New Phytologist

Research

Grass–herbivore interactions altered by strains of a native endophyte

Tammy Tintjer[1] and Jennifer A. Rudgers[2]

[1]Department of Science and Mathematics, St. Mary-of-the-Woods College, St. Mary-of-the-Woods, IN 47876, USA; [2]Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA

Author for correspondence:
Tammy Tintjer
Tel: +1 812 5355262
Fax: +1 812 5355133
Email: ttintjer@smwc.edu

Received: *12 November 2005,*
Accepted: *13 February 2006*

Summary

• Many plants support symbiotic microbes, such as endophytic fungi, that can alter interactions with herbivores. Most endophyte research has focused on agronomically important species, with less known about the ecological roles of native endophytes in native plants. In particular, whether genetic variation among endophyte symbionts affects herbivores of plant hosts remains unresolved for most native endophytes. Here, we investigate the importance of native isolates of the endophyte *Epichloë elymi* in affecting herbivory of the native grass host, *Elymus hystrix*.

• Experimental fungal isolate–plant genotype combinations and endophyte-free control plants were grown in a common garden and exposed to natural arthropod herbivory.

• Fungal isolates differed in their effects on two types of herbivory, chewing and scraping. Isolates exhibiting greater sexual reproduction were associated with greater herbivore damage than primarily asexual isolates. Endophyte infection also altered patterns of herbivory within plants, with stroma-bearing tillers experiencing up to 30% greater damage than nonstroma-bearing tillers.

• Results suggest that intraspecific genetic variation in endophytes, like plant genetic variation, can have important 'bottom-up' effects on herbivores in native systems.

Key words: fungal endophyte, *Elymus*, *Epichloë*, genetic variation, herbivory, bottom-up.

New Phytologist (2006) **170**: 513–521

doi: 10.1111/j.1469-8137.2006.01720.x

Introduction

Genetic variation within plant species has long been known to influence plants' interactions with herbivores, a 'bottom-up' effect of the plant (Denno & McClure, 1983; Fritz & Simms, 1992; Hunter & Price, 1992). Within most plants, however, there exists another layer of genetic variation in the form of symbiotic microbes, which can also influence plant–herbivore interactions. Understanding the ecological function of this microbial component of variation can help to predict the community-wide consequences of altering symbiont genotypes or creating new host plant–symbiont combinations. This research also improves general knowledge of the importance of genetic variation in affecting higher-order species interactions.

Here, we focus on genetic variation in systemic, endophytic fungi, which form associations with an estimated 20–30% of grass species worldwide, including many economically important forage and turf grasses (Leuchtmann, 1992). In these symbioses, the endophyte acquires nutrition and shelter (Thrower & Lewis, 1973), and, in exchange, some endophytes can increase host resistance to consumers through the production of bioactive alkaloids (reviewed by Latch, 1993; Breen, 1994; Clay, 1996; Clay & Schardl, 2002), enhance tolerance to drought stress (West, 1994), and/or improve host competitive ability (Clay *et al.*, 1993). However, as with many microbial symbionts, endophytes are also neutral or parasitic under some conditions (Saikkonen *et al.*, 1998; Faeth *et al.*, 2004).

Previous studies suggest that both interspecific and intraspecific variation in endophytes can influence interactions

with herbivores. First, different species as well as different isolates of endophytes vary in traits that may affect herbivores, such as alkaloid production (Clay & Schardl, 2002; Siegel *et al.*, 1990; TePaske & Powell, 1993), mycelial mass (Hiatt & Hill, 1997), and level of host phenotypic plasticity (Cheplick, 1998; Bultman *et al.*, 2004). Second, different plant–endophyte genotype combinations have been shown to differentially affect herbivores, including aphids, nematodes and flies in both cultivated grasses and in wild barley (Clement *et al.*, 1997, 2001, 2005; Wilkinson *et al.*, 2000; Timper *et al.*, 2001). In one example, lambs fed tall fescue grass experimentally infected with an endophyte isolate lacking ergot alkaloids were nearly 60% heavier than lambs fed tall fescue grass with an isolate producing ergot alkaloids (Bouton *et al.*, 2002). These same fungal isolates also varied in their effects on aphids (but not on weevils), with better aphid performance on the low alkaloid isolate (Bultman *et al.*, 2003; Bultman *et al.*, 2004).

Importantly, the majority of research has focused on endophyte variation in forage and turf grass systems. These systems differ from some native grasses in a fundamental way. In crops, such as tall fescue and perennial ryegrass, the endophytes reproduce exclusively asexually through vertical transmission to the seeds (there is no contagious spread) (reviewed by Hill, 1994). However, many native grasses host endophytes that can reproduce both asexually through vertical transmission and sexually by producing stromata (Leuchtmann & Clay, 1989; Clay & Schardl, 2002). In general, sexually reproducing endophytes are more antagonistic to hosts than asexual, noncontagious endophytes because fungal reproductive structures (stromata) prevent inflorescence production (Clay, 1990; Schardl, 1996; Saikkonen *et al.*, 1998; Clay & Schardl, 2002). Importantly, asexual and sexual endophytes may also have different effects on herbivores. For example, in a survey of 18 endophyte species, asexual species had higher antiherbivore alkaloid concentrations than sexual species (Leuchtmann *et al.*, 2000). In the native grass *Brachypodium sylvaticum*, which hosts genetically different isolates of *Epichloë sylvatica* within a single plant, insect herbivory was greater on tillers with a sexual isolate than on tillers with an asexual isolate (Brem & Leuchtmann, 2001). Whether endophyte reproductive strategy influences herbivory at the scale of the whole plant is unknown but important because this is the scale at which natural selection occurs.

