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Influence of fungal isolates infecting tall fescue on multitrophic interactions

Thomas L. BULTMAN[a,*], *Adilene AGUILERA*[b], *Terrence J. SULLIVAN*[a]

[a]*Biology Department, Hope College, Holland, MI, USA*
[b]*Richard Daley College, Chicago, IL, USA*

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ABSTRACT

The epichloae are ascomycetous fungi in the genera *Epichloë* and *Neotyphodium* that live within grasses. Some of these fungi produce alkaloids that can help protect the host from herbivores. The alkaloids may also travel up the food web and affect members of the third trophic level. In this way they can produce trophic cascades which are rippling effects when a trophic level impacts those above or below it. We briefly summarize the general patterns of multitrophic effects of endophytes and highlight the most recent studies on this topic. Further, we report on our study in which we tested if different fungal strains in tall fescue (cultivar Jesup) affect multitrophic interactions involving aphids and their parasitoid natural enemies. Using both the common strain of *N. coenophialum* and a novel isolate (AR577), we allowed potted plants to be colonized by aphids and parasitoids in a semi-natural setting. We found that endophyte infection of tall fescue resulted in greater vegetative growth of the plant. We also found that *N. coenophialum* modified bottom-up cascades by depressing both aphid and parasitoid densities. Finally, we found that multitrophic effects were modified by fungal isolate: the common strain had stronger negative impacts on aphid and parasitoid densities than did the novel isolate.

Introduction

The study of species interactions is at the heart of understanding the complexity of ecosystems. Most workers have generally focussed on interactions within one or between two trophic levels. However, there is growing awareness that more complex interactions are common and important in nature (Strauss 1991). Indeed, multitrophic interactions among organisms belonging to three or more trophic levels that occur through direct and often indirect pathways are increasingly gaining the attention of ecologists, as evidenced by recent symposia on the topic (Gange & Brown 1997; Wajnberg *et al.* 2001; Tscharntke & Hawkins 2002). One class of multitrophic interactions that has received considerable attention is the interaction between plants, herbivores and natural enemies of herbivores (Price *et al.* 1980; Heinrich & Collins 1983; Barbosa *et al.* 1991; Hunter & Schultz 1993; Abrahamson & Weiss 1997). The study of multitrophic interactions has helped to reveal the importance of trophic cascades in community structuring (Oksanen *et al.* 1981; Polis 1999). Bottom-up cascades occur when levels of resources and productivity at the base of the food web regulate productivity at higher levels, while top-down cascades occur when higher-level consumers are important in regulating levels below them.

Neotyphodium coenophialum is an ascomycetous fungus that lives endophytically within tall fescue grass and may be important in modulating multitrophic interactions and trophic cascades due to its production of alkaloids that are

* *Corresponding author.*
E-mail address: bultmant@hope.edu (T.L. Bultman).
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toxic to some herbivores (Clay 1990; Clement *et al.* 1994; Faeth & Bultman 2002; Popay & Bonos 2005). Alkaloids include the ergots and lolitrems which primarily affect vertebrates, and lolines and peramine which mainly influence invertebrates (Siegel & Bush 1996; Bush *et al.* 1997; Clay & Schardl 2002; Schardl *et al.* 2007). Through the toxic alkaloids the endophyte acts as a defensive mutualist with its host grass (Clay 1988).

While endophyte-produced alkaloids can negatively affect herbivores, they can also travel up the food web and affect members of the third trophic level (Faeth & Bultman 2002). We briefly summarize here the general patterns and highlight the most recent studies on this topic; the reader is directed to papers by Faeth & Bultman (2002); Bultman *et al.* (2009b); Hartley & Gange (2009) for thorough reviews of earlier work.

Effects of grass endophytes on individuals of the third trophic level

Effects of endophytes on members of the third trophic level are most often negative, yet the number of studies on this topic is quite low (Bultman *et al.* 2009b). Endophytes within perennial ryegrass infected with *Neotyphodium lolii* decreased development and survival of parasitoids of Argentine stem weevil (Barker & Addison 1996; Bultman *et al.* 2003, but see Urrutia *et al.* 2007). Similarly, parasitoids of fall armyworm caterpillars showed lower survival when hosts were fed tall fescue infected by some isolates of *N. coenophialum* (Bultman *et al.* 2009a). Further, entomopathogenic nematodes (*Steinernema carpocapse*) were less likely to successfully attack black cutworms (Kunkel & Grewal 2003) and fall armyworm (Richmond *et al.* 2004) when the herbivores had fed on perennial ryegrass infected with endophyte compared to grass lacking infection. In addition, Richmond & Bigelow (2009) found a similar pattern with nematodes attacking black cutworm larvae fed tall fescue. The authors of these studies suggested that the insect herbivores benefit from alkaloids in endophyte-infected plants by using them for defence against entomopathogenic nematodes (perhaps through reducing the pathogenecity of the nematode's lethal bacterial symbiont).

