Neotyphodium endophytes in grasses: deterrents or promoters of herbivory by leaf-cutting ants?

Abstract Endophytic fungi, particularly in the genus Neotyphodium, are thought to interact mutualistically with host grasses primarily by deterring herbivores and pathogens via production of alkaloidal mycotoxins. Little is known, however, about how these endophytes interact with host plants and herbivores outside the realm of agronomic forage grasses, such as tall fescue, and their livestock grazers or invertebrate pest herbivores. We tested the effects of Neotyphodium inhabiting introduced tall fescue and native Arizona fescue on preference, survival, and performance of the leaf-cutting ant, Acromyrmex versicolor, an important generalist herbivore in the southwestern United States. In a choice experiment, we determined preferences of foraging queens and workers for infected and uninfected tall fescue and Arizona fescue. In a no-choice experiment, we determined queen survivorship, worker production, and size of fungal gardens for foundress queens reared on diets of infected and uninfected tall fescue and Arizona fescue. In a no-choice experiment, we determined queen survivorship, worker production, and size of fungal gardens for foundress queens reared on diets of infected and uninfected tall fescue and Arizona fescue. Foraging workers and queens did not significantly prefer either uninfected tall fescue or Arizona fescue relative to infected tall fescue and more infected than uninfected Arizona fescue. Queen survivorship and length of survival was greater on uninfected tall fescue, and infected Arizona fescue than uninfected tall fescue and Arizona fescue. Foraging workers and queens did not significantly prefer either uninfected tall fescue or Arizona fescue relative to infected grasses, although ants tended to harvest more uninfected than infected tall fescue and more infected than uninfected Arizona fescue. Queen survivorship and length of survival was greater on uninfected tall fescue, uninfected Arizona fescue, and infected Arizona fescue than on infected tall fescue or the standard diet of palo verde and mesquite leaves. No queens survived beyond 6 weeks of the study when fed the infected tall fescue diet, in contrast to the effects of the other diets. Likewise, worker production was much lower and fungal garden size much smaller on infected tall fescue than in all other treatments, including the standard diet. In general, ant colonies survived and performed better on uninfected tall fescue and infected and uninfected Arizona fescue than standard diets of palo verde and mesquite leaves. The interaction of Neotyphodium with its host grasses is highly variable and these endophytes may increase, not alter, or even decrease resistance to herbivores. The direction of the interaction depends on host and fungal genotype, herbivore species, and environmental factors. The presence of endophytes in most, if not all, host plants suggests that endophytes may alter foraging patterns, performance, and survival of herbivores, such as leaf-cutting ants, but not always in ways that increase host plant fitness.

Key words Acromyrmex · Endophytic fungi · Festuca · Neotyphodium · Mutualism

Introduction

Endophytic fungi live asymptomatically and intercellularly within tissues of most plants and are thought to be plant mutualists, mainly by increasing resistance of the host plant to herbivores and plant pathogens (Clay 1988, 1990, 1991; Breen 1994; Saikkonen et al. 1998). Endophytes in the genus Neotyphodium (formerly Acremonium) inhabiting Pooidae grasses in the genera Bromus, Festuca, Lolium, Poa, and Stipa (Siegel et al. 1987) are well known for their anti-herbivore properties against many vertebrates and invertebrates (Clay 1988, 1990, 1991; Breen 1994; Schardl and Phillips 1997) and anti-microbial activity (Guo et al. 1992; Siegel and Bush 1997). The primary mechanism of increased resistance to herbivores and microbes is production of mycotoxins, such as loline, peramine, lolitrem, and ergovaline alkaloids, by the endophytic fungus (Siegel and Bush 1996, 1997).

The grasses Festuca arundinacea (tall fescue) and F. arizonica (Arizona fescue) are both infected by Neotyphodium, an asexual endophyte that does not sporulate and is only transmitted vertically by hyphae growing into seeds. N. coenophialum in F. arundinacea produces a variety of alkaloids that are active against mammalian,
avian, insect, nematode and microbial herbivores (Siegel et al. 1987, 1990; Siegel and Bush 1996, 1997). *F. arundinacea* is an Eurasian species which has been selectively bred and now widely distributed as a forage grass in North America (Hoveland 1993; Ball et al. 1993). Because of their agronomic importance, the anti-herbivore properties as well as benefits from increased drought resistance and competitive abilities resulting from the interaction between the endophyte *N. coenophialum* and its host, tall fescue, have been well studied (Bacon 1993; Elmi and West 1995; West et al. 1995). Conversely, *F. arizonica* is a cool-season grass that is native to mid to high elevations in southwestern North America (Hoveland 1993; Ball et al. 1993). From the interaction between the endophyte and its host, tall fescue, have been well studied (Bacon 1993; Elmi and West 1995; West et al. 1995). Conversely, *F. arizonica* is a cool-season grass that is native to mid to high elevations in southwestern North America (Hoveland 1993; Ball et al. 1993).