Here, we examine how endophyte variation affects arthropod herbivory using native endophyte isolates that vary in reproductive strategy. This work is among the first to explore the effects of endophyte isolate in a nonagronomic system. Experimental combinations of the grass *Elymus hystrix* and the endophyte *Epichloë elymi* plus endophyte-free control plants were grown in a common garden and exposed to natural arthropod herbivory. We addressed the following questions: (1) Does fungal isolate affect arthropod herbivory? (2) Does the reproductive strategy of the endophyte correlate with herbivore damage to the host, with increased herbivore damage on plants hosting sexually reproducing isolates? (3) How does the endophyte affect patterns of damage within plants and do stroma-bearing tillers experience greater damage than tillers lacking stroma?

Materials and Methods

Study system

Elymus hystrix L. (Poaceae) is a clump-forming, perennial, self-compatible woodland grass, native to temperate North America (Gleason & Cronquist, 1991). Near Bloomington, IN, USA, where our study was conducted, populations of *E. hystrix* range from 0 to 100% infection with *Epichloë elymi*, with a mean infection rate across populations of 25%. In *Epichloë elymi*, asexual reproduction (vertical transmission) occurs when the fungus infects host seeds and seedlings by clonal growth of hyphae. Sexual reproduction (horizontal transmission) occurs when the endophyte envelops the developing host inflorescence, aborting the inflorescence and forming a stroma. Fungal stromata produce sexual ascospores that can transmit the infection to uninfected seeds or adult plants (Chung & Schardl, 1997; Brem & Leuchtmann, 1999). *Elymus hystrix* infected with *E. elymi* exhibits continuous variation in stroma production; thus, there exists a mixture of vertical and contagious spread by the endophyte.

Experimental inoculations

To examine the effects of endophyte isolate on arthropod herbivory, we created experimental endophyte and host genotype combinations and assessed arthropod herbivore damage in a common garden. Naturally uninfected plants from four maternal families were inoculated with a single fungal isolate (one of 12 isolates cultured) or sham-inoculated (same procedure, but no endophyte present) as a control.

Seed source To obtain uninfected seeds, seeds from four naturally uninfected plants were collected from natural populations near Bloomington, Indiana (see Supplementary Material, Table S1). To minimize maternal effects on seedlings, during autumn 1999 seeds of these uninfected plants were grown in a 10×25 m random array at 0.5 m intervals within a common garden at the Indiana University Botany Experimental Field, Bloomington, IN, USA (39°10′26.6″ N, 86°30′23.2″ W). Resident vegetation was controlled by hand-weeding and mowing. Seeds were collected from plants in the common garden during autumn 2000 for use in experiments, and both maternal plants and a subset of the seeds were confirmed to be endophyte-free (Clark *et al.*, 1983).

Endophyte source Endophyte isolates were cultured from 12 host plants collected from natural populations near Bloomington, Indiana (see Supplementary Material, Table S1). Fungal

isolates were obtained from surface-sterilized nodal sections of plant stems following Clark *et al.* (1983). Data from isozymes and the β-tubulin gene (*tub2*) suggested genetic differentiation among some isolates (T. Tintjer and A. Leuchtmann, unpubl. data). Naturally infected clones of 11 of the original 12 source plants were also planted in the common garden in autumn 2003 (n = 3–5 clones per original source planted at random in a 12 × 13 m array). These common garden plants provided estimates of stromata production by the fungal isolates in their natural host that were not confounded with environmental variation associated with field conditions.

Inoculation procedure Seedlings from the four naturally uninfected maternal plant families were inoculated with one of the 12 endophyte isolates during spring 2002. At the time of inoculation, isolates were < 6 months old. Seeds were surface sterilized and germinated on H_2O agar in sealed Petri plates. When seedlings were 0.5–2.0 cm tall, we used a sterile hypodermic needle to create a small slit at the base of the seedling and inserted a small amount of fungal hyphae following Leuchtmann & Clay (1988). Inoculated seedlings were maintained at high humidity under artificial light (12 h light/12 h dark) for 7 d by sealing them inside Petri plates on agar for first 3 d and then covering with clear plastic wrap for an additional 4 d after seedlings were transplanted into individual pots (6.5 cm). After 7 d, seedlings were uncovered and moved to a glasshouse. Following 6 wk of glasshouse growth, leaf sheaths were stained and examined microscopically to determine inoculation success. We created nine replicates of each plant–endophyte combination. In addition, nine seedlings from each host maternal plant family were sham-inoculated to serve as uninfected controls.

Establishment of common garden During October 2002, we established a common garden at the Indiana University Botany Experimental Field. A total of 468 plants were transplanted (9 replicates × 4 maternal families × 13 (12 isolates + 1 control)) into a large array (15 × 40 m) comprising three blocks. Each block received three replicates of each treatment combination in a randomized design. Within a block, six rows were spaced 1.25 m apart, and *c.* 25 plants were positioned in each row at 0.5 m intervals. Control plants lacking endophytes did not acquire infection during the course of the experiment, as determined by testing both plant and seed material (Clark *et al.*, 1983), suggesting no or low contagious spread of *E. elymi* in the garden.

Assessing variation in fungal reproduction

For each fungal isolate, we obtained two estimates of fungal reproductive strategy. (1) In 2003, we recorded stroma formation on all replicates of the 12 experimentally infected host–endophyte combinations that were planted for the herbivore experiment (conducted in 2004). (2) In 2004, we determined stroma formation for all naturally infected clones of 11 of the original 12 source plants that we planted in the common garden. Although endophyte isolate is confounded with plant genotype in this second estimate, it gives an independent measure of stroma production in the natural host environment.

