Seed mass	Ratio seed/vegetative
Infection type	2, 142	0.24	2, 134	**0.02**	2, 122	**0.07**	**0.07**	**0.02**
Water	1, 142	0.23	1, 134	0.51	1, 122	0.17	**0.08**	0.55
Herbivory	1, 142	0.90	1, 134	0.51	1, 122	0.36	**0.06**	0.57
Infection type × water	2,	0.67	2,	**0.004**	2,	0.95	**0.06**	0.65

	142		134		122			
Infection type × herbivory	2, 142	0.96	2, 134	0.61	2, 122	0.74	**0.06**	**0.03**
Water × herbivory	1, 142	0.93	1, 134	0.93	1, 122	0.48	0.36	0.63
Infection type × water × herbivory	2, 142	0.75	2, 134	0.73	2, 122	0.63	0.11	0.90

Significant or marginally ($0.10 > P > 0.05$) significant P values are in bold
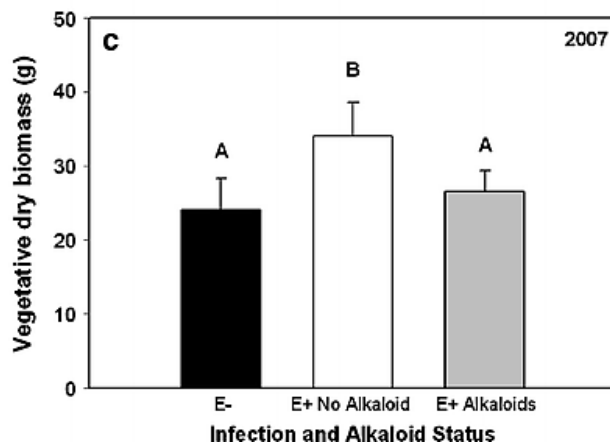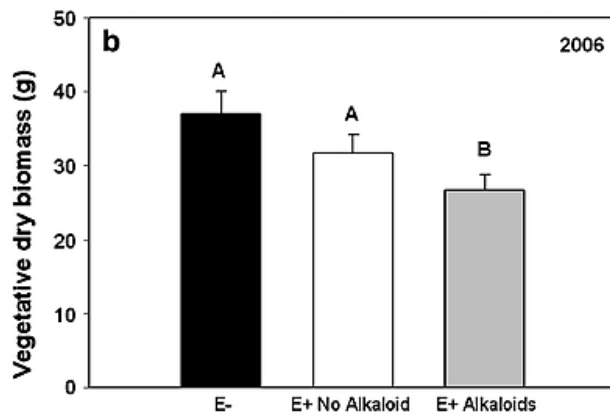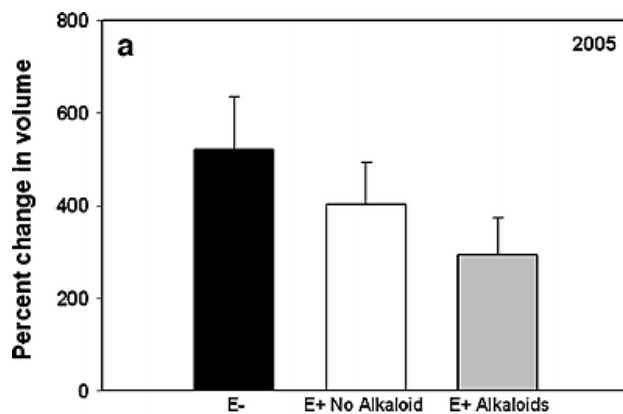
Figure 1 Mean (± SE) percent change in plant volume in 2005 (**a**) and in vegetative dry biomass in 2006 (**b**) and in 2007 (**c**) for E−, E+A−, and E+A+ plants. *Different letters above bars* indicate significant differences at $\alpha < 0.05$
