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Asexual *Neotyphodium* Endophytes in Arizona Fescue: A Test of the Seed Germination and Pathogen Resistance Hypothesis

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Abstract

Asexual, systemic, and seed borne endophytes that live symbiotically in pooid grasses are thought to interact mutualistically with their hosts. The mutualism should be most evident in seed and seedling stages, where host fitness is directly affected. We tested the hypothesis that the seed borne endophyte, *Neotyphodium*, increases seed germination success and seedling survival for a native grass host. We compared germination success and speed and seedling survival, between infected (E+) and uninfected (E–) of seeds from: 1) wild maternal plants and 2) four genotypes of E+ maternal plants from which the endophyte had been experimentally removed. In both experiments, infection did not increase germination success, as predicted. Wild E+ seeds harbored more weedy fungi, which were associated with reduced germination success and seedling survival and slower germination, than E– seeds. Contrary to prevailing notions, seed borne endophytes do not increase, and instead may decrease germination and seedling survival, and delay germination.

Keywords: Endophytes, fungal pathogens, germination, mutualism, *Neotyphodium*, seeds, seedlings, symbiosis

1. Introduction

Fungal endophytes are very diverse and abundant, and live internally,

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intercellularly and asymptomatically, at least for part of their life cycle, within aboveground tissues of their host plants. Although endophytes were once viewed collectively as plant mutualists (e.g., Carroll, 1988; Clay, 1988), accumulating evidence indicates that endophytic interactions with hosts range from pathogenic to mutualistic, and vary geographically and in evolutionary and ecological time (Saikkonen et al., 1998; Faeth, 2002; Faeth and Bultman, 2002). Nonetheless, one group of endophytes in pooid grasses remains ensconced as examples of extreme endophyte-plant mutualists (e.g., Clay and Schardl, 2002). These endophytes, in the genus *Neotyphodium*, are asymptomatic throughout their life stages, systemic, asexual and vertically-transmitted (e.g., Schardl and Phillips, 1997; Clay, 1990; but see White et al., 1996). Because fungal reproduction, and thus fitness, depends upon host reproduction, these specialized, systemic fungi are predicted by evolutionary theory to act as strong plant mutualists (Law, 1985; Frank, 1994; Connor, 1995; Clay, 1998). Empirical evidence, mostly drawn from agronomic grasses in North America introduced from Eurasia, usually supports this prediction. Infected grasses exhibit increased host resistance to herbivores (via endophyte produced alkaloids), pathogens, seed predators and drought, and display increased competitive abilities and germination success relative to uninfected counterparts (Clay, 1990; Saikkonen et al., 1998). The observation of high frequencies of *Neotyphodium* in both agronomic and natural grass populations is used as further support of endophytic mutualism with their host grasses (e.g., Clay, 1998). *Neotyphodium* infections can be lost via imperfect transmission into seeds (Ravel et al., 1997) or hyphal inviability (Siegel et al., 1984), but never gained by adult plants (but see White et al., 1996). If *Neotyphodium* interacts parasitically with the host, then selection should reduce infection frequencies; if the endophyte is neutral, then infection frequency should also decrease due to random loss of infection through imperfect transmission (Clay, 1998; Faeth and Sullivan, 2003). Instead, infection levels usually remain high in host grass populations, indicating a mutualistic relationship (Clay, 1998).

However, many of the purported benefits of *Neotyphodium* infection are not readily detectable in native grass populations. For example, the main benefit associated with infection, herbivore resistance via alkaloid production, rarely occurs among native grass species harboring *Neotyphodium* (Faeth, 2002). In addition, experimental studies show that the endophyte in Arizona fescue, a grass native to North America, often interacts parasitically, not mutualistically, at least during the adult life stages of the host. Infection generally decreases host growth and reproduction (Faeth and Sullivan, 2003), and resistance to herbivores (Saikkonen et al., 1999; Schulthess and Faeth, 1998; Tibbets and Faeth, 1999) and to abiotic stress such as low soil nutrients or moisture (Faeth and Fagan, 2002) and fire (Faeth et al., 2002a). Nevertheless, infection frequencies remain consistently high in this species and many other native grass populations, suggesting either 1) some other benefit of infection outweighs these costs or 2) infection is maintained by non-mutualistic mechanisms.

Benefits of endophytes that accrue at the seed and seedling stage should be particularly crucial to host fitness. Whereas endophyte-related benefits to the adult host are often indirect (increased competitive abilities eventually leading to increased seed production over the lifespan of the host) and perhaps superfluous (e.g., reduction of grazing for hosts, such as grasses, that are already tolerant of grazing, Faeth and Bultman, 2002), increases in seed or seedling survival directly affect host fitness (e.g., Wolock-Madej and Clay, 1991; Knoch et al., 1993; Faeth and Bultman, 2002). In both agronomic and native grasses, alkaloids, which are anti-microbial (White and Cole, 1985; Siegel and Bush, 1996), are usually highest in seeds than other plant parts (e.g., Leuchtmann et al., 2000), lending circumstantial support to the notion that protection of seeds may be the main route of mutualistic effects (Clay, 1998; Leuchtmann et al., 2000; Faeth and Bultman, 2002). However, tests of increased germination (e.g., Clay, 1987; Bacon, 1993) and resistance of seeds and seedlings to pathogens (Burpee and Bouton, 1993; Gwinn and Gavin, 1992) due to endophyte infection are limited for introduced agronomic grasses. To our knowledge, tests of pathogen resistance are non-existent for *Neotyphodium*-infected native grasses.

We tested the hypothesis that *Neotyphodium*-infected seeds in a widespread, native grass in North America, Arizona fescue (*Festuca arizonica*) differ in germination success and resistance to seed and seedling pathogens in two experiments. In the first, we compared germination success and speed of germination of infected (hereafter, E+) and uninfected (hereafter, E–) seeds from wild maternal plants. In the second experiment, we compared germination success and speed, and seedling mortality of seedlings due to seed pathogens of E+ and E– seeds from four maternal grass genotypes. The E– seeds were produced by experimentally removing the endophyte from clones of the four genotypes, such that plant genotype was controlled. Based upon the mutualistic concept of systemic grass endophytes, we predicted greater and faster germination, and higher survival of seedlings, due to reduced pathogenic fungi that co-occur within seed tissues.

2. Materials and Methods

Arizona fescue

Arizona fescue (*Festuca arizonica* (Vasey)) is a widespread, perennial bunch grass native to the southwestern USA and northern Mexico in semi-arid

Ponderosa pine-bunch grass communities at elevations between 2,300 m and 3,200 m (Kearney and Peebles, 1960). Arizona fescue reproduces by seed.

Neotyphodium endophyte

Neotyphodium (formerly *Acremonium* sect. Albo-lanosa Glenn et al., 1996) is a systemic, asexual endophytic fungus infecting cool-season grasses (Clay, 1990; Saikkonen et al., 1998). *Neotyphodium* endophytes are a group of clavicipitaceous fungi, which, unlike some of their sexual (teleomorph) relatives in the genus *Epichloë*, are strictly transmitted vertically by hyphal growth into the developing seeds of their host. The mycelia infect the meristem, leaf tissues and inflorescences of the host and utilize the host's cellular exudates as a nutritional resource (Christensen et al., 2002). *Neotyphodium* may produce varying amounts and types of alkaloids (Powell and Petroski, 1992; Siegel and Bush, 1996; Leuchtmann et al., 2000) depending upon host species and genotype, and fungal haplotype, as well as environmental factors. *Neotyphodium* in *F. arizonica* populations most closely resemble *N. starrii* (Faeth and Sullivan, 2003; Sullivan and Faeth, 2004) and produces only peramine, one of four major alkaloid types found in infected grasses, and usually at low levels (Faeth and Fagan, 2002; Faeth et al., 2002b).

Wild seed experiment

To test the hypothesis that the presence of the endophyte increases success and speed of germination, we collected seeds in September 1997 from 30 E+ and 30 E– adult plants from three natural populations located in Arizona on the Mogollon Rim (see Schulthess and Faeth, 1998 for a description of the study sites). Seeds were cold treated 30 days at 5°C and then stored at room temperature, which maintains viability of the endophyte (Faeth and Sullivan, 2003). Infection status of adult plants was previously determined from tissue print blot immunoassay and microscopic examination of seeds for the presence of distinct *Neotyphodium* hyphae concentrated in the aleurone layer (see Schulthess and Faeth, 1998; Saikkonen et al., 1998 for details).

In June 1998, 30 seeds were randomly-selected from each maternal plant and were first scarified with 50% sulfuric acid for 30 minutes, followed by three rinses with sterile water to maximize germination rates. All seeds were then surfaced sterilized (50% bleach for 30 minutes) to remove epiphytic microorganisms and placed for germination on three media types, water agar (WA), potato dextrose agar (PDA) and a high nutrient agar (HNA) containing 2% malt extract. Surface sterilization removes the many external microbes found on the surface of the seed but not internal, endophytic fungi. The internal fungi included not only *Neotyphodium* but also many other fungi, some of which are known pathogens, inside the seed tissues (Hamilton, 2002). We hereafter term these fungi as 'weedy' endophytic fungi because they are not systemic, not strictly seed borne, and grow rapidly from seeds and other plant tissues (e.g., Schulthess and Faeth, 1998) relative to slower growing *Neotyphodium*. Weedy fungi isolated from surface-sterlilized seeds included the genera *Alternaria*, *Aspergillus*, *Chaemtomium*, *Phoma*, *Cladosporum* and *Aureobasidium* (Hamilton, 2002). 300 E+ (10 from each of the 30 E+ maternal plants) and 300 E– (10 from each of the 30 E– maternal plants) were plated on each media type, for a total of 1,800 seeds. Each seed was placed on a separate plate with the appropriate media.

We examined seeds daily for germination (emergence of the root radicle) and the presence of fungi other than *Neotyphodium* growing from seeds for a period of 30 days (endophytic fungi, including slow-growing *Neotyphodium*, typically grow out from seeds within 2 weeks). Fungi growing from the seeds or seedlings were isolated in pure culture in the same media for identification. Fungal identification was based on conidia and conidiophore morphology as well as sexual characteristics (Farr et al., 1989) when present. Details of weedy fungal identification and taxonomy are in Hamilton (2002).

Endophyte-removed seed experiment

A similar experiment to test the seed germination and pathogen resistance hypothesis was conducted except that E– seeds came from plants from which the endophyte had been experimentally removed. In brief, *Neotyphodium* infections were removed from clones of four infected maternal plants (termed A, B, C, D) via hydroponic treatment with low levels of fungicide. These E– clones were then potted, split multiple times for replication, and then grown in the field for three years. Other clones from the four maternal plant genotypes were treated similarly but without fungicide, and thus remain infected (E+). Finally, clones of one plant genotype (A) were treated with fungicide, but remain infected (fungicide treatment removes the endophyte in ca. 50% of clones) and were used as controls (E+F) to test for any extraneous effects of the fungicide. Seeds were then collected from E– and E+ plants, as well as E+F plants in September 1999. Seeds were cold-treated for 30 days at 5°C and then stored at room temperature. All plants and seeds were continually re-tested to ensure infection status. Details of endophyte removal and assaying for the endophyte are in Faeth and Sullivan (2003).

At least 25 seeds from each infection status and genotype, plus the infected controls, were surface sterilized and scarified (as above) and placed on two media types, water agar (WA) and high nutrient agar (HNA) in December 1999

(PDA was not used because results from the first experiment showed no significant differences in germination or fungal growth between water agar and PDA). Each seed was placed on a separate plate with the appropriate media. Germination rate, time of root radicle emergence, pathogenic fungal growth, and survival of seedlings (up to the point where the shoot reached 4 cm. in length) was recorded daily for approximately 30 days.

Statistical analyses

To test the association of endophyte infection, the presence of weedy fungi, media type, and in the second experiment, plant genotype, with germination success, we used log linear analyses (SYSTAT 2000 Version 10.2). Log linear analysis finds the best fit (most parsimonious) model for multi-way frequency tables. In short, factors and their interactions are removed hierarchically from a more saturated model until the simplest model fits the data, such that the χ^2 for the model remains insignificant (SYSTAT 10.2).

In the first experiment with wild seeds, we used ANOVA (SYSTAT 10.2, GLM) to test for the effect of infection status and media type, and their interaction, on the speed of germination (days to root radicle emergence, square root transformed because the variable, days, was not normally distributed). The presence of other weedy fungi was used as a covariate in the analysis. In the second experiment with endophyte-removed seeds, we first used ANOVA to test for differences in speed of root and shoot emergence between E+ seeds and E+F (fungicide treated but still infected) seeds for plant genotype A. We then used ANOVA to test for the effect of plant genotype, endophyte infection, media type and their interactions, with the presence/absence of weedy fungi as a covariate on speed of root and shoot emergence (in days, square root transformed as above). If a factor or interaction was significant, we used Tukey HSD tests for multiple comparisons among means, and *post hoc* tests of hypotheses, with Bonferroni adjustments to α for multiple comparisons, respectively.

3. Results

Wild seed experiment

The best log linear model (χ^2=17.00, df=16, P=0.38) retained the following variables from the multi-way frequency table: the presence of weedy fungi, media type, the two way interaction between endophyte and weedy fungi, and the three way interaction between endophyte status, weedy fungi and media type. In short, seeds with weedy (non-*Neotyphodium*) fungi growing from them were associated with lower germination success (χ^2=22.67, df=1, P<0.001) than seeds without weedy fungi (Fig. 1). Furthermore, seeds with weedy fungi were positively associated (χ^2=51.24, df=1, P<0.0001) with E+ seeds (Fig. 2). More seeds germinated on water agar (67%) and PDA (63%) than the high nutrient agar (31%) (χ^2=62.63, df=2, P<0.0001).

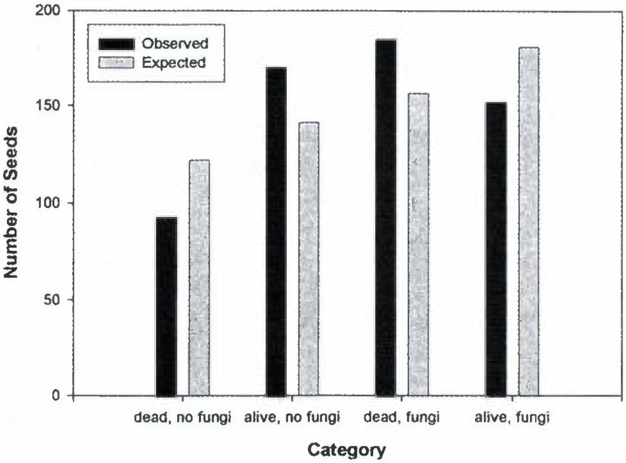

Figure 1. Observed (black bars) and expected (gray bars) number of seeds that were alive (germinated) or dead (no germination) with (fungi) and without (no fungi) infection by weedy fungi. Expected numbers were generated from multiway frequency tables.

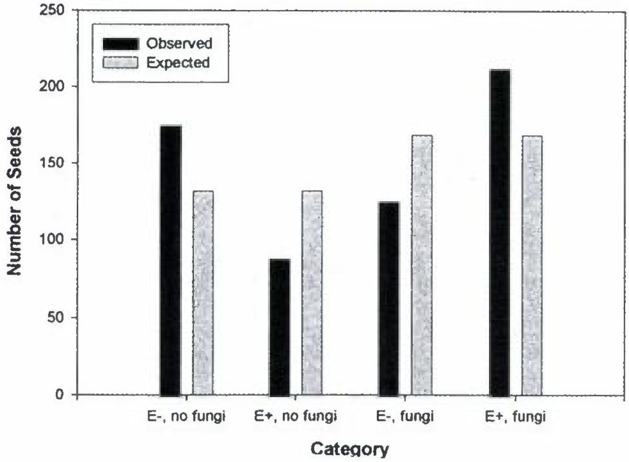

Figure 2. Observed (black bars) and expected (gray bars) number of E+ (infected by *Neotyphodium*) and E– (*Neotyphodium*-free) seeds with (fungi) and without (no fungi) infection by weedy fungi. Expected numbers were generated from multiway frequency tables.

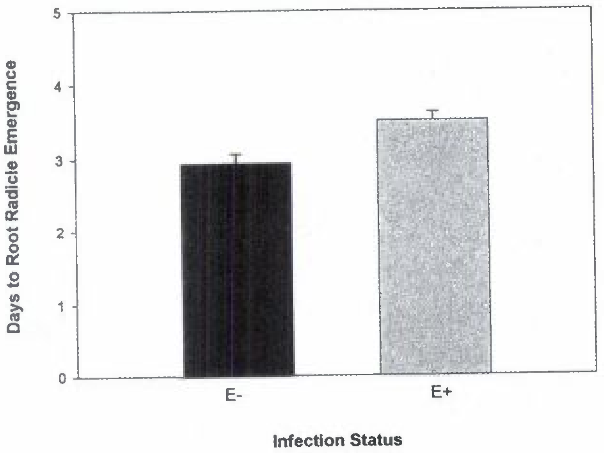

Figure 3. Mean (± S.E.) of the number of days to emergence of root radicles in germinating E– and E+ seeds.

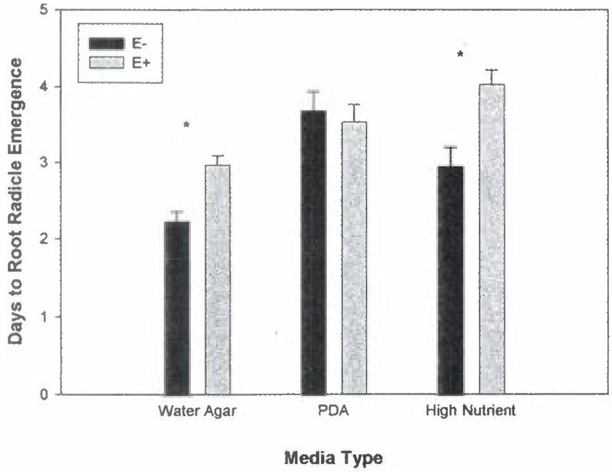

Figure 4. Interaction of endophyte status (E– and E+) with media type on the number of days to emergence of root radicles in wild seeds. Asterisks above bars indicate significant differences (*post hoc* hypothesis tests with Bonferroni adjustments to α).

E– seeds germinated faster (Fig. 3) than E+ seeds (F=17.01, df=1,479, P<0.001). The type of media also affected speed of germination (F=20.61, df=2,479, P<0.001), with seeds germinating faster on water agar than either PDA or the high nutrient media (Tukey HSD *post hoc* test, water agar vs. PDA and high nutrient, P<0.001). Finally, infection status and media type interacted to affect speed of germination (F=7.60, df=2, 479, P<0.01), with E–

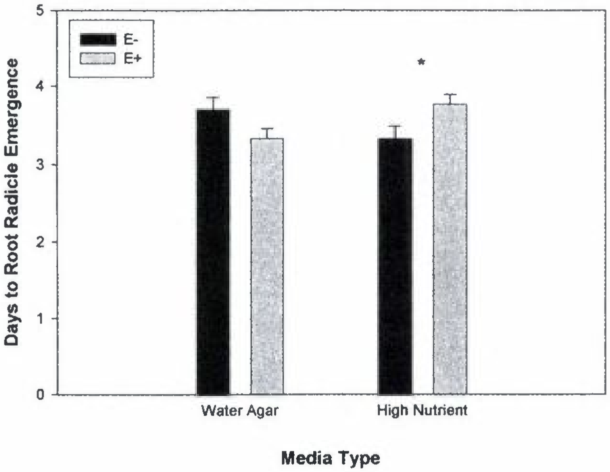

Figure 5. Interaction of endophyte status (E– and E+) with media type on number of days to emergence of root radicles in germinating seeds for the second experiment (endophyte-removed seeds). Asterisks above bars indicate significant differences (*post hoc* hypothesis tests with Bonferroni adjustments to α).

seeds germinating faster in the water agar (*post hoc* tests, p=0.001) and high nutrient media (P<0.001) than E+ seeds (Fig. 4). The presence of weedy fungi in seeds did not affect when seeds germinated (no covariate effect, F=0.11, df=1,479, P=0.60).

Endophyte-removed seed experiment

The best log linear model for germination success included only plant genotype (χ^2=6.74, df=3, P=0.08); neither endophyte status, media type nor any two or three way interactions between these factors significantly improved the log linear model. Unlike the first experiment, E+ and E– seeds did not differ in association with weedy fungi (χ^2=2.84, df=1, P>0.05). In general, weedy fungal growth was much reduced relative to wild seeds because the fungicide treatments probably removed fungal endophytes other than *Neotyphodium*. Nonetheless, the presence of weedy fungi was negatively associated with survival of seedlings to the 4 cm shoot stage (χ^2=31.31, df=1, P<0.001); only 28.8% of seedlings with weedy fungi survived, whereas 71.1% without fungi were alive at the 4 cm shoot stage.

Neither plant genotype (F=1.12, df=3,247, P=0.34), endophyte infection (F=0.01, df=1,247, p=0.93), nor media type (F=0.03, df=1,247, P=0.87), influenced the timing of the appearance of root radicles. However, infection status interacted significantly with media type (F=6.81, df=1,247, P=0.01); E+ seeds were slower to germinate on high nutrient media than E− seeds (*post hoc* test, P=0.04), but germination speed of E− and E+ seeds did not differ on the water agar media (P=0.11) (Fig. 5). The covariate, the presence of weedy fungi, did not affect the timing of root radicle emergence (F=0.96, df=1,247, P=0.33).

In terms of when shoots first emerged (no seeds produced shoots without root radicle emergence), E+ and E− seeds did not differ (F=0.29, df=1,227, P=0.59). However, day of shoot emergence varied by plant genotype (F=20.86, df=3,227, P<0.001) and media type (F=11.04, df=3,227, P=0.001). In general, genotype C had faster shoot emergence than genotype A, B or D (Tukey HSD test, p<0.001), and shoots emerged faster on water agar than high nutrient media. Infection status interacted with media type (F=17.60, df=1,227, P<0.001) in the same manner as root radicle emergence; E+ seeds produced shoots later than E− seeds on the high nutrient media than water agar (post hoc tests, P<0.001, figure not shown), but shoot emergence of E− seeds was equivalent on the two media types. Endophyte status also interacted with plant genotype (F=5.61, df=3,227, P=0.001). Shoot emergence of E+ seeds of genotype D were slower to emerge than E− seeds of the same genotype (*post hoc* test, P<0.001, figure not shown), but E+ and E− seeds from other genotypes did not differ (P>0.14). As with root emergence, the covariate, presence of weedy fungi, did not affect when shoots emerged (F=0.33, df=1,227, P=0.57).

E+F seeds (seeds from fungicide-treated maternal plants that retained the endophyte) did not differ in germination success from E+ seeds of the same plant genotype (χ^2=1.42, df=1, P>0.20), indicating no extraneous effects of fungicide treatment on germination success. Likewise, E+F seeds did not differ in the day of root radicle (F=0.14, df=1,108, P=0.71) or shoot emergence (F=2.54, df=1,96, P=0.11) from E+ seeds, indicating no spurious effects of fungicide treatment on germination speed or growth. We did not expect spurious effects of the fungicide because only clones of maternal plants were treated with fungicide in the greenhouse, and clones were continually split and re-potted, and then grown in the field for three years prior to seed collection.

4. Discussion

We found no support for the prediction that asexual, seed borne endophytes act mutualistically with their hosts by increasing germination success. Overall germination success did not differ between E+ and E− seeds, either from field-collected plants or from infected plants from which the endophyte had been experimentally removed. These results contrast with those from a previous study on agronomic tall fescue (*Festuca arundinacaea*) and perennial ryegrass (*Lolium perenne*), where seeds infected with *Neotyphodium coenophialum* and

N. lolii, respectively, showed about 10% higher germination rates than uninfected seeds (Clay, 1987). However, our results are similar to a later study of tall fescue where E+ and E– did not differ in germination success (Bacon, 1993).

Our results corroborate the only other previous studies comparing germination of E+ and E– seeds in a native grass. Neil et al. (2003) and Faeth et al. (2002) showed no differences in germination rates of E+ and E– Arizona fescue seeds under a wide range of osmotic potentials. Likewise, Faeth et al. (2002) found in field experiment that E+ and E– seeds germinated equally after a fire. Seed-borne endophytes potentially have their greatest impact on host plant fitness at the germination stage, yet apparently the presence of the endophyte in Arizona fescue seeds does not increase germination success, as has been proposed for grasses in general (e.g., Clay, 1987).

Moreover, the presence of the endophyte appears to delay germination in terms of both root radicle and shoot emergence. This delay is relatively small (ca. 12 hrs) and occurred in the laboratory under conditions that are ideal for germination (i.e., the high moisture content of media). Nevertheless, small lags in germination can translate into large asymmetric differences in growth of seedlings such that chances of survival in competitive environments are severely reduced (Baskin and Baskin, 1998; Weis and Hochberg, 2000). It may be particularly critical for grasses growing in semi-arid habitats, such as Arizona fescue, to germinate quickly when resources, such as soil moisture, are available, to gain a competitive edge over other seeds and seedlings. Germination of many grasses and herbaceous plants in semi-arid Pondersoa pine-grassland communities occurs in a brief pulse after seasonal summer rains in the southwestern USA (Faeth et al., 2002a). Infection by *Neotyphodium* in Arizona fescue seeds, with their associated delays in root germination and emergence of shoots, may render infected seeds less competitive during these bursts of germination, if germination of E+ seeds lags behind E– counterparts and other plant species.

Systemic endophytes in grasses, as well as more generalized, horizontally-transmitted endophytes in woody plants (e.g., Arnold et al., 2003) have been hypothesized to inhibit host plant pathogens and thus benefit their hosts (Clay, 1988, 1990; Siegel and Bush, 1996). *Neotyphodium* reduces pathogenic fungal growth in the laboratory, presumably from alkaloid production (White and Cole, 1985; Christensen, 1996; Yue et al., 2000). Because *Neotyphodium* hyphal densities and alkaloids are concentrated in seeds (Leuchtmann et al., 2000), and seeds harbor a wide array of potentially pathogenic fungi, protection of the seed and seedling stages from pathogens is viewed as an important, but largely untested, mechanism of the endophyte-host mutualism (Clay, 1987; Leuchtmann et al., 2000; Clay and Schardl, 2002). Clearly, inhibiting seed or seedling pathogens would provide direct benefits because of increased offspring survival.

We, however, found no support for the hypothesis that endophyte infections inhibit potential seed or seedling pathogens. Instead, *Neotyphodium* infections, at least in field-collected seeds, are positively associated with the presence of weedy and potentially pathogenic seed fungi, and these weedy fungi are associated with decreased survival of seedlings. These results contrast with studies showing increasing resistance to disease of infected tall fescue seedlings relative to uninfected seedlings (e.g., Gwinn and Gavin 1992).

In addition, *Neotyphodium* in adult plants appears negatively correlated with the presence of other, potentially pathogenic endophytic fungi in some endemic Asian grasses (Li et al., 1997). However, other studies found no increased resistance of agronomic tall fescue seedlings to disease (Burpee and Bouton, 1993; Trevathan, 1996). To our knowledge, our study is the first to test the effect of endophytes infection on seed borne weedy fungi in a native grass. We assume that these weedy fungi are indeed pathogens. At least some of the taxa (e.g., *Alternaria* and *Cladosporium*) are known as plant pathogens, and many of the taxa also are commonly found in soils. That decreased germination success was significantly and negatively associated with the presence of weedy fungi (Fig. 1), and survival of seedlings was negatively associated with these weedy fungi, suggests that at least some of these weedy fungi are indeed pathogenic. Thus, our results do not provide support for the seed or seedling pathogen hypothesis.

The positive association of *Neotyphodium* infections with other seed fungi suggests that harboring the endophyte incurs costs, rather than benefits, in terms of resistance to seed and seedling pathogens. It is possible that for a systemic infection such as *Neotyphodium* to persist within a host plant, the host immunological systems must be suppressed such that resistance to other fungal endophytes and pathogens is decreased. There is increasing evidence that systemic endophytes and their hosts are in dynamic conflict; for the former, manipulation or production of plant hormones favorable for fungal growth, and for the latter, limitation of fungal growth and branching via plant gene and signaling pathways (Christensen et al., 2002; Tan et al., 2001). This conflict may set the stage for other fungal interlopers and opportunists. Another possibility is that *Neotyphodium* endophytes only persist in plant lineages that are compatible with systemic infection, and these lineages are generally more susceptible to fungal infections. Evidence suggests that systemic endophyte and host plants are highly specialized at the species level (Leuchtmann, 1997; Schardl et al., 1997), and may also be specialized at the population level (Leuchtmann, 1992, 1997; Christensen, 1995; Sullivan and Faeth, 2004).

Maintenance of high Neotyphodium frequencies in natural populations

Infection by *Neotyphodium* does not provide benefits, as predicted, at the seed or seedling stage in terms of germination or protection from pathogens. Furthermore, systemic infection appears to delay germination, at least when germinated on media. Empirical support from previous studies of E+ and E– adult Arizona fescue stages counters the prevailing concept that asexual, systemic endophytes interact mutualistically with their hosts. For example, infection does not increase resistance to herbivores (Saikkonen et al., 1998; Tibbets and Faeth, 1999), generally reduces adult host growth and reproduction (Faeth and Sullivan, 2003), and does not increase competitive abilities (Faeth et al., 2004), except perhaps under extreme and prolonged drought conditions (Morse et al., 2002). Yet, evolutionary theory predicts that an asexual, vertically transmitted symbiont must act mutualistically to persist in populations (Law, 1985; Frank, 1994; Wilkinson and Schardl, 1997), especially if the symbiont is randomly lost from the host, as is *Neotyphodium* (e.g. Siegel et al., 1984; Ravel et al., 1997). For Arizona fescue, unless benefits accrued during prolonged and severe droughts (Morse et al., 2002) outweigh these costs of harboring infection at the adult (e.g., Faeth and Sullivan, 2003) and seed and seedling stages (this study), other explanations are needed to account for the high, but variable, frequencies of infection observed in natural populations (e.g., Schulthess and Faeth, 1998). These non-mutualistic explanations (Faeth and Sullivan, 2003) may include: 1) occasional horizontal transmission (White et al., 1996; Hamilton, 2002), 2) spatial structuring of host populations (Saikkonen et al., 2002) or 3) vertically transmitted endophytes acting as sexual parasites, similar to maternally transmitted *Wolbachia* bacteria in many arthropods (Werren and O'Neil, 1997; Hamilton, 2002; Faeth and Sullivan, 2003).

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