HERBIVORY MEDIATES GRASS–ENDOPHYTE RELATIONSHIPS

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Abstract. Endophytic fungi are plant symbionts living asymptomatically within plant tissues. *Neotyphodium* spp., which are asexual vertically transmitted systemic fungal endophytes of cool-season grasses, are predicted to be plant mutualists. These endophytes increase host plant resistance to environmental stresses and/or increase the production of alkaloid-based herbivore deterrents. The ubiquity of this defense mutualism is unclear, and a variety of alternative mechanisms may explain the observed variation in infection rates, levels of deterrence, and the maintenance of asexual endophytes in grass populations. We found that grass–endophyte interactions are variable and ordered along an herbivory gradient in an undisturbed subarctic alpine ecosystem. Native grass populations in grazed sites had significantly greater frequency of *Neotyphodium* infection compared to ungrazed sites. Tillers from grazed sites had significantly higher hyphal densities compared to ungrazed sites. The ability of grass–*Neotyphodium* constituents to deter vertebrate herbivory in natural systems is thought to be rare. In grazed meadows, we showed that endophyte infection resulted in the deterrence of grazing by native vertebrate herbivores. However, the same herbivores did not distinguish between infected and uninfected grass harvested from ungrazed areas. These results demonstrate that the relationship between vertically transmitted endophytes and grasses in the alpine tundra vary greatly within populations. This may be based in part on defense mutualism and is consistent, under varying levels of herbivory, with the predictions of optimal defense theory.

Key words: endophytic fungi; Festuca altaica; *herbivory; mutualism;* Neotyphodium; *optimal defense theory; subarctic; symbiosis.*

INTRODUCTION

Endophytes are remarkably diverse and found in most plant species surveyed (Arnold et al. 2000). Grass–endophyte interactions have generally been considered mutualistic, such that endophytes acquire protection and nutrients (Clay and Schardl 2002, Saikkonen et al. 2004) while host plants benefit from increased resistance to heat, drought, and herbivory stress (Vicari and Bazely 1993, Bazely et al. 1997, Malinowski et al. 1997). In the specialized case of *Neotyphodium* spp. (Glenn, Bacon and Hanlin), an asexual endophyte of cool-season grasses, proliferation is only by vertical transmission, or growing into seed (Clay 2001, Clay and Schardl 2002). Since vertical transmission links *Neotyphodium* survival directly to that of the host, evolutionary theory predicts the relationship to be mutualistic (Clay and Schardl 2002). This defense mutualism concept has been widely accepted to explain the often high frequencies of infection (Schardl and Phillips 1997, Saikkonen et al. 2000) and of alkaloid-based herbivory deterrents within pooid grass populations (Miles et al. 1998, Clay and Holah 1999). Grass populations not completely infected

are thought to be moving toward an equilibrium of 100% infection (Clay 1998).

Earlier studies supported defense mutualism under strong herbivory (Bazely et al. 1997), including those from the agricultural sector where various classes of alkaloids cause toxicosis, staggers, and many other negative effects in livestock at a cost of more than 6×10^8 US$ in lost revenue to agriculture in the United States (Hoveland 1993). While high infection frequency and toxicity levels as a result of intense grazing pressure have been demonstrated in agronomic systems (Clay 1998), recent evidence suggests that the beneficial effects of *Neotyphodium* spp. for cool-season pooid grasses, including benefits in relation to herbivory, are less clear (Faeth and Bultman 2002, Faeth and Sullivan 2003, Saikkonen et al. 2004). Important metrics of *Neotyphodium* infection in natural populations of grasses including infection frequency, toxicity levels, and hyphal densities are thought to be highly variable (Saikkonen et al. 2004) and influenced by multiple genetic (Leuchtmann et al. 2000) and environmental factors (Roylance et al. 1994, Faeth 2002, Faeth and Sullivan 2003).