For each plant, we estimated reproductive strategy by the percentage of tillers with stroma at anthesis. We are confident that estimates gave accurate information about the phenotypes of the fungal isolates because estimates of reproductive strategy were highly correlated across years (Experimental, 2003 vs 2004: r = 0.65, P = 0.02, n = 12; Natural, 2003 vs 2004: r = 0.87, P = 0.01, n = 7) and between natural and experimental hosts (Year 2003, r = 0.94, P = 0.0005, n = 8; Year 2004, r = 0.82, P = 0.0019, n = 11).

Assessing herbivore damage

We assessed herbivore damage from 4 to 22 June 2004 (just before plant senescence) in order to capture the peak of cumulative herbivore damage. For each plant, we visually estimated damage separately for nonstroma-bearing and stroma-bearing tillers (n = 3 tillers per type, chosen at random). Damage was estimated for three, fully expanded leaves per tiller because few tillers (< 25%) had more than three fully expanded leaves. Leaves were chosen consecutively starting with the oldest, nonsenescent leaf on the tiller.

Herbivory was visually estimated to the nearest 10% for each damage type and classified into one of three categories: chewing, scraping, or tracking (see Supplementary Material, Plate S1). Damage estimates were obtained blindly, without knowledge of the treatments. Orthopterans (*Melanoplus* and *Conocephalus* spp.) and caterpillars, including *Spodoptera* spp., were responsible for chewing damage. Scraping was similar to damage that is typically caused by two-spotted spider mites (*Tetranychus urticae*), Banks grass mites (*Oligonychus pratensis*), clover mites (*Bryobia praetiosa*), winter grain mites (*Penthaleus major*) and possibly others. Tracking damage was characteristic of flea beetles (Chrysomelidae), possibly the corn flea beetle (*Chaetocnema pulicaria*). We also scored damage by leaf miners, but percentage damage was very low (< 0.4%) and was not significantly affected by any treatment. We counted aphids and other herbivores nondestructively, but too few were observed to permit statistical analysis.

We determined *whole plant* estimates of damage with the following equation.

(mean damage for stroma tillers × number of stroma tillers) + (mean damage for nonstroma tillers × number of nonstroma tillers)/total number of tillers

This method prevented any bias in damage estimates resulting from uneven sampling of stroma-bearing vs nonstroma-bearing tillers within a plant. Across all experimental treatments,

plants had an average of 18.1 ± 0.6 SE total tillers, 6.0 ± 0.4 SE stroma-bearing tillers, and 12.1 ± 0.4 SE nonstroma-bearing tillers. Thus, our design sampled *c.* 33% of the whole plant, *c.* 50% of stroma-bearing tillers, and *c.* 25% of nonstroma-bearing tillers.

Visual estimates of damage were calibrated against grid-based estimates determined using a 1 cm^2 transparent grid. Calibrated estimates were conducted for a subset of 212 damaged leaves across a team of six observers, including the authors. Total leaf area was determined by counting the number of grid squares filled > 50% by the leaf. Percentage damage for each damage type was estimated to the nearest 1 cm^2 by counting grid squares filled > 50% by damaged tissue. Across observers, visual damage estimates were highly correlated with grid-based estimates (Pearson's $r = 0.81$, $P < 0.0001$; range across the eight observers $r = 0.76–0.93$; range across the three damage types $r = 0.55$ scraping $–$ 0.87 chewing).

Statistical analysis

Does fungal isolate affect arthropod herbivory? Because distributions of the data were non-normal, and nonnormality could not be substantially reduced through transformations, we used distribution-free randomization tests (with 9999 iterations) to evaluate the treatments (Edgington, 1987; Manly, 1991). A randomization test determines a *P*-value by comparing an observed test statistic (e.g. an *F*-ratio) with a distribution of the test statistic that is expected under the null hypothesis that the treatments have no effects. We applied a randomization test equivalent of mixed model ANOVA by encompassing Proc MIXED code within a SAS randomization macro program (Cassell, 2002). The model included the fixed effects of endophyte isolate, plant genotype, the plant–endophyte interaction, and the random effect of block. Here, we only used damage estimates at the scale of the whole plant, and we included control plants (endophyte-free). Because we hypothesized that endophyte isolate could influence specific consumers in different ways (e.g. grasshoppers vs flea beetles), the three response variables were percentage chewing, scraping and tracking damage. When fungal isolate or plant genotype effects were significant, we followed up with the randomization test equivalent of a Tukey HSD (honestly significantly different) test to determine which genotypes significantly differed, while controlling for multiple comparisons.

Does the reproductive strategy of the endophyte correlate with herbivore damage? We examined Spearman rank correlations (nonparametric) between whole plant damage estimates (chewing, scraping, tracking) and the reproductive strategy of the endophyte (i.e. the percentage of tillers with stroma) as estimated for each fungal isolate from (1) experimental or (2) natural hosts. *P*- values were Bonferroni corrected because correlations were examined for three estimates of damage ($P < 0.017$).

How does the endophyte affect patterns of damage within a plant? For endophyte-infected plants, we compared herbivore damage between stroma- and nonstroma-bearing tillers using a randomization test. The mixed model ANOVA included the fixed effects of endophyte isolate, plant genotype, and tiller status (stroma-bearing or nonstroma-bearing), all two- and three-way interactions and the random effect of block. We also examined correlations between fungal reproductive strategy and damage separately for stroma-bearing and nonstroma-bearing tillers.

Results

Does fungal isolate affect arthropod herbivory?

