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Fungal grass endophytes and arthropod communities: lessons from plant defence theory and multitrophic interactions

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ABSTRACT

Alkaloids produced by systemic fungal endophytes of grasses are thought to act as defensive agents against herbivores. Endophytic alkaloids may reduce arthropod herbivore abundances and diversity in agronomic grasses. Yet, accumulating evidence, particularly from native grasses, shows that herbivore preference, abundances and species richness are sometimes greater on endophyte-infected plants, even those with high alkaloids, contrary to the notion of defensive mutualism. We argue that these conflicting results are entirely consistent with well-developed concepts of plant defence theory and tri-trophic interactions. Plant secondary chemicals and endophytic alkaloids often fail to protect plants because: (1) specialist herbivores evolve to detoxify and use defensive chemicals for growth and survival; and (2) natural enemies of herbivores may be more negatively affected by alkaloids than are herbivores. Endophytes and their alkaloids may have profound, but often highly variable, effects on communities, which are also consistent with existing theories of plant defence and community genetics.

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Introduction

Fungal endophytes, fungi that live internally and asymptotically within plant tissues, are increasingly recognized as important contributors to the phenotype of the host plant (Redman *et al.* 2002; Cheplick & Faeth 2009). Some fungal endophytes, such as those in the genus *Neotyphodium*, are asexual and strictly vertically transmitted in the seeds of the host. Therefore, these endophytes are essentially inherited components of the host plant and thus increase not only phenotypic but also genotypic diversity of the host (Cheplick &

Faeth 2009; Jani *et al.* 2010). Because of vertical transmission mode of the fungi, fitness of the host and endophyte should be tightly linked, and thus these seed borne endophytes have been traditionally deemed as plant mutualists (e.g., Clay & Schardl 2002). Endophyte-mediated herbivore resistance of the host is by far the most well-known phenotypic change in the host plant associated with seed-borne endophytes (Cheplick & Faeth 2009). Increased herbivore resistance mainly results from the production of alkaloids by the endophytes. In addition, systemic endophytes have been reported to increase growth, reproduction, tolerance of drought,

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flooding, pathogens and enhance competitive abilities of the host grass (Clay 1990; Elbersen & West 1996; Clay & Holah 1999; Morse et al. 2007).

The observation in the 1970's that livestock feeding on cultivated grasses infected by *Neotyphodium* developed severe toxicosis was the spark for the explosion of research on endophyte-infected grasses and the alkaloids they produce (Bacon et al. 1977; Cheplick & Faeth 2009). Subsequent empirical laboratory and greenhouse preference and performance tests showed that *Neotyphodium* or *Epichloë* (the sexual ancestor of *Neotyphodium* that may cause choke disease in host grasses) endophytes often deterred or impaired growth and survival of invertebrate herbivores (e.g., Breen 1994; Saikkonen et al. 2004, 2006, 2010). Of these tests, the vast majority used agricultural invertebrate pest species, such as fall armyworm (*Spodoptera frugiperda*) and the bird cherry oat aphid (*Rhopalosiphum padi*), and various agronomic cultivars of two grass species, tall fescue (*Schedonorus arundinaceum* = *Festuca arundinacea* = *Lolium arundinaceum*) and perennial ryegrass (*Lolium perenne*). Tall fescue and perennial ryegrass are native to Eurasia, but selectively-bred cultivars have been introduced as pasture and turf grasses into North and South America, Australia and New Zealand. In addition, endophyte frequencies often rapidly increased to near 100% infectivity in grazed agricultural pastures (e.g., Clay 1998) and experimental plots in old fields (e.g., Clay et al. 2005), suggesting that resistance to both invertebrates and vertebrates increased fitness of infected agronomic grasses relative to uninfected ones. These subsequent empirical and observational studies cemented the earlier notion of grass endophytes as defensive mutualists (e.g., Clay 1988, 1990; Clay & Scharld 2002).