Faeth (2002) and Saikkonen et al. (2004) have argued that, in contrast to agronomic studies, in natural systems mutualism in grass–*Neotyphodium* associations are extremely rare and could only occur under very particular circumstances. Indeed, a toxic or deterrent effect of endophytes in native grasses on native vertebrate herbivores in a natural system has never been

reported (Faeth 2002). In the absence of a toxic effect, a condition thought to be important for mutualism and the persistence of *Neotyphodium* spp. in natural populations of grasses (Clay and Schardl 2002), infection in grasses could be maintained by the spatial structure of metapopulations, so that local-level selection favors infected plants relative to uninfected plants (Saikkonen et al. 1998). Although never demonstrated for vertically transmitted *Neotyphodium*, occasional horizontal transmission could theoretically allow infection frequencies to be sustained even when the relationship is parasitic or neutral (Faeth and Sullivan 2003). If variable frequencies in infection and levels of deterrence were a result of herbivory, then a pattern predicted by optimal defense theory should occur (Rhoades 1979, Zangerl and Rutledge 1996, Bazzaz and Grace 1997). Exposure to different levels of grazing is thought to be a determinant of the level of deterrent-based (constitutive) defenses (Rhoades 1979, Zangerl and Rutledge 1996), including alkaloid concentrations in grass–*Neotyphodium* constituents (Faeth 2002). Infected hosts with alkaloid-based deterrents would be favored where grazing was consistently high, while uninfected plants or infected plants that produce no deterrents would be favored where herbivory was low or absent and thus disadvantageous for the host to bear the costs of alkaloids. At the population level, grasses should have higher infection frequencies where grazing levels were high compared to populations where grazing was low or absent. In natural systems, variations in the grass–*Neotyphodium* relationship that affect the strength and degree of mutualism may feed back and be detectable as a measurable difference in herbivore responses. These relationships, at the population and individual levels, have rarely been demonstrated in a natural system with native grass and grass–*Neotyphodium* constituents in response to natural vertebrate herbivores. However, such relationships would demonstrate one important avenue by which the variation in infection frequencies and deterrent levels are sustained in natural populations (Faeth 2002) and significantly clarify the role of herbivores in mediating grass–endophyte relations in natural systems.

To investigate the response of grass populations and grass–*Neotyphodium* spp. constituents to different levels of herbivory, we examined rates of infection by endophytes (*Neotyphodium* spp.) in grass populations (*Festuca altaica* Trin.) and herbivory deterrence by *Neotyphodium* spp.–*Festuca altaica* constituents along a naturally occurring alpine grazing gradient.

Methods

Our study was conducted in subarctic alpine meadows in the Ruby Range, Yukon Territory, Canada (61°13′ N, 138°16′ W, elevation 1900 m). *Festuca altaica*, a perennial bunchgrass not known to be toxic to livestock (Damiran 2005), was ubiquitous. The common herbivores, *Ochotona collaris* (collared pikas) and *Marmota caligata* (hoary marmots), both generalists, were behav-

iorally constrained to distinct patches of boulder fields. Pikas and marmots concentrated their foraging in a narrow band of vegetation within 12 m from boulder fields, thereby producing a sharp grazing gradient. Pikas rarely ventured more than 10 m into meadows (McIntire and Hik 2002, Morrison et al. 2004) as a consequence of several factors including predation risk (Morrison et al. 2004) and thermoregulation (Smith 1974). Marmots spend 74% of their time at a mean distance of 11.6 m from boulder fields with a median distance of 5 m (Karels et al. 2004). Given that the physical aspects (location and size of boulder fields) of the study site were stable over hundreds of years (Price 1991), the spatial/behavioral constraints of the herbivores would have imposed differing selection pressures on grass–*Neotyphodium* pairings along the grazing gradient.

To quantify the intensity of vertebrate grazing on *F. altaica*, 21 line transects, running perpendicular to boulder fields at three locations in the valley, were established in 2004. These transects traversed the boulder fields and then continued into the meadows for 80 m. *Festuca altaica* tussocks ($N = 1174$) within 1 m of the transect lines were examined and the percentage of tillers from each tussock grazed in the current year were recorded. Tussocks were grouped into five categories reflecting decreasing levels of vertebrate grazing with distance from a boulder field (Karels et al. 2004, Morrison et al. 2004). We quantified invertebrate herbivory in 2005 at three distances from boulder fields: "on" boulder fields, 0–5 m; "near" boulder fields, 5–15 m; and "far" from boulder fields, 60–80 m, respectively. Marked tillers (total $N = 678$) were inspected for invertebrate grazing three times during the growing season at each distance. Of the 678 tillers marked in 2005 only two tillers presented indications that they were damaged by invertebrate herbivores. In two other cases it was not clear whether or not damage occurred by mishandling or invertebrates. We did not continue further analyses of invertebrate grazing given the extremely low number of occurrences. Hik et al. (2003) also reported extremely low occurrences of invertebrate herbivory on graminoids at the same study site.