Fungal isolates significantly differed in their effects on chewing damage. Plants with isolate 12 experienced 60–80% greater damage by leaf chewers than plants with isolates 2 or 3 (Fig. 1, Table 1). Scraping damage was also influenced by fungal isolate (Fig. 2, Table 1) and was marginally affected by plant genotype. Fungal isolate 12 resulted in 76–87% greater damage by leaf scrapers than isolates 1, 3, 5, or the control (Fig. 2b). By contrast, tracking damage was not significantly affected by fungal isolate or plant genotype (Table 1). Despite variation among fungal isolates, several isolates did not produce significantly different levels of herbivore damage than endophyte-free plants (Figs 1 and 2b). The exception was plants with isolate 12, which had significantly increased scraping herbivory (87% more) relative to endophyte-free plants (Fig. 2b).

Does the reproductive strategy of the endophyte correlate with herbivore damage? Greater sexual reproduction in the endophyte isolate was significantly, positively correlated with greater chewing and scraping damage (Fig. 3), but not significantly correlated with tracking damage ($r = 0.03$, $P = 0.6$). Correlations were the same direction and strength regardless of whether reproductive strategy was quantified via the natural host plant (Fig. 3) or via the experimental combinations (Experimental correlations: chewing $r = 0.11$, $P = 0.017$, $n = 424$; scraping $r = 0.12$, $P = 0.015$, $n = 424$; and tracking $r = –0.002$, $P = 0.97$, $n = 424$). Exclusion of plants with fungal isolate 12 (which had the highest percentage of stroma formation, 48%) did not significantly alter these results. Correlations between damage level and endophyte reproductive strategy did, however, depend on the stroma-status of tillers. Chewing herbivores were more sensitive to fungal isolate expression in tillers lacking stroma, whereas scraping herbivores were more sensitive to fungal isolate expression in tillers bearing stroma (Fig. 3).

How does the endophyte affect patterns of damage within a plant?

The endophyte strongly altered patterns of damage within plants. Herbivore damage of all types was significantly greater

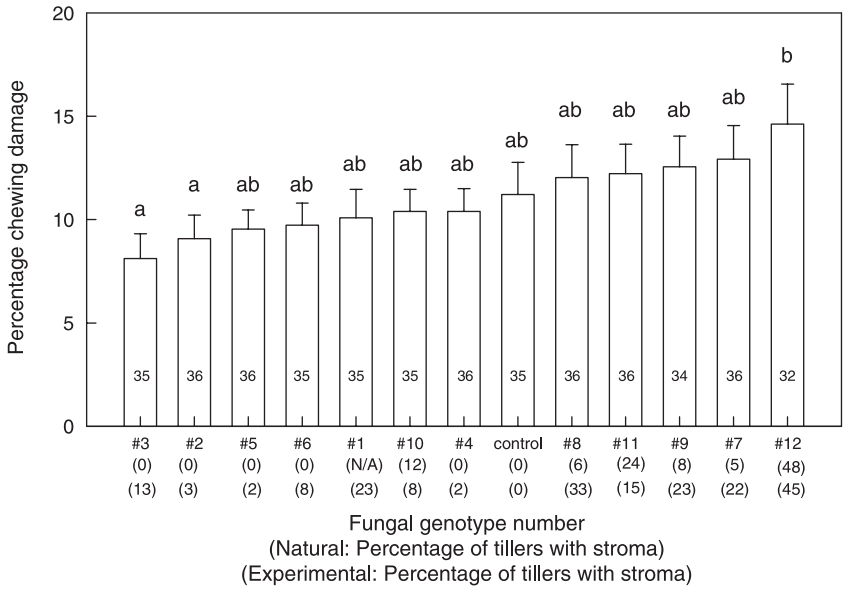

Fig. 1 Whole-plant percentage chewing damage for *Elymus hystrix* plants experimentally inoculated with one of 12 fungal isolates of the endophyte *Epichloë elymi* as well as endophyte-free controls. Data were averaged across plant genotypes because there was no significant plant genotype × endophyte isolate interaction. Bars show means ± SE, and bars with different letters are significantly different according to randomization test version of a Tukey HSD test. Sample sizes are given inside the open bars. Percentages of tillers with stroma are shown below the *x*-axis.

