

Are endophytic fungi defensive plant mutualists?

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Endophytic fungi, especially asexual, systemic endophytes in grasses, are generally viewed as plant mutualists, mainly through the action of mycotoxins, such as alkaloids in infected grasses, which protect the host plant from herbivores. Most of the evidence for the defensive mutualism concept is derived from studies of agronomic grass cultivars, which may be atypical of many endophyte-host interactions. I argue that endophytes in native plants, even asexual, seed-borne ones, rarely act as defensive mutualists. In contrast to domesticated grasses where infection frequencies of highly toxic plants often approach 100%, natural grass populations are usually mosaics of uninfected and infected plants. The latter, however, usually vary enormously in alkaloid levels, from none to levels that may affect herbivores. This variation may result from diverse endophyte and host genotypic combinations that are maintained by changing selective pressures, such as competition, herbivory and abiotic factors. Other processes, such as spatial structuring of host populations and endophytes that act as reproductive parasites of their hosts, may maintain infection levels of seed-borne endophytes in natural populations, without the endophyte acting as a mutualist.

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Endophytic fungi usually live asymptotically within tissues of their host plants and have attracted great attention in the past few decades for two main reasons. First, growing evidence indicates that endophytes are found in all plants, are extremely abundant and are often very diverse (Stone and Petrini 1997, Schulthess and Faeth 1998, Arnold et al. 2000). Most of these endophytes form internal localized infections in foliage, roots, stems and bark and are horizontally transmitted via spores. A much smaller fraction, mostly found in pooid grasses, form systemic infections in above-ground tissues. Some of these are vertically transmitted via hyphae growing into seeds (Saikkonen et al. 1998). Second, endophytes may produce mycotoxins, or otherwise alter host physiology and morphology. Endophytic mycotoxins are thought to benefit their woody plant hosts as 'inducible defenses' against insect herbivores (Carroll 1988, 1991) and their grass hosts as 'acquired plant defenses' (Cheplick and Clay 1988) against both vertebrate and invertebrate herbivores. Endophytes

may also alter other physiological, developmental or morphological properties of host plants such that competitive abilities are enhanced, especially in stressful environments (Clay 1988, 1990, Bacon 1993, Malinowski and Belesky 1999). This wide array of purported benefits has led to the conclusion that many, if not most, endophytes in host plants are strong plant mutualists (Carroll 1988, Clay 1988, 1990, Schardl and Clay 1997).

The mutualistic view of endophytes has been particularly promulgated for systemic and symbiotic endophytes of cool season grasses. The 'defensive mutualism' concept (hereafter termed DMC) (Cheplick and Clay 1988, Clay 1988, Vicari and Bazely 1993, White et al. 2001) has been generally accepted as the foundation for widespread occurrence of systemic endophyte infections among pooid grass species (Clay 1988, 1990, 1991a, Vicari and Bazely 1993, Schardl and Phillips 1997) and high frequencies of infection within populations (Clay 1998). In pooid grasses, endophytes

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in the closely-related genera *Epichloë* and *Neotyphodium* are often, or for the latter, always, transmitted vertically by hyphae growing into seeds. Vertical transmission is predicted by evolutionary theory to be associated with strong mutualistic interactions (Law 1985, Ewald 1994) since microbial symbiont and host survival and reproduction are closely linked. Evidence from agronomic grasses appears to support this prediction: seed borne *Epichloë* species appear to benefit the host more than species that are transmitted horizontally (through spore-producing stromata) (Bucheli and Leuchtman 1996, Schardl and Clay 1997, Schardl et al. 1997). *Neotyphodium*, alternatively, is considered the ultimate plant mutualist – always transmitted vertically and often producing high levels of a diverse suite of alkaloids that deter herbivores, root-feeders, and pathogens and often improving growth and reproduction of the host (Clay 1988, 1990). Because it is vertically transmitted through the host seeds, *Neotyphodium* has been traditionally viewed as a ‘trapped’ symbiont that must increase host fitness to persist (Wilkinson and Schardl 1997, White et al. 2001).

Here, I argue that evidence for the DMC is weak or usually absent for horizontally transmitted endophytes in woody plants and grasses. Surprisingly, the same generally holds for systemic and vertically transmitted endophytes in native grasses. Endophyte benefits related to increasing competitive abilities of their grass hosts appear more common than defensive mutualisms. Even these benefits, however, are highly variable in native grasses. For certain host-endophyte genotypes in some environments, the costs of harboring endophytes may exceed any benefits.

Defensive mutualisms against vertebrates

Endophytic fungi are unique among the many plant microbial symbionts because they are the only microbial group thought to benefit their hosts through direct defense against natural enemies of the plant (Carroll 1988, Clay 1988, Faeth and Bultman 2002). Defensive mutualisms also appear to be absent from the myriad symbioses involving microbes and animals. Instead, when microbial symbionts benefit their plant or animal hosts, they usually do so through acquisition of limiting nutrients and increasing competitive abilities (Paracer and Ahmadjian 2000). The view of endophytes as defensive mutualists has stemmed mainly from studies of systemic endophytes in domesticated grasses, especially asexual, vertically transmitted *Neotyphodium*, which are well known for causing toxicosis, staggers, intoxication, narcosis, gangrene and other severe negative effects on vertebrate grazers (Clay 1989, 1990, 1991a, Ball et al. 1993, Schardl and Phillips 1997). These toxic and detrimental effects are attributable to endophyte production of alkaloids, especially ergot and indole diterpene-type

(e.g., lolitrem B) in infected perennial ryegrass (*Lolium perenne*) and pyrrolizidine (lolines) and ergot alkaloids in tall fescue (*Festuca arundinacea*) (Siegel and Bush 1996, Bush et al. 1997). Infected agronomic grasses may also negatively affect native populations of small vertebrates (Coley et al. 1995, Clay and Holah 1999). Frequencies of infected, noxious agronomic grasses, like tall fescue, often increase rapidly to 100% under intense grazing by livestock, as predicted by the DMC (Clay 1996, 1998).

However, strong toxic effects on vertebrates of *Neotyphodium*-infected native grasses appear to be the exception, not the rule. There are only nine known cases of vertebrate toxicity, and one of these (*Lolium temulentum*) may not be related to a fungal endophyte (Table 1). Notably, all cases of livestock toxicity in native grasses were observed a century or more ago (Table 1) undoubtedly because of their dramatic pharmacological effects on, and economic losses to, livestock, although the causal link of the toxicity to *Neotyphodium* endophytes was established much later (Bacon et al. 1977). The cool season pooid grasses are historically important forage grasses for livestock. All but the most remote regions of the world inhabited by these grasses have been intensively grazed by livestock. Therefore, it is unlikely that few, if any, additional examples of strong toxicity, at least to vertebrate herbivores, will be discovered. Leuchtman (1992) conservatively estimated that 30% of approximately 3000 pooid grasses, or 900 species, harbor systemic endophytes. Therefore, the 8 or 9 cases with strong toxic properties against vertebrates represent only about 1% of all grasses harboring systemic endophytes. Additionally, at least some of these noxious grasses are only toxic in a small fraction of their range (Table 1), indicating much wider variability in toxic properties in these natural populations than previously thought. Indeed, evidence suggests that some of the noxious infected grasses in native populations only increased to noticeable frequencies when consistently overgrazed by livestock (Miles et al. 1998, Nan and Li 2001). All the evidence for toxicity of these native grasses is based upon introduced livestock as herbivores; there are no reported cases of these infected native grasses harming or deterring native vertebrate grazers, although infected domesticated grasses may harm native vertebrates when planted extensively (Clay and Holah 1999).

At least an equal number of native grasses infected with *Neotyphodium* endophytes are not toxic or deterrent to either native or domestic vertebrates (Table 1), and sometimes infection even decreases herbivore resistance (Saikkonen et al. 1998, Schulthess and Faeth 1998, Saikkonen et al. 1999). Unfortunately, relatively few native grasses with non-toxic *Neotyphodium* have been thoroughly studied relative to their effects on herbivores. There is little economic reason to document or study endophyte infections in grasses that are

Table 1. Reported toxicity and non-toxicity of native grasses (with common names when known) infected with asexual *Neotyphodium*. Domesticated grasses and commercial or greenhouse grass cultivars of native grasses and those infected by non-*Neotyphodium* endophytes are excluded. Only results from studies of native populations of these grasses are shown here.