To determine the rate of *Neotyphodium* infection of *F. altaica*, in 2003 and 2004 we established transects at three locations in the valley at "on," "near," and "far" distances from boulder fields. At each of the distances, we flagged individual *F. altaica* tussocks and selected one tiller from the edge of a tussock. The bottom 1 cm of the youngest fully expanded leaf from the tiller was removed, cut into multiple subsamples, and fixed in acidified alcohol. One set of subsamples was tested for endophytic infection using a field immunoblot assay to detect monoclonal antibodies specific to *Neotyphodium* (Phytoscreen Immunoblot Kit #ENDO7973, Agrinostics, Watkinsville, Georgia, USA; see also Koh et al. 2006). The ability of the field immunoblot assay to detect *Neotyphodium* in *F. altaica* (and other grass species) was found to be highly consistent with staining and micros-
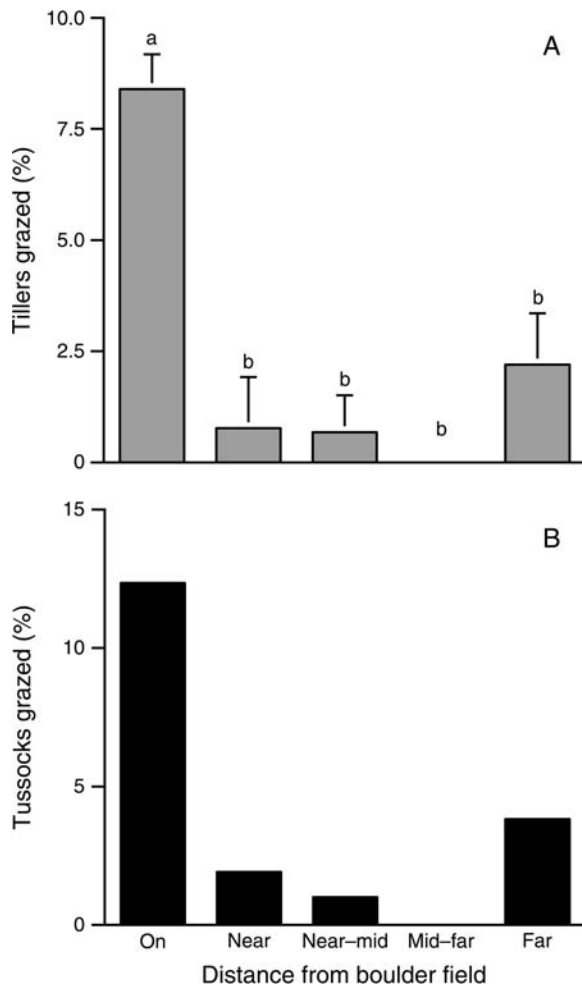
FIG. 1. Distances from boulder fields and numbers of *Festuca altaica* sampled: on the boulder field, 0–5 m (N = 340 plants); near, 5.1–15 m (N = 157); near–mid, 15.1–37.4 m (N = 301); mid–far, 37.5–60 m (N = 219); far, 60.1–80 m (N = 157). (A) Percentage of tillers grazed within tussocks (mean + SE; means with different lowercase letters are significantly different at $P < 0.0001$); and (B) mean percentage of tussocks grazed at increasing distances from boulder fields. Note the different y-axis scales for tillers and tussocks.