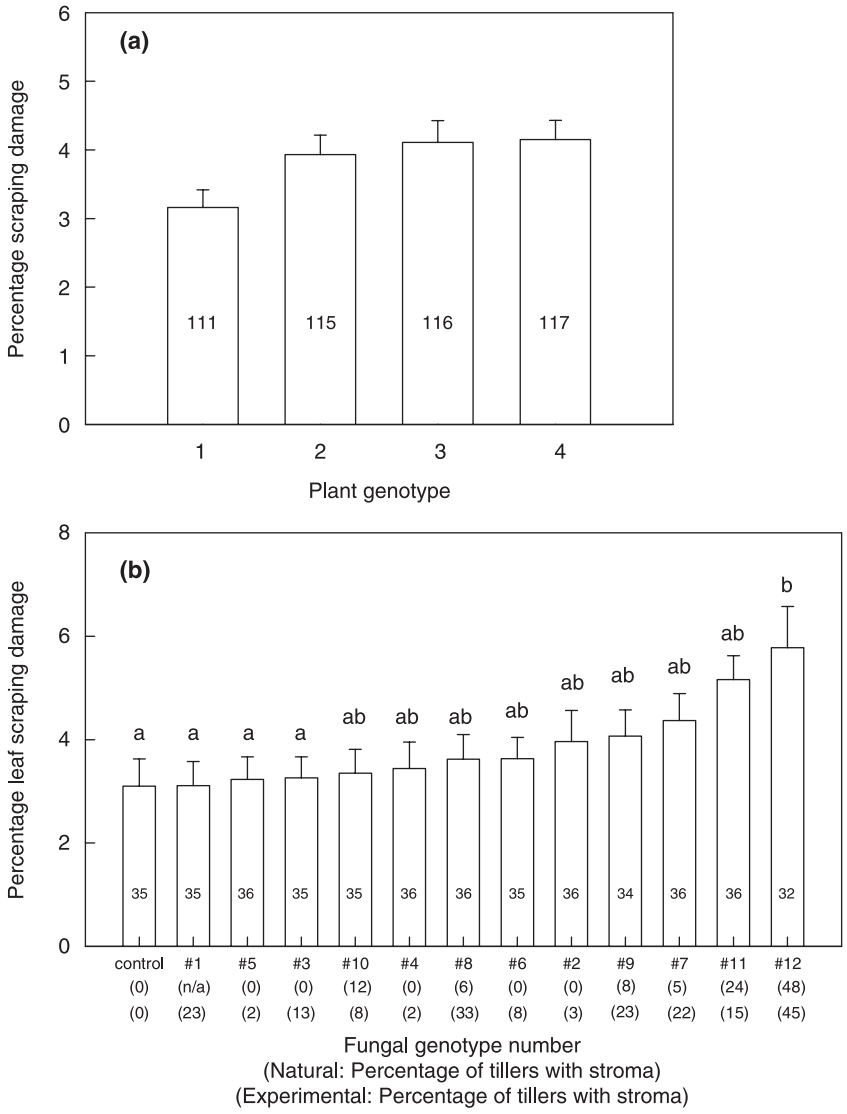

Fig. 2 Whole-plant percentage scraping damage for (a) four *Elymus hystrix* plant genotypes (data were averaged across fungal isolates because there was no significant plant genotype–endophyte isolate interaction) and (b) plants experimentally inoculated with one of 12 fungal isolates of the endophyte *Epichloë elymi* as well as endophyte-free controls (data were averaged across plant genotypes because there was no significant plant genotype–endophyte isolate interaction). Bars show means ± SE, and bars with different letters are significantly different according to randomization test version of a Tukey HSD test. Sample sizes are given inside the open bars. For fungal genotypes, percentages of tillers with stroma are shown below the *x*-axis.

Table 1 *P*-values from randomization test analysis of *Elymus hystrix* whole-plant damage estimates for three types of damage (chewing, scraping and tracking)

Effect	Chewing	Scraping	Tracking
Plant genotype	0.2761	0.0639	0.6458
Endophyte isolate	**0.0371**	**0.0080**	0.1181
Plant × endophyte	0.3722	0.7988	0.6985
Block	**0.0000**	**0.0432**	0.5802

$P < 0.05$ given in bold type. Sample sizes shown in Figs 1, 2.

on tillers with stroma than on tillers without stroma, with a 28% difference for chewing, 31% for scraping, and 17% for tracking (Fig. 4, Table 2). Differences between tiller types were consistent across both plant genotypes and fungal isolates, as indicated by the lack of significant interactions between plant or isolate and tiller status (Table 2). Results from this model should not be used to infer genotype or isolate effects at the whole plant scale.

Discussion

Experimental inoculations of the endophyte *Epichloë elymi* into *Elymus hystrix* plants showed that fungal isolates contribute significantly to variation among plants in the amount of arthropod herbivory. These results demonstrate that variation in natural, native microbial symbionts of plants can make an important contribution to variation among plants in levels of herbivore damage – a 'bottom-up' effect.

The importance of microbial variation to herbivory may commonly depend on the herbivore's degree of specialization and mode of feeding on the host plant. In our study, leaf chewers and scrapers (generalist grasshoppers, caterpillars and mites) were more responsive to variation among endophyte isolates than were tracking insects (monocot specialist flea beetles). In this system, the flea beetles also consume tissue in a manner different from leaf chewers and scrapers, by eating small streaks or 'windowpane' patches in the leaves (see Supplementary Material, Plate S1). Other studies have yielded similar results, with generalist chewing herbivores exhibiting greater sensitivity to the presence of microbes than herbivores with more specialized diets (Barbosa *et al.*, 1991; Gehring & Whitham, 2002). Finally, herbivores also varied in response to stroma-bearing vs nonstroma-bearing tillers within infected plants: leaf chewers showed the greatest response and tracking herbivores showed the least. Because only damage levels were measured, these patterns may reflect either differential preference or differential performance of the herbivores.