However, even as studies affirming the defensive role of endophytes in cultivated turf and pasture grasses accrued (see Cheplick & Faeth 2009, Table 3.1), concurrent studies of native grasses and herbivores showed much more variable outcomes (e.g., Faeth & Saikkonen 2007). Studies of how infected native grasses influence vertebrate herbivory are uncommon. The few existing studies are largely correlative and show increased frequency of infection with grazing intensity (Bazely et al. 1997, Hirta island) or preference by livestock (Jones et al. 2000) and native mammals (Koh & Hik 2007) for uninfected grasses, as predicted by the defensive mutualism hypothesis (DMH). But in other studies, the frequency of endophyte infection does not increase with greater livestock (Bazely et al. 1997, Rum and Benbecula islands) and native vertebrate (Rambo & Faeth 1999) grazing as predicted by DMH. In general, Faeth (2002) argued that relatively few grasses infected by seed borne endophytes are toxic to vertebrates. The few that are toxic have been well-studied relative to non-toxic endophytes because of their negative economic effects on livestock production.

Studies of infected native grasses and invertebrate herbivores are far more numerous than those with vertebrates. The outcomes of these studies span the range of negative to neutral to positive effects of endophyte infection. Some studies involving native grasses show reduced herbivory or increased insect deterrence on infected grasses relative to uninfected grasses (Christensen & Latch 1991; Brem & Leuchtman 2001; Gonthier et al. 2008; Clement et al. 1997, 2005; Tintjer & Rudgers 2006; Afkhami & Rudgers 2009),

whilst other studies showed no difference (Christensen & Latch 1991; Lopez et al. 1995; Clement et al. 1997; Tintjer & Rudgers 2006; Afkhami & Rudgers 2009), depending on herbivore species and endophyte strain. Note that most of these studies used generalist insect pest species, such as the armyworm and bird oat cherry aphid, in bioassay tests. At the other end of the spectrum, native invertebrate herbivores sometimes prefer and perform better (e.g., Saikkonen et al. 1999; Tibbets & Faeth 1999; Afkhami & Rudgers 2009) or reached higher abundances (Faeth & Shochat 2010; Jani et al. 2010) on infected native grasses, in direct contradiction to the predictions of DMH. Thus, support for the DMH hypothesis appears much more equivocal in native grasses than in agronomic grasses, especially when native insect herbivores are considered.

In addition, the frequency of infection among populations of most native grasses is highly variable, ranging from 0% to 100% (see Cheplick & Faeth 2009; Table 5.5), unlike agronomic grasses where infection frequencies are usually near 100%. Likewise, unlike agronomic infected grasses where alkaloid levels tend to be uniformly high, concentrations of alkaloids are also highly variable both within and among infected native grass species. Many infected grasses produce either no alkaloids at all, or alkaloids at only very low levels. Very few produce the high levels found in cultivated grass cultivars (Faeth 2002; Faeth et al. 2002; Piano et al. 2005). Finally, when more complex, natural communities are considered, the effects of endophytes and their alkaloids become much less predictable. For example, in a recent meta-analysis, Saikkonen et al. (2010) reported that a defensive role of endophyte is supported in agronomic grasses, but not well in other grasses, and largely disappears when other trophic levels are considered. This and other sources of variability (e.g., Faeth & Saikkonen 2007) in the effects of endophytes suggest that selective pressure from invertebrate and vertebrate herbivores in native grass populations does not always lead to increases in infection frequency nor in consistently high alkaloids levels, as originally proposed by the defensive mutualism hypothesis (Clay 1988). Instead, accumulating studies with native grasses show a wide range of outcomes of the effects of fungal endophytes.

In this paper, we argue that these variable and seemingly contradictory results are easily explained and reconciled within the conceptual and empirical framework of plant defence theory (hereafter PDT). PDT provides a long-standing and well-developed conceptual framework accompanied by a rich literature for explaining the ecology and evolution of plant–herbivore interactions. For unknown reasons, PDT has been, and still is (e.g., Clay 2009), largely ignored in explaining host endophyte–grass–herbivore interactions, despite early and repeated calls (e.g., Saikkonen et al. 1998; Faeth 2002; Faeth & Bultman 2002; Cheplick & Faeth 2009) for applying existing ecological theory to endophyte–host grass interactions.