Grass species	Endophyte	Herbivores	Effect	Cont. ¹	Year ² toxicity 1st report	Toxicity among popul.	Source
1. <i>Achnatherum (Stipa) robustum</i> (sleepygrass)	<i>Neotyphodium</i>	Horses/sheep	Narcosis/toxicosis	NA	1903, 1929	Rare, 6 of 54 popul.	Bailey 1903, Marsh and Clawson 1929, Jones et al. 2000
2. <i>Achnatherum inebrians</i> (drunken horse grass)	<i>Neotyphodium</i>	Cattle	Narcosis/toxicosis	AS	1876	Unknown	Hance 1876, Miles et al. 1998
3. <i>Echinopogon ovatus</i>	<i>Neotyphodium</i>	Cattle/sheep	Staggers/toxicosis	AU, NZ	1911, 1926	Variable	Henry and Massey 1911, Seddon and Carne 1926, Miles et al. 1998
4. <i>Elymus canadensis</i> (wild ryegrass)	<i>Neotyphodium</i>	Livestock	None	NA	N/A	Absent	Vinton et al. 2001
5. <i>Elymus cylindricus</i>	<i>Neotyphodium?</i>	Livestock	None	AS	N/A	Unknown	Nan and Li 2001
6. <i>Elymus tangutorum</i>	<i>Neotyphodium?</i>	Livestock	None	AS	N/A	Unknown	Nan and Li 2001
7. <i>Festuca alata</i>	<i>Neotyphodium?</i>	Livestock	None	AS	N/A	Unknown	Nan and Li 2001, L. Nan, pers. comm.
8. <i>Festuca argentina</i>	<i>Neotyphodium?</i>	Livestock	Staggers/narcosis	SA	1909	Unknown	Rivas and Zanolli 1909
9. <i>Festuca arizonica</i> (Arizona fescue)	<i>Neotyphodium starrii</i>	Native grasshoppers, leafcutting ants, native vertebrates, cattle	None or positive	NA	N/A	Absent	Schulthess and Faeth 1998, Tibbets and Faeth 1999, Saikkonen et al. 1999
10. <i>Festuca arundinacea</i> (tall fescue)	<i>Neotyphodium coenophialum</i>	Aphid pests	Reduced numbers	EU,AS	N/A	Variable	Clement et al. 2001
11. <i>Festuca hieronymi</i> (la tembladera)	<i>Neotyphodium tembladera</i>	Cattle	Staggers/narcosis	SA	1909	Unknown	Rivas and Zanolli 1909, Cabral et al. 1999
12. <i>Hordeum bogdanii</i>	<i>Neotyphodium?</i>	Livestock	None	AS	N/A	Unknown	Nan and Li 2001, Z. Nan, pers. comm.
13. <i>Hordeum violaceum</i>	<i>Neotyphodium?</i>	Livestock	None	AS	N/A	Unknown	Nan and Li 2001, Z. Nan, pers. comm.
14. <i>Lolium perenne</i> (perennial ryegrass)	<i>Neotyphodium lolii</i>	Livestock Seedling pest	Staggers None	EU,AS	1898	Variable	Guérin 1898, Lewis and Clement 1986, Lewis et al. 1997
15. <i>Lolium temulentum</i> (darnel) ³	<i>Neotyphodium oculans</i> ³	Livestock ³	Poisonous ³	EU,AS	1898, 1904	Variable ³	Vogl 1898, Freeman 1904, Moon et al. 2000
16. <i>Melica descumbens</i> (drunk grass)	<i>Neotyphodium</i>	Cattle	Staggers/narcosis	AF	1873	Unknown	Shaw 1873, Clay 1988
17. <i>Poa huecu</i>	<i>Neotyphodium tembladera</i>	Cattle	Staggers/narcosis	SA	1909	Unknown	Rivas and Zanolli 1909, Cabral et al. 1999
18. <i>Poa poecilia</i>	<i>Neotyphodium</i>	Cattle	None	SA	N/A	Absent	Cabral et al. 1999
19. <i>Poa rigidifolia</i>	<i>Neotyphodium</i>	Cattle	None	SA	N/A	Absent	Cabral et al. 1999

¹ Continental codes: AF = Africa, AU = Australia, AS = Asia, EU = Europe, NA = North America, SA = South America.

² The year of the first known literature report of livestock toxicity to what is now known is a *Neotyphodium*-infected grass.

³ *Neotyphodium* has not been linked to toxic effects; instead a non-fungal endophyte may be responsible (C. Schardl, pers. comm.).

palatable and non-toxic to livestock. However, as interest in basic research of endophyte-grass interactions intensified in the past decade, discoveries of non-toxic *Neotyphodium*-infected native grasses increased concomitantly (Table 1). Interestingly, no new cases of toxic infected grasses have been uncovered in this period of intensified research (Table 1). The absence of new reports of *Neotyphodium* toxicity, now stretching into the past century, despite increased basic and applied research efforts, suggests the many unstudied *Neotyphodium*-infected grasses will also prove to be non-toxic. For example, of the 1028 Poaceae species in 210 genera in China, all but one appear palatable and non-toxic to livestock (Table 1, Nan and Li 2001, Z. Nan, personal communication). Likewise, some native grasses infected with seed borne *Epichloë* also do not confer resistance to native (Brem and Leuchtman 2001) and only occasionally to introduced vertebrates (Bazely et al. 1997).

Defensive mutualism against invertebrate herbivores, nematodes and pathogens

Horizontally transmitted endophytes in woody plants were originally proposed as plant mutualists by providing increased resistance to insect herbivores of the host (Carroll 1988). However, many, if not most, of these endophytes (Carroll 1992, Faeth and Hammon 1997, Saikkonen et al. 1998, Faeth and Bultman 2002) do not generally deter or reduce performance of invertebrate herbivores, except for a few cases involving sedentary insects. Some woody plant endophytes decrease resistance to herbivores (Saikkonen et al. 1998). Because herbivores may promote horizontal transmission and infection via damage or gut passage, one expects selection for tolerance or facilitation of herbivory rather than resistance (Faeth and Hammon 1997). Very few horizontally transmitted endophytes have been studied relative to their vast diversity (Arnold et al. 2000). Nonetheless, I predict that very few of these endophytes should act as defensive mutualists of their hosts.

On the other hand, vertically transmitted endophytes in grasses are predicted by the DMC to strongly increase resistance to invertebrate herbivores (Clay 1990). This prediction is generally supported for *Neotyphodium*-infected, agronomic grasses, tall fescue and perennial ryegrass (Clay 1987a, 1988, 1989, 1991a, Cheplick and Clay 1988, West et al. 1988, Gwinn and Bernard 1990, Kimmons et al. 1990, Rowan et al. 1990, Dahlman et al. 1991, Clay et al. 1993, Latch 1993, Breen 1994), and *Epichloë*-infected *F. rubra*, a European forage grass (Bazely et al. 1997). However, increased resistance to invertebrates even in the agronomic grasses is by no means universal, and there are counterexamples of either no, or even positive, effects of infection (Lewis and Clement 1986, Saikkonen et al. 1998). Moreover, nearly all tests on these grasses have involved introduced,

generalist pests. These pests may not well represent invertebrate herbivores in natural populations, because most phytophagous insects are specialists on one or a few plant species (Futumya and Mitter 1996). Similarly, infections in agronomic grasses increase resistance to root-feeding nematodes and some plant pathogens (West et al. 1988, Kimmons et al. 1990, Gwinn and Gavin 1992, Elmi et al. 2001) but not to others (Burpee and Bouton 1993, Trevathan 1996). Moreover, the proportion of studies showing increased resistance to either invertebrate herbivores or pathogens in these agronomic species may be skewed because neutral or negative results are less likely to be published (Csada et al. 1996).

Very few *Neotyphodium*-infected grasses from natural populations have been tested for resistance to invertebrate herbivores, root-feeders or pathogens, especially native ones (Table 1). *Neotyphodium* infections in native Arizona fescue do not increase, and instead often decrease, resistance to several native invertebrates (Lopez et al. 1995, Saikkonen et al. 1999, Tibbets and Faeth 1999). Furthermore, *Neotyphodium* infections appear to increase, not decrease, susceptibility to seed and seedling pathogens in Arizona fescue (Faeth et al. 2001) and systemic seedling pathogens in *Achnatherum robustum* (C. Hayes and S. Faeth, unpubl.), another southwestern US native grass reputed as highly toxic to vertebrates (Petroski et al. 1992, Kaiser et al. 1996, Jones et al. 2000). In contrast, Clement et al. (2001) found that most infected native populations of *Festuca arundinacea* were resistant to at least generalist, aphid pests. Cheplick and Clay (1988) found resistance of greenhouse cultivars of some native grasses to non-native pests. However, Louis and Clement (1986) showed that infection in wild populations of perennial ryegrass had no effect on resistance to a seed pest.

Support for the DMC is sporadic and also conflicting in *Epichloë*-infected grasses. Brem and Leuchtman (2001) found weak deterrent effects and decreased performance of a noctuid generalist pest on the native grass, *Brachypodium sylvaticum*, infected with *Epichloë*. Native invertebrates preferred plant parts with stromata caused by sexual forms of *Epichloë* compared with parts infected with asexual forms, consistent with the DMC, at least within infected plants. Clay and Brown (1997) found lower *Epichloë* infections in one pasture treated with insecticides, and presumably lower invertebrate herbivore pressure, supporting the DMC, but not in another. Collectively, these studies suggest that systemic, seed borne endophytes have widely varying effects on invertebrate herbivores, ranging from negative to positive.

Why are defensive mutualisms rare?

Overall, despite the wide acceptance of systemic endophytes as defensive mutualists of their host grasses against herbivores, supporting evidence is not over-

whelming for native grasses (Table 1), and is not universal even for introduced, domesticated grasses (Saikkonen et al. 1998). How can the lack of strong and consistent anti-herbivore effects in native grasses be reconciled with the remarkably toxic properties of some infected agronomic grasses and a few natives ones?

There are several reasons why infected native grasses differ from agronomic grasses in herbivore resistance. Herbivore defense stems largely from the types and levels of alkaloids produced by the endophyte. Infected agronomic tall fescue and perennial ryegrass typically produce two to three different alkaloid types, often at high levels (Siegel and Bush 1996, Bush et al. 1997), whereas infected native grasses usually produce only one to two types and usually at much lower levels (Saikkonen et al. 1998, Leuchtman et al. 2000, Faeth and Bultman 2002) and sometimes none at all (Leuchtman et al. 2000, Faeth and Fagan 2002). Alkaloid production depends on host and endophyte genotype and environmental conditions (Royle et al. 1994, Leuchtman et al. 2000, Wilkinson et al. 2000). The high concentrations of multiple types of alkaloids in agronomic grasses may have resulted from limited plant and endophyte genetic diversity in original accessions (Saikkonen 2000) and subsequent inbreeding of cultivars relative to native grasses (Braverman 1986), combined with selection by intense and consistent grazing. Generally, native populations of the perennial ryegrass and tall fescue have much more variable, and often reduced, levels, and fewer types of alkaloids compared with their domesticated counterparts (Leuchtman et al. 2000, Bony et al. 2001).