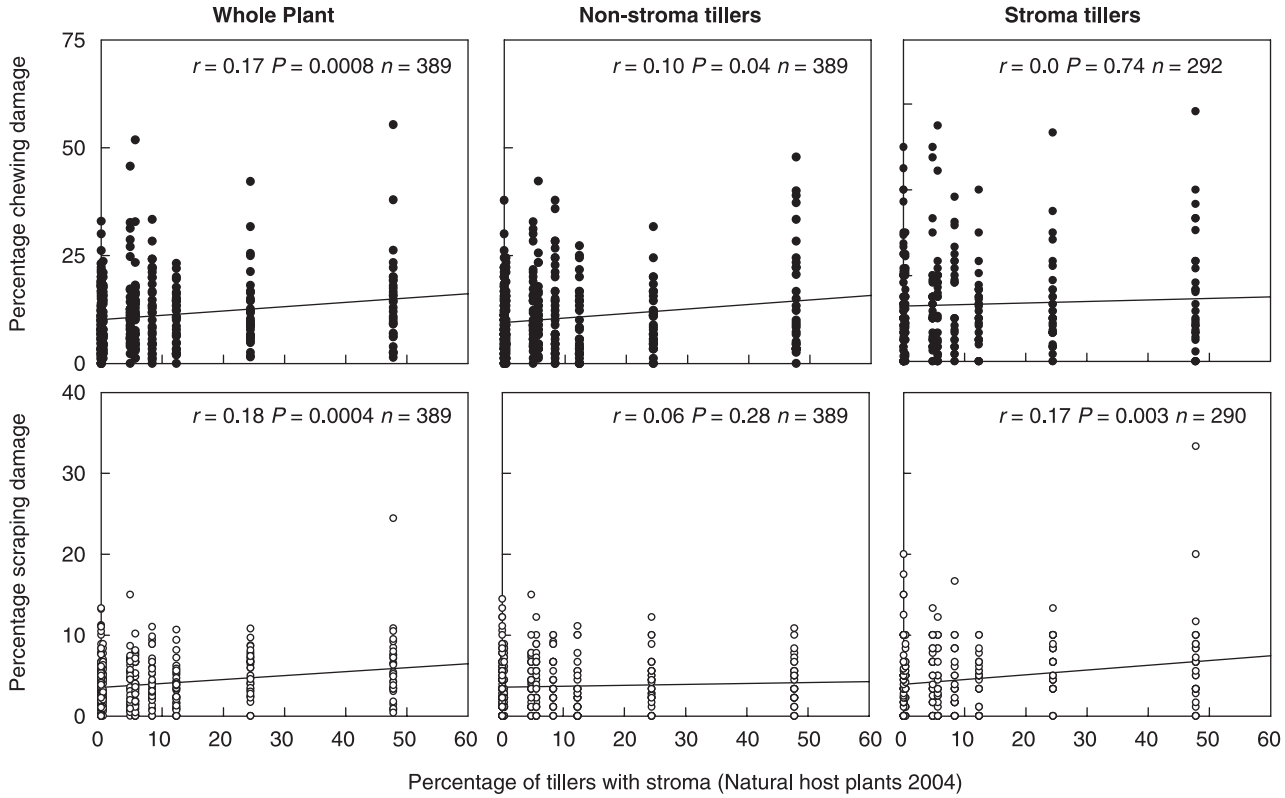

Fig. 3 Correlations between the degree of sexual reproduction by the endophyte *Epichloë elymi* in *Elymus hystrix* plants as determined by the percentage of tillers bearing stroma and the amount of herbivore damage. Herbivore damage is chewing (closed circles) or scraping (open circles). Correlations are shown for the whole-plant estimate of damage and for damage estimates separated by tiller status, nonstroma- or stroma-bearing. Results of Spearman rank correlation analysis are given for each relationship.

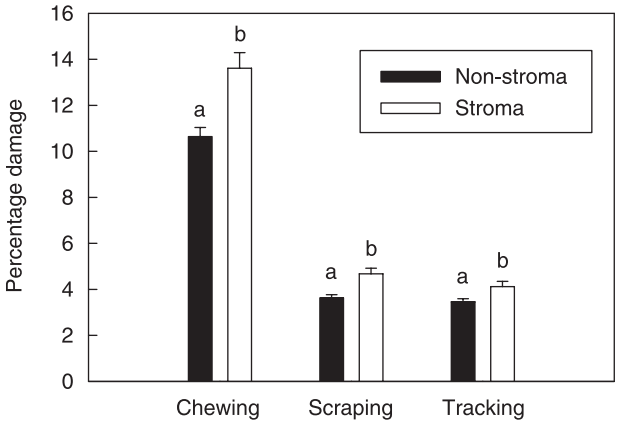

Fig. 4 Differences in damage levels between stroma-bearing (open bars) and nonstroma-bearing (closed bars) tillers within plants of *Elymus hystrix*, including the three most common types of arthropod herbivory: chewing, scraping, and tracking. Bars show means ± SE and, within a damage type, bars with different letters are significantly different according to randomization test version of a Tukey HSD test.

Table 2 *P*-values from randomization test analysis of *Elymus hystrix* tiller-level damage estimates for three types of damage (chewing, scraping and tracking)

Effect	Chewing	Scraping	Tracking
Plant genotype	0.0853	0.4074	0.6618
Endophyte isolate	0.2176	**0.0375**	0.3774
Tiller status	**0.0003**	**0.0000**	**0.0098**
Plant × endophyte	**0.0415**	0.0807	**0.0472**
Plant × tiller	0.1892	0.5568	0.3511
Endophyte × tiller	0.2838	0.4202	0.3962
Plant × endophyte × tiller	0.4617	0.9815	0.6357
Block	**0.0000**	**0.0000**	0.4807

$P < 0.05$ given in bold type.

Overall, we did not detect a benefit of the endophyte to *E. hystrix* in terms of increased herbivore resistance. While some fungal isolates resulted in less herbivory to hosts than uninfected controls, in no case were these differences statistically significant. For scraping damage one isolate (12) had significantly greater damage than the controls, suggesting a potential cost of the endophyte to the plant via increased herbivory. Because many endophytes have been shown to reduce herbivory (Clay & Schardl, 2002), the conclusion that one endophyte strain primarily increases herbivory relative to uninfected plants is surprising. Other potential benefits (or costs) of endophyte symbiosis, such as increased nutrient acquisition (Malinowski *et al.*, 2000) or enhanced drought tolerance (Elmi & West, 1995) remain to be investigated in this system.

Evolutionary theory predicts that increased vertical transmission of symbionts (here, asexual reproduction of the endophyte) should reduce the cost of the symbiont to its host (Ewald, 1987; Lipsitch *et al.*, 1995; Yamamura, 1996). Reported correlations were consistent with this prediction: Endophyte isolates with a greater percentage of asexual reproduction were less costly to plants, as measured by herbivore damage by leaf chewers and scrapers. To our knowledge, this is the first experimental study on endophytes to show such a correlation at the whole plant level. Ongoing work is examining fitness responses of the host plant to the endophyte isolates, an important next step in understanding the complex interactions among the host, endophyte, and herbivores in this system (T. Tintjer and K. Clay, unpubl. data).