copy methods (Koh et al. 2006). Tussocks sampled in 2003 were not resampled in 2004. A subset of infected individuals was flattened, stained with a dye selective for the endophyte (aniline blue; Shipton and Brown 1962), and wet mounted. We determined the hyphal densities for the infected subset by randomly selecting three fields of view with a compound microscope at 200× magnification. A visual transect was run across the diameter of the field of view (0.7 mm) perpendicular to the long axis of the parenchyma cells, and hyphae that crossed this transect were counted. To reduce observer bias, visual hyphal counts were conducted "blind" without knowledge of the origin of the sample. Slides were re-coded before the test and back referenced afterwards with a code key. Because these data were not distributed

normally, hyphal densities among distance categories were compared using Kruskal-Wallis signed-rank tests. Post hoc tests between means (on vs. near and on vs. far) were performed using Bonferroni-adjusted Wilcoxon signed-rank tests with significance set at $P = 0.025$.

To determine if grass–*Neotyphodium* constituents with different histories of grazing and infection status differ in deterring herbivory we used open cafeteria-style feeding trials (Morrison et al. 2004) in 2004. For each trial, wild collared pikas were presented with 10 g of infected and uninfected freshly harvested *F. altaica* placed in separate white polyvinylchloride tubes (10 × 15 cm) positioned together on boulders at the center of their territories. This fresh material was obtained from tussocks with known infection status marked during the frequency of infection survey (N = 526), from either "far" from boulder fields (N = 9 trials) or from "on" and "near" boulder fields (N = 10 trials). Grasses on and near were pooled (and mixed in equal proportions in trials) because overall infection rates were similar at those distance categories over the two years (see Fig. 2A). Once the trial began, cumulative selection by pikas for infected or uninfected grass was tabulated at one-minute intervals for a maximum of one hour. Rodgers' index of preference (Morrison et al. 2004) was calculated for each feeding trial. Because these data were not distributed normally, preference for either infected or uninfected grasses (for each distance category) was compared using Wilcoxon signed-ranks tests.

To determine if forage quality of *F. altaica* was a selection factor during the cafeteria trials, we randomly selected a subset of tillers from far, near, and on distance categories from tussocks originally sampled for infection status and hyphal densities. Infected and uninfected tillers were analyzed for percentage nitrogen using a Dumas dry combustion on a Carlo-Erba 1500 NC analyzer (Strada Rivol Tana, Milan, Italy). Data were analyzed with a two-factor ANOVA with distance and infection status as fixed factors. For tillers for which we had both nitrogen data and hyphal density counts, we used Pearson's correlation coefficient to determine if there was a relationship between the two variables.

RESULTS

The mean percentage of tillers grazed by vertebrates per tussock decreased significantly with distance from boulder fields (Kruskal-Wallis test, $\chi^2_{0.05,4} = 73.4844$ [first subscript gives the α level of significance, followed by df], $P < 0.0001$; Fig. 1A). A significantly greater percentage of tussocks less than 5 m from boulder fields ("on") was grazed compared to all other distances from boulder fields (2 × 5 contingency table: 2003, $\chi^2_{0.05,1} = 69.5$, $P < 0.0001$; Fig. 1B).

