Occam's Razor Cuts Both Ways: Endophytes, Resource Allocation, Herbivory, and Mutualism: A Reply to Rudgers et al.

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Abstract:

Fungal endophytes and their grass hosts have attracted growing research interest as systems in which to examine the ecological and evolutionary consequences of maternally inherited symbioses. The lion's share of research for these endophytic symbioses has been focused on *Neotyphodium* endophytes in three introduced agronomic grasses (but especially one, tall fescue; see Faeth and Saikkonen 2007; fig. 7.1 in Cheplick and Faeth 2009), and much of the conventional wisdom about endophyte-host interactions has been developed from these agronomic grass systems. However, accumulating studies from wild grasses suggest important species-specific differences between host grass–endophyte associations and therefore challenge concepts based on a few agronomic grass systems. My long-term study (Faeth 2009) of how asexual *Neotyphodium* endophytes affect resource allocation and herbivore loads in a wild grass, Arizona fescue (*Festuca arizonica*), is one of these, and Rudgers et al. (2010) challenge both my methods and my interpretation.

Keywords: endophytes | herbivory | mutualism | Neotyphodium | resource allocation | tolerance

Article:

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asexual *Neotyphodium* endophytes affect resource allocation and herbivore loads in a wild grass, Arizona fescue (*Festuca arizonica*), is one of these, and Rudgers et al. (2010) challenge both my methods and my interpretation.

I thank these authors for their interest in my article. Their main objections are whether I presented adequate evidence that the endophyte acts parasitically rather than mutualistically and whether infection alters host resource allocation and increases herbivory. They present several major methodological and interpretive criticisms within these larger objections. In this response, I (1) address each of their main criticisms, (2) present additional data supporting my original interpretation, (3) argue that the mutualist-versus-parasite dichotomy is unproductive, and (4) show that the Rudgers et al. (2010) alternative and purportedly more parsimonious explanations rely on the same lines of evidence that they criticize in my study.

Response to Criticisms

My study was a long-term (4-year) study of changes in vegetative and reproductive growth of individual infected (E+) and uninfected (experimentally removed; E−) native Arizona fescue plants, where plant genotype, herbivory, and a limiting resource, soil moisture availability, were carefully controlled and manipulated in a common-garden field experiment. The main objective of my study was to test the effects of endophyte infection, plant genotypic variation, and environmental factors (herbivory and water) and their interactions on host resource allocation and herbivore loads over plant ontogeny. In short, E+ plants showed greater seed biomass in the second year and greater reproductive effort in the first two years, flowered earlier, and had greater vegetative biomass than E− plants across all three years. Furthermore, E+ plants had significantly greater herbivore loads than E− plants in early ontogeny, contrary to the long-held concept that endophytes act as defensive mutualists (e.g., Clay 1988).

Rudgers et al. (2010) first question whether ontogenetic shifts in growth and reproduction in E+ grasses relative to E− grasses decrease lifetime fitness of the host and my interpretation that endophytic symbionts might act parasitically in this grass. Note that I was careful to offer my interpretation only as a conditional possibility (Faeth 2009, p. 563): if this shift reduces lifetime host fitness, then the endophytes may be acting as reproductive parasites (symbionts that alter host reproduction function or sex ratios to increase their fitness at the expense of the host fitness; e.g., Werren 1997). Rudgers et al. (2010) also state that I provided no evidence that endophyte-mediated early reproduction in the host alters life-history traits that negatively affect host lifetime fitness.

This criticism is well taken and is the crux of their arguments. Whereas the costs of reproduction in general and shifts to early reproduction specifically are typically assumed to reduce lifetime fitness in perennial plants, especially in stressful or resource-limited environments (e.g., Kozłowski and Uchmański 1987; Vuorisalo and Mutikainen 1999), actual experimental tests over the life span of perennial hosts are exceedingly difficult and fraught with complicating