Potential mechanisms underlying the observed correlations are also of interest. Specifically, what traits of the sexually reproducing endophyte strains make hosts more attractive to herbivores? Sexually reproducing endophyte strains may alter plant allocation patterns, perhaps increasing leaf nutritional value for herbivores. It also remains possible that stroma-producing endophytes can interfere with intrinsic plant-based defenses against herbivory, as is the case with some plant pathogens (Thaler *et al.*, 2002). Alternatively, the simple increase in stress to the host plant caused by stroma formation could increase host attractiveness to herbivores (White *et al.*, 1993). Because increases in herbivory (rather than declines) were observed, it is highly unlikely that endophyte-derived, anti-herbivore alkaloids are responsible for the observed patterns. In addition, in no case were infected plants more resistant to herbivory than uninfected plants. Ultimately, further experimental manipulations will be required to determine whether the correlation between endophyte reproductive strategy and herbivory results from sex expression itself, or from another trait correlated with reproductive strategy of the endophyte (e.g. altered nutritional value of leaf tissues).

In addition to variation among fungal isolates in herbivory, we also found that the endophyte altered patterns of herbivory within plants, with stroma-bearing tillers experiencing greater damage than nonstroma-bearing tillers. Several plant- or endophyte-mediated mechanisms may explain differential herbivory on developmentally different tiller types, including a plant stress response in stroma-bearing tillers or a reduction in alkaloid production by the endophyte during the rapid growth phase of stroma formation. Plants may also actively reduce allocation to the defense of tillers already attacked by the endophyte, a 'cutting your losses' strategy. Similar patterns were reported for the native grass, *Brachypodium sylvaticum*, where tillers bearing stroma-forming isolates received greater damage by microherbivores (mainly insects) than tillers with nonstroma-forming isolates (Brem & Leuchtmann, 2001). However, the difference between stroma and nonstroma tillers in our study was exclusively developmental, rather than genetic, because only a single isolate of *E. elymi* infected each plant. Our results also differ from the grass, *Agrostis hyemalis* infected by *Epichloë amarillans*, where the leaf nearest the stroma was avoided more by generalist fall armyworm larvae (*Spodoptera*

frugiperda) than were nonstromal leaves (White *et al.*, 1993). Thus, how endophytes affect patterns of damage within plants appears to vary across systems.

Overall, this study provides experimental evidence that variation among fungal isolates can have an important 'bottom-up' effect on arthropod herbivory on host plants. In addition, endophyte isolates with a greater degree of sexual reproduction were correlated with higher rates of herbivory, a pattern that fits evolutionary predictions about the relationship between parasite reproductive strategy and cost of symbiosis to the host plant.

Acknowledgements

Many thanks to J. Stuaan, B. Fishman, K. Mack, and B. Thompson for assistance in the field. Thanks to K. Clay for assistance in many aspects of the study. Comments from K. Reinhart, J. Koslow, T. Bultman and A. Leuchtmann greatly improved this manuscript. Work was funded by NSF DBI#0200485 to J.A.R. and NSF DEB#0309240 to T.T.

References

Barbosa P, Krischik VA, Jones CG. 1991. *Microbial mediation of plant–herbivore interactions.* New York, NY, USA: Wiley.

Bouton JH, Latch GCM, Hill NS, Hoveland CS, McCann MA, Watson RH, Parish JA, Hawkins LL, Thompson FN. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. *Agronomy Journal* **94**: 567–574.

Breen JP. 1994. *Acremonium*/endophyte interactions with enhanced plant resistance to insects. *Annual Review of Entomology* **39**: 402–423.

Brem D, Leuchtmann A. 1999. High prevalence of horizontal transmission of the fungal endophyte. *Epichloë e sylvatica. Bulletin of the Geobotanical Institute ETH* **65**: 3–12.

Brem D, Leuchtmann A. 2001. *Epichloë* grass endophytes increase herbivore resistance in the woodland grass *Brachypodium sylvaticum. Oecologia* **126**: 522–530.

Bultman TL, Bell G, Martin WD. 2004. A fungal endophyte mediates reversal of wound-induced resistance and constrains tolerance in a grass. *Ecology* **85**: 679–685.

Bultman TL, McNeill MR, Goldson SL. 2003. Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. *Oikos* **102**: 491–496.

Cassell DL. 2002. A randomization-test wrapper for SAS PROCs. In: SAS Institute Inc., eds. *Proceedings of the Twenty-Seventh Annual SAS Users Group International Conference* Cary, NC, USA: SAS Institute, Inc.

Cheplick GP. 1998. Genotypic variation in the regrowth of *Lolium perenne* following clipping: Effects of nutrients and endophytic fungi. *Functional Ecology* **12**: 176–184.

Chung KR, Schardl CL. 1997. Sexual cycle and horizontal transmission of the grass symbiont, *Epichloë typhina. Mycological Research* **101**: 295–301.

Clark EM, White JF, Patterson RM. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *Journal of Microbial Methods* **1**: 149–155.

Clay K. 1990. Fungal endophytes of grasses. *Annual Review of Ecology and Systematics* **21**: 275–297.

Clay K. 1996. Interactions among fungal endophytes, grasses and herbivores. *Researches on Population Ecology* **38**: 191–201.

Clay K, Marks S, Cheplick GP. 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology* **74**: 1767–1777.