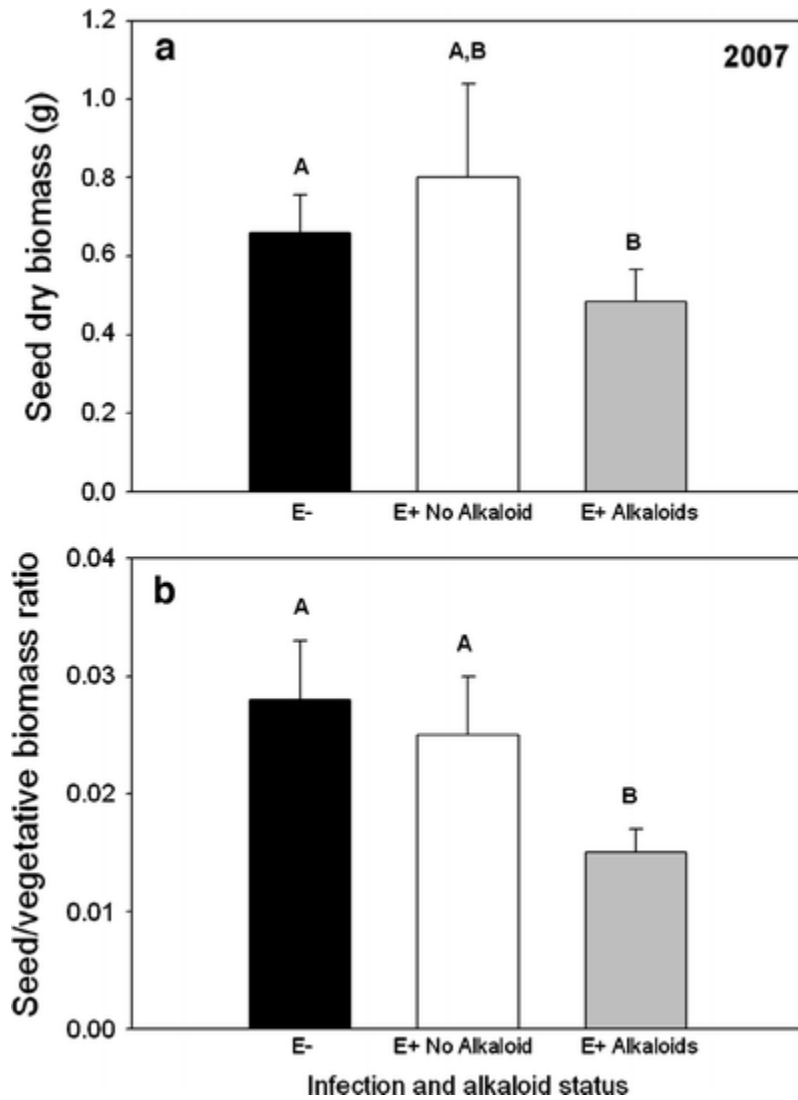
Figure 2 Mean (± SE) of seed biomass (**a**) and the ratio of seed to vegetative dry mass (reproductive effort; **b**) for E−, E+A−, and E+A+ plants. The ratio of seed to vegetative dry biomass was arcsine square root transformed; *data in graphs* show untransformed data. *Different letters above bars* indicate significant differences at $\alpha < 0.05$

The herbivory and water treatments affected seed dry biomass in 2007 (Table 1). Seed biomass was reduced in the full herbivory treatment (mean = 0.44 ± 0.06 g) compared to the reduced herbivory treatment (mean = 0.78 ± 0.14 g). Seed biomass was greater in the ambient water treatment (mean = 0.71 ± 0.13 g) relative to the supplemented water treatment (mean = 0.50 ± 0.08 g).