factors (e.g., Obeso 2002; Worley et al. 2003). Instead, indirect methods are often used to indicate some cost of reproduction (e.g., Obeso 2002). These indirect methods do indeed, however, indicate a cost of early reproduction in E+ grasses. For example, Olejniczak and Lembicz (2007) showed, in a field study with a wild grass, that infected plants reproduced earlier at the expense of reproduction and vegetative growth a few years later. Cheplick (see fig. 5.4 in Cheplick and Faeth 2009) showed that reproductive spikelet production in agronomic E+ perennial ryegrass in one year was negatively correlated with reproduction in the following year, suggesting a cost to reproduction. Moreover, in a previous study with Arizona fescue, Faeth and Hamilton (2006) found experimentally that E+ and E− plants showed significant differences in survival curves, with E+ plants showing lower survival in earlier life stages (Rudgers et al. [2010] incorrectly summarize the results of this study). In the survey part of this study, adult E+ plants had lower survival rates than adult E− plants, although the difference was not significant because of the very low overall mortality rates during the 7-year course of the study. Thus, *Neotyphodium* infection tends to reduce host survival in Arizona fescue and certainly does not increase survival, as would be expected under Rudgers et al.'s (2010) endophytes-as-mutualists hypothesis. These studies support the assumption that an endophyte-mediated shift to early reproduction is costly in terms of later growth and reproduction and longevity, especially in the low-productivity environments where Arizona fescue is found.

Rudgers et al. (2010) also claim two methodological problems: (1) the "harvest of all aboveground biomass at the each growing season could introduce artifacts that disrupt the demographic transitions of perennial hosts and alter the effects of experimental treatments" and (2) "host age and temporal environment were confounded." Concerning the former criticism, I harvested aboveground biomass from all plants at the end of the growing season, after aboveground plant parts had senesced and dried. This biomass is naturally lost after plants senesce in the winter and before regrowth in May, so it is highly unlikely that end-of-season harvesting affected the experiment. As for the latter criticism, I carefully acknowledged this complicating factor in my article (Faeth 2009, p. 562). However, all plants were subject to the same background variation, and there were consistent differences between E+ and E− plants in reproductive effort across all controlled soil moisture treatments (reduced, ambient, and supplemented; fig. A3) and across all plant genotypes (fig. A2). Given that precipitation is a driving factor in plant growth and herbivore abundance in these semiarid grasslands, the fact that the differences between E+ and E− plants were consistent across all three soil moisture treatments, despite change in ambient precipitation, provides strong evidence that changes in resource allocation were affected by infection status and not by background environmental variation. It is virtually impossible to control background environmental variation in a field experiment where the intent is to include realistic selective pressures, including native herbivores, and their effects on grass performance. There have been no studies to date, including studies involving agronomic grasses, that have controlled background environmental variation.

However, I agree with Rudgers et al. (2010) that changes to resource allocation and their effects on life-history traits can be complex. The ideal way to confirm the effects on life-history traits and fitness, positively or negatively, is via lifetime studies of host and endophyte fitness, a challenging task for perennial grasses that may live for decades. Nonetheless, my study also provides some additional, albeit indirect, evidence. First, seed production in one year was negatively correlated with change in vegetative biomass in the following year (Pearson correlation: $r = -0.38$, $P < .0001$), suggesting a cost of reproduction. Second, the endophytes-as-reproductive-parasites hypothesis predicts that the endophyte-mediated shift to early reproduction and growth in early ontogeny should begin to negatively affect growth and reproduction in later years. I have continued to maintain experimental treatments and to measure vegetative and reproductive growth. Here, I present results from two additional years (fig. 1). Although vegetative dry mass remained higher for E+ than for E− plants in 2008, the relative differences decreased with time and then disappeared in 2009 (fig. 1A). Notably, seed dry mass was significantly higher for E+ plants only in 2006, and by 2009, E+ seed mass was significantly less than that of E− plants (fig. 1B). Most importantly, the reproductive effort of E+ plants was significantly less than that of E− plants in 2008 and 2009 (fig. 1C). Contrary to Rudgers et al.'s (2010) contention that "symbiosis never reduced plant growth or net reproduction," these results indicate that the endophyte-mediated shift to early growth and reproduction comes at a reproductive, and perhaps vegetative, cost in later ontogeny and is consistent with the endophytes-as-reproductive-parasites hypothesis.
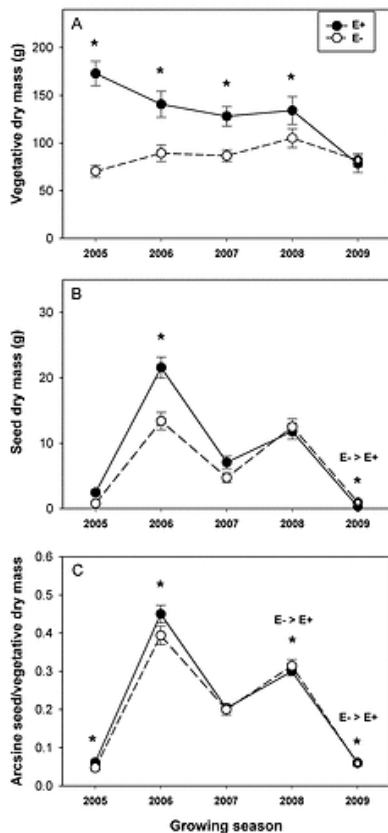
Figure 1: Effect of endophyte infection on vegetative dry mass (*A*), seed dry mass (*B*), and the ratio of seed to vegetative dry mass (reproductive effort, *C*) when herbivores are experimentally reduced, showing the fourth (2008) and fifth (2009) years of results. Asterisks indicate significant differences between E+ and E− plants for vegetative dry mass (2008: E+ > E−, $F_{1,87} = 8.76$, $P = .004$), seed biomass (2009: E+ > E−, $F_{1,87} = 4.59$, $P = .029$), and reproductive effort (2008: E− > E+, $F_{1,87} = 4.52$, $P = .04$; 2009: E− > E+, $F_{1,82} = 3.95$, $P = .05$). Vegetative dry mass in 2009 and seed dry mass in 2008 did not differ between E+ and E− plants ($P > .70$). See Faeth (2009) for details of statistical analyses.