Plant defence theory and endophyte–grass–herbivore interactions

Plants produce a bewildering array of allelo- or secondary chemicals – compounds that are not involved in primary

metabolism and perform secondary functions such as storage and defence (e.g., [McKey 1974](#)). For example, plants produce many different types of alkaloids without the assistance of fungal endophytes, and many other classes of compounds that potentially increase resistance to herbivores such as glucosinolates, phenolic glycosides, and terpenes. Plant defence theory (also called optimal plant defence theory) states, in brief, that plants have limited resources to allocate to growth, reproduction and defence (e.g., [Herms & Mattson 1992](#); [Bazzaz & Grace 1997](#)). PDT predicts that allocation of resource to defence concomitantly reduces resources available for growth and reproduction. These allocation costs are substantial although often difficult to detect (e.g., [Orians et al. 2010](#)). This is particularly true for alkaloids, which are nitrogen-rich compounds, one of the key limiting factors for plant growth and reproduction. Alkaloids require a sizeable fraction of metabolic plant resources to produce (e.g., [Ohnmeiss & Baldwin 1994](#)). In addition to metabolic and nutrient costs (allocation costs), there may also be ecological costs to resistance, such as deterrence of pollinators or natural enemies of herbivores (e.g., [Strauss et al. 2002](#)). Given these phylogenetic, physiological and ecological constraints, plants should evolve and maintain chemical defences only when herbivory exerts a strong selective pressure and when these chemical defences effectively reduce herbivory.

Endophytic alkaloids in grasses are functionally the same as plant-derived alkaloids or the other myriad secondary chemicals found in plants. Although many fungal alkaloids (e.g., the ergot alkaloids) are distinct from those produced by plants, they should, nonetheless, have similar biological activity against herbivores. Therefore, fungal-based alkaloids in plants fall generally under the purview of PDT. We should expect that like plant-based alkaloids, endophytic alkaloids are also costly to produce in terms of tradeoffs with growth and reproduction ([Faeth 2002](#)). Maintenance of endophytic alkaloids in grasses may even decrease host fitness if herbivores are absent or if they are ineffective at deterring herbivory because uninfected plants or infected plants without alkaloids should be better competitors ([Faeth 2002](#)). The cost of endophyte infection and associated alkaloids are also difficult to detect because of the long-lived nature of the perennial grass hosts. Nonetheless, recent studies suggest a cost of infection in terms of re-growth ([Sullivan et al. 2007](#); [Saari et al. 2010](#)) and competitive abilities ([Faeth & Sullivan 2003](#)), and long term studies show that the cost and benefits change over the lifespan of the host ([Faeth 2009](#); [Faeth & Shochat 2010](#)).

Because of the cost of maintaining constitutive chemical defences (those always present in plant tissues), plants have also evolved inducible defences, where secondary chemicals are produced 'on-demand' when plants (or their neighbours) are under attack by herbivores (e.g., [Karban & Baldwin 1997](#)). Endophytic alkaloids can also be induced when plants are under attack, at least in agronomic grasses (e.g., [Bultman et al. 2004](#)). Like constitutive defences, the genetic bases for these 'on-demand' defences, particularly for alkaloids, are well-established for both plants (e.g., [Anssour & Baldwin 2010](#)) and for endophytic fungi ([Sullivan et al. 2007](#); [Zhang et al. 2009](#)). So, again PDT includes induced responses and applies directly to endophyte-mediated resistance to herbivores.

Why endophytes do not always act as defensive mutualists

Accumulating evidence points to widely variable outcomes of endophyte infection on resistance to herbivores, especially in natural grassland communities. We now explore the reasons for this observed variability within the context of PDT and other related ecological models.

Plants evolve chemical defences but herbivores evolve countermeasures

The classic example of coevolution is the arms race between plants and herbivores: plants evolve chemical defences and herbivores counter with the evolution of detoxifying mechanisms ([Ehrlich & Raven 1964](#)). Moreover, specialist insect species often evolve not only to detoxify plant secondary chemicals but to also require them as cues for host plant location, oviposition and phagostimulation, and may sequester them as their own defence against natural enemies. Indeed, [Ehrlich & Raven's \(1964\)](#) classic paper relied upon observations of butterfly species that specialize on groups of plants based upon their relatedness and hence secondary chemistry. Because insect generation time and mutation rates are much faster than their host plants, insects are usually viewed as being ahead in the 'arms race' ([Ehrlich & Raven 1964](#)).

