The Effects of Endophytes on Seed Production and Seed Predation of Tall Fescue and Meadow Fescue

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Abstract:

Fungal endophytes of grasses are often included in agricultural management and in ecological studies of natural grass populations. In European agriculture and ecological studies, however, grass endophytes are largely ignored. In this study, we determined endophyte infection frequencies of 13 European cultivars and 49 wild tall fescue (*Schedonorus phoenix*) populations in Northern Europe. We then examined seed production and seed predation of endophyte-infected (E+) and endophyte-free (E−) tall fescue (in wild grass populations and in a field experiment) and meadow fescue (*Schedonorus pratensis*; in a field experiment only). Endophytes were detected in only one of the 13 cultivars. In contrast, >90% of wild tall fescue plants harbored endophytes in 45 wild populations but were absent in three inland populations in Estonia. In three wild tall fescue study sites, 17%, 22%, and 56% of the seeds were preyed upon by the cocksfoot moth. Endophyte infection did not affect seed mass of tall fescue in the field experiment. However, seed predation was lower in E+ than E− grasses in the two tall fescue populations with higher predation rates. For meadow fescue, the mean number of seeds from E+ plants was higher than E− plants, but E− and E+ seeds had equal rates of predation by the moth. Our results suggest that the effects of grass endophytes on seed production and cocksfoot moth seed predation vary considerably among grass species, and the effects may depend on herbivore pressure and other environmental conditions.

Keywords: endophytes | tall fescue | meadow fescue | cocksfoot moth | seed production | seed predation

Article:

Introduction

Systemic fungal endophytes of grasses have received increasing worldwide attention since the 1970s when agricultural scientists found that some livestock disorders, such as fescue toxicosis, were attributable to endophyte-produced alkaloids in the widely planted tall fescue (*Schedonorus phoenix* ((Scop.) Holub. ex.*Lolium arundinaceum,* ex. *Festuca arundinacea*)) cultivar “Kentucky 31” [*34*]. The associated economic losses to the livestock industry in North America were estimated at $609 million annually as of 1993 [*13*] and are undoubtedly much higher today. The responsible endophyte in Kentucky 31 is *Neotyphodium coenophialum*, a systemic and seed borne fungal endophyte. In seed borne endophyte infections, hyphae grow internally and intercellularly throughout the above-ground tissues of the host plant and into the developing inflorescence and seeds. Thus, the fungus is transmitted vertically from maternal plant to offspring seeds and seedlings. Because of the heritable nature of the fungal infection in maternal plant lineages, infection rate of the endophytes is considered a cultivar character, and seed companies are required to declare this rate for grass cultivars in the USA and New Zealand. Although fungal endophytes are commonly detected in most of the fescue species [*30*], tall fescue and their systemic endophytes are still largely ignored in Europe where these fescues are native, perhaps because tall fescue is not a preferred livestock forage grass. In Europe, pastures are usually sown as mixtures of different grass species, and there is low incidence of livestock toxicosis [*38*].

In the ecological literature, seed borne, systemic grass endophytes are generally considered to be mutualists [e.g., *2*, *4*]. The fungus subsists entirely on the host's resources and is not free-living, and endophytes often confer benefits to their hosts through increased herbivore resistance [*32*, *33*], growth, reproduction, tolerance of drought and flooding, mycotoxin-based pathogen resistance, and enhanced competitive abilities [*5*, *7*, *9*, *19*, *25*]. However, accumulating evidence suggests that the nature of the relationship between grasses and endophytes may vary from antagonism to mutualism depending on such factors as availability of nutrients in the soil and complexity of the food web [*32*, *33*]. Accordingly, endophyte infection frequencies of grasses are highly variable among natural grass populations and species [*2*, *27*, *30*,*37*].

In this study, we examined tall fescue and its close relative meadow fescue {*Schedonorus pratensis*[((Huds.) P. Beauv.) (ex. *Lolium pratense* (Huds.) Darbysh)]}. The latter is a commonly used forage grass in Northern Europe. First, we recorded systemic endophyte infection frequencies of 13 commercially available European cultivars and 49 wild tall fescue populations in the northern distributional limits of this species in Finland, Sweden, and Estonia. In previous studies on meadow fescue in Northern Europe, systemic endophyte infections were commonly detected both in cultivars and wild populations [*24*, *28*, *30*]. The majority of cultivars were infected, while infections were detected in less than half of the wild meadow fescue populations that were examined [*30*]. Furthermore, infection levels of cultivars of meadow fescue were higher and less variable than natural populations [*30*].