Infection frequencies of *F. altaica* populations by *Neotyphodium* (Fig. 2A) decreased significantly with distance from boulder fields in both years (2 × 3 contingency table for 2003, $\chi^2_{0.05,1} = 33.87$, $P < 0.0001$; for 2004, $\chi^2_{0.05,1} = 23.10$, $P < 0.0001$). While infection
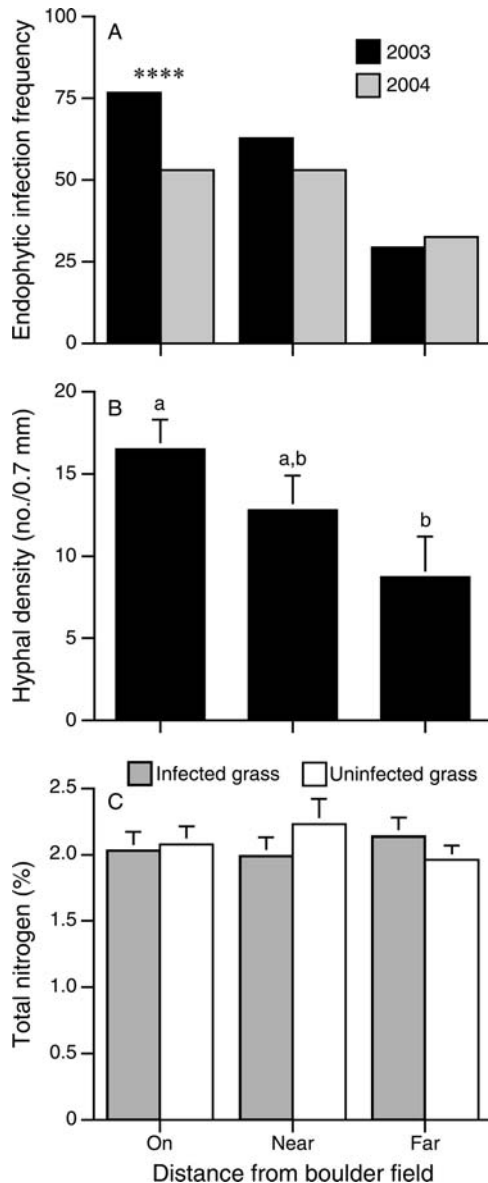
FIG. 2. (A) Infection rates of *Festuca altaica* by endophytic *Neotyphodium* spp. at increasing distances from boulder fields during 2003 (black bars) and 2004 (gray bars). Sample sizes: on boulder fields, $N = 90$ in 2003, $N = 162$ in 2004; near, $N = 110$ in 2003, $N = 179$ in 2004; far, $N = 58$ in 2003, $N = 221$ in 2004. Years were significantly different ($P < 0.0001$) only for plants on the boulder field. (B) Hyphal density (mean no. of hyphae per 0.7 mm + SE; see *Methods*) of *Neotyphodium* from *Festuca altaica* tillers sampled at increasing distances from boulder fields. Sample sizes: on, $N = 13$; near, $N = 10$; far, $N = 7$. Means that were significantly different are indicated by different letters ($P < 0.025$) as determined by Wilcoxon signed-ranks test with Bonferroni-adjusted P values. (C) Total percentage nitrogen content (mean + SE) of *Festuca altaica* infected (gray bars) and uninfected (white bars) with *Neotyphodium* at increasing distances from boulder fields. Sample sizes: on, infected $N = 13$, uninfected $N = 14$; near, infected $N = 13$, uninfected $N = 7$; far, infected $N = 13$, uninfected $N = 20$. See Fig. 1 legend for details on the distance from boulder field measurements.

frequencies "near" and "far" from boulder fields remained the same between 2003 and 2004 (2×2 contingency table for far, $\chi^2_{0.05,1} = 0.229$, $P = 0.633$; near, $\chi^2_{0.05,1} = 2.60$, $P = 0.107$), infection frequencies at "on" boulder fields dropped from 76% in 2003 to 53% in 2004 (2×2 contingency table: on, $\chi^2_{0.05,1} = 14.12$, $P = 0.0002$).

Relative hyphal densities of *Neotyphodium* inside *F. altaica* (Fig. 2B) decreased significantly with distance from boulder fields (Kruskal-Wallis test, $\chi^2_{0.05,2} = 6.08$, $P = 0.0479$). "Far" and "on" boulder field samples were significantly different from each other (Wilcoxon signed-ranks test, $\chi^2_{0.05,1} = 5.655$, $P = 0.0174$), "on" and "near" samples were not significantly different from each other (Wilcoxon test, $\chi^2_{0.05,1} = 2.036$, $P = 0.1535$).

Nitrogen content of tillers did not vary with distance (ANOVA, $F_{2,74} = 0.095$, $P = 0.9$; Fig. 2C) or infection status (ANOVA, $F_{1,74} = 0.081$, $P = 0.777$), nor were there any significant interactions between distance and infection status (ANOVA, $F_{2,74} = 1.004$, $P = 0.371$). There were no correlations between nitrogen content and hyphal densities (Pearson correlation, $r = 0.093$, $P = 0.267$).