It is not happenstance that much of the support for the defensive mutualism hypothesis of grass endophyte associations is based upon studies of generalized insect pests feeding on infected tall fescue and perennial ryegrass. For example, the fall armyworm has a very wide host range, feeding on more than 80 different plant species, and although it prefers grasses, will feed on peanut, soybean, tobacco, citrus, apple, and sugar beet ([Peairs & Davidson 1956](#)). The other common bioassay organism in endophyte studies, the bird cherry-oat aphid, is less generalized than the armyworm and is largely restricted to feeding on grasses and *Prunus* trees (its alternative host), but still feeds on all major cereal crops, pasture and turf grasses ([Dixon 1971](#); [Lehtonen et al. 2006](#)). In comparison, about 75 % of native insect herbivores feed on only one or a few host plants ([Bernays & Chapman 1994](#)). Deterrence of generalized, but not specialized, arthropod herbivores, is expected from PDT. Most insect herbivores in these natural communities are likely to be specialists (e.g., [Strong et al. 1984](#)). Thus, based upon PDT, we expect that many of these herbivores have evolved to detoxify, require, and even sequester endophytic alkaloids ([Faeth 2002](#)). Thus it follows from PDT that the outcomes of endophyte–grass–herbivore interactions in native grass communities are much more variable or even reversed relative to studies of cultivated grasses and generalist insect pests.

Plants evolve tolerance instead of chemical defences

Plants may avoid the cost of chemical defences altogether by evolving tolerance to herbivory. Tolerance is an evolved plant strategy in which plants compensate, or even over-compensate, for herbivory by re-growth, without any

measurable decline in fitness (e.g., Strauss & Agrawal 1999). Tolerance is an alternative to constitutive or induced chemical defences, but like chemical defences, is also assumed to have costs and to be constrained by energy and nutrients (e.g., Fornoni *et al.* 2004). There is some limited evidence that fungal endophytes also modulate host plant tolerance to herbivory but in varying directions (Cheplick 1998; Bultman *et al.* 2004; Saari *et al.* 2010). In a recent study, Faeth & Shochat (2010) showed that despite higher herbivore loads, infected Arizona fescue (*Festuca arizonica*), a native grass, produced more seeds than uninfected grasses, at least in early ontogeny. This suggests that fungal endophyte infection increased host tolerance to herbivory in this grass. Hence, models of plant tolerance to herbivory also well encompass endophyte–host–herbivore interactions and may explain why endophyte infections do not always deter herbivores. Indeed, Faeth & Shochat (2010) argued that Arizona fescue fungal endophytes may facilitate, rather than deter, herbivores so that host grasses produce more infected seeds in early ontogeny because infections may be lost from adult plants.

Natural enemies often nullify or reverse chemical defences

Feeding on plants with a battery of secondary chemicals is not the only challenge for plant herbivores. They must also contend with low nutrient content of most plant tissues and natural enemies, predators and parasites. Many invertebrate herbivores tolerate or compensate for suboptimal nutritional diets (e.g., Simpson & Simpson 1990; Slansky 1993) and others store, sequester and use plant noxious compounds for their own defence against natural enemies (e.g., Bowers 1992; Soetens *et al.* 1998; Dobler 2001). For example, sawflies and leaf beetles either accumulate or use secondary metabolites from the host plant as precursors of their own chemical defence against predators (Pasteels *et al.* 1983; Soetens *et al.* 1998; Dobler 2001). Bernays & Graham (1988) argued that defence from generalist predators was more important than toxicity of secondary chemicals in evolution of specialization of insects on host plants. Thus, a more expansive view of plant defence theory includes not just the interaction between plants and herbivores but also interactions with natural enemies of herbivores, commonly termed tri- or multi-trophic interactions (e.g., Price *et al.* 1980).