Endophytic alkaloids may be costly to the host

The DMC assumes that endophytic alkaloids benefit host grasses by deterring herbivores, but endophyte production of alkaloids may also exact significant costs from the host plant. The theory of optimal defense and resource allocation for plants (McKey 1974, Bazzaz and Grace 1997, Hamilton et al. 2001) predicts that allocation to allelochemical defenses decreases growth and reproduction because of production, opportunity and storage costs (Bazzaz and Grace 1997). Because alkaloids are nitrogen-rich compounds and costly to produce (Ohnmeiss and Baldwin 1994), synthesis by the endophyte may compete with other basic plant growth and reproductive functions (Faeth and Bultman 2002, Faeth and Fagan 2002). Additional costs may result from toxicity of alkaloids to the host plant itself, as suggested for mycotoxins in woody plants (Carroll 1991) and known for constitutive alkaloid defenses in plants (Karban and Baldwin 1997).

Costs of endophytic alkaloids should be particularly acute when resources available for growth and reproduction are low, as predicted by the optimal defense

theory. Tests of performance of infected vs. uninfected domesticated grasses (Cheplick et al. 1989, Marks et al. 1991) and native grasses (Ahlholm et al. 2002, Faeth and Fagan 2002, Faeth and Sullivan *subm.*) support the idea that endophyte infection and associated alkaloids are costly, because infected grasses may outperform uninfected ones only under enriched resource environments. This evidence contrasts sharply with earlier views that the costs of systemic endophyte infections are 'nil' (Bacon and Hill 1996). In agronomic settings where nutrients are usually supplemented, the cost of alkaloids may be ameliorated relative to infected grasses in natural habitats where resources are more variable and limiting. These shifting costs and benefits may partially explain not only the generally lower and more variable frequencies of endophyte infections within and among natural grass populations (Latch et al. 1987, Lewis et al. 1997, Schulthess and Faeth 1998, Zabalgoeazcoa et al. 1999, Jones et al. 2000, Spyreas et al. 2001), but also the wide range of toxicity found in the few cases of native grasses with endophytes that strongly deter herbivores (Table 1, Jones et al. 2000).

Herbivory is a weak selective force on grasses

Herbivory may be a relatively transient and weak selective force, especially on native grasses. The rationale for the DMC is that grasses lack their own allelochemical defenses and therefore have enlisted endophytes to provide surrogate defenses (Clay 1988, 1990). The assumption that grasses lack their own allelochemicals is not entirely correct, since grasses produce a wide array of noxious allelochemicals (Chapman 1996), although less frequently than most other plants. An alternative explanation for lack of chemical defenses is that herbivory is not a strong negative selective pressure and grasses therefore rely upon other strategies to tolerate, or even promote (Tibbets and Faeth 1999), herbivory. Hawkes and Sullivan (2001), in a recent meta-analysis of the effects of herbivory in different resource environments, showed that monocots, including grasses, benefit from herbivory via overcompensation in resource-rich (high light, water and nutrients) environments, whereas other plants (dicot herbs and woody plants) do not. Alternatively, herbivory has a net negative effect on monocots in low resource, and, presumably, highly competitive environments. Whereas there is still considerable debate about herbivory as a selective force on plants in general (Crawley 1983, Marquis 1992, Strauss and Agrawal 1999, Hawkes and Sullivan 2001), grasses have generally adapted to grazing, with positioning of basal meristems near or below ground (Stebbins 1981, Hawkes and Sullivan 2001). Thus, the cost of chemical defenses for many native grasses, either from the host grasses themselves or from their endophytic symbionts, may be prohibitively high relative to benefits, except

under intensive herbivory and in certain resource environments. Certainly, intensive and consistent grazing can alter competitive hierarchies in plant communities (Harper 1977), but herbivory by both invertebrate and vertebrates in heterogeneous natural grasslands is generally less pronounced and more sporadic than by livestock or introduced invertebrate pests in agronomic monocultures. Thus, grasses harboring the most toxic endophytes may have been artificially, and often unintentionally (Ball et al. 1993), selected under cultivation.

Other roles of alkaloids

The DMC has generally focused on endophytes as protective mutualists of host plants at the adult stage via alkaloids. However, endophyte-related alkaloids in adult plants of native grass populations and species, except for relatively rare cases (Table 1), are often absent, or at levels (Siegel et al. 1990, Leuchtman et al. 2000, Faeth and Fagan 2002) that are ineffective against most herbivores, especially specialists. This wide variation in alkaloid levels suggests that selective pressures in addition to, or exclusive of, herbivory on adult host plants have maintained this variability. Reduction of seed and seedling predation is one such selective force. Faeth and Bultman (2002) predicted that an endophyte-associated defense against natural enemies of the host grass is more likely to occur at the seed (Cheplick and Clay 1988, Wolock-Madej and Clay 1991, Knoch et al. 1993) and seedling stage, where predation, pathogen attack and herbivory have more direct effects on plant fitness. Generally, the theory of optimal plant defense predicts that chemical defense should be allocated to plant parts that have the highest value in terms of fitness (Bazzaz and Grace 1997). Documentation of higher levels of alkaloids in some seeds and seedlings than adult plants (Siegel et al. 1990, Bush et al. 1993, Welty et al. 1994, Leuchtman et al. 2000) is consistent with this prediction.

Another intriguing possibility is that alkaloids function not only as deterrents to natural enemies of the host as envisioned by the DMC, but also, and perhaps in some cases, primarily, as allelopathic agents. Alkaloids from infected domesticated grasses may be leached into the soil and inhibit growth of competing grasses (Peters 1968, Peters and Zam 1981, Matthews and Clay 2001). Siemens et al. (2002) proposed that constitutive alkaloids of *Brassica rapa* have dual functions as herbivore defenses and as allelopathic agents that increase performance during plant competition. Their 'defense-stress benefit' hypothesis implies that the costs of alkaloid production may not necessarily increase in resource-poor or competitive environments, as predicted by the theory of optimal plant defense, if alkaloids also function as allelopathic agents. *Neotyphodium* endophytes, at least in tall fescue and perennial ryegrass, are well known for enhancing competitive abilities of their host grasses (De Battista et

al. 1990, Hill et al. 1990, Marks et al. 1991, Elbersen et al. 1994, Richardson et al. 1992, 1993, Cheplick and Clay 1988, Cheplick et al. 1989, Clay 1990, Bacon 1993, Clay et al. 1993, Latch 1993). Increased competitive ability is usually linked mechanistically to increased drought resistance, at least in tall fescue (Richardson et al. 1992, 1993, Latch 1993, Elbersen et al. 1994, Elbersen and West 1996, Bacon and White 1994, Elmi and West 1995, Elmi et al. 2001) and some native grasses (Marlatt et al. 1997) or enhanced nutrient uptake mediated by the endophyte (Malinowski and Belesky 1999, Malinowski et al. 1999). The 'defense-stress benefit' hypothesis (Siemens et al. 2002) suggests that alkaloid may act in concert with these mechanisms. If so, then the cost of alkaloid production in low resource or competitive environments may not exceed the costs. Interestingly, alkaloid production in infected tall fescue increases under moderate drought stress (Arachavaleta et al. 1992), and under low resource conditions in Arizona fescue (Faeth et al. 2002).

Variable infection and alkaloid levels – mutualistic predictions

Recent evidence suggests that natural grass populations are often a mixture of uninfected hosts and infected plants with no, low and high concentrations of alkaloids (Leuchtman et al. 2000, Faeth and Fagan 2002; Faeth et al. 2002). Variation in alkaloid levels originates from various endophyte strains or genotypes (Leuchtman et al. 2000, Wilkinson et al. 2000) living within different host plant genotypes (Siegel et al. 1990) and influenced by local environments (Royle et al. 1994). Even if native grass populations harbor only one or a few haplotypes (= genotypes for asexual endophytes) of *Neotyphodium* (Sullivan and Faeth 2001), these haplotypes are reshuffled into different plant genotypes at each host reproductive event, which in turn alters alkaloid levels (Faeth et al. 2002). Given this variation, under what conditions should uninfected hosts and infected plants with different alkaloids levels be favored by natural selection?

I propose one scenario (Table 2) that is based upon the assumptions that 1) alkaloids are costly (i.e. optimal

Table 2. Predictions for when uninfected and infected grasses should be favored under different competitive and herbivory environments, based upon alkaloid levels (none, low or high). This schematic is based upon assumptions described in the text.

	Low herbivory	High herbivory
Low competition	E- or E+ (no alkaloids)	E+ (low alkaloids)
High competition	E+ (low alkaloids)	E+ (high alkaloids)

defense theory, Bazzaz and Grace 1997), 2) alkaloids also function as allelopathic agents (Siemens et al. 2002) and, 3) the effects of herbivory are most negative for grasses during competition for limiting resources (Hawkes and Sullivan 2001). Infected hosts with high levels of alkaloids should be favored in environments with intense and consistent herbivory and competition (Table 2). In these environments, the effect of herbivory may be particularly severe, if competition results in limiting resources (Hawkes and Sullivan 2001). Alternatively, uninfected plants, or infected plants that produce no alkaloids, should be favored when herbivory and competition are low or absent, because the benefits related to alkaloid-mediated reduction of competition or herbivory are not realized, while the costs of alkaloids remain (Table 2). Predictions for the intermediate environments (Table 2) are less clear, but one might expect selection for infected hosts with lower and more variable levels of alkaloids. These predictions obviously depend on the simplifying assumptions, which may or may not hold in nature. For example, levels of resource availability does not necessarily equate to intensity of competition, as plants may grow overdispersed in low resource environments with no competition (Siemens et al. 2002). In this case, infected plants with no alkaloids may be favored if infection confers resistance to drought (Richardson et al. 1992, 1993, Latch 1993, Elbersen et al. 1994, Elbersen and West 1996, Bacon and White 1994, Elmi and West 1995, Elmi et al. 2001) or if infection increases acquisition of limiting nutrients (Malinowski and Belesky 1999, Malinowski et al. 1999). Nevertheless, this scenario, based upon optimal plant defense theory, may provide a starting point for explaining the observed variation in infection levels and the wide range of alkaloid levels found in infected grasses within and among native populations (Jones et al. 2000, Leuchtman et al. 2000, Faeth and Fagan 2002).

Hamilton et al. (2001), in refining the theory of optimal plant defense (McKey 1974), noted that the benefit of a chemical defense is notoriously difficult to measure because the chemical defense usually cannot be eliminated from the plant, so that the fitness change due to loss of herbivory (Hamilton et al. 2001) or allelopathy (Siemens et al. 2002). Systemic endophytes and their host grasses, however, may provide ideal model systems to test optimal plant defense theory. Infected maternal grasses that vary in alkaloid levels can be easily divided into genetically identical ramets, and the endophyte, and its associated alkaloids, removed via fungicides. Ramets of plants with and without their endophytes then can be grown in common garden experiments, where resources, competition and herbivory are manipulated (Faeth and Sullivan, *subm.*).

Variable infection levels – non-mutualistic explanations

The previous section assumes that asexual, seed-borne endophytes, such as *Neotyphodium*, have net mutualistic interactions with their hosts in most environments, as predicted by evolutionary theory (Law 1985, Ewald 1994, Wilkinson and Schardl 1997, Schardl and Clay 1997). Thus, under most circumstances (Table 2), infected plants should be favored and high infection frequencies should be maintained in natural populations. Indeed, the observed high frequencies of asexual endophytes in domesticated and natural populations has been used as *de facto* evidence that endophytes interact mutualistically with their host grasses (Clay 1998). *Neotyphodium* symbionts, and some species of *Epichloë*, are thought to be strictly asexual and vertically transmitted (Clay 1988, Schardl and Phillips 1997). Vertical transmission via seeds, however, may be less than 100% because hyphae fail to grow into some seed heads or tillers (imperfect transmission, Ravel et al. 1997) or hyphae can lose viability in seeds or plants (Siegel et al. 1984). Hence, if *Neotyphodium* is selectively neutral, then the frequency of infected plants should decline with time because of random loss of the endophyte (Ravel et al. 1997); if parasitic and strictly seed borne, then infected host plants should be at a selective disadvantage, and infected plants should decline over time (Leuchtman and Clay 1997, Schardl and Clay 1997, Clay 1998).

Yet, evidence for detectable benefits of harboring systemic endophytes is wanting from recent empirical studies of asexual endophytes in some native grasses. Native Arizona fescue (*Festuca arizonica*) for example, grows and reproduces better when its *Neotyphodium* is removed, regardless of plant genotype or environment (Faeth and Sullivan, *subm.*). Furthermore, infection decreases rather than increases resistance to herbivores (Tibbets and Faeth 1999, Saikkonen et al. 1999) and plant pathogens (Faeth et al. 2001), and infection decreases competitive abilities (Faeth, Helander and Saikkonen, *subm.*). Finally, *Neotyphodium* infection has no effect on germination success, another benefit of infection reported for agronomic perennial ryegrass (Clay 1987b), in this native grass under varying osmotic potentials (Neil et al. *subm.*). Even *Neotyphodium* in agronomic grasses does not always benefit the host grass. West et al. (1995), for example, found that *Neotyphodium* can act parasitically in tall fescue depending on cultivar and degree of water stress. Likewise, infection by *Neotyphodium* did not increase performance of agronomic perennial ryegrass in different environments; instead plant genotype determined performance (Cheplick et al. 2000). Asexual endophytes may also inhibit mycorrhizal colonization and effectiveness in both agronomic grasses (Chu-Chou et al. 1992, Guo et al. 1992) and native Arizona fescue (K. Neil and

S. Faeth, unpubl), and thus may reduce or offset gains in nutrient uptake by altered root structure or chemistry (Malinowski and Belesky 1999). Collectively, these studies suggest that asexual endophytes may interact parasitically, at least in some circumstances, with their host grasses, much like sexual endophytes (Schardl et al. 1997, McCormick et al. 2001) and some mycorrhizae (Johnson et al. 1997) and microbial symbionts of animals (Douglas and Smith 1983). Yet *Neotyphodium* infections are still found at high, but variable, frequencies in natural populations (Schulthess and Faeth 1998, Saikkonen et al. 2001). There are, however, several ways that asexual endophytes may be maintained at high frequencies, without acting as mutualists.

Horizontal transmission of asexual endophytes

The *Neotyphodium* symbiont, and some species of closely related *Epichloë* species, are generally thought to be asexual and strictly vertically transmitted (Clay 1988, Schardl and Phillips 1997). A neutral or parasitic symbiont may be maintained at high frequencies in populations if the endophyte is occasionally transmitted horizontally, either through conidia and spore formation or hyphal transmission to other plants. If horizontally transmitted, then high frequencies of *Neotyphodium* may be explained without mutualistic interactions, such as those in woody plants (Faeth and Hammon 1997). White et al. (1996, 2001) suggested this possibility based upon the observation of epiphyllous nets and conidia formation by *Neotyphodium* in the leaf surface tissues of some infected grasses (Moy et al. 2000). Secondly, hyphae, at least from seeds grown in culture, occasionally produce external hyphae and hyphae from pure cultures can be inoculated into some uninfected seedlings under sterile laboratory conditions (Christensen 1995). To maintain endophyte frequencies, rates of horizontal transmission would have to at least equal rates of imperfect transmission and exceed them if infections are parasitic. To my knowledge, there has been no confirmation of horizontal transmission of *Neotyphodium* in any field population of native grass in the field. Nevertheless, if horizontal transmission occurs, then concepts of grass-endophyte interactions based on solely on mutualism will require revision.