Pikas did not discern a difference between infected and uninfected grasses "far" from boulder fields (Wilcoxon test, $\chi^2_{0.05,1} = 0.0556$, $P = 0.8136$; Fig. 3). However, pikas showed a significant preference for grass free of *Neotyphodium* when presented with infected and uninfected grasses from "on–near" boulder fields (Wilcoxon test, $\chi^2_{0.05,1} = 11.7833$, $P = 0.0006$).

DISCUSSION

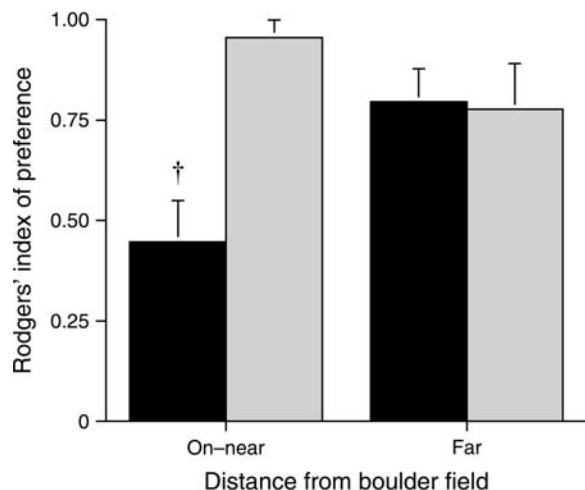We examined the dynamics between vertically transmitted endophytes and native grasses along a distinct

FIG. 3. Preference by collared pikas (*Ochotona collaris*) for *Festuca altaica* infected (black bars) and uninfected (gray bars) with *Neotyphodium* spp., based on Rodgers' index of preference (mean + SE). Trials were run separately for *F. altaica* gathered at each boulder field distance category (on–near, 0–15 m, $N = 10$; far, 60.1–80 m, $N = 9$). Means that were significantly different, as determined by a Wilcoxon signed-ranks test, are indicated by a dagger ($P = 0.0006$).

and quantifiable natural grazing gradient. Earlier studies to assess grass–*Neotyphodium* and native vertebrate herbivore relations that incorporated exclosures (e.g., Saikkonen et al. 1999) may have measured grass–endophyte pairs selected under grazing pressure that were subsequently released from herbivory. It is possible that any grazing-tolerant grass–endophyte pairs would perform relatively poorly in exclosures compared to uninfected plants in the absence of herbivory if there were costs to the grass to harbor endophytes (Faeth and Bultman 2002). In contrast, grass–*Neotyphodium* pairs at our study site would have experienced consistent but different selection pressures (depending on location) since the major herbivores were constrained to boulder fields (Morrison et al. 2004) that were stable over space and time (Price 1991). Thus we were able to account for the long-term responses at the population level that could have resulted from specific genotypic pairings between grasses and *Neotyphodium* in situ. Our results suggest a consistently positive relationship between grazing, frequencies of infection, and *Neotyphodium* hyphal densities, consistent with the suggestion that herbivory can mediate grass–*Neotyphodium* relationships at the population level as predicted by both defense mutualism (Vicari and Bazely 1993, Clay and Schardl 2002) and optimal defense theory (Zangerl and Rutledge 1996). Our results suggest that vertebrate herbivory is one means by which frequency of infection can be made to vary within natural populations of grasses. Infection frequencies from "on" boulder fields may be more variable from year to year because of variability in the spacing of individual tussocks, herbivores, and cover from predators. Consequently, individual tussocks sampled from "on" boulder fields between years are likely to have been exposed to different levels of herbivory. At relatively lower grazing levels away from boulder fields, this variability would probably be reduced.