In their second major criticism, Rudgers et al. (2010) question my results showing higher herbivore loads yet increased seed production in E+ plants in early ontogeny. They then criticize my conclusions that (1) endophytes may mediate host tolerance or overcompensation to herbivory and (2) endophyte-mediated resistance to herbivory changes with ontogeny, with reduced resistance in early ontogeny. They first argue that herbivore abundances do not necessarily scale to herbivore damage. I agree that quantifying herbivore damage is important, and I stated clearly in my article that this experiment did not directly measure herbivore damage. Measuring herbivore damage for grasses, especially since many herbivores are sucking insects, is exceedingly difficult, and I know of no endophyte–host grass study, including those with agronomic grasses, that has done so. Nevertheless, my long-term results clearly show an effect of the reduced- and ambient-herbivory treatments. Plants in the greatly-reduced-herbivory treatment (via insecticide and caging) had significantly increased seed biomass and reproductive effort in the first two years and significantly increased vegetative biomass in the last two years relative to the ambient herbivory treatment (see table 1 in Faeth 2009). Much of this effect was due to strong negative effects of herbivory on E− plants in early ontogeny. Furthermore, the strong negative effects of herbivory (reduced aboveground and seed biomass) for plants in the full-herbivory treatment have continued in 2008 and 2009 (data not shown). I know of no other field studies of endophyte-host interactions where herbivory was controlled, including studies of agronomic grasses, such that the effect of endophyte-mediated changes in herbivory on plant growth and reproduction could be ascertained. Yet previous studies have concluded that endophytes are defensive mutualists on the basis of abundance data alone (e.g., Rudgers and Clay 2008). My results clearly show that increased herbivore loads generally decrease growth and reproduction in this grass and that insect abundance and biomass accurately reflect the effects of herbivory on the host.

Rudgers et al. (2010) then question my interpretation that endophytes may mediate plant tolerance and instead argue that my results support the alternative hypothesis that increased seed production under higher herbivore loads supports "endophyte-mediated protection." I emphasized in my article (Faeth 2009, p. 561) that this experiment was not designed specifically to test for host plant tolerance or overcompensation. Nonetheless, my results clearly show that in early ontogeny, E+ plants allocate more to seeds under full herbivory and have greater vegetative and seed biomass than E− plants, despite higher herbivore loads. These results strongly suggest

endophyte-mediated tolerance or overcompensation in early stages. Whether endophyte-mediated tolerance or overcompensation does indeed occur and, if so, how it positively or negatively affects host and symbiont fitness depend on a number of factors, such as the probability of herbivore encounter and available resources, and cannot be ascertained without additional long-term experiments.