Leaf-cutting ants of the genus *Acromyrmex* (Hymenoptera: Formicidae: tribe Attini) are generalized, and often dominant, herbivores commonly ranging from neotropical, desert, to grassland regions and occasionally temperate habitats (Fowler et al. 1990). We hypothesized that leaf-cutting ants and their fungal gardens should be particularly susceptible to either fungal endophytes per se or their alkaloidal mycotoxins, because the ants forage selectively for plant material for cultivation of specialized fungal gardens which, in turn, provide food for the colony (Weber 1972). Leaf-cutting ant species in the genera *Atta, Acromyrmex, Trachymyrmex* and *Seriomyrmex* cultivate similar fungi species (Chapela et al. 1994; North et al. 1997). Success of the fungal gardens directly determines survival of founding queens and her larvae and workers (Rissing et al. 1996). We hypothesized that fungal endophytes may inhibit growth of these fungal gardens through (1) direct toxicity to foraging queens and workers, (2) reduction of foraging activity by queens and workers, (3) resource competition of endophytes with garden fungi, or (4) inhibition of fungal garden growth by endophytic mycotoxins. Previous studies of tropical leaf-cutting ants show that workers forage selectively based on variation in plant allelochemistry and nutrition (Hubbell et al. 1984; Howard 1987, 1988, 1990; Knapp et al. 1990; Vasconcelos and Fowler 1990; J. Weser, unpublished work) and workers produce antibiotics in metapleural glands to inhibit growth of competing microorganisms, presumably to promote growth of the fungal garden (Nascimento et al. 1996). In nature, leaf-cutting ant species likely encounter numerous fungal endophyte species and, potentially, their mycotoxins and metabolic byproducts, in foraging for plant material, since all plant species examined to date harbor fungal endophytes (Petrini 1991). Often a single woody plant species harbors more than 40 species of fungal endophytes (Petrini et al. 1992; Gaylord et al. 1996; Saikkonen et al. 1996; Faeth and Hammon 1997).

We tested the hypothesis that *Neotyphodium* endophytes in *F. arundinacea* (tall fescue) and *F. arizonica* (Arizona fescue) alter foraging activities and fungal garden growth and thus colony success of *Acromyrmex versicolor* (Pergande), a widespread, leaf-cutting ant species in the southwest United States and northern Mexico. To test the effect of the endophyte on preference, we presented workers in foraging colonies choices between infected and uninfected tall fescue and Arizona fescue. To determine the anti-herbivore (e.g., Siegel et al. 1987; Bush et al. 1997; Siegel and Bush 1997) and anti-fungal (e.g., Guo et al. 1992) attributes of *Neotyphodium* infections on this ant species, we reared foundress queens on diets of infected and uninfected tall fescue and Arizona fescue. We predicted that *Neotyphodium* in these grasses should decrease colony survival and performance in terms of fungus garden size and number of workers produced by founding *A. versicolor* queens.

**Materials and methods**

**Study grasses and fungi**

*Tall fescue* is a perennial Eurasian grass introduced into North America as a pasture grass and is now widespread (Ball et al. 1993; Hoveland 1993). *Neotyphodium*-infected tall fescue has increased in pastures because of increased resistance to grazing and to drought stress, and increased competitiveness (Hoveland 1993). Cattle that feed on tall fescue infected with *N. coenophialum* show reduced weight gain, decreased milk production, increased respiration and, in extreme cases, dry gangrene in hooves (Siegel et al. 1987), and other mammalian herbivores show similar symptoms of toxicosis (e.g., Siegel and Bush 1996, 1997; Kaiser et al. 1996). Tall fescue infected with *N. coenophialum* also reduces performance and survival of many invertebrate herbivores, including fall armyworm, crickets, flour beetles, stem weevils, and sod webworms (see references in Siegel et al. 1987; Breen 1994; Bush et al. 1997), and inhibits microbial growth (Guo et al. 1992). Most of these herbivores are introduced generalists and agricultural pests.

*Arizona fescue* (*F. arizonica*) is a perennial bunchgrass inhabiting Ponderosa pine-grassland habitats in Arizona at elevations above 1500 m (Kearney and Peebles 1960). Arizona fescue reproduces primarily by seed (United States Department of Agriculture 1988). *Neotyphodium* is common in Arizona fescue populations (Schultz and Faeth 1998), with frequency of infected plants ranging from 50 to 100% in populations examined to date.

The *Neotyphodium* endophyte grows intercellularly within grass tissues and produces no external signs of infection. *Neotyphodium* is the asexual (anamorphic) stage and is transmitted vertically to plants by hyphae growing into seeds. The sexual stage, *Epichloë typhina*, sporulates and produces disease symptoms (external hyphae that reduce flowering) or may also remain asexual and be transmitted vertically via seed (Schardl et al. 1997). Species in the genus *Neotyphodium* have evolved from *Epichloë* multiple times (Schardl and Tsai 1992) and recent evidence suggests that *N. coenophialum* and *E. typhina* undergo hybridization events (Schardl et al. 1994; Tsai et al. 1994).

*Tall fescue* (*F. arundinacea*, variety Kentucky 31) was grown from seed of known infection status (seed provided by M. Siegel, University of Kentucky) in the greenhouse in potting soil supplemented with nitrogen fertilizer. Arizona fescue (*F. arizonica*) was grown from plants of known infection status that were transplanted from field sites near Flagstaff, Arizona to the greenhouse and grown as described for tall fescue. All plants of tall and Arizona fescue were tested for *Neotyphodium* infections by microscopy and by tissue print immunoblot (modified from Gwinn et al. 1991) to confirm infection status.