Likewise, invertebrates feeding on endophyte-infected grasses may also sequester and use endophytic alkaloids in their defence against their predators and parasites (Faeth 2002; Faeth & Bultman 2002; Cheplick & Faeth 2009). Endophytic alkaloids may negatively affect growth, development and survival of parasites (e.g., Barker & Addison 1996; Goldson *et al.* 2000; Omacini *et al.* 2001; Bultman *et al.* 2003;) and predators (e.g., de Sassi *et al.* 2006; Härrä *et al.* 2008) of herbivorous insects. Although the effects of endophytes on higher trophic levels are still poorly understood (Saikkonen *et al.* 2010), recent evidence suggests that endophytic alkaloids in grasses may affect natural enemies of herbivores more than the herbivores themselves, thus creating ‘enemy-free space’ (Jeffries & Lawton 1985) on infected plants. In studies of native grasses, Faeth & Shochat (2010) and Jani *et al.* (2010) found that parasite and predator richness declined on infected Arizona fescue and sleepygrass (*Achnatherum robustum*), respectively, and

herbivore abundances were greater on infected plants. Evidence that endophytic alkaloids cascade through trophic levels to alter attack by natural enemies, and in some cases, result in higher, rather than lower herbivore loads, is in keeping with the broader view of PDT.

Plant–herbivore–natural enemy interactions vary in time and space

Just as plants and herbivores coevolve through time with adaptive plant defences and herbivore countermeasures, tri-trophic interactions also vary in ecological time and space. For example, plants show dramatic seasonal and ontogenic changes in host plant quality and types and levels of secondary chemistry (e.g., Karban 1992; Boege & Marquis 2005). Also, host plant quality and secondary chemistry vary with environmental factors such as soil nutrients, amount of light, and precipitation. This temporal and spatial variation in nutrition and secondary chemistry alters the probability of being eaten (e.g., Karban & Baldwin 1997). Herbivore species and abundances also change dramatically by season and year and by local and regional environments. Therefore, short bioassay experiments in the laboratory or greenhouse showing a negative effect of a plant secondary compound on preference and performance of an herbivore often do not translate into negative changes in natural herbivore populations (Karbon 1992). Likewise, plant tolerance to herbivory also varies with plant ontogeny, available resources and plant genetics (Boege & Marquis 2005).

Variation in time and space clearly applies to endophyte–host grass interactions because the majority of seed borne endophytes are found in long-lived perennial grasses that are widely distributed across various habitats. Yet, tests of the defensive mutualism hypothesis have traditionally relied upon short-term, laboratory or greenhouse bioassays comparing preference and performance on herbivores on infected and uninfected tall fescue and perennial ryegrass in the absence of any natural enemies (e.g., Saikkonen *et al.* 2006, 2010). Long-term field studies are rare, especially those involving native grasses. Using plots in old field communities in Indiana that were heavily seeded with infected and uninfected agronomic tall fescue years earlier, Rudgers & Clay (2008) showed that herbivore abundances were reduced in endophyte-infected plots (Table 1). However, Faeth & Shochat (2010) and Jani *et al.* (2010) showed the opposite – herbivore abundances were higher on infected native Arizona fescue and sleepygrass plants in natural grassland communities (Table 1). However, for Arizona fescue, this relationship changed with plant ontogeny – the increase in herbivore abundances disappeared as plants matured (Faeth 2009; Faeth & Shochat 2010). A recent meta-analysis by Saikkonen *et al.* (2010) suggests that negative effects of endophytes on herbivores disappear in long-term experiments and in more natural field conditions where natural enemies are included. Again, these varying outcomes are expected based on PDT and tri-trophic level interactions.

Varying community-wide effects of endophytes

Recent studies suggest that endophytes, despite their relatively small biomass, may have profound effects at the

Table 1 – Summary of community-level studies of the effects of *Neotyphodium* endophytes on arthropod abundance and diversity.

System	Herbivore abundance	Herbivore richness	Parasite richness	Predator richness	References
Tall fescue K-31 cultivar (introduced)	Decreased	Decreased		Decreased	Finkes <i>et al.</i> (2006); Rudgers & Clay (2008)
Perennial ryegrass cultivar (introduced)	Decreased	Increased coexistence			Härri (2007), Meister <i>et al.</i> (2006)
Ital. ryegrass cultivar (introduced)	Decreased	Unchanged	Slightly decreased		Omacini <i>et al.</i> (2001)
AZ fescue (native)	Increased	Unchanged	Decreased	Unchanged	Faeth & Shochat (2010)
Sleepygrass (native)	Increased	Increased	Unchanged	Decreased	Jani <i>et al.</i> (2010)