Rudgers et al. (2010) also question my method of sampling arthropod abundances, claiming that the method is biased by the exclusion of large, mobile taxa (they provide no citations supporting this contention). To the contrary, the sampling device, a Burkhard Vortis insect suction sampler, is specifically designed to sample insects from vegetation. Complete details of arthropod taxa collected are presented in a companion paper (app. A in Faeth and Shochat 2010). The collections included large mobile taxa such as grasshoppers and leafhoppers and eight different taxa of large mobile spiders, as well as other large insect predators. Many insects collected were indeed small sucking insects, such as aphids and thrips, but these compose the bulk of many arthropod communities in these native semiarid grasslands (e.g., Rambo and Faeth 1999). Furthermore, we estimated biomass to ensure that effects of infection on abundance and biomass corresponded (abundance and biomass results did generally correspond, but note that for some taxa, such as predators, infection effects on biomass and abundances differed; see Faeth and Shochat 2010). Thus, I believe that my results indisputably show much higher herbivore loads on E+ plants during the first two years and then no differences in the third year. That herbivore loads are either higher or equivalent on E+ plants refutes the key prediction of the defensive-mutualism hypothesis, that E+ plants should have significantly reduced herbivore loads relative to E− plants. Moreover, these results are entirely consistent with multiple bioassay (e.g., Tibbets and Faeth 1999) and field experiments (e.g., Saikkonen et al. 1999) showing that insects preferred and performed better on E+ than on E− Arizona fescue.

Rudgers et al. (2010) imply that evidence against the defensive-mutualism hypothesis is based on evidence from a "single wild host, Arizona fescue," compared to three agronomic species (but primarily tall fescue). This is simply not the case. Accumulating evidence from multiple infected wild species (e.g., Faeth 2002; Saikkonen et al. 2006; Hartley and Gange 2009) indicates great variability of the effects of infection and alkaloids on herbivores (Saikkonen et al. 2010). In a recent study with another wild grass, sleepygrass (*Achnatherum robustum*), Jani et al. (2010) found, via field observations and experiments, that herbivore abundances and species richness were greater on E+ plants with high alkaloids than on E+ plants with no alkaloids or on E− plants, also failing to support the defensive-mutualism hypothesis. In natural populations, there are several ecological explanations for higher herbivore abundances on E+ plants with alkaloids that are consistent with plant-insect interactions in general (see Faeth 2002; Cheplick and Faeth 2009; Saikkonen et al. 2010).

It is important to note that two previous studies by the authors (Omacini et al. 2001; Rudgers and Clay 2008) used visual estimates and sweep netting, respectively, to estimate abundances of

insect herbivores and carnivores and did not report biomass of these groups. Both methods have their own biases, and abundances of certain groups that vary greatly in size (e.g., predators) do not necessarily equal insect biomass. Nor did those studies control herbivory, measure alkaloids, or assess plant damage in any fashion, as Rudgers et al. (2010) claim is necessary to show that changes in herbivore abundances translate into effects on host plants. In Rudgers and Clay's (2008) study, insects were sampled from entire plots with multiple plant species in addition to E+ or E− tall fescue, so these other plant species confounded determinations of herbivore abundances on E+ and E− plants. Yet both studies used abundance data, as have other studies involving infected agronomic grasses (see references in Saikkonen et al. 2006), to support the notion of endophytes as defensive mutualists of host grasses.

This, then, begs the question: if Rudgers et al. (2010) claim that indirect evidence, such as decreased herbivore abundances, supports the defensive-mutualism hypothesis, then why does the similar and perhaps more compelling evidence in my article and other studies showing increased or equal herbivore abundances on E+ plants not refute this hypothesis? The most parsimonious explanation for these results is that inherited symbionts do not act as protective mutualists, at least in these wild grasses. If increased or equal herbivore abundances on E+ Arizona fescue plants is somehow "most consistent" with the defensive-mutualism hypothesis, as the authors claim, then the defensive-mutualism hypothesis becomes an irrefutable panchreston, which by explaining everything explains nothing.