Festuca altaica from "far" areas would have encountered very low rates of herbivory compared to grasses from "on–near" areas where herbivores were active (McIntire and Hik 2002, Karels et al. 2004, Morrison et al. 2004). Since there may be metabolic costs to harbor endophytes and to produce secondary metabolites (Rhoades 1979, Faeth and Bultman 2002), there could be selection against a deterrent system for infected grasses where there is no herbivory (Rhoades 1979, Zangerl and Rutledge 1996). In contrast, where grazing was high (on and near boulder fields), grass–endophyte constituents that produced herbivore deterrents would be favored over both grass–*Neotyphodium* constituents that did not produce deterrents and uninfected grasses. This should not be surprising in the northern alpine tundra where plants growing in relatively poorer soils and harsher environments with relatively shorter growing seasons incur a greater cost for tissue lost to herbivory (Coley et al. 1985). While the specific nature of the deterrent produced by *Neotyphodium* was not identified in our experiment (the results of a preliminary screening for ergot alkaloids in infected and uninfected grasses were negative), the pikas, as a bioassay, only demonstrated avoidance of infected *F. altaica* tillers when the tillers originated from boulder fields where grazing was relatively greater and levels of endophyte hyphae concentrations higher. Since nitrogen content, an important determinant in forage preference for herbivores, of grass was similar among infected and uninfected tillers from all distances from boulder fields, we ruled out food quality as a basis for selection during the feeding trials. Since densities of hyphae in infected grasses decreased with distance from boulder fields we suggest there may be a link between the strength of a yet to be determined deterrent and the variation in grazing pressure associated with proximity to the boulder fields. Rasmussen et al. (2007) demonstrated that concentrations of the alkaloids peramine, lolitrems, and janthitrems increased with hyphal concentrations of *Neotyphodium lolii* in perennial ryegrass, *Lolium perenne* (however, see Dahlman et al. [1991]). Further screening for at least six other common chemical deterrents produced by endophytes (Clay and Schardl 2002) will be necessary to identify the compound or combination of compounds responsible for the forage preferences of pikas.

Variations at the population level in the deterrence ability of infected tillers, frequency of infection, and hyphal densities are thought be produced by multiple factors (genetic and environmental) including differences in grazing pressure (Faeth 2002). We propose that the differences in infection metrics and herbivore avoidance of infected grass from only one of two distances from boulder fields were a consequence of the distinct longer term grazing history of the grasses and how that history influences the relationship (Rhoades 1979, Zangerl and Rutledge 1996) between grasses and *Neotyphodium*. Our findings demonstrate that the relationship between vertically transmitted endophytes and grasses in the alpine tundra vary greatly within populations and may be based in part on defense mutualism (Vicari and Bazely 1993, Clay and Schardl 2002) and optimal defense theory (Rhoades 1979).

Literature Cited

Arnold, A. E., Z. Maynard, G. S. Gilbert, P. D. Coley, and T. A. Kursar. 2000. Are tropical fungal endophytes hyperdiverse? Ecology Letters 3:267–274.

Bazely, D. R., M. Vicari, S. Emmerich, L. Filip, D. Lin, and A. Inman. 1997. Interactions between herbivores and endophyte-infected *Festuca rubra* from the Scottish islands of St. Kilda, Benbecula and Rum. Journal of Applied Ecology 34: 847–860.

Bazzaz, F. A., and J. B. Grace. 1997. Plant resource allocation. Academic Press, San Diego, California, USA.

Clay, K. 1998. Fungal endophyte infection and the population biology of grasses. Pages 255–285 *in* G. P. Cheplick, editor. The population biology of grasses. Cambridge University Press, Cambridge, UK.

Clay, K. 2001. Symbiosis and the regulation of communities. American Zoologist 41:810–824.

Clay, K., and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science 285:1742–1744.

Clay, K., and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist 160:S99–S127.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895–899.

Dahlman, D. L., H. Eichenseer, and M. R. Siegel. 1991. Chemical perspectives on endophyte–grass interactions and their implications to insect herbivory. J. Wiley and Sons, New York, New York, USA.

Damiran, D. 2005. Palatability of Mongolian rangeland plants. Eastern Oregon Agricultural Research Center, Oregon State University, Union, Oregon, USA.

Faeth, S. H. 2002. Are endophytic fungi defensive plant mutualists? Oikos 98:25–36.

Faeth, S. H., and T. L. Bultman. 2002. Endophytic fungi and interactions among host plants, herbivores and natural enemies. Pages 89–123 *in* T. Tscharntke and B. A. Hawkins, editors. Multitrophic interactions. Cambridge University Press, Cambridge, UK.

Faeth, S. H., and T. J. Sullivan. 2003. Mutualistic asexual endophytes in a native grass are usually parasitic. American Naturalist 161:310–325.