community level, altering not only abundances and diversity of herbivores (e.g., Rudgers & Clay 2008; Jani *et al.* 2010) but also of other plants (e.g., Clay & Holah 1999), natural enemies (e.g., Omacini *et al.* 2001), and detritivores (e.g., Lemons *et al.* 2005; Faeth & Shochat 2010). Once again, the presumed primary mechanism for these community-wide effects is endophytic alkaloids that may modulate abundances and diversity of consumers (herbivores and detritivores), competitors (other conspecific or heterospecific plants) and consumers of plant consumers (predators and parasites). However, endophytic infections also cause other phenotypic changes in the host grasses in terms of growth and reproductive allocation (Faeth 2009), physiology (e.g., Morse *et al.* 2002), water, nutrient and metabolite content (e.g., Rasmussen *et al.* 2008), and oxidative stress protection (e.g., White & Torres 2010), all of which may also affect associated animal and plant communities. Furthermore, alkaloids are rarely measured in studies of the effect of endophyte infection at the community level (but see Jani *et al.* 2010).

Here, we review the outcomes of studies of the community-level effects of *Neotyphodium* endophyte infection on abundance and diversity of associated arthropod communities (Table 1). We limit our review to arthropods because we know of no studies of the effects of endophytes on vertebrate community properties. Most community-level studies have been conducted with three imported agronomic grasses – tall fescue, perennial ryegrass and Italian ryegrass (*Lolium multiflorum*) (Table 1). These studies collectively show reduced abundances of herbivores on infected grasses. Effects on herbivore species richness are less clear, with some studies showing decreased richness but others indicating that endophytes may increase herbivore coexistence and thus species richness of herbivores. Only three studies examined effects on higher trophic levels. Omacini *et al.* (2001) reported slightly reduced parasitoid richness (loss of one species) in infected Italian ryegrass grown outdoors in containers while Finkes *et al.* (2006) and Rudgers & Clay (2008) found reduced predator richness in infected agronomic tall fescue plots in old fields.

Endophytes also alter community properties in native grasses, but with very different outcomes than the existing studies of agronomic grasses. Faeth & Shochat (2010) and Jani *et al.* (2010), using common garden experiments in natural habitats with Arizona fescue and sleepygrass, showed increased herbivore abundances on infected plants (Table 1). In addition, parasite and predator abundances and species richness were reduced on infected Arizona fescue and sleepygrass,

respectively. Thus, for these two grass species, it appears that endophytes and their alkaloids have greater negative effects on the natural enemies of the herbivores than the herbivore themselves, resulting in enemy-free or enemy-reduced space (Jani *et al.* 2010) for herbivores on infected plants.

These variable outcomes, especially in native grass communities, where many herbivores are specialists and predators and parasites are diverse, are entirely consistent with expectations from PDT and tri-trophic interactions. Plant secondary compounds may reduce herbivore abundances and richness, especially generalist herbivore species, but they may also increase abundances of specialist herbivores and negatively impact abundances and diversity of natural enemies of herbivores. In a broader community perspective, fungal endophytes and their phenotypic host changes can be viewed within the context of community genetics (Cheplick & Faeth 2009). Whitham *et al.* (2003) proposed that genetic variation among individuals within species at one trophic level alters properties and processes at other trophic levels. For example, heritable secondary chemical variation in plants may be amplified across trophic levels, affecting not only herbivores but also efficacy of natural enemies and rate of decomposition (Whitham *et al.* 2003). As asexual, maternally-inherited components that radically alter host properties, seed borne endophytes increase plant genotypic and phenotypic richness (Jani *et al.* 2010). This endophyte-mediated phenotypic variation often has profound effects on community structure, diversity and function. Thus, endophyte–host interactions and their community effects are encompassed within both theories of plant defence and broader concepts of community genetics (Cheplick & Faeth 2009; Jani *et al.* 2010).

When should we expect endophyte-mediated defensive mutualisms?