Rudgers et al.'s (2010) third main criticism is that my study provided no data on endophyte fitness. Measuring how shifts in resource allocation affect symbiont lifetime fitness is perhaps even more difficult than determining effects on host fitness (e.g., Ryan et al. 2008). One necessary component of the endophytes-as-reproductive-parasites hypothesis is that early host reproduction benefits endophytes because infections can be lost in later host ontogeny. Loss of infection in adult plants is now well documented in many infected native grasses (e.g., Afkhami and Rudgers 2008). Arizona fescue plants lose infection at substantial rates, as shown by previous experimental (e.g., Saikkonen et al. 1999) and observational studies. For example, Faeth and Hamilton (2006) tracked 1,633 Arizona fescue plants, most of which were infected, in natural habitats for survival and infection status over a 7-year period. About 4% of infected plants lost infection during that time period. This study was not designed to explicitly test for time-dependent infection loss, but these observations further support the idea that early reproduction may benefit transmission of the endophyte, perhaps at the expense of long-term host fitness.

Is the Mutualism Hypothesis More Parsimonious?

Rudgers et al. (2010) argue that because class 1 fungal endophytes are vertically transmitted, their persistence seems paradoxical "if the symbionts reduce host fitness." However, as they note, there are at least two other theoretical ways for endophytes to persist without conferring measurable benefits (metapopulation model; Saikkonen et al. 2002) or with benefits that are

"exceedingly small" or "undetectable" (transmission efficiency model; Gundel et al. 2008). In addition, there is at least one other way that has also some observational support (Cheplick and Faeth 2009, pp. 129–134). Faeth et al. (2007) showed analytically that small levels of horizontal transmission could result in the persistence of neutral or parasitic asexual endophytes, could compensate for imperfect transmission (failure of infected plants to produce 100% infected seeds), and could be difficult to detect. Whereas there has yet to be unequivocal confirmation, observation of epiphyllous nets and conidia on the leaves of some infected grasses suggest some horizontal transmission (e.g., White et al.1996; Moy et al. 2000). Also, *Neotyphodium* can be readily inoculated into uninfected agronomic grasses in the laboratory (e.g., Christensen 1995). Similar transmission may also occur in nature via insect herbivore vectors, which transmit other nonsystemic endophytes, or by root-to-root contact (Cheplick and Faeth 2009, p. 132). Rudgers et al. (2010) focus on mode of transmission as the sole factor determining the interaction outcome between symbionts and their hosts. Both theory and empirical studies show that other factors, including transmission rate, symbiont diversity within populations, and the geographic mosaic, affect the virulence or avirulence of symbionts (e.g., Thompson 2005).

Whereas vertical transmission increases the probability of mutualistic outcomes, it is certainly no guarantee (Thompson 2005; Cheplick and Faeth 2009). There are vertically transmitted symbionts that are parasitic and persist in populations, defying the conventional notion that vertically transmitted symbionts must be mutualistic to persist in nature, such as maternally inherited *Wolbachia* (e.g., Werren1997) and Flavobacteria (e.g., Hurst et al. 1999) that alter sex ratios of invertebrates. Likewise, vertically transmitted, clonal fungi domesticated by leaf-cutting ants retain control over the fungal garden even though such control may be detrimental to the host (Poulsen and Boomsma 2005). One of the authors (Rudgers) herself suggested that "grass-endophyte symbioses may function like insect-*Wolbachia* interactions" (Afkhami and Rudgers 2008, p. 413) on the basis of an apparent shift in allocation to infected seeds in several native grasses. Although Afkhami and Rudgers (2008) interpreted their results as supportive of the endophytes-as-mutualists hypothesis, they are also consistent with the endophytes-as-reproductive-parasites hypothesis, first suggested by Faeth and Sullivan (2003). Increased allocation to infected seeds is also congruent with endophytes manipulating host plants to increase their own transmission at the expense of the host, as suggested in my article (Faeth 2009, p. 563).

Clearly, there are some inherited symbionts that appear to be locked into a mutualistic mode, such as *Buchnera* in insects, which exhibit a highly reduced genome and hosts that are reproductively dependent on them (e.g., Moran 2007). However, others, like*Wolbachia*, remain largely parasitic, while still others vary their interactions with the host, depending on host species and host and symbiont genetics and environment (e.g., Moran 2007). Given (1) this spectrum of possible interaction outcomes of inherited symbionts, (2) that *Neotyphodium* retains its full genome size, often exceeding that of its sexual and parasitic counterpart by frequent hybridization (e.g., Schardl and Craven 2003), (3) that host grasses are not dependent on the

symbiont for reproduction (E− grasses reproduce and persist in natural populations), and (4) that the closely related, sexual ancestor *Epichloë* itself acts as a reproductive parasite by dramatically altering host reproduction (e.g., Clay 1991) and resource allocation (e.g., Pan and Clay 2003), the endophytes-as-reproductive-parasites hypothesis is a reasonable alternative to the endophytes-as-mutualists hypothesis. The question of which hypothesis is more "parsimonious" or "consistent" seems to be a red herring that is irresolvable at this point, given that the critical long-term demographic experiments touted by Rudgers et al. (2010) as necessary to pinpoint the direction of the interaction between endophytes and host grasses have not been yet performed, even in agronomic grass systems.