Infection type interacted with water to affect vegetative biomass in 2006 and seed biomass in 2007 (Table 1). In 2006, E− plants produced more vegetative biomass with supplemented water than ambient water, but E+A+ plants responded oppositely, producing more vegetative biomass under ambient than supplemented water (Fig. 3a). The effect of increased water reducing seed dry biomass in 2007 appears largely due to E+A− responding differently than E− and E+A+ to change in soil moisture (Fig. 3b).
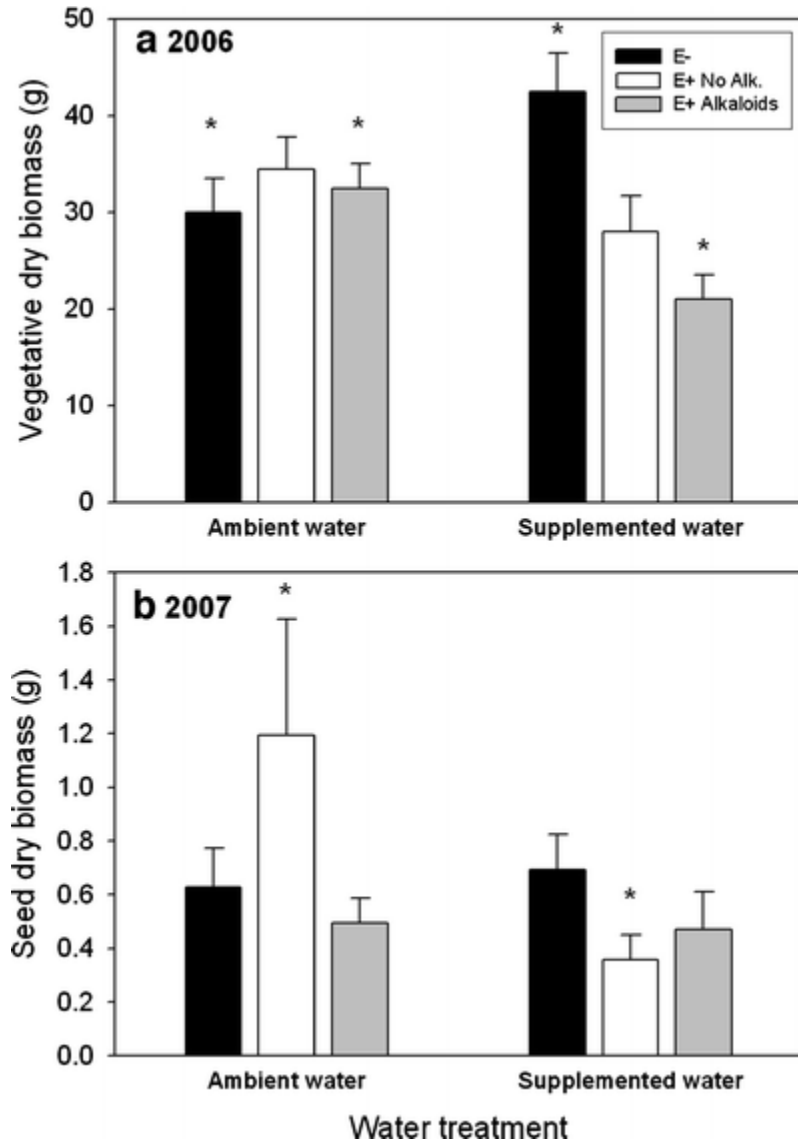
Figure 3 The interaction between type of infection (E−, E+A−, and E+A+) and the two soil moisture treatments for mean (± SE) vegetative dry biomass in 2006 (**a**) and mean (± SE) seed dry biomass in 2007 (**b**). *Asterisks above bars* indicated significant differences at $\alpha < 0.05$ between the same infection type in the two different water treatments