Hik, D. S., M. Brown, A. Dabros, J. Weir, and J. F. Cahill. 2003. Prevalence and predictability of handling effects in field studies: results from field experiments and a meta-analysis. American Journal of Botany 90:270–277.

Hoveland, C. S. 1993. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plants. Agriculture Ecosystems and Environment 44:3–12.

Karels, T. J., L. Koppel, and D. S. Hik. 2004. Fecal pellet counts as a technique for monitoring an alpine-dwelling social rodent, the hoary marmot (*Marmota caligata*). Arctic, Antarctic, and Alpine Research 36:490–494.

Koh, S., M. Vicari, J. P. Ball, T. Rakocevic, D. S. Hik, and D. R. Bazely. 2006. Rapid detection of fungal endophytes in grasses for large-scale studies. Functional Ecology 20:736–742.

Leuchtmann, A., D. Schmidt, and L. P. Bush. 2000. Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloe/Neotyphodium* endophytes. Journal of Chemical Ecology 26:1025–1036.

Malinowski, D., A. Leuchtmann, D. Schmidt, and J. Nosberger. 1997. Growth and water status in meadow fescue is affected by *Neotyphodium* and *Phialophora* species endophytes. Agronomy Journal 89:673–678.

McIntire, E. J. B., and D. S. Hik. 2002. Grazing history versus current grazing: leaf demography and compensatory growth of three alpine plants in response to a native herbivore (*Ochotona collaris*). Journal of Ecology 90:348–359.

Miles, C. O., et al. 1998. Endophytic fungi in indigenous Australasian grasses associated with toxicity to livestock. Applied and Environmental Microbiology 64:601–606.

Morrison, S., L. Barton, P. Caputa, and D. S. Hik. 2004. Forage selection by collared pikas, *Ochotona collaris*, under varying degrees of predation risk. Canadian Journal of Zoology 82:533–540.

Price, L. W. 1991. Subsurface movement on solifluction slopes in the Ruby Range, Yukon Territory, Canada—a 20-year study. Arctic and Alpine Research 23:200–205.

Rasmussen, S., et al. 2007. High nitrogen supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. New Phytologist 173:787–797.

Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Pages 1–55 *in* G. A. Rosenthal and D. H. Janzen, editors. Herbivores: their interactions with secondary plant metabolites. Academic Press, Orlando, Florida, USA.

Roylance, J. T., N. S. Hill, and C. S. Agee. 1994. Ergovaline and peramine production in endophyte-infected tall fescue–independent regulation and effects of plant and endophyte genotype. Journal of Chemical Ecology 20:2171–2183.

Saikkonen, K., J. Ahlholm, M. Helander, S. Lehtimaki, and O. Niemelainen. 2000. Endophytic fungi in wild and cultivated grasses in Finland. Ecography 23:360–366.

Saikkonen, K., S. H. Faeth, M. Helander, and T. J. Sullivan. 1998. Fungal endophytes: a continuum of interactions with host plants. Annual Review of Ecology and Systematics 29: 319–343.

Saikkonen, K., M. Helander, S. H. Faeth, F. Schulthess, and D. Wilson. 1999. Endophyte–grass–herbivore interactions: the case of *Neotyphodium* endophytes in Arizona fescue populations. Oecologia 121:411–420.

Saikkonen, K., P. Wali, M. Helander, and S. H. Faeth. 2004. Evolution of endophyte–plant symbioses. Trends in Plant Science 9:275–280.

Schardl, C. L., and T. D. Phillips. 1997. Protective grass endophytes: Where are they from and where are they going? Plant Disease 81:430–438.

Shipton, W. A., and J. F. Brown. 1962. A whole-leaf clearing and staining technique to demonstrate host–pathogen relationships of wheat-stem rust. Phytopathology 52:1313.

Smith, A. T. 1974. Distribution and dispersal of pikas: influences of behavior and climate. Ecology 55:1368–1376.

Vicari, M., and D. R. Bazely. 1993. Do grasses fight back? The case for antiherbivore defenses. Trends in Ecology and Evolution 8:137–141.

Zangerl, A. R., and C. E. Rutledge. 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. American Naturalist 147:599–608.

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