In natural populations, the distribution of endophytes and infection frequencies largely depend on the reproductive success of infected hosts compared to their uninfected conspecifics.

Therefore, we also correlated the presence of endophytes with seed production and seed predation caused by cocksfoot moth (*Glyphipterix simpliciella* Stephens) in field collected samples and in common garden experiments involving tall fescue and meadow fescue. Several smaller data sets were combined to make one large study. Based on the hypothesis that grass endophytes are plant mutualists [*4*, *5*, *7*, *8*], we predicted that (1) systemic, seed borne endophytes should be common in tall fescue; (2) infection frequencies of cultivars are less variable than infections in wild populations because of selective breeding; and (3) the presence of systemic, seed borne endophytes increase seed production and protect the host grass from seed predation [*6*, *16*, *18*].

Materials and Methods

Tall Fescue

We selected tall fescue as one of the study species because the potential effects of endophytes on tall fescue have received relatively little attention in ecological research and agricultural management in Europe. In the USA, tall fescue is non-native but is the most commonly used grass in endophyte host studies and is of great agronomic importance [*2*]. Tall fescue is a long-lived perennial bunchgrass, commonly infected by systemic *N. coenophialum* (Morgan-Jones & Gams) Glenn, Bacon & Hanlin endophyte [*30*]. Typically, tall fescue has a long growing season, and it is 60 to 120 cm tall in the stage of producing seeds. Tall fescue is a wind pollinated and a self-incompatible grass species which spreads through seed transmission only—not by stolons or rhizomes, which is common in many other grass species. However, tall fescue may have numerous sterile shoots that extend the width of each bunch. Tall fescue is native in Europe, and it has been introduced in North and South America, Australia, New Zealand, and south and east Africa because of its value as a pasture grass [*10*]. Plant breeders in Finland and Scandinavia are increasingly planting commercial cultivars of tall fescue in pastures [*20*]. Tall fescue is distributed throughout Europe north to 62° in Scandinavia where it is mainly limited to coastal areas around the Baltic Sea [*10*].

Meadow Fescue

We chose meadow fescue as another study species because it is more commonly used in pastures and as forage compared to tall fescue in Europe. Meadow fescue is a close relative of tall fescue and commonly infected by systemic *Neotyphodium uncinatum* (Gams, Petrini & Schmidt) Glenn, Bacon, Price & Hanlin endophyte [*28*, *30*]. Meadow fescue is one of the most important forage grasses of Finland and Scandinavia. It is a wind-pollinated and self-incompatible grass species that is native to Europe, also commonly occurring outside of agronomic use in meadows, roadsides, and wastelands [*15*].

Cocksfoot Moth

Cocksfoot moth Stephens was chosen in this study because the larvae of the moth are common seed predators of different fescue species in Europe and may cause serious reductions in seed production of grasses [*1*]. Effects of *Neotyphodium* endophytes on this seed predator have not been studied previously, although the effects of *Neotyphodium* in tall fescue on some other seed predators are known [e.g., *2*]. Larvae feed in the florets of the host plant, hollowing out the kernels of the seeds. Each larva consumes about ten seeds before becoming fully grown. The fully grown larva moves down the culm and bores a hole into the stem where it spins a cocoon and overwinters. Adult cocksfoot moths hatch in summer and lay eggs in florets of the host plant. Larvae hatch in 10–15 days [*1*].

Endophyte Infection Frequencies in Tall Fescue

To determine frequencies of endophyte infections in tall fescue cultivars, we microscopically examined ~100 seeds [*29*] from 13 cultivars ("Arminda," "Barbitzon," "Barcel," "Barfelix," "Bariane," "Bonnet," "Cochise," "Elfina," "Kora," "Kord," "Max," "Retu," and "Wrangler") for their infection status. All of these cultivars originate from Central Europe, except for "Retu" which is a Finnish cultivar. The seeds were obtained from the Plant Production Inspection Centre, Seed Testing Department, Loimaa, Finland.

To determine endophyte infection frequencies within and among different wild populations of tall fescue from various sites in Northern Europe, tall fescue seeds were collected from 49 populations from Sweden (Södermanland and Gotland) and Finland (Åland islands) in the summer of 2003 and from Estonia in 2004 (Fig. 1). Seeds were haphazardly collected from five to 22 wild populations from each site and from two to 64 plant individuals from each population (depending on the size and number of the populations; Fig. 1). Three seeds per parental plant were stained and examined microscopically for endophyte status [*29*].

Figure 1 Tall fescue (*S. phoenix*) populations sampled in Sweden (coast of mainland in Södermanland and Gotland), in Finland (Åland islands), and in Estonia. *Squares* represent the populations where seeds were collected to study endophyte infection frequencies. *Circles* represent the populations where seeds were collected to study both endophyte infection frequencies and cocksfoot moth (*G. simpliciella*) seed predation

Seed Production

Seed production was determined for individual plants of tall fescue and meadow fescue in long-term common garden experiments at the Turku Botanical Garden, University of Turku and at MTT Agrifood Research Finland, Jokioinen, respectively. For tall fescue, we used the bulk seed of E− and E+ plants collected from Finland (Åland) and Sweden (Gotland and Södermanland) in 2003 and seeds of two cultivars, “Kentucky-31” (KY-31: E− and E+ seeds) and the E− cultivar “Retu.” The cultivar “Retu” is a Finnish cultivar free from endophytes, and thus only E− seeds of this cultivar were used. Seeds were germinated and planted in mixture of potting soil and sand in individual pots in the greenhouse in summer 2004. A field plot was tilled without nutrient applications and fenced to prevent large herbivores from browsing the experimental plants. In August 2004, ten seedlings of E− and E+ plants of each wild site (Åland, Gotland, and Södermanland) and “Kentucky-31” cultivar and ten plants of “Retu” cultivar (only E−) were assigned to a randomized complete block design with ten replicates. We use “site” to refer to both physical location (Gotland, Södermanland, and Åland) and agronomic cultivars (“KY-31” and “Retu”) further on in the article. Plants with two to three tillers each were planted into the field 0.5 m apart from other plants. The space between the experimental plants was either hand weeded or sprayed with herbicide (glyphosate Roundup®Bio) two times during the growing season to prevent interspecific competition between weeds and experimental plants. In the summer of 2005, the infection status of all the experimental plants was double-checked. We sampled a leaf from each plant for immunoblot assay to detect monoclonal antibodies specific to *Neotyphodium* (Phytoscreen ImmunoblotKit #ENDO7973, Agrinostics, Watkinsville, GA, USA). In addition, at the end of the summer, three seeds from each plant were stained [*29*], and endophytic infections were checked by microscopic examination. All plants were of the expected endophyte status. At the end of August 2007, when seeds had matured but before the seeds shattered, we collected all the inflorescences of each experimental plant, removed the seeds, and weighed them separately for each plant.

For meadow fescue, E− and E+ seed lots of cultivar “Kasper” (one of the most widely used meadow fescue cultivars in Finland) were grown in a common garden experiment. Seeds were obtained from the Plant Production Inspection Centre, Seed Testing Department, Loimaa, Finland. Infection frequencies of the E− and E+ seed lots were determined to be 0% and 95%, respectively, by staining 50 seeds per each lot and examining them microscopically. In 2002, E+ and E− seed lots were randomly assigned and sown in five plot pairs so that E+ seeds were sown to one plot and E− seeds to the other plot within each plot pair (2 × 5 array, each 15 × 20 m). Before sowing, the field was tilled, and a commercial fertilizer (16:9:22 (N:P:K) with micronutrients, Kemira, product number 0647334) was applied. In July 2007, seeds of five to eight randomly selected meadow fescue plants from each plot were collected, and infection status of the plants was checked microscopically from two seeds per plant [*31*], and the number of seeds was counted separately for each plant.

Seed Predation Caused by Cocksfoot Moth

For tall fescue, we determined natural seed predation in wild grass populations from the seed samples collected from grasses with known endophyte infection status (see frequencies of endophyte infections in tall fescue) from Finland (Åland) and Sweden (Södermanland and Gotland) in 2003 (from eight to 25 grasses from each of the three to five populations in each site; Fig. 1). Seeds collected from E− and E+ plants from each site were combined into bulk E− and E+ seed. We randomly selected ten groups of 100 seeds from each bulk seed lot (1,000 seeds total for each bulk seed lot). We then ascertained seed predation by examining each seed for characteristic *G. simpliciella* bore holes in the surface of the seed coat [*3*].

For meadow fescue, we randomly selected five to eight plant individuals from each plot in the common garden experiment in July 2007. We examined all the seeds from each plant for bore holes.

Statistical Methods

All statistical analyses were performed using SAS 9.1 (Enterprise Guide 4.0), with the MIXED procedure. Endophyte status, site, and endophyte status × site interaction were fixed effects in the analysis of seed predation of wild tall fescue populations. Endophyte status, site, and endophyte status × site interaction were fixed effects, and block was random effect in the analysis of tall fescue seed biomass. Endophyte was a fixed effect, and replicate and replicate × endophyte interaction were random effects in the analysis of meadow fescue seed production and seed predation. All response variables except seed predation of wild tall fescue populations were square root transformed to meet the assumptions of normality. All pairwise comparisons were calculated using paired *t* test.

Results

Endophyte-infected seeds were detected only in one ("Wrangler") out of the 13 tall fescue cultivars that we examined. Furthermore, infection level was <10% in the infected cultivar "Wrangler." In contrast, 46 of the 49 wild populations that we sampled harbored endophyte infection. Furthermore, endophyte frequencies were high (>90%) in all of the populations that harbored the endophyte. The three endophyte-free populations were clustered in inland Estonia (Figs. 1 and 2). These infection-free populations in Estonia may be descendants of agronomical tall fescue cultivars, which may explain lack of endophytes in these populations.

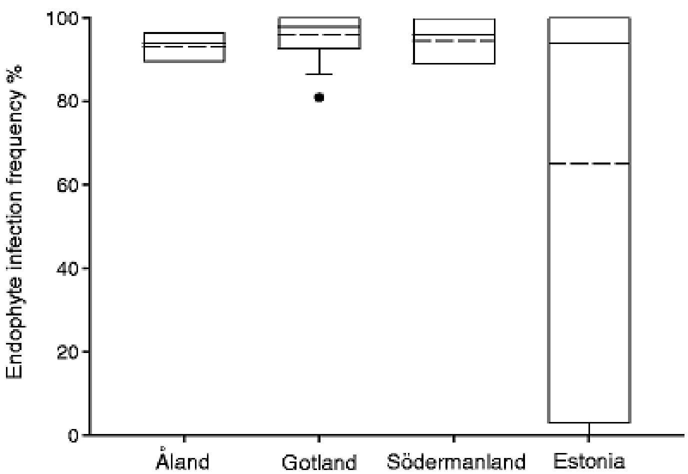

Figure 2 Endophyte infections in wild tall fescue (*S. phoenix*) populations. *Dashed lines* denote mean values and*solid line* medians. The *upper* and *lower boundaries of boxes* represent upper and lower quartiles, respectively. *Vertical bars* represent the tails of distribution. *Dots* represent outliers

Seed mass of tall fescue did not differ between E− and E+ plants (E− vs. E+, $F_{1,79} = 0.35$, $p = 0.59$), and infection status did not interact with plant site (infection status × site, $F_{3,79} = 0.35$, $p = 0.79$). However, seed biomass varied significantly among sites ($F_{4,79} = 3.82$, $p = 0.007$). Seed mass of tall fescue was greatest in the E− Finnish cultivar "Retu" (mean = 52 g, SE = 7.45, $n = 10$) and second greatest in E+ "Kentucky 31" grasses (mean = 43 g, SE = 13.20, $n = 10$; Fig. 3). Although the mean seed mass was 37% higher in E+ compared to E− "Kentucky 31" grasses, the difference remained statistically insignificant because of high variance in seed production among E+ grasses ($t_{79} = 0.65$, $p = 0.52$; Fig. 3). In contrast to tall fescue, the mean number of E+ meadow fescue seeds was marginally higher than E− ($F_{1,57} = 3.76$, $p = 0.058$; back-transformed estimates of least square means, E+ 224 (95% CI = 171 − 285) and E− 152 (95% CI = 109 − 202)).

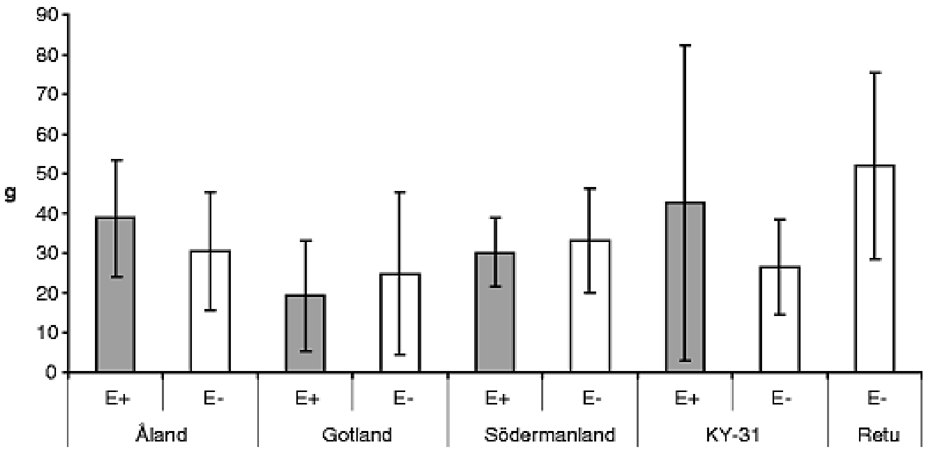

Figure 3 Seed mass of endophyte-infected (E+) and endophyte-free (E−) tall fescue (*S. phoenix*; means ± SD)

Tall fescue E+ seeds from wild populations showed less seed predation by cocksfoot moth than E− seeds ($F_{1,54} = 32.20$, $p < 0.001$). Percent of seed predation also varied significantly among the sites ($F_{2,54} = 141.83$, $p < 0.001$); 17%, 22%, and 56% seeds were preyed upon in Åland, Gotland, and Södermanland, respectively. E+ tall fescue seeds from the two populations with heavy seed predation showed less seed predation than seeds from E− grasses (infection status × site, $F_{2,54} = 4.46$, $p = 0.016$; Åland $t_{54} = 0.95$, $p = 1.0$; Gotland $t_{54} = 5.07$, $p < 0.0001$; and Södermanland $t_{54} = 3.81$, $p < 0.0053$; Fig. 4).

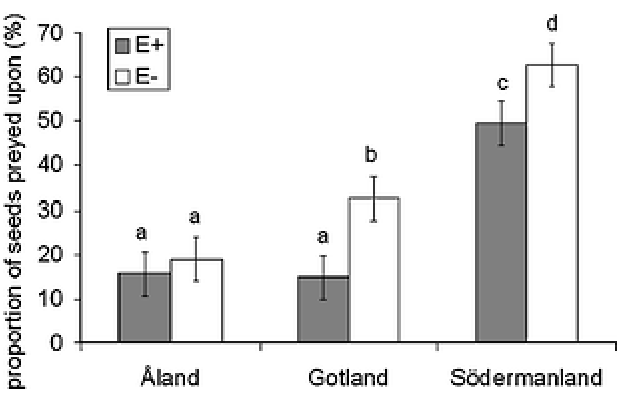

Figure 4 The proportion of tall fescue (*S. phoenix*) seeds preyed upon by cocksfoot moth (*G. simpliciella*; estimates of least square means and 95% confidence limits). Different *letters* indicate significant ($p < 0.05$) differences between groups

In contrast to tall fescue, only 15% of the meadow fescue seeds were preyed upon. Furthermore, there was no difference in percent of seed predation on E+ and E− seeds ($F_{1,57} = 1.83$, $p = 0.18$; back-transformed estimates of least square means, E+ 29.0 (95% CI = 18.5 − 41.9) and E− 18.9 (95% CI = 10.7 – 29.4)).

Discussion

The high endophyte infection frequencies in wild tall fescue populations corroborate past studies, suggesting that infection may provide some selective advantage to tall fescue host grasses in Europe [*4*, *7*, *30*, *35*, *37*]. However, endophyte infection frequency does not necessarily reflect selective pressures in nature. This may be true for instance if *Neotyphodium* endophytes are horizontally transmitted from plant to another [*2*, *31*]. Endophyte infections among tall fescue populations are common, and infection frequencies appear to be very high across Europe up to the northernmost grass populations at 62° in Scandinavia [*10*, *21–23*, *30*]. Despite the exceptionally high infection frequencies in tall fescue populations throughout its native distribution range in Europe [*10*], native tall fescue is not an aggressive competitor in Europe nor a dominant species. Instead, tall fescue occurs sporadically in damp pastures, wet meadows, and coastal areas [*10*]. Tall fescue was introduced from Europe to the USA in 1800s and then planted widely as the cultivar K-31 in the mid-twentieth century and is now considered by some [*7*] as a tenacious invasive species that may threaten native biodiversity of semi-natural grasslands.