Infection type interacted with herbivory to affect seed dry biomass and the ratio of seed to vegetative biomass (reproductive effort) in 2007 (Table 1). Both E− and E+A− plants produced

more seed biomass when herbivory was reduced relative to full herbivory, but seed biomass of E+A+ plants was unaffected by changes in herbivory treatments (Fig. 4a). Reproductive effort was reduced in E+A− plants under full herbivory, but reproductive effort of E− and E+A+ plants was unaffected by changes in herbivory (Fig. 4b).
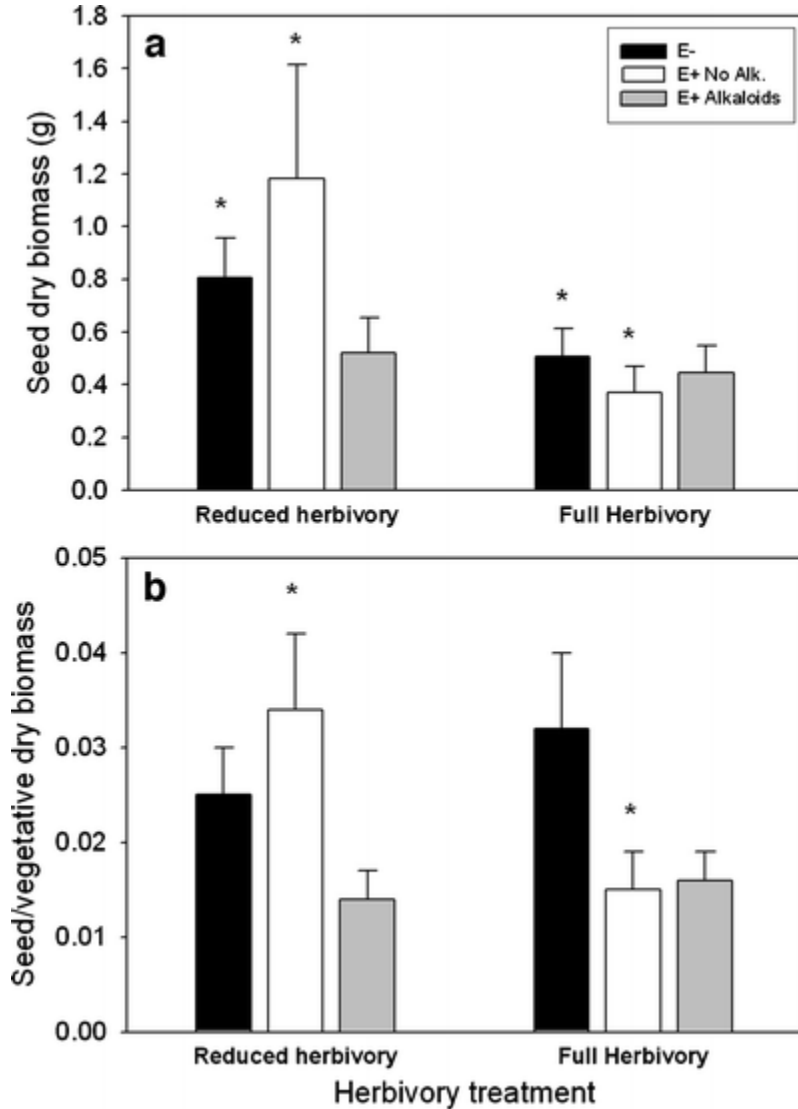
Figure 4 The interaction between type of infection (E−, E+A−, and E+A+) and the two herbivory treatments (reduced and full or ambient herbivory) in 2007 for mean (± SE) seed dry biomass (**a**) and the ratio of seed to vegetative dry mass (reproductive effort; **b**). The ratio of seed to vegetative dry biomass was arcsine square root transformed; *data in graphs* show untransformed data. *Asterisks above bars* indicated significant differences at $\alpha < 0.05$ between the same infection type in the two different herbivory treatments

Discussion

The benefits of asexual endophyte infections with few, if any, costs in introduced, agronomic grasses such as tall fescue and perennial ryegrass are well established (e.g., [2]). As such, infection frequencies in these agronomic grasses often increase to near 100% over time in agronomic and old fields, especially under chronic herbivory from vertebrates or invertebrates [5, 7]. However, these agronomic grasses generally exhibit less variability in endophyte-related properties than infected native grasses because infection is usually by a single endophyte haplotype, plant genotypic variability is usually reduced due to selective breeding, and resources are often less variable in agroecosystems [34]. Grasses in natural populations typically harbor multiple endophyte haplotypes [2, 12, 15]. Furthermore, studies now show that the interaction between asexual endophyte infection and their native hosts vary by endophyte and plant genotype (e.g., [27]) and prevailing biotic and abiotic environments [11, 13, 14, 16, 23]. Thus, the costs and benefits of endophyte infection should depend on endophyte haplotype with its associated alkaloids and the intensity of herbivory and the availability of resources [8].

Our results support this prediction. In our experiment with sleepygrass, there was no clear benefit of infection per se, but instead, the effects of infection were dependent on the endophyte haplotype and varied through time and by biotic and abiotic environmental conditions. Generally, plants harboring endophytes that produced high levels of alkaloids grew slower and produced less vegetative biomass than uninfected plants and infected plants that produced no alkaloids in the first two growing seasons. In the third growing season, growth of E− plants exceeded the other two plant types, indicating that the relative cost and benefits are variable through time. Likewise, E+A+ plants had lower seed biomass than E− plants and generally allocated less to reproductive effort than E− or E+A− plants.

We used only one maternal genotype of each plant type in this experiment, and although these represent the spectrum of sleepygrass plants in natural populations (i.e., uninfected and infected with no and high alkaloids [19]), caution is necessary in interpreting our results. We would expect that additional variation would be apparent among different maternal plant genotypes harboring the same endophyte haplotype or among different maternal E− plants. In addition, our experiment could not completely separate the effects of fungal and plant genotype. Nevertheless, most studies to date of endophyte effects on host performance are of short duration, use only E+ and E− agronomic grasses, and do not consider variation among endophyte haplotypes or the resulting variation in alkaloids (e.g., [12, 19]). Different endophyte haplotypes have recently been experimentally transferred into agronomic grass cultivars (e.g., [18]), resulting in changes in host physiology, growth, and herbivore resistance properties, suggesting, like our results here, that endophyte haplotype or strain can dramatically alter host traits.

Given that alkaloids are metabolically expensive to produce (e.g., [8]), one would predict that E+A+ plants would perform better in high resource environments and under high herbivore pressure. However, that does not seem to be the case here. Under supplemented water, the limiting resource in these semiarid habitats [11], E+A+ plants grew less than E+A+ plants under ambient soil moisture. Similarly, seed biomass of E+A+ plants was not different in ambient and

increased soil moisture treatments. Only E+A− plants responded to changes in soil moisture in terms of seed biomass. In this case, more seeds were produced when under ambient than supplemented water conditions, suggesting a benefit of harboring endophytes that do not produce alkaloids when available resources are restricted. We caution here that we only tested one limiting factor, soil moisture availability, and manipulation of other abiotic factors, such as soil nitrogen, may have produced different results.

Endophytes are traditionally thought to act mutualistically with their hosts by producing alkaloids they act as defenses against herbivores (e.g., [5, 6]). If alkaloids protect sleepygrass from herbivory and thus provide benefits relative to E− or E+A− grasses as postulated by the defensive mutualism hypothesis [1], we would expect to see increased vegetative and seed biomass of E+A+ plants in the full herbivory treatment relative to E− and E+A− plants. Although full herbivory reduced overall seed biomass, this effect was due to reduction of seed biomass of E+A− plants, not an increase of seed biomass of E+A+ plants (Fig. 4a) as predicted. Seed biomass of E− and E+A+ plants did not change under full and reduced herbivory. Our results here suggest no clear advantage of host grasses harboring alkaloid-producing endophytes under full herbivory, at least by invertebrates and small mammals during the growing season. Additional studies [13,19] also show that abundance of insect herbivores is greater on E+A+ plants than E− or E+A− native grasses contrary to the notion that alkaloids are defensive. These studies suggested at least two explanations for increased abundances on infected plants: (1) insect herbivores in natural communities are often specialized to feed on plants with allelochemicals and may evolve counter defenses to endophyte-based alkaloids or even require them for oviposition cues or development or (2) natural enemies of herbivores may be more affected than the herbivores themselves, thus rendering chemical defenses ineffective or even counterproductive [2, 8, 17]. E+A+ plants thus may provide enemy-reduced space for herbivores.