Aphids are common pests of grasses and are often attacked by natural enemies. The performance of these enemies is often influenced by the presence of endophytes. For example, parasitiods of the aphid, *Metopolophium festucae*, had prolonged development time (Harri *et al.* 2009) and their offspring had reduced reproduction (Harri *et al.* 2008) when reared from hosts fed endophyte-infected perennial ryegrass. Similarly, ladybird beetles preying on the aphid, *Rhopalosiphum padi*, exhibited reduced fecundity when endophyte-infected perennial ryegrass served as the food source for aphids (de Sassi *et al.* 2006). This study suggests predators, like parasitoids, are negatively affected by grass endophytes, at least in food webs with agronomic grasses at their base.

A few studies have shown that members of the third trophic level benefit from the presence of endophytes. For example, larvae of the Japanese beetle feeding on endophyte-infected tall fescue were more susceptible to the entomopathogenic nematode *Heterorhabditis bactenophora* (Grewal *et al.* 1995). The investigators suggested that reduced beetle vigor, perhaps due to endophyte-produced alkaloids, made them more susceptible to nematodes. A recent study showed that third trophic level effects are not limited to invertebrates. Saari *et al.* (2010) found that voles fed endophyte-infected meadow fescue suffered more predation by weasels. They concluded that the voles' antipredatory behavior was negatively affected by consuming endophyte-infected grass, however, the nature of this affect was unknown.

Effects of grass endophytes on populations of the third trophic level

A few studies have been conducted in which grasses, herbivores and natural enemies have been allowed to interact together over time so that population-level effects of endophytes could be assessed. Omacini *et al.* (2001) studied the insect food webs established on *Neotyphodium*-infected and uninfected Italian ryegrass (*Lolium multiflorum*) in field plots in Argentina. They found that aphid populations were reduced on endophyte-infected plants, apparently due to poor food quality (i.e., alkaloids) of those grasses. They also found that parasitism was greater on aphids feeding on uninfected grass, but that the endophyte reduced the rate of parasitism by secondary parasitoids (hyperparasitoids and mummy parasitoids of primary parasitoids) and decreased the overall complexity of the food web. Their findings fit nicely into what is called a bottom-up trophic cascade in which the quality or productivity of the producer (Italian ryegrass) regulates the productivity of the trophic levels above it.

Another study used a multifactorial design in which fertilizer, endophyte infection and plant cultivar were manipulated (Krauss *et al.* 2007). Perennial ryegrass with or without infection by *N. lolii* was established at an experimental field site. Aphids and their parasitoids were allowed to naturally colonize the grasses. They found that endophyte infection did not reduce aphid or parasitoid abundances; endophytes did not cause a bottom-up cascade, in contrast to the Omacini *et al.* (2001) study. The authors suggested that relatively low levels of peramine found in their infected plants may explain the lack of plant resistance to aphids (*N. lolii* does not produce lolines).

Finkes *et al.* (2006) worked in replicated, successional fields in which endophyte infection (*N. coenophialum*) in tall fescue was manipulated. They found the number of spider (obligate predators of insect herbivores) families and morphospecies was higher in the absence of endophyte. They also found a shift in spider species composition showing that the invasive endophyte-infected tall fescue can alter the structure of these grassland food webs. They attributed the effects on spiders to reductions they found in abundances of some spider prey in plots with endophyte-infected grass.

In contrast to the studies above, Faeth (2009) conducted a field experiment on a native grass, Arizona fescue (*Festuca arizonica*). He manipulated genotype of the grass, herbivory, water availability and infection status over 4 yr. He found infected plants had *greater* herbivore abundance in early stages of the study (a trophic cascade opposite to that found in studies cited above) and increased allocation to reproduction. He suggested that the endophyte may act more as a reproductive parasite than as a mutualist with its host. Faeth