

# Water availability alters the tri-trophic consequences of a plant-fungal symbiosis

Kelsey M. Yule · James B. Woolley ·  
Jennifer A. Rudgers

Received: 3 May 2010 / Accepted: 2 November 2010 / Published online: 4 December 2010  
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**Abstract** Plant–microbe protection symbioses occur when a symbiont defends its host against enemies (e.g., insect herbivores); these interactions can have important influences on arthropod abundance and composition. Understanding factors that generate context-dependency in protection symbioses will improve predictions on when and where symbionts are most likely to affect the ecology and evolution of host species and their associated communities. Of particular relevance are changes in abiotic contexts that are projected to accompany global warming. For example, increased drought stress can enhance the benefits of fungal symbiosis in plants, which may have multi-trophic consequences for plant-associated arthropods. Here, we tracked colonization of fungal endophyte-symbiotic and aposymbiotic *Poa autumnalis* (autumn bluegrass) by *Rhopalosiphum padi* (bird-cherry-oat aphids) and their parasitoids (*Aphelinus* sp.) following manipulations of soil water levels. Endophyte symbiosis significantly reduced plant colonization by aphids. Under low water, symbiotic plants also supported a significantly higher proportion of aphids that were parasitized by *Aphelinus* and had higher above-ground biomass than aposymbiotic plants, but these endophyte-mediated effects disappeared under high water. Thus, the multi-trophic consequences of plant-endophyte symbiosis were contingent on the abiotic context,

suggesting the potential for complex responses in the arthropod community under future climate shifts.

**Keywords** Climate change · Defensive mutualism · Fungal endophyte · Herbivory · Indirect interaction · *Neotyphodium*

## Introduction

Symbionts often provide benefits to host organisms to aid in defense against enemies (Haine 2008; White and Torres 2009). These defenses can complement and bolster the host's intrinsic mechanisms of defense, generating effects not only on host ecology but also on host-associated communities. For example, the bacterial symbiont *Hamiltonella defensa* can provide protection to pea aphids (*Acyrtosiphon pisum*) by increasing resistance to parasitoid wasps (*Aphidius ervi*) (Oliver et al. 2005). Similarly, fungal endophytes, such as *Neotyphodium*, can increase host plant resistance to herbivory via the production of secondary metabolites (Wilkinson et al. 2000; Clay and Schardl 2002). These effects may cascade to other members of the community, such as the parasitoids or predators of herbivores (Omacini et al. 2001; Rudgers and Clay 2008).

Understanding factors that cause context-dependency in the outcome of symbioses will improve predictions on when and where protection symbioses are most likely to affect host species and their associated communities. As in other positive species interactions (Bronstein 1994), the benefits conferred by protective symbionts may be conditional on several factors, including the abundance and identity of the host's natural enemies (e.g., Clay et al. 2005; Afkhami and Rudgers 2009) as well as resource availability and the abiotic environment (reviewed by Cheplick

Handling editor: Robert Glinwood.

K. M. Yule (✉) · J. A. Rudgers  
Department of Ecology and Evolutionary Biology,  
Rice University, Houston, TX 77005, USA  
e-mail: kmy1@rice.edu

J. B. Woolley  
Department of Entomology, Texas A&M University,  
College Station, TX 77843-2475, USA

and Faeth 2009). Protection interactions are perhaps more likely to be context-dependent than symbioses providing direct (e.g., nutritional) rewards because the presence/abundance of a third species (the host enemy) is required for benefits to accrue, thus forming an indirect species interaction (Bronstein 1998; Rudgers et al. 2010). However, relatively few studies have investigated factors affecting the strength of benefits conferred by most types of protection symbioses, with the exception of ant-plant interactions (reviewed by Bronstein 1998). Furthermore, the ecological outcomes of protection symbioses can become very complex when embedded in natural food webs. For example, in plants, foliar fungal endophytes can defend against herbivores, but may have more complicated effects if they also influence the natural enemies of herbivores, such as predatory or parasitic arthropods (Grewal et al. 1995; Bultman et al. 1997; Finkes et al. 2006; Härril et al. 2009). Abiotic conditions could additionally alter the outcomes of these multi-trophic, biotic interactions.

In grasses, fungal endophytes are common, intercellular symbionts found within the above-ground tissues of host plants (Clay 1990; Rodriguez et al. 2009). Past research has shown that endophyte-produced alkaloids can increase host plant resistance to herbivores (reviewed by Clay 1996; Rudgers and Clay 2007; Schardl et al. 2007). However, the protective benefits of endophytes to host plants can be conditional on abiotic and biotic factors. For example, increased soil nutrient content has been shown to boost the defenses of endophyte symbiosis against the aphid *Rhopalosiphum padi* in *Lolium pratense* (Lehtonen et al. 2005). Additionally, in the host grasses *Festuca subverticillata* and *L. arundinaceum*, endophyte-mediated resistance to insect herbivores depended on herbivore identity (Bultman and Bell 2003; Afkhami and Rudgers 2009; Crawford et al. 2010). Most notably, the relationship between grasses and endophytes is sensitive to water availability. Experimental manipulations of endophyte presence have shown stronger endophyte benefits to hosts under reduced water availability (reviewed by Malinowski and Belesky 2000; Cheplick and Faeth 2009), and endophyte frequencies in natural host populations have been positively associated with regional drought propensity (Lewis et al. 1997; Novas et al. 2007; Saona et al. 2010). Drought stress has also been associated with increased concentrations of the endophyte-produced loline alkaloids, which can confer herbivore resistance (Belesky et al. 1989; Bush et al. 1993).

Symbiont-mediated protection can have consequences that extend beyond the host and its parasites or herbivores. There is some evidence to suggest that negative impacts of fungal endophytes on the behavior, life span, and growth of herbivores can be magnified in herbivores' natural enemies (Faeth and Bultman 2002; Chaneton and Omacini 2007).

For example, rates of secondary parasitism by parasitoid wasps significantly declined in the presence of an endophyte in *L. multiflorum*, resulting in a food web of reduced complexity in comparison to systems in which the grass was endophyte-free (Omacini et al. 2001). These tri-trophic effects do not appear to be driven solely by endophyte-mediated reductions in the abundance of herbivorous prey. For example, ladybird beetles (*Coccinella septempunctata*) fed aphids grown on plants with an endophyte displayed reduced reproductive success, longer developmental time, and decreased survival in comparison to beetles fed the same number of aphids reared on endophyte-free plants (de Sassi et al. 2006).

Environmental conditions may complicate the outcomes of tri-trophic interactions among symbiotic plants, herbivores, and predators or parasitoids. Changing climatic conditions (IPCC 2007) make it particularly important to understand the potential impacts of the abiotic environment on multi-trophic systems (Hoover and Newman 2004), and environmental conditions are well known to alter the outcomes of tri-trophic interactions in systems lacking symbiont-mediated protection. For example, elevated CO<sub>2</sub> levels increased survivorship of the cotton aphid, *Aphis gossypii*, while lengthening the developmental time of its lady beetle predator, *Propylaea japonica* (Gao et al. 2008).

To our knowledge, no prior study has investigated whether the abiotic environment affects the tri-trophic interactions among an endophyte-symbiotic grass, herbivore, and natural enemy. In this study, we tested the question: *Does water availability alter the tri-trophic interactions among an endophyte-symbiotic grass, aphids, and parasitoids?* In a greenhouse experiment, we assessed the rate of colonization by the aphid *Rhopalosiphum padi* on both endophyte-symbiotic and endophyte-free *Poa autumnalis* plants. Plants were exposed to high or low levels of water availability to manipulate the abiotic environment. *Aphelinus* wasps were allowed to parasitize aphids freely, and we compared rates of parasitism among the treatments. In general, we predicted that the benefits of the endophyte to the plant would be greatest under low water availability, possibly due to increased production of anti-herbivore loline alkaloids under water stress. Alternatively, the opposite effect might be observed if stress were severe enough to inhibit plant resistance to herbivores. Under low water, we expect that aphids feeding on endophyte-symbiotic plants would be better protected against parasitism due to both increased production of fungal alkaloids and magnification of the alkaloid's effects for higher trophic levels. Alternatively, symbiotic plants under low water may have altered volatile profiles or compromise aphid behavior in ways that make aphids more susceptible to parasitoid attack.

## Materials and methods

### Study system

Autumn bluegrass (*Poa autumnalis*) is a perennial  $C_3$  grass native to forested habitats, and particularly abundant in the southeastern USA (Barkworth et al. 2006). In eastern Texas, *P. autumnalis* grows frequently in pine and mixed pine-hardwood forests and flowers from March to May (Gould 1975). The endophyte *Neotyphodium* PauTG-1 (Moon et al. 2004) occurs commonly in *P. autumnalis* and is known to produce loline alkaloids (Siegel et al. 1990; Schardl et al. 2007). Our surveys of naturally occurring field populations showed a mean endophyte frequency (% of individuals with the endophyte per population) of 95.6% and range of 87–100% across 14 Texas sites (Rudgers et al. 2009). In this experiment, seeds were collected on 4 May 2006 from a natural population in the Stephen F. Austin Experimental Forest, Nacogdoches, Texas (31°29'52" N, 94°45'57" W), where the natural endophyte frequency was 90%. Seeds were stored at 4°C until we began the experiment.

### Endophyte treatment

Endophyte disinfection was achieved by heating a subset of seeds to 60°C in a convection oven for 7 d (see also Rudgers and Swafford 2009). Control seeds were placed at room temperature during this period. Next, all seeds were removed from the lemma and palea and surface sterilized for 1 min in 50% commercial bleach. For germination, seeds were placed on 9.5 cm diameter Petri plates filled with 1% water agar on 2 May 2007. Plates were placed in an incubator with a 14 h light cycle and a 22°C/10.2°C day/night temperature cycle. We transferred seedlings into 70 mL plastic pots after 1 mo., and then into 10.2 cm square plastic pots after 2 mo.

The heat treatment successfully eliminated the endophyte. We removed thin sections of the inner leaf sheath for 2–3 leaves per plant, stained them with lactophenol cotton blue, and examined tissue under a microscope at 200× (Leica Microsystems, Wetzlar, Germany) (Bacon and White 1994) to check the effectiveness of the treatment. Endophyte frequency in seedlings from control seeds was 63% ( $n = 140$  seedlings) and in heat-treated seeds was 11% ( $n = 92$  seedlings). Naturally endophyte-symbiotic (naturally E+) or successfully disinfected plants (disinfected E−) were then used in the experiment. In addition, we included a subset of naturally endophyte-free plants (naturally E−) from the control seed batch for comparison with the endophyte-disinfected treatment. To minimize potential side effects of the heat treatment, the plants (parental greenhouse stock) were grown in a common

greenhouse environment for approximately 1 year (no supplemental lighting, 24°C) prior to initiation of the experiment.

Plants for the experiment were cloned from the parental greenhouse stock. During 15–16 May 2008, two tillers were split from each of 20 plants per treatment (naturally E+, naturally E−, disinfected E−) and transplanted into 15.4 cm diameter plastic pots containing 1,800 mL soil. The soil mix consisted of 50% ProMix (Premier Horticulture Inc., Quakertown, PA) and 50% Premium Play Sand (Quickrete International Inc., Atlanta, GA). Plants were watered daily with 90 mL using automatic drip emitters (Rain Bird, San Diego, CA) until initiation of the water treatment.

### Water treatment

Beginning in July 2008, half of the plants from each endophyte treatment ( $n = 10$  plants) were subjected to a water reduction treatment (45 mL daily); the remaining plants received regular water (90 mL daily). Water levels were chosen to achieve daily soil saturation versus a regular drying down of the soil without causing plants to wilt. In December 2008, watering was increased to 180 mL per day for the low water treatment and to 360 mL per day for the high water treatment because of the increase in plant size.

### Aphid and parasitoid wasp responses

Plants were colonized naturally by bird cherry-oat aphids (*Rhopalosiphum padi*) in the greenhouse during January 2009. Parasitoid wasps (*Aphelinus* sp.) subsequently parasitized the aphids. Specimens of *Aphelinus* sp. were slide-mounted in Canada balsam (Noyes 1982) and deposited in the Texas A&M University Insect Collection as Voucher Specimen Series #683. The specimens represent a species in the *mali* species group of *Aphelinus*, but they are not referable to any described species. On 3 February, 19 February, 3 March, and 13 March 2009, we conducted censuses of aphids and parasitism. During all four censuses, we counted the number of live aphids (nymphs and adults) and parasitized aphids on each of five tillers. Tillers were chosen haphazardly and blindly within each plant. To correct aphid responses for the size of individual tillers, and therefore to obtain aphid densities per plant, we measured the length of each tiller to the nearest mm. Parasitized aphid numbers were assessed by identifying ‘mummies,’ which appeared brown and bloated and often displayed a hole from which a parasitoid had emerged. During the third and fourth censuses, we also counted separately the number of early aphid instars, defined as the first three of four nymphal stages ( $\leq 1$  mm in length). At

these early instars, the status of parasitism was not apparent, as the developing parasitoid kills the aphid only in the final (fourth) instar or adult stage (Cohen et al. 2005).

### Plant growth response

On 1 April 2009, 10.5 mo. after beginning the water treatments, we harvested above-ground plant material at the soil surface. Biomass was dried in a convection oven (60°C, 48 h) and weighed to the nearest 0.01 g.

### Statistical analysis

We conducted ANOVA to test for treatment effects. However, the distributions of the residuals were often non-normal, and non-normality could not be substantially reduced through transformations. Therefore, we used distribution-free randomization tests with 10,000 iterations (Edgington 1987; Manly 1991). A randomization test determines a *P* value by comparing an observed test statistic with a distribution of the test statistic that is expected under the null hypothesis that the treatment had no effect. We applied a randomization test equivalent of repeated measures ANOVA by encompassing Proc Mixed code within a SAS v. 9.1 (SAS Institute, Cary, NC, USA) randomization macro program (Cassell 2002). The following responses were analyzed: live aphids per tiller length, parasitized aphids per tiller length, and the percentage of total aphids that were parasitized. Percentage parasitism required arcsin square-root transformation to satisfy the assumption of homogeneity of variances. Our models included the following fixed factors: endophyte status (naturally E+, naturally E−, or disinfected E−), water treatment (high or low), the endophyte × water interaction, and all interactions with the repeated effect of census. The effects of treatments on the percentage of aphids that were classified as early instars (data collected on the final two censuses only) were also evaluated using a randomization procedure to test whether observed differences in parasitism rates were simply due to differences between treatments in herbivore size or age.

We used factorial ANOVA to detect effects of our treatments on above-ground plant biomass. Naturally endophyte-free and experimentally disinfected plants did not significantly differ in above-ground biomass or in their response to water availability (ANOVA contrasts for natural E− v. disinfected E−: low water  $F_{1,53} = 0.04$ ,  $P = 0.84$ , high water  $F_{1,53} = 0.17$ ,  $P = 0.69$ ). Thus, these endophyte-free treatments were combined in the final analysis of plant response to increase statistical power. Because we expected the endophyte to improve plant performance under low water, we specifically tested two a priori contrasts in the plant biomass analysis: whether

endophyte-symbiotic and endophyte-free plants differed under low water availability and whether endophyte-symbiotic and endophyte-free plants differed under high water availability.

## Results

### Aphid densities

On average through time, aphid densities were 83% lower on endophyte-symbiotic plants than on endophyte-free plants (Fig. 1a, b, Endophyte Treatment, Table 1). Differences between the endophyte treatments in the densities of live aphids were most apparent on the first two census dates (91 and 58% endophyte-mediated reductions, respectively), before aphid abundance had been substantially reduced by parasitism, as indicated by a significant census × endophyte treatment interaction (Fig. 1a, b; Table 1). Across dates, live aphid responses did not significantly differ between naturally endophyte-free and endophyte-disinfected plants (Fig. 1a, b,  $P = 0.972$ ).

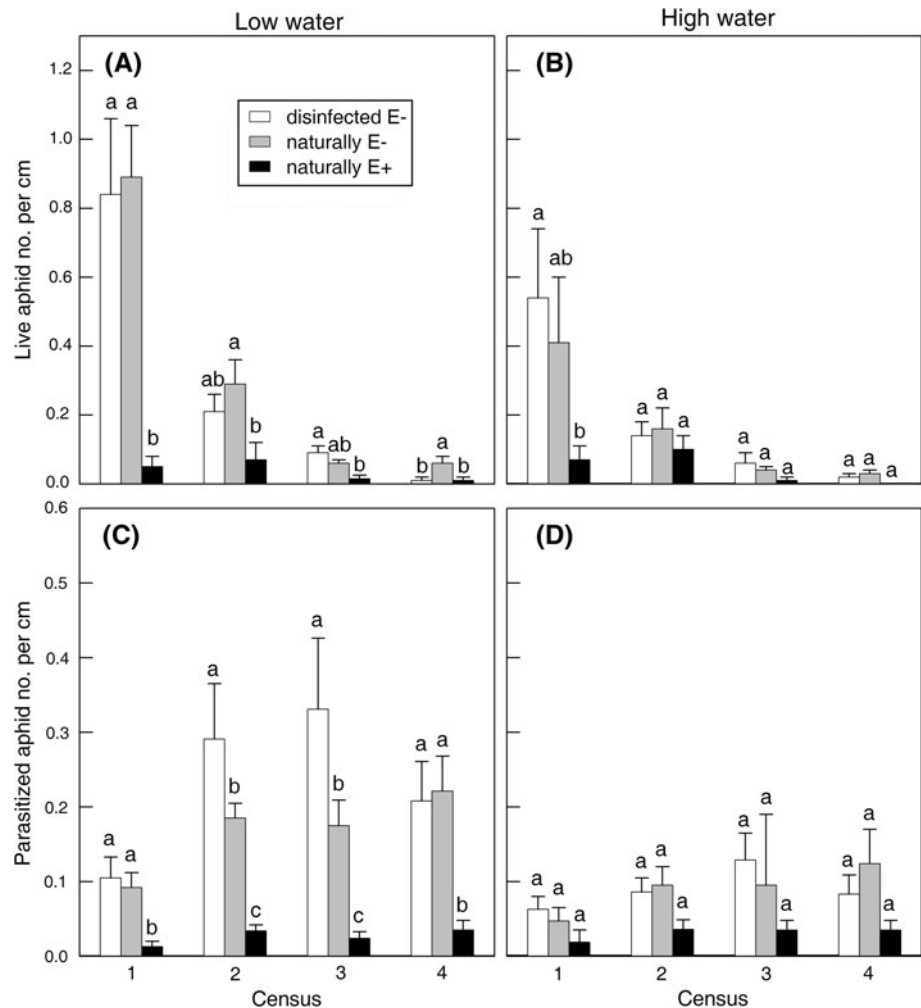
Densities of live aphids were higher on plants exposed to low water than to high water (Fig. 1a, b, Water Treatment, Table 1). This effect was mainly driven by aphid responses on the first census date, for which aphids were 82% more abundant on endophyte-free plants in the low water treatment than in the high water treatment (Fig. 1a, b).

To test whether the effects of treatments on live aphids were attributable to differences in aphid age (parasitism is not apparent during the first three instars), we tracked the percentage of aphids that were early (first to third) instars for the final two census dates. There were no significant differences in the relative fractions of early instar aphids between the endophyte treatments or the water treatments (Table 1).

### Parasitism by *Aphelinus*

Across censuses, endophyte-symbiotic plants had 80% lower densities of parasitized aphids than endophyte-free plants, as indicated by a significant main effect of the endophyte treatment (Fig. 1c, d; Table 1). Differences between endophyte treatments were stronger under low water (87% endophyte-mediated reduction) than under high water (65% reduction) (Endophyte × Water Treatment, Table 1). Within the low water treatment, naturally endophyte-free plants supported 36% lower densities of parasitized aphids on census 2 and 47% lower densities on census 3 relative to endophyte-disinfected plants, suggesting that the initial disinfection treatment may have affected parasitoids or that differences exist in the

**Fig. 1** Live aphid density (aphids/cm of tiller) on *Poa autumnalis* by the endophyte symbiosis treatment and census (13 February 2009, 219 February 2009, 33 March 2009, 413 March 2009) for **a** the low water treatment and **b** the high water treatment. Parasitized aphid density by endophyte symbiosis and census for **c** the low water treatment and **d** the high water treatment. E− = endophyte-free and E+ = endophyte-symbiotic. Bars show means ± SE.  $n = 10$  for all treatments except for naturally E+ under low water availability where  $n = 9$ . Letters indicate significant differences among endophyte treatments within each water level according to post-hoc Tukey HSD tests for each census



**Table 1** Results from repeated measures randomization tests evaluating the effects of the endophyte treatment and water treatment on responses of the aphid, *Rhopalosiphum padi*, and the parasitoid wasp, *Aphelinus* sp.

	<i>df</i>	Live aphids per cm	Parasitized aphids per cm	<i>df</i>	% Early aphid instars	<i>df</i>	% Aphids parasitized
Endophyte treatment	2,53	<b>&lt;0.0001</b>	<b>0.0001</b>	2,48	0.0991	2,52	0.1397
Water treatment	1,53	<b>0.0463</b>	<b>0.0014</b>	1,48	0.3962	1,52	0.5647
Endophyte × water	2,53	0.2465	<b>0.0293</b>	2,48	0.5852	2,52	<b>0.0426</b>
Census	3,159	<b>&lt;0.0001</b>	<b>0.0001</b>	1,50	0.1734	3,138	<b>&lt;0.0001</b>
Census × endophyte	6,159	<b>&lt;0.0001</b>	<b>0.0024</b>	2,50	0.4715	6,138	0.2253
Census × water	3,159	<b>0.0239</b>	0.0880	1,50	0.8086	3,138	0.1935
Census × endophyte × water	6,159	0.3639	0.2791	2,50	0.5089	6,138	0.2574

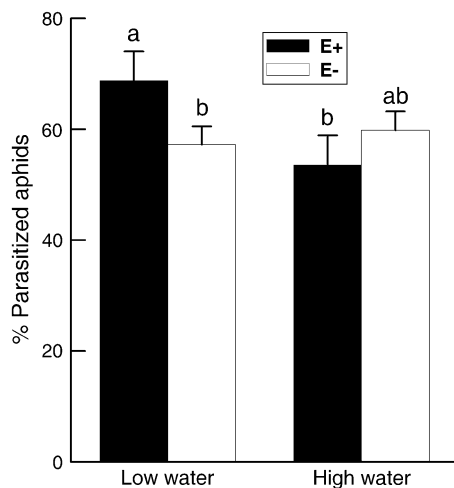
*P* values < 0.05 are shown in bold. Data for early aphid instars were only collected on the last two of four census dates. Data for % aphids parasitized (and % early aphid instars) had lower sample sizes (*df*) because only plants with aphids (or live aphids) were included in the analysis

genotypes of naturally endophyte-free and naturally symbiotic plants (Fig. 1c; Table 1).

Water availability also affected the density of parasitized aphids with 98% higher densities of parasitized aphids in the low water than in the high water treatment (Water Treatment, Table 1). This effect was stronger for

parasitized aphids on endophyte-free plants (Endophyte × Water Treatment, Table 1), which showed, on average, 123% higher densities under low water than under high water (Fig. 1c, d). This difference was particularly large on the second and third census dates, when parasitized aphids were 163 and 126% more abundant in the low





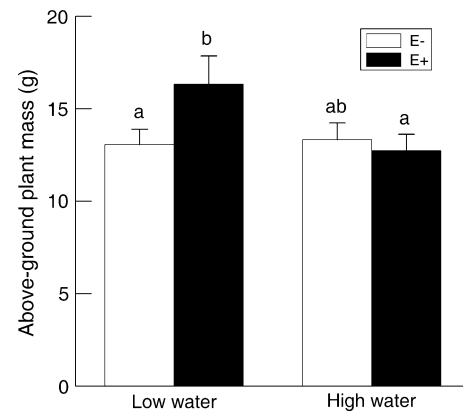
**Fig. 2** Percentage of parasitized aphids for endophyte symbiotic (E+) and endophyte-free (combined naturally E- and disinfected E-) *Poa autumnalis* at high and low water levels. Bars show means across all census dates  $\pm$  SE. E- = endophyte-free and E+ = endophyte-symbiotic.  $n = 20$  for the combined E- plants,  $n = 9$  for E+ under low water availability and  $n = 10$  for E+ under high water availability. Letters indicate significant differences among treatments according to a post-hoc Tukey HSD test encompassed in the randomization test procedure

water treatment than in the high water treatment, respectively. In contrast, water availability had no significant effect on the abundance of parasitized aphids for endophyte-symbiotic plants (Fig. 1c, d,  $P = 0.930$ ).

The density of parasitized aphids is relevant for inferring the community-level consequences of our treatments, but is not independent of the density of live aphids. This relationship was supported by a significant positive correlation between live aphid density and parasitized aphid density across all treatments (Spearman  $r = 0.55$ ,  $P < 0.0001$ ). Thus, it is also important to examine the percentage of aphids parasitized. These data revealed a different pattern. The percentage of aphids that were parasitized was greatest for endophyte-symbiotic plants under the low water treatment (Fig. 2), as indicated by a significant interaction between the endophyte  $\times$  water treatments (Table 1). Thus, there was a synergism between endophyte symbiosis and low water availability in increasing the rate of aphid parasitism. Despite temporal variation in the abundances of aphids and parasitoids, treatment effects on the percentage of parasitized aphids showed no significant variation through time (no significant interactions with census, Table 1).

#### Plant biomass

Endophyte-symbiotic plants produced 25% more above-ground biomass than endophyte-free plants, but only under low water availability (Fig. 3, a priori contrast low water:



**Fig. 3** Above-ground biomass (g) for endophyte symbiotic (E+) and endophyte-free (combined naturally E- and disinfected E-) *Poa autumnalis* at high and low water levels. Bars show means across all census dates  $\pm$  SE.  $n = 20$  for the combined E- plants,  $n = 9$  for E+ under low water availability and  $n = 10$  for E+ under high water. Letters indicate significant differences according to a post-hoc Tukey HSD test

E+ v. E-,  $F_{1,55} = 4.5$ ,  $P = 0.039$ ). Under high water levels, the presence of the endophyte had no significant effect on plant mass (Fig. 3, a priori contrast high water: E+ v. E-,  $F_{1,55} = 0.2$ ,  $P = 0.688$ ), demonstrating that endophyte benefits were conditional on water availability and supported by a marginal interaction term ( $F_{1,55} = 3.3$ ,  $P = 0.077$ ). Unexpectedly, plants in the high water treatment produced lower average biomass than plants in the low water treatment (Fig. 3), although this effect was not statistically significant (water treatment  $F_{1,55} = 2.4$ ,  $P = 0.125$ ). Endophyte-symbiotic plants under low water availability had greater average biomass than their high-water counterparts (Fig. 3).

#### Discussion

Our results provide some of the first evidence that the effects of plant symbionts on tri-trophic interactions can vary with abiotic conditions. Most notably, the presence of the fungal endophyte only increased rates of parasitoid attack on aphids when water availability to plants was low. Increases in plant biomass resulting from endophyte-symbiosis also occurred only when water availability was low, although endophyte presence reduced aphid abundance at both levels of water availability. This demonstration that abiotic conditions can alter arthropod responses to plant-microbe symbioses may prove important for improving agronomic practices and for predicting the community-level consequences of climate change in both natural and managed systems (see also, Gregory et al. 2009).

In our study, symbiotic plants not only experienced lower aphid densities, but, when water-limited, these plants

also supported a higher incidence of parasitism, which should further suppress aphid populations through time. Mechanisms responsible for the increased proportion of parasitized aphids in water-limited, endophyte-symbiotic plants are not yet resolved. One possibility is that the parasitoids are attracted to larger or higher quality host plants. Alternatively, parasitoids of aphids can respond strongly to host plant volatiles as a signal of herbivore presence or plant quality (Hatano et al. 2008; Arimura et al. 2009), and some evidence suggests endophyte symbiosis can alter the volatile profiles of grasses (Yue et al. 2001; Jallow et al. 2008). Another hypothesis is that aphids feeding on endophyte-symbiotic plants have altered behavior that makes them more susceptible to parasitism by wasps. For example, some endophyte-produced alkaloids, including lolines, increase in concentration under water-related stress (Belesky et al. 1989; Bush et al. 1993). These compounds could reduce the ability of aphids to avoid parasitoid attack, by slowing escape behaviors such as dropping from leaves (Losey and Denno 1998; Villagra et al. 2002; Francke et al. 2008) or by reducing the aphid's ability to encapsulate parasitoids (Carver and Woolcock 1985; Carver et al. 1988). We observed very few winged aphid morphs in our study, but similar work with *R. padi* on *L. arundinaceum* has shown that endophyte presence reduces the formation of winged morphs in the presence of ladybird beetles, suggesting the endophyte could reduce this escape mechanism in aphid populations (Züst et al. 2008). In contrast, the parasitoids in our study did not appear to be responding to aphid abundances via positive density-dependence, because there would have been higher rates of parasitism on endophyte-free plants due to greater aphid densities. Similarly, aphids are more likely to show dropping behavior from plants of lower quality (Losey and Denno 1998), which would not explain the higher rate of parasitism on lower quality symbiotic plants.

By evaluating the differences between treatment groups for above-ground plant biomass, our study provides the first estimate of the net benefits of endophyte symbiosis to the native grass *P. autumnalis*: Under low water availability, vegetative growth was 25% higher in plants with the endophyte than in aposymbiotic plants. This result contributes to a growing body of literature demonstrating benefits of fungal endophytes in native grasses (see also Bazely et al. 1997; Clement et al. 2005; Gonthier et al. 2008; Kannadan and Rudgers 2008; Crawford et al. 2010). However, the benefit to *P. autumnalis* was highly context-dependent, and only present when water availability was low. Surprisingly, the low water treatment did not significantly reduce above-ground biomass in our study. Perhaps water was not limiting enough to alter biomass production but was sufficient to alter plant chemistry or stress responses in such a way as to affect the food web.

Additionally, past research on the physiological effects of endophyte-symbiosis for *Poa* grasses has shown greater effects on biomass below-ground than above-ground (Kannadan and Rudgers 2008). Currently, we cannot tease apart direct benefits of this symbiosis from the benefits derived from altered food web interactions because neither aphids nor parasitoids were directly manipulated. Future studies that experimentally control the presence of aphids and of parasitoid wasps would help to determine the degree to which benefits of the symbiont can cascade back to the plant through an altered trophic structure. It is also worth considering the role of aphid genotype in influencing the magnitude of endophyte effects (Bieri et al. 2009).

Vertically transmitted symbionts, such as the *Neotyphodium* endophytes in grasses, rely on their hosts not only for resources, but also for reproduction. As their fitness is directly tied to the fitness of their hosts, symbionts can increase in frequency within populations by conferring net benefits to host individuals, such as protection from natural enemies (Sachs et al. 2004; Gundel et al. 2008; Rudgers et al. 2010). In accordance with previous experimental results for other host grass species (reviewed by Clay 1996; Cheplick and Faeth 2009), we found that endophyte presence strongly reduced the density of aphids attacking the native grass *Poa autumnalis*. Endophyte-symbiotic plants had both lower densities of live aphids and lower densities of parasitized aphids, indicating a bottom-up, community-level response to endophyte symbiosis, consistent with previous work (Faeth and Bultman 2002; Finkes et al. 2006; Härrä et al. 2008a; Rudgers and Clay 2008). For example, in a similar study on the introduced grass species, *Lolium multiflorum*, Omacini et al. (2001) found a decline in secondary parasitism that led to a less complex arthropod community structure in the presence of an endophyte.

It remains unclear what the ultimate consequences of endophyte symbiosis may be for populations of *Aphelinus* wasps. Prior work has shown that aphids fed endophyte-symbiotic plants can have negative effects on the development time, reproduction, and survival of their predators and parasitoids, relative to aphids fed diets of endophyte-free plants (de Sassi et al. 2006; Härrä et al. 2008a, c, 2009). Conducting similar feeding trials with parasitoids in the *P. autumnalis* system could help to further elucidate mechanisms underlying the endophyte and water-mediated increase in the rate of parasitism (see also Härrä et al. 2008b).

Due to increases in climate disruptions (IPCC 2007), a clear need exists for improved understanding of the potential effects of shifting abiotic conditions on multi-trophic systems. Here, we show that the abiotic condition of water availability alters the effects of plant-associated symbionts on plant-arthropod interactions.

**Acknowledgments** This work was funded by the Godwin Assistant Professorship and NSF-DEB#054278 to J.A.R. and by the Rice Century Scholars Fund to K.M.Y. We would like to thank Alex Gorischek, Liz Seifert, and Sami Hammer for assistance with the experiments.

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