Faeth (2002) proposed that because alkaloids are costly, in terms of metabolic (e.g., Ohnmeiss & Baldwin 1994) and ecological costs (e.g., Strauss *et al.* 2002) like plant secondary chemicals, selection should favour persistence of endophyte–host combinations with high levels of alkaloids when herbivory is chronic, herbivores are mainly generalists, and nutrients are relatively plentiful. If endophytic alkaloids have other functions, such as allelopathic agents that enhance competitive abilities (e.g., Matthews & Clay 2001), then grasses with high alkaloids should be favoured when both plant

competition and herbivory are intense, assuming alkaloids deter herbivores. These predictions logically follow from PDT. Because the aforementioned conditions often occur in agroecosystems, it should not be surprising that the most consistent support for the defensive mutualism hypothesis comes from studies of agronomic grasses (Saikkonen *et al.* 1998, 2006).

Most empirical studies of the effects of endophytic alkaloids have involved invertebrates, although the original basis for defensive mutualism was derived from observations of deterrence and toxicity of *Neotyphodium*-infected grasses on livestock (Bacon 1995). Others have argued that endophytic alkaloids may be more effective against grazing vertebrates (e.g., Clay 2009). Infection frequencies of some endophyte-infected, toxic grasses are well known to increase under heavy and chronic grazing by livestock in agronomic pastures (e.g., Clay 1998). However, it is unlikely that native vertebrate grazers consume more biomass and exert more selective pressure than invertebrate herbivores. Unlike intense and persistent cattle grazing in enclosed pastures, vertebrate grazing in natural grass communities is sporadic and seasonal, and some native grasses have coevolved with some vertebrate grazers to tolerate grazing (Faeth 2002). Furthermore, experimental studies show that invertebrates significantly reduce both tree (e.g., Marquis & Whelan 1994) and grass fitness (e.g., Faeth 2009) via chronic and persistent herbivory, and insect consumption of plant biomass is equivalent or greater than that from grazing vertebrates (e.g., Gibson *et al.* 1987). In addition, invertebrate herbivores, unlike vertebrates, are well-known vectors of many severe plant diseases, and deterrence of insect vectors via endophytic alkaloids has been shown to reduce infection by barley yellow dwarf virus (Lehtonen *et al.* 2006). This disease-reducing aspect of invertebrate deterrence may be as important, or more important, than reducing consumption. Finally, unlike livestock grazers, invertebrates consume significant below-ground plant biomass and some specialize to consume seeds. Certainly, vertebrate herbivory can influence the evolution of plant secondary compounds and, as an extension, endophytic alkaloids. However, just as the coevolution of herbivores and plants was primarily based upon invertebrate herbivores (Ehrlich & Raven 1964), it is likely that direct or indirect selective pressures from invertebrate herbivores are largely responsible for the few cases of infected grasses maintaining high alkaloid levels in natural populations.

Conclusions

Defensive mutualism via endophyte alkaloids or defence via plant secondary chemistry is not an all-or-none proposition (e.g., Faeth 2010), as it has been traditionally and more recently portrayed (Clay 2009). Yet, many research papers involving endophytes begin with the explicit assumption that fungal endophytes of grasses are defensive mutualists (Faeth 2002; Clay 2009). Most ecologists who studied plant–herbivore interactions, when asked if any given plant secondary chemical is ‘defensive’ against herbivores would respond that it depends on the plant and herbivore species, the suite of natural enemies that are present and the particular environment. Plant–herbivore researchers long ago recognized the

question of plant chemical defences cannot be answered with a simple ‘yes or no’ but rather ‘yes and no’. Evidence that endophyte-infected plants with alkaloids have more (or the same) herbivores than uninfected plants does not refute the totality of the defensive mutualism hypothesis. Similarly, evidence of reduced herbivore abundances on infected plants does not confirm the universality of defensive mutualism via endophytes. Outcomes are species, system, time and place specific, just as are plant–herbivore–natural enemy interactions. Defence against herbivores is just one of several explanations for the plethora of plant secondary compounds (as well as endophytic alkaloids (Faeth 2002)).

The more interesting question is not whether or not endophytes and their alkaloids are defensive but rather what ecological selective pressures maintain high alkaloids in some infected grass species and populations but not in others (Faeth 2002; Cheplick & Faeth 2009).

When should we expect ‘defensive’ chemicals to be worth the physiological and ecological costs to produce and maintain them? How do natural enemies influence whether alkaloids are effective against herbivores or not? How is alkaloid production constrained not just by costs, but also by plant and endophyte phylogenies and genotypes and prevailing environment factors? These questions remain largely unanswered for systemic, seed borne endophytes of grasses.

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