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Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes

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Abstract

We assessed how *Neotyphodium* infection influenced the biomass production and growth of Arizona fescue (*Festuca arizonica* Vasey), a dominant understory grass in Ponderosa pine (*Pinus ponderosa*) forests of the southwest USA, by growing potted infected (E+) and uninfected (E−) plants under a high and a low water availability regime for 87 days. We measured growth analysis parameters, leaf net photosynthesis (P_n), chlorophyll fluorescence parameters, conductance to water vapor (g_l) and water potential (Ψ), to provide explanations for differences in biomass production under these treatments. Under high water availability, E− plants produced more biomass and had greater relative growth rates (RGR; rate of biomass gain per biomass); higher RGR of E− plants was correlated with higher P_n as well as production of less dense, presumably thinner leaves, which provided more leaf area per leaf biomass, and greater LAR (leaf area ratio; leaf area per total plant biomass). Under low water availability, E+ plants produced more aboveground biomass and had greater RGR; higher RGR of E+ plants was correlated with higher net assimilation rates, as well as production of less dense, leaves and greater LAR. