Endophyte infection of *Festuca eskia* enhances seedling survival to drought and cutting at the expense of clonal expansion

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Abstract

**Aims**
Symbiotic relationships between fungal endophytes and grass species are known to increase stress resistance in the grass host; however, there is little evidence to suggest that the positive effects occur early in the grass life cycle. In this study, we explored the effects of the endophyte *Epichloë festucae* on the growth and survival of *Festuca eskia* seedlings under drought and frequent cutting stress.

**Methods**
*Festuca eskia* seedlings were collected from the western part of the plant repartition area in a non-symbiotic population located in a mesic and heavily grazed site (W-NS) and from the eastern part in a symbiotic population from a xeric and lightly grazed site (E-S). The E-S population was experimentally freed from its endophyte (E-F). Two greenhouse experiments were conducted to compare growth and survival between the three seedling types under drought stress and frequent cutting. In the first experiment, 126 seedlings per seedling type (n = 378) were grown for 6 weeks under non-limiting conditions before the cessation of watering. After 3 weeks without irrigation, full irrigation was restored for 10 days to measure the survival rate. Leaf length, leaf elongation rate and survival rate were assessed per population. In the second experiment, 156 seedlings per seedling type (n = 468) were grown under non-limiting conditions. All seedlings were cut to 3 cm high, twice a week, during the first month of growth. Leaf elongation and tillering were monitored on 52 seedlings per seedling type. For each type, seedling survival rate was determined by the number of plants alive after 10 days of regrowth, without cutting.

**Important Findings**
The drought experiment revealed a phenotypic differentiation to drought in 30 *F. eskia* populations, suggesting adaptive differentiation: the eastern seedlings showed the highest survival rate. A trade-off between growth and survival was highlighted: the highest drought survival rate was associated with the lowest leaf elongation rate under non-limiting water conditions. Endophyte presence in the eastern population increased seedling drought survival. In contrast, cutting survival rate was similar between W-NS and E-S because the presence of the endophyte increased seedling survival to frequent cutting. However, this positive effect came with a cost: the endophyte reduced seedling tillering rate.

**Keywords:** *Epichloë festucae* • water stress • cutting • fitness • native grass • mutualism

Received: 3 September 2010 Revised: 17 December 2010 Accepted: 23 January 2011

INTRODUCTION

All plants in natural ecosystems are thought to be symbiotic with mycorrhizal and/or endophytic fungi (Burnett 2003; Petrini 1996). In addition, recent studies suggest these symbioses facilitated land colonization by plants (Simon et al. 1993) and continue to promote adaptation of plants and other organisms to harsh or resource deficient environments (see e.g. Johnson et al. 2010; Redman et al. 2002; Rodriguez et al. 2008). Symbioses are now considered essential for the development and persistence of all living systems (Douglas 2010).

Hereditary symbioses between endophytic fungi and grasses are well-known examples of symbiosis that may be implicated in plant adaptations to their environment. The primary benefits include resistance to herbivores via production of fungal alkaloids (Cheplick and Faeth 2009b; Popay and Bonos 2005) and enhancement of drought and mineral stress tolerance (Cheplick and Faeth 2009a; Malinowski et al. 2005).
Vertically transmitted fungal endophytes grow symptomatically (i.e., without external sign of infection) in aerial plant tissues and endophyte transmission occurs via host seed, directly coupling the reproduction and propagation of both symbiotic partners. In natural ecosystems, 20–30% of grass species are estimated to be associated with a fungal endophyte therefore, the endophyte/grass symbiosis is suspected to have a substantial impact on plant communities and population dynamics (Leuchtmann 1992; Rudgers et al. 2010).

In comparison to numerous studies with cultivated grass species, only a few have been devoted to endophyte symbioses in naturally occurring grass populations (Ahlholm et al. 2002; Kannadan and Rudgers 2008; Morse et al. 2007; Rudgers and Swafford 2009). The beneficial effects of endophytic fungi have principally been reported from studies on cultivars of two agronomically important grasses (Festuca arundinacea and Lolium perenne) and may not necessarily occur in wild grasses (Brem and Leuchtmann 2002; Faeth and Sullivan 2003; Faeth et al. 2004). A growing amount of literature reports that the nature of the endophyte/grass symbiosis is not always mutualistic and varies depending on environmental conditions and endophyte/grass genotypes (Müller and Krauss 2005; Saikkonen et al. 2006).

The percentage of symbiotic plants in spontaneous grass populations has been reported to exhibit the entire range of endophytic status from 0 to 100% (Bazely et al. 2007; Gonzalo-Turpin et al. 2010; Lewis et al. 1997; Novas et al. 2007; Rudgers et al. 2009; Saona et al. 2010; Zabalgogeazcoa et al. 1999). Among the biotic and abiotic environmental factors, drought and herbivory are thought to be the two major forces driving this range in spontaneous plant populations; it decreases in grass populations with water availability (Gonzalo-Turpin et al. 2010; Lewis et al. 1997; Novas et al. 2007; Saona et al. 2010) and increases with grazing pressures (Bazely et al. 2007; Clay et al. 2005; Koh and Hik 2007; Koh and Hik 2008).

Theoretical modeling analysis of the dynamics of endophyte infection in annual grass populations yielded that an exceedingly small advantage, which may be very difficult to measure, is sufficient to maintain high rates of infection (Clay 1990; Gundel et al. 2008; Ravel et al. 1997). Plants are especially vulnerable to environmental constraints during the early life stages. However, the role of endophytes in drought and grazing resistance in its host has been extensively studied in nature but relatively neglected in early plant stages. Recent studies have revealed the effects of Epichloë endophytes on fine fescue seedling growth, which are dependent on the nature of the endophyte/grass symbiosis, half of the seeds collected from the W-NS population were released from their endophytic fungus and formed the third seedling type (E-F). This treatment allowed the partitioning of population locality and symbiotic status effects in the eastern population.

MATERIALS AND METHODS

Study system

_Epichloë festucae_ Leuchtmann, Schardl and Siegel (Ascomycota: Clavicipitaceae) is a fungal endophyte that forms systemic intercellular infection in cool-season grasses. The endophyte grows symptomatically in aerial tissues of plants and is transmitted vertically by seeds (asexual reproduction) and/or horizontally by ascospores (sexual reproduction). However, sexual reproduction has not been observed in its host species Festuca eskia. Associated with _Festuca_ sp., _E. festucae_ is known to produce alkaloids that deter vertebrate and invertebrate herbivory (Clay and Schardl 2002; Jensen et al. 2007; Wilkinson et al. 2000), and induces plant drought resistance (Schardl 1996).

_Festuca eskia_ Ramond ex DC. (Poaceae) is an endemic native perennial grass of the Pyrenees and Cantabric Mountains that harbors an asexual form of the endophytic fungus _E. festucae_ (Gonzalo-Turpin et al. 2010). _Festuca eskia_ is a dominant species of the subalpine and alpine Pyrenean communities (>1 500 m) and forms the foundation of many subalpine species-rich plant assemblages of the Pyrenees (Tosca 1986). According to European Economic Community laws (92/43/EEC habitat directives 1992), the vegetation dominated and structured by this species must be locally preserved because of its high ecological and patrimonial value. _Festuca eskia_ (2n = 14) is described as outcrossing and wind pollinated (Baudier et al. 1973), and occupies large areas forming dense south-oriented swards. The species is structured in two genetically distinct eastern and western populations (Gonzalo-Turpin et al. 2010). Eastern populations experience a drier climate and higher infection rates by _E. festucae_ than western populations, suggesting an endophyte role in drought resistance.
The *F. eskia* life cycle can be broken down into four life stages: (i) seeds, (ii) seedlings, corresponding to plants less than 1 year old, (iii) juveniles, plants older than 1 year that never reproduce and (iv) adults that reproduce at least once.

**Seedling type sampling**

From June to August 2006, we collected 2 800 seeds from 30 maternal plants representing two genetically distinct populations in the Pyrenees (Gonzalo-Turpin et al. 2010). The 1 000-seed weight was determined for both populations. The two populations are located on the two edges of the Pyrenean Massif. The W-NS population is located in the western part of the massif at Ansabe`re (42°90′N–1°85′W) and the E-S population in the eastern part at Puymorens (42°58′N–1°85′E). These populations present contrasting endophytic frequencies and environmental conditions. The W-NS population exhibits <7% of symbiotic plants, whereas all plants from the E-S population were naturally symbiotic (Gonzalo-Turpin et al. 2010). The western population corresponds to a mesic site (water balance: +1 100 mm; i.e. precipitation—evapotranspiration in Météorologie Nationale Bessemoulin 1989), with a high level of grazing (summer stocking rate: 120–200 ewes and 20 cows; proportion of grazed plants: 50%). In contrast, the eastern population is exposed to a more xeric habitat (water balance: +200 mm), with low grazing pressure (summer stocking rate: 6–10 horses, proportion of grazed plants: <5%).

**Endophyte screening and elimination**

We compared the following three seedling types for our experiments: (i) naturally non-symbiotic seedlings from the western population (W-NS); (ii) naturally symbiotic seedlings from the eastern population (E-S) and (iii) fungicide-treated seedlings from the eastern population (E-F). For W-NS and E-S seedlings, endophyte infection status was screened using the Phytoscreen tissue print immunoblot kit in five seedlings from each maternal plant, i.e. 150 seedlings per population (Agrinostics Ltd, Inc., Watkinsville, GA, USA) (Koh et al. 2006). This method was validated by endophyte visualization under the microscope using histological staining (Hatt et al. 1999). Imperfect transmission of endophytes from the maternal plant to seeds occurs in plant grass. Consequently for the experiment, the seedlings used were only from maternal plants that exhibited either 0 or 100% endophyte infection in tested seedlings.

E-F seedlings were obtained by eliminating the endophyte in one half of the symbiotic seeds of the E-S population using a standard fungicide treatment, without phytotoxic effects at low concentration (prochlorazene; BASF Society Agro SAS; 0.50 g kg⁻¹ of seed) (Leyronas et al. 2006). Fungicide was applied with a batch seed treatment machine HEGE11 (HEGE, Germany). Each seed batch corresponding to each seedling type was treated according to the slurry method. Slurry was applied at 20 l kg⁻¹ of seed. After the chemical application, the seeds were dried over a period of 5 days in ambient conditions and stored at 5°C until the beginning of the experiment. To assess treatment success, we grew 90 seedlings from chemically treated seeds and scored 88 to be endophyte free (98%). We tested germination rates between treated and untreated seeds of the naturally non-symbiotic population (W-NS) to evaluate the presence of phytotoxicity in 100 seeds. Fungicide phytotoxicity was not detected as no significant difference between treated and untreated seeds of W-NS populations was observed (P < 0.05). However, long-term effects on seedling growth were not tested.

**Greenhouse experiments**

Two experiments were conducted under greenhouse conditions to assess the effects of the host’s genetic background and endophytic status on *F. eskia* seedling growth and survival to drought stress and frequent cutting. Maternal effects were partially determined by measuring 1 000-seed weight per population. In both experiments, the seeds were cold treated at 4°C for 4 weeks, subsequently sown on moist filter paper in Petri dishes and incubated at 20°C for 2 weeks in a growth chamber. Germination rates were recorded for the three seedling types. Seedlings were transplanted to pots once the leaves were twice the length of the seeds.

In the first experiment, 126 seeds per seedling type (n = 378) were sown as single seedlings in 8 x 8 x 7.5 cm deep plastic pots, filled with 80% sand, 10% loam and 10% clay. After potting, seedlings were grown in a greenhouse for 6 weeks with a 14 h length day, light intensity of 12.5 MJ m⁻² day⁻¹ and a day/night temperature of 20°C/5°C. During this period, seedlings were fertilized weekly (N:P:K = 19:2.6:10) to avoid nutrient deficiency. The pots were randomly arranged in the greenhouse. During 6 weeks, seedlings were grown under non-limiting water conditions. Soil water content (SWC, gH₂O/g dry soil, %) was determined by gravimetry in all pots twice a week, allowing to maintain all pots at 80% of soil capacity. The drought stress was initiated once the plant’s roots had reached the bottom of the pots. At that time, seedlings exhibited on average 3–4 tillers. Plants were subsequently cut to 5 cm high to prevent any difference in plant transpiration. Irrigation was ceased at the same time for all seedlings and as a consequence, daily increasing water deficits were experienced. Following 3 weeks without irrigation, full irrigation was restored for 10 days to measure survival rates. The survival rate in each seedling type was measured by the number of seedlings that revived during 10 days following rehydration. Every 3 days for the last 2 weeks under non-limiting conditions, and during the drought stress period until leaf growth ceased, leaf elongation was measured on the youngest expanded leaves of 32 seedlings per seedling type. Leaf elongation was expressed in mm per growing degree days (GDD) in order to take into account the slight variation in temperature that occur at plant level (Hazard et al. 2006). The response of leaf elongation to temperature in *F. eskia* was considered linear from 0 to 20°C. GDD was the thermal time corresponding to the sum of average daily temperature above 0°C from the beginning of the
experiment. The leaf length of the last adult leaf was also measured on 32 seedlings per seedling type under non-limiting conditions.

In the second experiment, 156 seedlings (n = 468) per seedling type were sown as single seedlings in 10 × 10 × 10 deep plastic pots, filled with 60% loam and 40% sand. Seedlings were grown in a greenhouse for 6 weeks, under the same experimental non-limiting conditions as the first experiment. All seedlings were cut at 3 cm high, twice a week, during the first month of growth. Leaf elongation was monitored on 52 seedlings per seedling type. Leaf elongation (as measured in the first experiment) and the number of tiller under non-limiting conditions and at the end of cutting stress were measured on 52 seedlings per seedling type. For each seedling type, the survival rate was determined by the number of plants alive 10 days after the final cutting.

Statistical analyses

The data were analyzed using S-plus 6.0 software (Lucent Technology, Inc.). Significant differences in 1 000-seed weight and germination rates between W-NS and E-S populations were analyzed using the Student’s t-test. Deviance analysis was performed to test the effect of ‘seedling type’, ‘experiment’ (cutting or drought stress) and their interaction on seedlings survival. One-way analysis of variance (ANOVA) was used to test the effect of seedling types on adult leaf length and the initial and final number of tillers. Post hoc Tukey tests were performed to detect any significant differences among means. For each experiment, analysis of covariance (ANCOVA) was used to evaluate significant differences between seedlings type in leaf elongation, dependent on SWC or GDD as the covariate. Normality of residuals and homogeneity of variance were verifed.

RESULTS

Germination rates and 1 000-seed weight

Germination rates were significantly different between W-NS and E-S populations (P = 0.002; W-NS: 29 ± 1%; E-S: 86 ± 0.4%; mean ± SE). No significant difference in 1 000-seed weight was detected between the W-NS and the E-S populations (P = 0.073; W-NS: 2.11 ± 0.05, E-S: 1.85 ± 0.15; mean ± SE).

Seedling survival rates

Analysis of deviance shows significant effects of experiment (P = 0.0001) and seedling types experiment interaction (P = 0.0001) on seedling survival but no global effect of seedling types (P = 0.171). Seedling survival was significantly higher following the frequent cutting than in the drought stress experiment (79 vs. 35%). Under drought stress, E-S and E-F seedling survival rates were significantly higher (2-fold) than the W-NS seedlings (P = 0.025; Fig. 1). Among the eastern population, drought survival was significantly higher in E-S than in E-F seedlings (P = 0.035; Fig. 1). Under cutting stress, seedling survival varied according to endophytic status: E-F seedling survival was significantly lower (2-fold) than that of E-S and W-NS seedlings (P = 0.0001; Fig. 1).

Seedling growth

One-way ANOVA shows that in the drought stress experiment, adult leaf length under non-limiting conditions was significantly different between seedling types (P = 0.0001). E-S seedlings had significantly smaller adult leaves than W-NS seedlings (E-S: 68 mm ± 4 vs. W-NS: 109 mm ± 4, P = 0.0005) but longer adult leaves than E-F seedlings (49 mm ± 2; mean ± SE, P = 0.0001).

In the frequent cutting experiment, the one-way ANOVA shows also a significant effect of seedling types on the initial (P = 0.002) and final (0.0049) number of tillers. Post hoc test shows that under non-limiting conditions, the E-F seedlings exhibited a significantly higher number of tillers than E-S seedlings (E-F: 5.26 ± 0.2 vs. E-S: 3.06 ± 0.6; mean ± SE, P = 0.049). However, E-F and E-S tiller numbers were not significantly different from W-NS (W-NS: 4.62 ± 0.8, P = 0.096). These differences remained the same at the end of the cutting experiment. In the same way than under non-limiting conditions, the E-F seedlings had a higher number of tillers than E-S (E-F: 7.76 ± 0.54 vs. E-S: 5.8 ± 0.45; mean ± SE, P = 0.025) and E-F and E-S were not significantly different from W-NS (W-NS: 6.8 ± 0.59, P = 0.089).

For the drought stress experiment, ANCOVA shows the significant effects of SWC (P = 0.0001), seedling type (P = 0.0001) and their interaction (P = 0.0001) on leaf elongation. Leaf elongation decreased significantly during drought stress. The slope and the intercept of the linear regressions between leaf elongation rate and SWC were significantly different between W-NS and E-S seedlings (Fig. 2a). The leaf elongation rate of
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Figure 2: relationship between (a) seedling leaf elongation rate (mm GDD-1) or (b) SWC (%) under drought stress and GDD (°C) under frequent cutting in Festuca eskia (circle: non-symbiotic western seedlings; closed triangle: symbiotic eastern seedlings; open triangle: endophyte free eastern seedlings). Linear regression: solid line [non-symbiotic western seedlings (a) \( r^2 = 0.99 \), (b) \( r^2 = 0.87 \)]; dotted line [symbiotic eastern seedlings (a) \( r^2 = 0.90 \), (b) \( r^2 = 0.97 \)] and dashed line [endophyte free eastern seedlings (a) \( r^2 = 0.95 \), (b) \( r^2 = 0.87 \)]. Mean ± SE (a) \( n = 32 \), (b) \( n = 52 \).

W-NS seedlings was higher under non-limiting conditions and decreased more rapidly under drought stress than in the case of E-S seedlings. In contrast, the slope and the intercept of linear regressions carried out for the E-F and E-S seedlings were not significantly different (Fig. 2a).

For the cutting experiment, ANCOVA shows significant effects of GDD \( (P = 0.0001) \), seedling types \( (P = 0.0001) \) and their interaction \( (P = 0.007) \). As in the drought stress experiment, the slope and the intercept of linear regressions between leaf elongation rate and SWC were significantly different between W-NS and E-S seedlings and not different between E-F and E-S seedlings (Fig. 2b).

**DISCUSSION**

*Festuca eskia* exhibited phenotypic divergence between the populations collected on both ends of the Pyrenean climatic range. Survival rate under drought stress was higher in E-S seedlings coming from the xeric site than in W-NS seedlings collected in the mesic site. In addition, when released from their endophyte, under drought conditions E-F seedlings exhibited a drought survival rate higher than W-NS seedlings. These results suggest that some kind of local adaptation on the part of the plant, rather than on the part of endophytic symbiosis, is responsible for the highest rate of survival in the E-S plants. This result is congruent with prior reports, where endophyte presence was found to reinforce adaptive differentiation to drought stress between natural populations of *L. perenne* (Gibert et al., submitted for publication). Endophyte presence increased seedling drought survival in the eastern population, which suggests phenotypic divergence is reinforced in E relative to the W-NS population. In contrast, Faeth and Hamilton (2006) found that seedlings of *Festuca arizonica*, submitted to a severe and prolonged drought, had a higher survival rate in non-infected plants than in infected plants, although infected and non-infected plants showed no difference in long-term survival. Our results support the hypothesis that summer drought may select for endophytic symbiosis in native populations. Neutral genetic differentiation observed in *F. eskia* by Gonzalo-Turpin et al. (2010) is likely associated with an adaptive differentiation to drought stress reinforced by endophytic symbiosis.

Initially, the two populations of *F. eskia*, although collected from two locations that differed in grazing intensity, did not show differentiation to frequent cutting. Seedlings from the E-S population collected from the lightly grazed site had a similar seedling survival rate to the W-NS seedlings, which originated from the heavily grazed site. However, the endophyte presence in E-S plants significantly increased seedling fitness: the E-S population exhibited an increased survival rate with frequent cutting relative to the E-F population. Consequently, the symbiosis in the E-S population compensates for decreased survival due to frequent cutting in contrast to the W-NS population. Therefore, not only may fungi endophytes induce herbivore defense in grass species via deterrence to herbivores but they may also enhance tolerance to herbivory. Tolerance to herbivory is the ability of a plant to compensate for tissue loss by rapid regrowth without loss of fitness (Del-Val and Crawley 2005; Strauss and Agrawal 1999). Previous studies concluded that endophyte infection decreases plant tolerance to frequent cutting based on a decrease in plant regrowth following artificial cutting in infected plants compared to non-infected plants (Belesky and Fedders 1996; Bultman et al. 2004; Cheplick 1998; Sullivan et al. 2007). Thus, the endophyte is viewed as a mediator to changes in plant strategy by promoting defensive processes (Sullivan et al. 2007). However, conclusions from these studies were made based on adult plants and not seedlings. Our study demonstrated that these tolerance processes occurred also at early plant developmental stages. Furthermore, all former studies tested the impact of infection on tolerance in terms of vegetative regrowth following artificial cutting. However, tolerance is defined in terms of host fitness, and our study determined host survival to be a major component of fitness, not just regrowth.
Seedling growth differs according to environmental conditions at population localities. The seedlings in our study seemed to show morphogenetic adaptation to the specific environment. Whereas there was no difference in number of tiller between E-S and W-S seedling types under non-limiting conditions, E-S seedlings from the xeric and lightly grazed site had a significantly lower leaf elongation rate regardless of the experiment and lower adult leaf length than the W-NS seedlings distributed in the mesic and heavily grazed site. This differentiation follows the well-known trade-off between growth under optimal conditions and survival rates under drought stress (Bazzaz 1996). Seedling drought survival in *F. eskia* is associated with a low growing potential under non-limiting conditions, described by Chapin et al. (1993) as the stress-resistant syndrome. In all seedlings, diminutive leaf elongation during drought stress is likely to be followed by low plant transpiration, leading to increased water maintenance in plant tissues (Reymond et al. 2003). In the same way, the ability of W-NS seedlings to produce the longest leaves with the highest leaf elongation rate likely result from positive fitness effects in the locality. A rapid production of long leaves is selected under competition for light in mesic grasslands (Hazard et al. 2001). It is also an investment strategy found in competitive species growing in more favorable environments (cf. Grime 1977). Under grazing pressures, competitive-like plants are tolerant to frequent cutting and compensate for tissues through fast regrowth (Del-Val and Crawley 2005; Strauss and Agrawal 1999; Tiffin and Rauscher 1999). Thus, in the W-NS population, data suggested plants were selected to compensate for herbivory. Although the W-NS and E-S populations exhibited a similar survival rate to frequent cutting, W-NS seedlings seem better adapted to intensive grazing due to a higher capacity for rapid regrowth.

Endophytic fungi significantly affected seedling leaf length and tiller numbers. The presence of endophytic fungi increased adult leaf length but significantly decreased tiller numbers, compensating for morphogenetic differentiation between E-F and W-NS populations. Leaf length expansion may be explained by a positive relationship with higher light competition, whereas the decrease in tiller number appears to be a cost of symbiosis, under non-limiting conditions. Indeed, tillering affects plant fitness through both seed production and plant persistence through exploration of the environment for limiting nutrients (Cook 1985; de Kroon and Hutchings 1995; Oborny and Cain 1997), and survivorship by spreading the risk of mortality among tillers (Cook 1979; de Steven 1989). Furthermore, while most alpine plants exhibit a strategy favoring clonal multiplication over sexual reproduction (Körner 2003), the endophyte seems to counteract this strategy. In contrast, leaf elongation did not differ between E-F and E-S seedlings ($P > 0.05$), regardless of growing conditions. This result was congruent with prior reports on native grasses, where endophyte presence did not affect (Morse et al. 2007) or increase plant growth and biomass compared to uninfected plants under low water availability (Kannadan and Rudgers 2008; Morse et al. 2002). However because phytotoxicity analyses were only carried out on seed germination rate in our study, we cannot definitely discard the possibility of a long-term effect due to the fungicide treatment. The fungicide may have been phytotoxic and weakened seedling growth or may have killed pathogenic fungi and promoted their growth.

In the Pyrenees, water availability and grazing pressure of natural rangelands vary across the range of *F. eskia*, eliciting different selective pressures on the species. Mesic rangelands under an oceanic influence sustain much higher stocking rates than xeric rangelands under a Mediterranean climatic influence. Our results suggest that the pattern of *E. festucae* frequencies in *F. eskia* populations is a combined effect of selective advantage to drought stress and grazing pressures, results congruent with Saona et al. (2010) in *F. rubra*. However, our study supports a hypothesis that drought is even a stronger selective force driving endophytic frequencies than grazing in *F. eskia* populations.

Our study clearly demonstrates that phenotypic differentiation occurs in *F. eskia*, which impacts seedling survival. Furthermore, a symbiotic relationship with a fungal endophyte contributes to differentiation in the species that is likely adaptive. Our work highlights the beneficial nature of endophyte/ grass interaction on seedling stress survival but also reveals the cost of symbiosis that reduces plant clonal expansion. Our results show that abiotic and biotic factors influence endophyte symbioses in native grasses and suggest that field experiments, such as reciprocal transplants, are needed to confirm the adaptive effect of endophyte symbioses in plant local adaptations.

ACKNOWLEDGEMENTS

We are grateful to Drs. Chuck West and Heloise Gonzalez-Turpin for their useful comments, Dr. Robert Fairve for his help with statistical analyses and Dr. Johanna Schultz from WrightScience Right and Ailis Hill for the English corrections. We thank Paul Laurent and Nathalie Seguin for their technical assistance in this research.

Conflict of interest statement. None declared.

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