The Mutualism-versus-Parasitism Question: A Superfluous Dichotomy?

We now know that the direction and strength of the interaction between inherited endophytic symbionts vary with plant (e.g., Faeth 2009) and endophyte (e.g., Morse et al. 2007) genotype, environmental factors, and their interactions (e.g., Cheplick and Faeth 2009), even in agronomic grasses where interactions can become parasitic (e.g., West et al. 1995). Furthermore, these interactions and their effects on host plants clearly change over time, as shown in my study, and over geographic space (e.g., Sullivan and Faeth 2008), like other species interactions (Thompson 2005). Therefore, the question of whether inherited endophytes are mutualists (or more specifically, defensive mutualists), as posed by Rudgers et al. (2010), is far too simplistic and detracts from advancing research on far more relevant and important ecological and coevolutionary questions. For example, at least two genetic strains of *Neotyphodium* co-occur in native sleepygrass populations. One haplotype produces extraordinarily high levels of ergot alkaloids, whereas the other produces no alkaloids (Faeth et al. 2006). Similar disparities in alkaloid levels are found among different endophyte haplotypes infecting wild tall fescue and perennial ryegrass populations in Europe (see Cheplick and Faeth 2009, pp. 55–56). It seems pointless to assume that each of these endophyte haplotypes must be a protective mutualist, or a mutualist in general, simply because they are vertically transmitted. The defensive-mutualist and reproductive-parasite hypotheses are not mutually exclusive. The direction of interactions between inherited symbionts and hosts can change over ecological and evolutionary timescales and with symbiont and host species and genetics, other interacting species, and environmental factors (e.g., Thompson 2005). The mutualism-versus-parasitism dichotomy diverts research attention from more important ecological and coevolutionary questions such as these: What changing selective pressures maintain E− plants and E+ grasses with different endophyte strains and varying alkaloid levels in wild populations? How do the interactions vary with plant genotype, available resources, and climate? How do the interactions vary through ecological and evolutionary time and as geographic mosaics (Thompson 2005)? How do other interacting plant, herbivore, and natural enemy species affect the direction and strength of the interaction?

Conclusion

It is perplexing that Rudgers et al. (2010) criticize my long-term study, which is of the very type that they prescribe to test the nature of interaction of endophytes with their hosts. It is equally puzzling that no such studies exist, to my knowledge, for the systems of introduced agronomic grasses in which the authors work. For example, Clay (1990) performed a 3-year demographic study of E+ and E− tall fescue, but this study was relatively short-term, did not measure seed production, lifetime survival, or age-specific endophyte transmission, and did not control environmental factors, background environments, or plant genotype, falling far short of the authors' experimental prescription. Yet for more than 20 years, seed-borne endophytes have repeatedly been proclaimed as mutualists without these definitive, long-term demographic studies and on the basis of evidence that is similar to, and arguably often less compelling than, that presented in my article. Rudgers et al. (2010) demand a standard of experimental evidence for demonstrating the lifetime fitness consequences of infection for the host and symbiont that they themselves have not yet met. I agree with them on one key point: life span demographic experiments are essential to test how vertically transmitted endophytes alter life-history traits and the consequences for lifetime host fitness. I also agree that it is necessary to clearly define what lifetime fitness means for each organism and the appropriate ways for measuring it (e.g., Ryan et al. 2008). For experimental studies, it is also essential to control and manipulate plant and endophyte genotype and environmental variation, including herbivory. We now know that host-endophyte interactions vary with these factors and over time and space (see references in Cheplick and Faeth 2009), like other species interactions (e.g., Thompson 2005). I believe that my experiment is a step in the right direction. My research group plans to continue to maintain treatments and monitor lifetime resource allocation and survival of the plants and endophyte transmission efficiency in this unique long-term experiment.

Acknowledgments

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