However, our results do indicate that E+A+ may provide an advantage in overwintering survival. E+A+ survived better than E+A− plants during the winter of 2004 to 2005. Unfortunately, we could not compare survival of E− plants during this period because they were not planted until May 2005. We cannot be certain of the mechanism for increased overwintering survival of E+A+ plants but there are possibilities. First, although above-ground parts senesce during the winter, alkaloids in roots, litter, or surrounding soils may deter winter feeding invertebrates or vertebrate herbivores. Caged (reduced herbivory) plants also had higher survival, regardless of plant type, suggesting that protection from herbivory during the winter increases survival. Second, E+A+ may allocate more to root than shoot growth and thus increase winter survival. Because we did not measure root biomass, we cannot address this latter hypothesis without further experimentation.

Our experiment indicates tradeoffs based not only upon the absence or presence of endophyte infection but also upon the type of infection and their dependence on local biotic and abiotic environments. Furthermore, our results suggest that these tradeoffs vary through time, across

growing seasons and winter senescence. We suggest that most natural grass populations are mosaics of E− and E+ grasses that vary by endophyte and plant genotype because there are tradeoffs in cost and benefits of infection and infection type. The tradeoffs that vary spatially and temporally act to maintain the observed heterogeneity found in most wild grass populations.

Acknowledgments

References

1. Cheplick GP, Clay K (1988) Acquired chemical defenses in grasses: the role of fungal endophytes. Oikos 52:309–318

2. Cheplick GP, Faeth SH (2009) The ecology and evolution of the grass-endophyte symbiosis. Oxford University Press, Oxford

3. Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16

4. Clay K (1990) Fungal endophytes of grasses. Annu Rev Ecol System 21:275–297

5. Clay K (1998) Fungal endophyte infection and the population dynamics of grasses. In: Cheplick GP (ed) Population biology of grasses. Cambridge University Press, Cambridge, pp 255–285

6. Clay K, Schardl CL (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:S99–S127

7. Clay K, Holah J, Rudgers JA (2005) Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. Proc Nat Acad Sci 102:12465–12470

8. Faeth SH (2002) Are endophytic fungi defensive plant mutualists? Oikos 98:25–36

9. Faeth SH (2009) Asexual fungal symbionts alter reproductive allocation and herbivory over time in their native perennial grass hosts. Am Nat 173:554–565

10. Faeth SH, Bultman TL (2002) Endophytic fungi and interactions among host plants, herbivores, and natural enemies. In: Tscharntke T, Hawkins BA (eds) Multitrophic level interactions. Cambridge University Press, Cambridge, pp 89–123

11. Faeth SH, Sullivan TJ (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. Am Nat 161:310–325

12. Faeth SH, Saikkonen K (2007) Variability is the nature of the endophyte–grass interaction. In: Popay AJ, Thorn ER (eds) Proc. 6th Internat. Symp. Fungal Endophytes of grasses. New Zealand Grassland Assoc, Dunedin, pp 37–48

13. Faeth SH, Shochat E (2010) Inherited microbial symbionts in a native grass increase herbivore abundances and alter diversity and community structure of arthropod communities. Ecology (in press)

14. Faeth SH, Helander ML, Saikkonen KT (2004) Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. Ecol Lett 7:304–313

15. Faeth SH, Gardner DR, Hayes CJ, Jani A, Wittlinger SK, Jones TA (2006) Temporal and spatial variation in alkaloid levels in *Achnatherum robustum*, a native grass infected with the endophyte *Neotyphodium*. J Chem Ecol 32:307–324