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

Clement SL, Elberson LR, Bosque-Perez NA, Schotzko DJ. 2005. Detrimental and neutral effects of wild barley–*Neotyphodium* fungal endophyte associations on insect survival. *Entomologia Experimentalis et Applicata* **114**: 119–125.

Clement SL, Elberson LR, Youssef NN, Davitt CM, Doss RP. 2001. Incidence and diversity of *Neotyphodium* fungal endophytes in tall fescue from Morocco, Tunisia, and Sardinia. *Crop Science* **41**: 570–576.

Clement SL, Wilson AD, Lester DG, Davitt CM. 1997. Fungal endophytes of wild barley and their effects on *Diuraphis noxia* population development. *Entomologia Experimentalis et Applicata* **82**: 275–281.

Denno RF, McClure MS, eds. 1983. *Variable plants and herbivores in natural and managed systems.* New York, NY, USA: Academic Press.

Edgington ES. 1987. *Randomization tests.* New York, NY, USA: Marcel Dekker.

Elmi AA, West CP. 1995. Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. *New Phytologist* **131**: 61–67.

Ewald P. 1987. Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Faeth SH, Helander ML, Saikkonen KT. 2004. Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. *Ecology Letters* **7**: 304–313.

Fritz RS, Simms EL, eds. 1992. *Plant resistance to herbivores and pathogens.* Chicago, IL, USA: University of Chicago Press.

Gehring CA, Whitham TG. 2002. Mycorrhizae–herbivore interactions: population and community consequences. In: van der Heijden M, Sanders I, eds. *Mycorrhizal ecology.* Heidelberg, Germany: Springer-Verlag, 295–320..

Gleason HA, Cronquist A. 1991. *Manual of vascular plants of northeastern United States and adjacent Canada.* New York, NY, USA: The New York Botanical Garden.

Hiatt EEI, Hill NS. 1997. *Neotyphodium coenophialum* mycelial protein and herbage mass effects on ergot alkaloid concentration in tall fescue. *Journal of Chemical Ecology* **23**: 2721–2736.

Hill NS. 1994. Ecological relationships of Balansiae-infected graminoids. In: Bacon CW, White JF, eds. *Biotechnology of endophytic fungi of grasses.* Boca Raton, FL, USA: CRC Press, 59–71.

Hunter MD, Price PW. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**: 724–732.

Latch GCM. 1993. Physiological interactions of endophytic fungi and their hosts: biotic stress tolerance imparted to grasses by endophytes. *Agriculture Ecosystems and Environment* **44**: 143–156.

Leuchtmann A. 1992. Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins* **1**: 150–162.

Leuchtmann A, Clay K. 1988. Experimental infection of host grasses and sedges with *Atkinsonella hypoxylon* and *Balansia cyperi* (Balansiae, Clavicipitaceae). *Mycologia* **80**: 291–297.

Leuchtmann A, Clay K. 1989. Experimental evidence for genetic variation in compatibility between the fungus *Atkinsonella hypoxylon* and its three host grasses. *Evolution* **43**: 825–834.

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

Lipsitch M, Nowak MA, Ebert D, May RM. 1995. The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society of London Series B, Biological Sciences* **260**: 321–327.

Malinowski DP, Alloush GA, Belesky DP. 2000. Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. *Plant and Soil* **227**: 115–126.

Manly BFJ. 1991. *Randomization and Monte Carlo methods in biology*. London, UK: Chapman & Hall.

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

Schardl CL. 1996. *Epichloë* species: fungal symbionts of grasses. *Annual Review of Phytopathology* **34**: 109–130.

Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Bacon CW, Johnson MC. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. *Journal of Chemical Ecology* **16**: 3301–3316.

TePaske MR, Powell RG. 1993. Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. *Journal of Agricultural and Food Chemistry* **41**: 2299–2303.

Thaler JS, Fidantsef AL, Bostock RM. 2002. Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. *Journal of Chemical Ecology* **28**: 1131–1159.

Thrower LB, Lewis DH. 1973. Uptake of sugars by *Epichloë typhina* (Pers. Ex Fr.) Tul. in culture and from its host, *Agrostis stolonifera* L. *New Phytologist* **72**: 501–508.

Timper P, Gates RN, Bouton JH. 2001. Nematode reproduction in tall fescue infected with different endophyte strains. *Phytopathology* **91**: S144.

West CP. 1994. Physiology and drought tolerance of endophyte-infected grasses. In: Bacon CW, White JF, eds. *Biotechnology of endophytic fungi of grasses*. Boca Raton, FL, USA: CRC Press, 87–99.

White JF, Glenn AE, Chandler KF. 1993. Endophyte host associations in grasses. 18. Moisture relations and insect herbivory of the emergent stromal leaf of *Epichloë*. *Mycologia* **85**: 195–202.

Wilkinson HH, Siegel MR, Blankenship JD, Mallory AC, Bush LP, Schardl CL. 2000. Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. *Molecular Plant–Microbe Interactions* **13**: 1027–1033.

Yamamura N. 1996. Evolution of mutualistic symbiosis: a differential equation model. *Researches on Population Ecology* **38**: 211–218.

Supplementary Material

The following supplementary material is available for this article online:

Table S1. Location of natural populations of seeds and/or plant collections for uninfected plants and endophyte isolates.

Plate S1. Photographs of common arthropod damage to the grass *Elymus hystrix*. (A) Chewing damage (note: entire leaf section is missing), (B) Scraping damage, (C) Tracking ('windowpane') damage, (D) Leaf mine encompassing the tip of a leaf with visible insect frass inside. Approximate scale is given on each panel.

This material is available as part of the online article from http://www.blackwell-synergy.com