In contrast to high infection frequencies in nature, tall fescue cultivars were almost endophyte-free, suggesting that the cultivars have either lost infections during breeding programs or the endophyte has been intentionally removed by breeders. Endophyte infections can be lost from seeds during long storage or unfavorable conditions or if the fungus fails to grow from the maternal plant into all host tillers and seeds [*26*,*36*]. Such a partial loss of infection should however result only in variable infection levels in some of the cultivars, rather than a complete loss, especially if infection confers a selective advantage. Thus, we propose that an overarching absence of infections in the examined tall fescue cultivars suggests that the endophyte may negatively correlate with desired traits that are selective bred into the cultivars, such as rapid establishment and growth of the grass or palatability to livestock.

Our results suggest that endophyte infection benefits tall fescue host grasses by enhancing host resistance to seed predators rather than through enhanced reproductive effort. The mean seed mass of tall fescue did not differ between E− and E+ plants. However, seeds from E+ plants showed significantly lower proportions of seed predation compared to seeds from E− plants, at least in the two populations with severe losses to seed predators. This result, combined with previous evidence that *N. coenophialum* increases tall fescue resistance to both vertebrate and invertebrate herbivores [*32*, *33*], suggests that seed predation and herbivory may select for E+ tall fescue plants in northern European populations. Endophyte-mediated resistance to herbivory is largely ignored in agricultural management of pastures in Europe, in contrast to the USA and New Zealand, probably because botanically diverse permanent pastures allow livestock to choose other pasture species over tall fescue and thus avoid fescue toxicosis [*10*, *38*]. Also, the colder, wetter climates of Northern Europe may ameliorate the toxic effects of endophyte-mediated mycotoxins on livestock compared to warmer, drier climates where tall fescue has been imported [*11*, *12*]. Plant breeding programs in Europe have mainly operated on plant characters other than herbivore resistance of grasses. These characters include seed and biomass production, which correlate with plant genotype as indicated by the significant effect of site [see also *21*, *22*].

In contrast to tall fescue, E+ meadow fescue plants produced marginally more seeds than E− plants in our experiments. More frequent endophyte infections in meadow fescue [*24*, *28*, *30*] relative to tall fescue cultivars may reflect differences in breeding programs for tall fescue and meadow fescue and more intensive use of meadow fescue as a forage grass in Europe.

In contrast to natural tall fescues, seed predation by cocksfoot moth on cultivated meadow fescue did not differ between seeds from E+ and E− plants. However, only 15% of meadow fescue seeds were preyed upon, in contrast to much higher rates on tall fescue. The difference in predation rates between E+ and E− tall fescues only became apparent under more severe seed predation pressure. Thus, endophyte-mediated resistance to seed predators of meadow fescue could become important when predation rates are higher. Furthermore, previous studies have revealed that endophytes can increase meadow fescue resistance to herbivores, including aphids and voles [*14*, *17*, *28*]. Thus, herbivory may also be important factor in maintaining endophyte infections in meadow fescue.

Our results suggest that the effects of endophytes vary among grass species and their cultivars and may depend on seed predation pressure and other environmental conditions. This variability in the effects of endophytes may result from several sources. In wild grasses with high genetic variation, endophyte-mediated effects on the host may be more variable than in genetically more uniform cultivars [*2*]. Second, the different endophyte species that infect tall fescue and meadow ryegrass may have very different effects on various vertebrate and invertebrate herbivores [*2*]. Third, mycotoxin production, notably alkaloids, by endophytes is conditional on environmental factors such as soil nutrients or weather conditions [*12*, *32*, *33*] and plant and endophyte genotype [*2*]. Thus, successful breeding programs that aim to maximize yield production, improve forage quality, and minimize pest damage by increasing resistance require a comprehensive view of how endophytic fungi and their host grasses co-evolve and respond as a unit to prevailing environmental conditions. Our study also emphasizes the importance of adapting forage grass breeding programs for regional environmental selective pressures.

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