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# Plant population and genotype effects override the effects of *Epichloë* endophyte species on growth and drought stress response of *Achnatherum robustum* plants in two natural grass populations

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## Abstract

***Aims***
In cool-season grasses, systemic and vertically transmitted *Epichloë* infections often provide a suite of benefits including increased growth, reproduction and competitive abilities. However, these effects of *Epichloë* endophytes on their hosts often depend upon host and endophyte genotype and environmental factors.

***Methods***
*Achnatherum robustum* (sleepygrass) harbors at least two *Epichloë* species within natural populations in the Southwest USA. We tested the effects of endophyte infection and species, host population and plant genotype (by experimentally removing the endophyte), and soil moisture (a key limiting factor) on growth and drought stress response of infected *A. robustum* plants from two populations (Weed and Cloudcroft) in the Sacremento Mountains of New Mexico, USA).

***Important Findings***
Although the two populations harbor distinct *Epichloë* species each with very different chemoprofiles, neither endophyte status (infected vs. uninfected) nor endophyte species affected most growth parameters at 8 or 25 weeks of the experiment, except for leaf length. In high water treatment, infected plants from the Weed population had longer leaf length compared with uninfected plants. In contrast, the population of origin affected all growth parameters, including plant height, leaf number, length and width, tiller number and shoot and root biomass, as well as wilting time. Grasses from the Cloudcroft population generally showed greater growth than grasses from the Weed population. Endophyte infection did affect wilting time, with infection in the Weed population generally reducing time to wilting under low and high water, whereas infection in the Cloudcroft population reduced time to wilting only under high water conditions. Our results suggest that plant population and their associated plant genotypes may play a much larger role in endophyte–host grass interactions in varying environments than previously thought. Asexual *Epichloë* species may be compatible with only specific host genotypes within populations such that the phenotypic effects due to population may be greater than phenotypic changes influenced by variation in the endophyte.

***Keywords:*** endophyte genotype, *Epichloe* infection, growth parameters, sleepygrass, wilting time

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## INTRODUCTION

Fungal endophytes are abundant and diverse microbial symbionts that inhabit the above-ground parts of various plants (Cheplick and Faeth 2009). In cool-season pooid grasses, some fungal endophytes in the genus *Epichloë* are asexual (previously placed in genus *Neotyphodium*, Leuchtmann *et al.* 2014) and are strictly vertically transmitted via hyphae growing

into developing seeds. These symbionts live systemically and asymptomatically in the intercellular spaces within grass tissues (Clay 1990; Saikkonen *et al.* 1998; Schardl *et al.* 2004). Asexual endophytes are closely related to, and derived from, their *Epichloë* sexual ancestors, which can be transmitted vertically or horizontally, depending on the strain and environmental conditions (Schardl *et al.* 2004).

*Epichloë* infections, especially asexual ones, may profoundly alter host phenotypes chemically, physiologically and morphologically (Cheplick and Faeth 2009). *Epichloë* endophytes may provide a suite of benefits to their hosts including increased growth, reproduction and competitive abilities, increased resistance to biotic and abiotic stresses and enhanced nutrient uptake (Müller and Krauss 2005). Endophytic fungi are well known to improve drought resistance in agronomically important forage and turf grasses, such as perennial ryegrass (*Lolium perenne* L.) and tall fescue (*L. arundinaceum* Darbyshire ex. Schreb.) as well as some wild grasses (Cheplick and Faeth 2009).Because endophytes provided beneficial effects to their hosts, particularly under stressful biotic and abiotic conditions, the symbiosis has often been characterized as mutualistic (Assuero *et al.* 2000; Hesse *et al.* 2003). Endophyte-mediated changes in host grasses may reverberate throughout the entire community due to enhanced performance of infected host grasses relative to other species present in the community (e.g. Rudgers *et al.* 2010).

However, comparatively few studies have investigated effects of endophytes on their wild host grass species in non-agricultural settings (Brem and Leuchtmann 2001; Craig *et al.* 2011; Gonthier *et al.* 2008; Kannadan and Rudgers 2008), where endophyte effects may vary from mutualism to parasitism or commensalism (Faeth *et al.* 2004; Morse *et al.* 2002; Müller and Krauss 2005). These variable outcomes of endophyte–host interactions in natural populations are hypothesized to result from variation in host and endophyte genotypes and environmental factors such as soil moisture (Cheplick and Faeth 2009; Faeth and Fagan 2002; Meijer and Leuchtmann 2000).

Research on variable *Epichloë*–grass interactions in both agronomic and wild grasses has focused on variation in the endophyte genotype. Endophyte species or genotype is presumed as the main driver of the interaction between host and endophyte, with host genotype and environmental factors playing lesser roles (Cheplick and Faeth 2009). For example, endophyte genotype within the same grass species may differ greatly in endophyte-mediated changes in host phenotypes, such that variation among host traits with different endophyte haplotypes may be greater than that between infected and endophyte-free plants (Morse *et al.* 2007). In agronomic grasses, the endophyte species or strain is typically manipulated against a common host genetic background to achieve desired properties of the host grass (Bouton and Easton 2008). Recent molecular genetic evidence confirms enormous genetic variation within and among endophyte species inhabiting wild grasses (Leuchtmann *et al.* 2014; Schardl *et al.* 2013; Takach *et al.* 2012). Unlike most endophyte infected agronomic grass cultivars which are infected with a single *Epichloë* genotype, wild grass species usually harbor multiple *Epichloë* species or genotypes, sometimes within the same population (Cheplick and Faeth 2009). Less attention has focused on environmental factors, and especially, plant population and genotype, as determinants of the direction and strength of endophyte–host interactions. Relatively few studies have simultaneously tested the effect of endophyte, plant population and genotype, and environmental factors on host performance (Cheplick and Faeth 2009; Oberhofer *et al.* 2014).

To test the effect of infection, endophyte and host population and a key environmental factor, water availability on growth and response to drought, we performed an experiment comparing infected (E+) and uninfected (E−) plants from two different populations of grass, *Achnatherum robustum* (sleepygrass) that is native to the Southwestern (SW) USA and northern Mexico. From previous and ongoing studies, we knew that one population (Weed, NM) was infected with *E. funkii* (Moon *et al.* 2007), whereas a nearby population (Cloudcroft NM, 22 km from Weed) was infected with an undescribed *Epichloë* species. Both populations are found in semi-arid, Ponderosa-pine grasslands where water is limiting to plant growth, with the Weed population persisting in lower elevation areas that are drier and warmer than the Cloudcroft population. Sleepygrass is an important native forage grass in the semi-arid SW USA forests and has been targeted for restoration projects (Jones *et al.* 2000). But some populations have long been known to be highly toxic to livestock and native vertebrate grazers due to high levels of endophytic alkaloids. More recent evidence shows the toxicity is highly localized because of different endophyte species (Shymanovich *et al.* 2015). Understanding how the different endophyte species affect host performance in different environments should provide valuable insights into best practices for restoring this wild grass in various habitats while minimizing any undesirable effects on livestock.

Our main goal was to determine how the effects of endophyte infection vary between plant populations growing in different environments. Increasing evidence indicates that, although endophytes can have profound effects on host phenotypes and hence fitness, these direction and strength of these effects are contingent upon endophyte species, plant genotypic variation and local environments and their interactions, especially for wild grasses (Cheplick and Faeth 2009). Understanding these complex interactions should provide insight into the basic ecology and evolution of microbial symbiont–host plant interactions as well as practical implications for grassland management.

We tested the effect of endophyte infection, plant genotype and water availability in a randomized block design where individual infected (E+) and uninfected (E−, the endophyte experimentally removed) plants from the Weed and

Cloudcroft population were grown under two levels of water. To estimate host performance, we measured various growth parameters as well as wilting response to drought. Because the endophytes in the two populations are different species with very different genetic backgrounds (Shymanovich *et al.* 2015), we predicted that variation in the endophyte would override plant genotype and environmental factors in determining growth properties and drought response of the host grass.

## MATERIALS AND METHODS

### The host plant—sleepygrass

*Achnatherum robustum* (Vasey) Barkworth [= *Stipa robusta* (Vasey) Scribn. = *Stipa vaseyi* Scribn.] (Pooideae: Tribe Stipeae) is a cool-season, perennial native bunchgrass found at high elevations throughout the western and SW USA in semi-arid pine grasslands (Jones *et al.* 2000). Sleepygrass is an obligate outcrossing species and reproduces by seed (Faeth *et al.* 2010). The common names of *A. robustum* are robust needlegrass and sleepygrass. The latter name is derived from its long known toxic and narcotizing properties to livestock (e.g. Marsh and Clawson 1929). It was much later discovered that this toxicity was caused by infection with an asexual *Epichloë* endophyte (formerly *Neotyphodium*; Kaiser *et al.* 1996).

### The *Epichloë* endophytes

Sleepygrass in natural populations is often infected by an asexual *Epichloë* endophyte that is vertically transmitted by hyphae growing in culms and eventually into seeds (Faeth *et al.* 2006). In sleepygrass, there are at least two endophyte species which produce very different alkaloids (Faeth *et al.* 2006). Recent evidence shows that *Epichloë* from Cloudcroft populations (N: 32°57.452′, W: 105°43.092) is a new and yet undescribed species (Shymanovich *et al.*, unpublished work). This undescribed species is of hybrid origin, possesses genes for ergot alkaloids and may produce high levels of the ergot alkaloids ergonovine and lysergic acid amide. Sleepygrass is derived from the narcotic effects of these ergot alkaloids on livestock. However, these toxic effects on livestock are restricted to a small part of the range of *A. robustum* near Cloudcroft NM. In other, often nearby populations of *A. robustum*, another asexual endophyte, *E. funkii* (formerly *N. funkii*) was described by Moon *et al.* (2007) from a population in Colorado. *E. funkii*, also of hybrid origin but with different ancestral strains than the Cloudcroft endophyte, infects sleepygrass in populations near Weed, NM (N: 32°47.691′, W: 105°35.659′), only 22 km from Cloudcroft. This endophyte harbors genes for producing chanoclavine, an ergot alkaloid, the indole-diterpene alkaloids paspaline and terpendoles and possibly peramine (Shymanovich *et al.* 2015).

The Weed and Cloudcroft populations also differ in their habitats. Although both are semi-arid Ponderosa-pine grasslands where water is limiting to plant growth, the Weed population is at lower elevation (2265 m), receives less precipitation (mean rainfall: 51.8 cm per year) and is more exposed (fewer large trees) than the Cloudcroft population (2591 m; mean rainfall: 77.0 cm per year). Generally, *A. robustum* plants at the Weed site are smaller than those in Cloudcroft, reflecting poorer growing conditions (Faeth *et al.* 2006).

### The experiment

To test the effects of infection status (E+ or E−), endophyte species, plant population, water availability on host growth, biomass production and wilting response, we designed a growth chamber experiment, where water availability was controlled. We used *Epichloë* infected sleepygrass seeds collected from an experimental plot in Arboretum at Flagstaff in 2010 and stored at −20°C from plants originating from the Cloudcroft and Weed sleepygrass populations, NM. We used seeds from six infected maternal plants from each population. Infection status and species type was checked by DNA extraction for PCR testing and genetic characterization of mating types and alkaloid gene analyses (see methods in Shymanovich *et al.* 2015 and Takach *et al.* 2012). We removed the endophyte from half of seeds from each maternal plant via heat treatment. We soaked the seeds in water for 4 h and incubated in 1.5-ml tubes in a water bath at 55°C for 35 min. This temperature and duration was the most efficient for removing the endophyte without affecting germination rates after several preliminary trials. Heat treatment is a standard method for effectively removing the endophyte from seeds (see Cheplick and Faeth 2009).

Seeds were sown in 300-ml pots with potting soil (Garden Pro Company) in 27 March 2013, watered and allowed to germinate. Pots were placed in a growth chamber under a 25/15°C (day/night) temperature regime with a 16-h photoperiod, during which time they received 400 μmol·m$^{-2}$·s$^{-1}$ photosynthetically active radiation from a combination of cool white fluorescence tubes and incandescent bulbs. Three weeks after germination, E+ and E− seedlings of each endophyte type of a similar size were selected for the experiment, resulting in a total of 120 plants.

We randomly selected 60 plants of Cloudcroft (30 E+ and 30 E−) and 60 plants of Weed (30 E+ and 30 E−) for the experiment from a pool of individuals of similar size. Sixty (E+ and E−) plants from Cloudcroft and 60 (E+ and E−) plants from Weed were randomly assigned into each of the two water treatments: high water availability (HW; watered three times per week; 80 ml per watering), or low water availability (LW; watered once a week; 40 ml per watering). There were 15 E+ and 15 E− replicates of each treatment and population in our experiment. These levels of water have been used previously to achieve differences in plant growth in growth chambers (e.g. Saari and Faeth 2012). The water treatments began on 12 April 2013.

Plant infection status was confirmed by using Phytoscreen Immunoblot Kit (Agrinostics) at the end of the experiments. To re-confirm several unclear immunoblot results (12 plants), we also extracted DNA with Plant/Fungus DNA Extraction Kit

(Zymo Research) from the bottom tiller parts from the questionable experimental plants and several with known infections. We then used a Step One Plus real-time PCR machine (Applied Biosystems) and Power SYBR Green PCR Master Mix according to manufacturer instructions with tubulin B primers IS-NS-5′ (GAG CGT ATG AGT GTC TAC TTC AA) and TUB-2W-3′ (contra-sence reversed GTT GTT GCC AGA AGC CTG TCA C; Dombrowski *et al.* 2006) to compare fungal DNA extracted from experimental plants with that from plants of known infection status. The PCR run method used was as follows: 95°C for 10 min, 40 cycles: (95°C for 15 s, 58°C for 1 min), melting curve stage (95°C for 15 s, 58°C for 1 min and gradual warming up to 95°C for 15 s). Samples were considered infected if there was amplification within 40 PCR cycles and melting peak temperatures were matching with positive control samples. PCR testing is more sensitive for endophyte detection than immunoblotting, and thus, we were confident of infection status of our experimental plants.

### Growth, wilting time and biomass production

We recorded plant height and leaf size parameters two times per week for the first 8 weeks. To test the drought stress response, we stopped watering all plants on 30 May 2014 and recorded wilting time. Wilting time was estimated as the time required for all leaves on a plant to show to wilt. Plants were then cut 2 cm above the soil level, water was added to all plants and they were allowed to re-grow for 2 weeks. We dried the removed plant material (3 days at 65°C) and then determined dry above-ground biomass. After 2 weeks of recovery, we continued the original water treatments. And at the end of 25 weeks from the beginning of experiment, plants were harvested. We divided above-ground from below-ground parts and washed roots of all soil. Shoot and root material was placed in individual paper bags and heated in a drying oven at 65°C for 3 days. Following this drying period, we measured dry above-ground re-growth biomass and dry below-ground biomass.

### Statistical analyses

For statistical analysis, we used mixed-effects, nested design because the two endophyte species differ and both species are not found within the same plant populations. Endophyte infection was nested within the plant population or genotype in our experimental design and analyses. Endophyte and water treatments were considered fixed factors, whereas population was considered a random factor. Thus, in our statistical analyses, we compared the variation in growth and biomass production of uninfected plants and plants infected with one of the two endophytes from each population. Analyses of variance (ANOVA; SYSTAT 13.0 software) were used to examine the effects of infection status (E+ or E−) in each population, population (Weed or Cloudcroft) and water treatment effects on leaf parameters (number of leaves, leaf length and width, plant height and number of tillers, shoot biomass and wilting time) at 8 weeks. A similar ANOVA was used to test the effects of infection status (E+ or E−) in each population, population (Weed or Cloudcroft) and water treatment effects on shoot biomass re-growth and root and total dry biomass at the end of the experiment (25 weeks) when plants were completely harvested. We tested and met all assumptions of normality and homogeneity of variances.

Furthermore, we can indirectly test for differences in plant genotype by assuming that plant genotypes within the two populations differ from one another. This is a reasonable assumption, given that the populations are isolated from each other by 22 km and dispersal of *A. robustum* seeds are largely by wind and limited by topography. Additionally, asexual endophytes in wild grasses likely have high fidelity to, and compatibility with, specific host genotypes (Cheplick 2008; Saikkonen *et al.* 1998). However, we also directly tested for the contributions of plant genotypes from these two populations to growth parameters and drought stress response by comparing only E− (experimentally removed) plants from the two populations. Without their respective endophytes, any differences in growth or drought response are thus attributable to differences between plant genotypes from the populations.

## RESULTS

### Plant vegetative traits

For 8-week-old plants, number of leaves, mean leaf length and width, plant height and number of tillers varied by population origin, with the grasses from Cloudcroft showing greater growth responses than grasses from Weed (Table 1, Fig. 1a–d). Although plants generally grew better under higher water as expected, the only significant effect of higher water on plant growth or morphology was on mean leaf width. Leaves in the HW treatment were wider than those in the LW treatment (Table 1).

The only effect of endophyte infection or species on growth at 8 weeks was that on leaf length (Table 1). This effect, however was not uniform in both populations. In the Cloudcroft population, E+ and E− plants did not differ in leaf length ($P > 0.05$). Although the Weed population tended to have shorter overall leaf length than the Cloudcroft population, E+ plants had longer leaf length (mean = 56.66 cm ±2.35 SE) than E− plants (mean = 49.77 cm ± 2.10 SE) in this population.

### Shoot biomass and wilting time at 8 weeks

As expected, dry shoot biomass at 8 weeks in HW treatment was greater for the HW treatment than LW treatment for both populations (Table 2). Likewise, plants from both populations in the HW treatment had longer wilting times than those in the LW treatment (Table 2, Fig. 2). HW treatment plants would have more soil moisture when water was withheld. As with growth parameters at 8 weeks, populations differed in both wilting time and dry biomass. Cloudcroft plants had greater biomass than Weed plants (Fig. 1e), but Weed plants

**Table 1:** ANOVA results for the effect of endophyte infection in each population, drought stress and population on vegetative traits of sleepygrass at 8 weeks

| Source | | Plant height | | Leaf number | | Leaf length | | Leaf width | | Tiller number | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Population | 1 | 22.03 | **<0.01** | 25.788 | **<0.01** | 22.809 | **<0.01** | 15.521 | **<0.01** | 6.080 | **0.015** |
| Water | 1 | 0.96 | 0.329 | 0.303 | 0.583 | 1.739 | 0.190 | 6.314 | **0.013** | 0.186 | 0.667 |
| Water*population | 1 | 0.29 | 0.589 | 0.004 | 0.953 | 0.556 | 0.458 | 1.341 | 0.250 | 0.000 | 0.991 |
| Endophyte (population) | 2 | 2.55 | 0.083 | 0.311 | 0.733 | 3.460 | **0.035** | 0.934 | 0.396 | 0.432 | 0.651 |
| Water*endophyte (population) | 2 | 0.31 | 0.736 | 1.906 | 0.154 | 0.366 | 0.694 | 0.616 | 0.542 | 0.106 | 0.900 |
| Error | 106 | | | | | | | | | | |

Significant *P*-values are in bold.

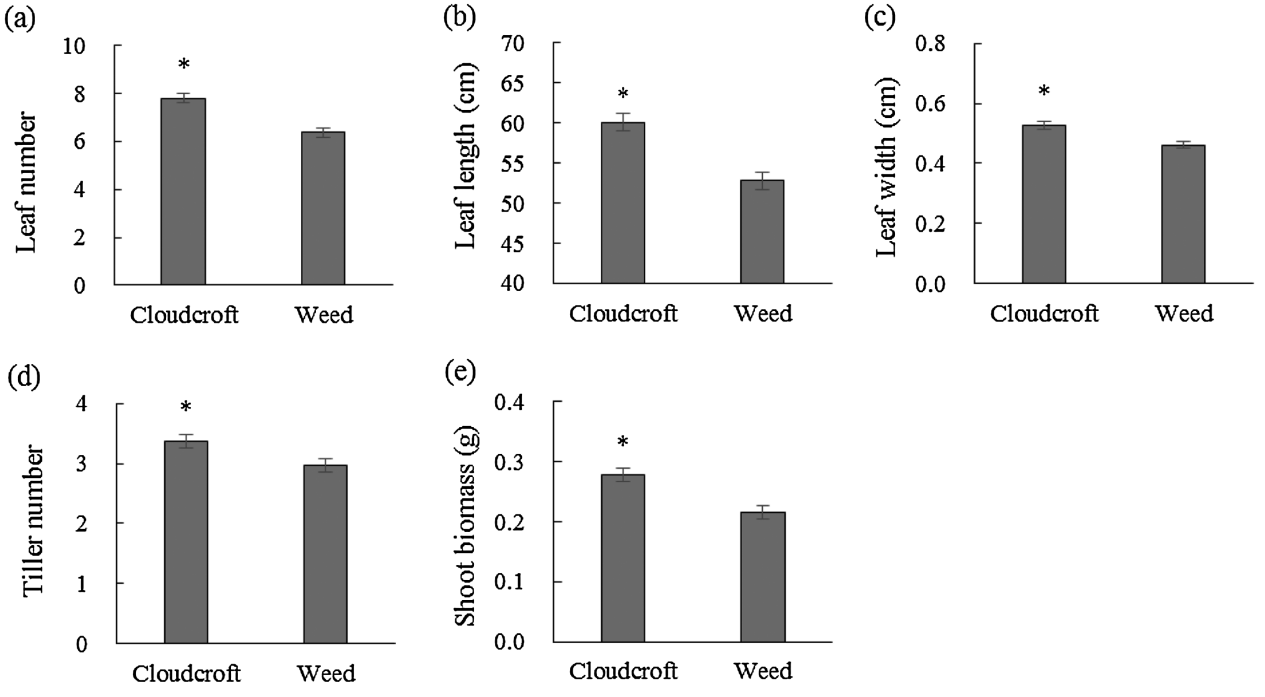


**Figure 1:** mean (±SE) of vegetative traits, wilting time and shoot biomass of sleepygrass from the different populations at 8 weeks. An asterisk denotes significance at $P < 0.05$. (a) Leaf number, (b) leaf length, (c) leaf width, (d) tiller number and (e) shoot biomass.

overall had longer wilting times than Cloudcroft plants when water was eliminated (Table 2, Fig. 2).

Endophyte infection within the populations affected wilting time, but not shoot dry mass, after the 8-week period (Table 2). For the Cloudcroft population, endophyte infection had no effect on wilting time in the LW treatments, but infection reduced wilting time in the HW treatment (Fig. 2). In contrast, E+ plants in the Weed population wilted faster than E− plants in both the HW and LW treatments (Fig. 2).

### Re-growth parameters

At 25 weeks (the end of the experiment), plant shoot, root and total biomass increased in the HW treatment compared with the LW treatment, as expected. Biomass allocation to roots (root:shoot ratio) was also influenced by water treatments with plants in the LW treatment having a greater root:shoot biomass ratio (more allocation to roots) than plants in the HW treatments (Table 3). Similar to the 8-week data, population had strong effects on growth parameters, including shoot dry biomass, root dry biomass and total dry biomass (Table 3; Fig. 3). However, endophyte infection had no effect on any of these growth parameters (Table 3).

## DISCUSSION

### Effects of endophyte and plant population on growth

Infection by *Epichloë* endophytes in many agronomic and some wild grasses is well known for altering growth and

**Table 2:** ANOVA results for the effect of endophyte infection in each population, drought stress and population on wilting time and shoot biomass of sleepy grass at 8 weeks

| Source | | Wilting time | | Shoot dry biomass | |
|---|---|---|---|---|---|
| | *df* | *F* | *P* | *F* | *P* |
| Population | 1 | 30.203 | **<0.01** | 17.679 | **<0.01** |
| Water | 1 | 21.350 | **<0.01** | 7.695 | **<0.01** |
| Water*population | 1 | 0.320 | 0.573 | 0.642 | 0.425 |
| Endophyte (population) | 2 | 4.530 | **0.013** | 0.720 | 0.489 |
| Water*endophyte (population) | 2 | 1.272 | 0.285 | 0.048 | 0.953 |
| Error | 106 | | | | |

Significant *P*-values are in bold.

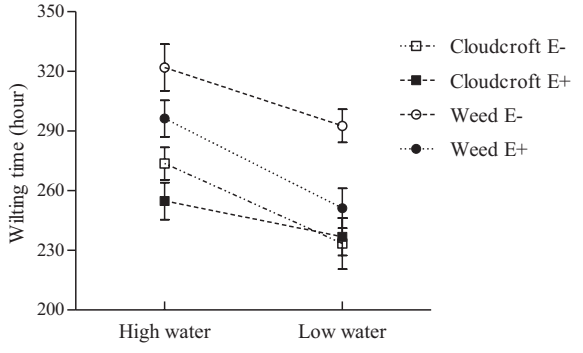


**Figure 2:** mean (±SE) wilting time of endophyte infected (E+) or endophyte-free (E−) sleepygrass from different populations under two conditions of water availability.

reproduction, often in a positive direction (e.g. Clay 1988; Kannadan and Rudgers 2008; Latch *et al.* 1985; Malinowski *et al.* 1997; Pan and Clay 2002; Vila-Aiub *et al.* 2003). However, more recent studies of agronomic and wild grasses have found that the effects of infection *per se* on host growth is often modified or even subsumed by endophyte and host genotype, environmental factors and the complex interactions among them (Ahlholm *et al.* 2002; Cheplick *et al.* 2000; Elbersen and West 1996; Gibert *et al.* 2012; Oberhofer *et al.* 2014). In a previous study with a co-occurring SW USA native grass, Arizona fescue, Morse *et al.* (2007) found that endophyte genotype largely dictated plant physiological and growth responses to water availability, even more so than infection status itself. Given the large genetic divergence between the Cloudcroft and Weed population endophytes (different *Epichloë* species, rather than simply different genotypes of the same species) and their dissimilar chemoprofiles (see below), we expected endophyte status and endophyte species would strongly influence growth parameters. However, we found that endophyte status and species had either no or relatively weak effects on growth and did not interact with a key environmental factor, water availability, to alter host growth in two sleepygrass populations.

Instead, our results indicate that host grass differences between the two populations largely outweigh effects of endophyte status and species, at least in terms of the growth parameters that we measured. Few studies have included variation in plant population and genotype in determining the relative effects of endophyte infection on host growth. Those that have included plant population and genotype usually find that the cultivar for agronomic grasses and populational genotypes for wild grasses often modulate the effects of endophyte infection. For example, Cheplick (2004) and Hesse *et al.* (2004) found that growth response in agronomic cultivars and wild populations, respectively, of perennial ryegrass (*L. perenne*) depended largely on host genotype–endophyte combinations. In meadow fescue (*Schedonorus pratensis*), Wali *et al.* (2008) found that the benefits of *Epichloë* infection were cultivar dependent and varied with soil nutrients. Like these studies, we found strong effects of host grass population. However, unlike these studies, we did not find any interactions between population and water availability.

## Effects of endophyte and plant population on wilting time and post-drought growth

In this study, infection decreased wilting time under pre-drought LW and HW treatments for the Weed population but only for pre-drought HW treatments for the Cloudcroft population. Again, there was a strong effect of plant genotype associated with each population on wilting time (Table 2, Fig. 2). Many previous studies have also reported endophyte-mediated amelioration of drought stress and enhanced re-growth after drought stress in both agronomic (e.g. Arachevaleta *et al.* 1989; Cheplick *et al.* 2000; Elbersen and West 1996) and wild grass systems (Craig *et al.* 2011; Gonthier *et al.* 2008; Kannadan and Rudgers 2008). In these studies, infection often decreased wilting time and increased leaf rolling, and was associated with increased growth and biomass after recovery from drought (Cheplick and Faeth 2009). Increased leaf rolling and decreased wilting times may preserve water retention in the leaf sheath and therefore protect the internal growing zone from lethal desiccation (Elbersen and West 1996). Other presumed mechanisms for endophyte-mediated drought resistance are varied and range from decreased stomatal conductance, higher water use efficiency and enhanced osmotic regulation (Cheplick and Faeth 2009). These

**Table 3:** ANOVA results for the effect of endophyte infection in each population, drought stress and population on re-growth biomass allocation of sleepygrass at 25 weeks

| Source | | Shoot dry biomass | | Root dry biomass | | Root: Shoot | | Total dry biomass | |
|---|---|---|---|---|---|---|---|---|---|
| | *df* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Population | 1 | 17.584 | **<0.01** | 7.997 | **<0.01** | 0.062 | 0.804 | 14.468 | **<0.01** |
| Water | 1 | 19.011 | **<0.01** | 29.754 | **<0.01** | 76.552 | **<0.01** | 22.702 | **<0.01** |
| Water*population | 1 | 0.091 | 0.763 | 0.001 | 0.979 | 0.155 | 0.694 | 0.040 | 0.842 |
| Endophyte (population) | 2 | 1.707 | 0.187 | 1.029 | 0.362 | 1.048 | 0.355 | 1.466 | 0.236 |
| Water*endophyte (population) | 2 | 0.007 | 0.993 | 0.118 | 0.889 | 0.872 | 0.421 | 0.010 | 0.990 |
| Error | 91 | | | | | | | | |

Significant *P*-values are in bold.

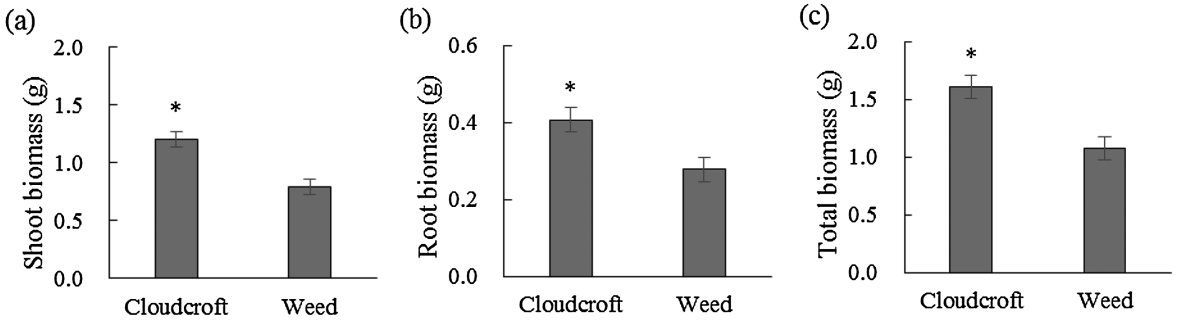


**Figure 3:** mean (±SE) re-growth biomass allocation of sleepygrass from Cloudcroft and Weed. An asterisk denotes significance at $P < 0.05$. (a) Shoot biomass, (b) root biomass and (c) total biomass.

mechanisms mediated by endophyte infection or selection of more drought-tolerant plant genotypes, or a combination of both, may be particularly important for grasses growing in semi-arid conditions, like sleepygrass.

We also did not find that endophyte status or species enhanced post-drought shoot or root biomass or differential allocation to roots and shoots as reported for some endophyte–host grass interactions (e.g. Belesky and Fedders 1996; Cheplick and Faeth 2009; Hahn *et al.* 2008) but not others (e.g. Oberhofer *et al.* 2014). We also did not find any interaction between plant population and genotype or endophyte species and environmental factors as has been reported in studies of re-growth after clipping for perennial ryegrass (e.g. Cheplick 1998). Once again, however, plant population and genotype affected re-growth and final root and shoot biomass.

The consistent and overriding effects of plant population suggest that sleepygrass genotypes in the two populations are very different from each other and have evolved separately, despite being separated by only 22 km. However, environmental factors can vary greatly and plant populations can be easily isolated by topographically factors, such as ridges and drainages basins, even over short distances in the mountainous regions of New Mexico. The Weed habitat is lower in elevation, considerably drier and more exposed (fewer trees). Therefore, plant genotypes in this population may be more adapted to drought than the Cloudcroft population, where soil moisture is generally higher and plants are more shaded. Generally, the Weed population grasses had much longer wilting times than the Cloudcroft population in both of the pre-drought treatments. This response of the Weed plants may allow longer periods without wilting when exposed to drying conditions and thus longer photosynthesis episodes.

We note that because asexual *Epichloë* endophytes are thought to be only vertically transmitted (but see Oberhofer *et al.* 2014), there is likely high fidelity among endophyte species and maternal plant genotypes. Thus, even though we detected strong plant population effects, these effects may not be completely separated from endophyte infection because maternal plant genotypes and infection in each population may be tightly linked. We did not test naturally uninfected plant genotypes in either population because these are generally rare (Faeth *et al.* 2006).

We also caution that other selective factors besides water availability may drive differences between the populations and select for association with different endophytes. For example, infected *A. robustum* plants from the Cloudcroft population are well known for their toxic and narcotizing effects on vertebrates due to extremely high levels of ergot alkaloids (Jones *et al.* 2000; Petroski *et al.* 1992). The Cloudcroft endophyte is a new species and has genes for, and also produces, three ergot alkaloids and paspaline, an indole-diterpene alkaloid (Shymanovich *et al.* 2015). The endophyte from the Weed population, identified as *E. funkii*, harbors peramine, ergot and indole-diterpene genes and

produces one ergot and several indole-diterpene alkaloids (Shymanovich *et al.* 2015). Thus, differences in herbivore pressure and the cost of producing nitrogen-rich alkaloids may explain the persistence of infected plants in each population (Faeth *et al.* 2010). We also caution here that we only tested one limiting factor, soil moisture availability in growth chamber experiments, and manipulation of other abiotic factors, such as soil nitrogen, and under field conditions where herbivores are present, may have produced different results.

With these caveats in mind, our results suggest that in some natural grass populations, differences among grass populations may override effects of endophyte infection and endophyte species in terms of host plant growth and response to drought. *Epichloë* endophytes are known to profoundly alter grass phenotypes and competitive abilities. These changes in the host grass may then, in turn, cascade to influence plant and consumer community structure and diversity (e.g. Cheplick and Faeth 2009). In many previous studies of the role of *Epichloë* endophytes, plant genotype is often ignored or randomized. Our results, plus those of other recent studies (e.g. Vesterlund *et al.* 2011), indicate that host grass origin may subsume endophyte effects in the response of grasses to abiotic and biotic selective pressures.

Because *Epichloë* endophytes infect many important forage grasses in the subfamily Pooideae (Cheplick and Faeth 2009), our results have implications for managing and restoring wild grasses. Although increasing attention has been devoted to manipulation of the endophyte in managing wild grasses (e.g. Cheplick and Faeth 2009), this should not come at the expense of minimizing the role of plant population origin or genotypes. Our results underscore the complexity of endophyte genotype, host plant population and genotype and environment interactions in determining performances and fitness of wild grasses.

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