Asexual endophytes manipulate host sex allocation and reproduction

Maternally-inherited symbionts can be maintained in host populations without conferring benefits to the their hosts, contrary to the notion that asexual microbial symbionts must be mutualists (Werren and O'Neill 1997). For example, *Wolbachia*, a maternally-transmitted cytoplasmic bacterium, persists in many invertebrate

populations at high frequencies without providing benefits to the host by acting as a reproductive parasite (Werren 1997). *Wolbachia* reduces male function and male:female sex ratio, increases feminization, and decreases fitness of uninfected hosts (through cytoplasmic incompatibility) in a wide range of invertebrates, thereby increasing the frequency of infected offspring (Werren 1997, O'Neill et al. 1997). *Neotyphodium*, and other asexual endophytes, could similarly persist at high frequencies by manipulating maternal function or sex ratio. Increasing female, or alternatively reducing male, function or flowers, would increase transmission of maternally-transmitted endophytes. Altering sex ratio may or may not benefit the host, depending on pollen limitation (Campbell 2000), but should increase the frequency of infected plants over time.

Currently, there is no direct evidence for the reproductive parasite hypothesis for asexual endophytes but several observations hint that it is possible. The sexual stages of systemic endophytes, at least, are well known for radically altering host reproduction by reducing or destroying inflorescences (Clay 1990, 1991b) and sexual stages of the fungus *Balansia cyperi* induces pseudovivipary in their sedge hosts (Clay 1986). In the native grass *Brachypodium sylvaticum* infected with vertically transmitted *Epichloë*, self-fertilization is high relative to other species, suggesting that infection promotes selfing and thus maintenance of favorable plant genotypes for fungal infection (Meijer and Leuchtman 2001). In Arizona fescue, infection by *Neotyphodium* increases the number of filled seeds per unit mass of reproductive tissue (panicles, culms, flowers) relative to uninfected plants (S. H. Faeth, unpubl), suggesting increased allocation to female at the expense of male function.

Whether asexual endophytes have retained the capacity of their sexual ancestors to alter reproduction function and therefore act as reproductive parasites of their hosts remains to be tested. If so, grasses infected with asexual, seed-borne endophytes should display a wide range of changes in reproduction that promote transmission, including shifts from male to female flowers or floral structures, vivipary or pseudovivipary (Elmqvist and Cox 1996) or other forms of apomixis (Asker and Jerling 1992). This prediction necessitates a new view of asexual endophytes as symbionts that can manipulate and exploit their hosts despite the loss of sexual reproduction. Instead, like *Wolbachia*, these maternally-inherited symbionts may influence host growth and reproduction that increases their transmission and persistence in populations, but also conflicts with fitness of their host.

Spatial structuring of grass populations

Because of their immobility and relatively low dispersal rates, grass populations may be highly structured

spatially in terms of plant and endophyte genotype and infection status, and thus probably exist as metapopulations (Saikkonen et al. 1998). Infected and uninfected host plants may persist indefinitely in patches or zones within populations, even if endophytes have net negative or positive effects on their hosts (Hochberg et al. 2000). Limited dispersal, such as seed shadows near mother grass plants, may promote local pockets of either infected or uninfected plants where natural selection based simply on relative costs and benefits may be denied. The direction and strength of endophyte-host interactions probably also varies at larger spatial scales (e.g. geographically among populations) and with phylogenies of endophyte and host, as do other species interactions (Thompson 1994).

Conclusion

Increasing evidence suggests that mutualistic concepts derived from systemic endophytes in agronomic grasses may not hold for those inhabiting native grasses, especially the concept of defensive mutualism. Because of limited genetic diversity and artificial selection, chronic grazing and altered growing environments of agronomic grasses, views of endophyte interactions with hosts may have been skewed towards strong mutualistic interactions. Caution should be exercised from extrapolating general ecological concepts from endophyte interactions with domesticated grasses. Although studies and experiments involving native grasses are currently limited, a different picture of endophyte-host interactions is emerging. The magnitude and direction of the interaction greatly depends on plant and endophyte genotype, and biotic and abiotic environments, which vary greatly in time and space. These factors, in turn, may result in the observed coexistence of uninfected and infected plants with widely varying levels of alkaloids within and among grass populations. Clearly, long-term experiments and observations of native grasses which considered not only whether hosts are infected or not, but also plant and endophyte genotype, alkaloid production, and environmental factors, are required to determine the effects of systemic endophytes on lifetime host fitness. The persistence of even strictly asexual endophytes in populations need not involve consistent mutualistic interactions because there are other ways that asexual symbiotic microbes can persist in host populations.

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