16. Hamilton CE, Faeth SH, Dowling TE (2009) Distribution of hybrid fungal symbionts and environmental stress. Microb Ecol 58:408–413

17. Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Ann Rev Entomol 54:323–342

18. Hunt MG, Newman JA (2005) Reduced herbivore resistance from a novel grass–endophyte association. J Appl Ecol 42:762–769

19. Jani AJ, Faeth SH, Gardner D (2010) Asexual endophytes and associated alkaloids alter arthropod community structure and increase herbivore abundances on a native grass. Ecology Letters 13:106–117

20. Jones TA, Ralphs MH, Gardner DR, Chatterton NJ (2000) Cattle prefer endophyte-free robust needlegrass. J Range Manag 53:427–431

21. Kaiser WJ, Bruehl GW, Davitt CM, Klein RE (1996) *Acremonium* isolates from *Stipa robusta*. Mycol 88:539–547

22. Koh S, Hik DS (2007) Herbivory mediates grass–endophyte relationships. Ecology 88:2752–2757

23. Lehtonen P, Helander M, Saikkonen K (2005) Are endophyte-mediated effects on herbivores conditional on soil nutrients? Oecologia 142:38–45

24. Leuchtmann A, Schmidt D, Bush LP (2000) Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloë/Neotyphodium* endophytes. J Chem Ecol 26:1025–1036

25. Marsh CD, Clawson AB (1929) Sleepy grass (*Stipa vaseyi*) as a stock-poisoning plant. USDA Tech Bull 114, Washington, DC

26. Moon CD, Craven KD, Leuchtmann A, Clement SL, Schardl CL (2004) Prevalence of interspecific hybrids amongst asexual fungal endophytes of grasses. Mol Ecol 13:1455–1467

27. Morse L, Day TA, Faeth SH (2007) *Neotyphodium* interactions with a wild grass are driven mainly by endophyte haplotype. Funct Ecol 21:813–822

28. Müller CB, Krauss J (2005) Symbiosis between grasses and asexual fungal endophytes. Curr Opin Plant Biol 8:450–456

29. Petroski RJ, Powell RG, Clay K (1992) Alkaloids of *Stipa robusta* (Sleepygrass) infected with an *Acremonium* endophyte. Nat Toxins 1:84–88

30. Reekie EG (1999) Resource allocation, tradeoffs, and reproductive effort in plants. In: Vuorisalo TM, Mutkainen PK (eds) Life history evolution in plants. Kluwer Acad, Boston, pp 173–193

31. Root RB (1996) Herbivore pressure on goldenrods (*Solidago altissima*): its variation and cumulative effects. Ecology 77:1074–1087

32. Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Ann Rev Ecol System 29:319–343

33. Saikkonen K, Helander M, Faeth SH, Schulthess F, Wilson D (1999) Endophyte–grass–herbivore interactions: the case of *Neotyphodium* endophytes in Arizona fescue populations. Oecologia 121:411–420

34. Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth SH (2006) Model systems in ecology: dissecting the endophyte–grass literature. Trends Plant Sci 11:428–433

35. Schardl CL, Craven KD (2003) Interspecific hybridization in plant-associated fungi and oomycetes: a review. Mol Ecol 12:2861–2873

36. Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Ann Rev Plant Biol 55:315–340

37. Siegel MR, Bush LP (1996) Defensive chemicals in grass–fungal endophyte associations. Rec Adv Phytochem 30:81–119

38. Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Bacon CW, Johnson MC (1990) Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. J Chem Ecol 6:3301–3315

39. Sullivan TJ, Faeth SH (2004) Gene flow in the endophyte *Neotyphodium* and implications for coevolution with *Festuca arizonica*. Mol Ecol 13:649–656

40. Tibbets TM, Faeth SH (1999) *Neotyphodium* endophytes in grasses: deterrents or promoters of herbivory by leaf-cutting ants? Oecologia 118:297–305

41. United States Department of Agriculture (1988) Range plant handbook. Dover, New York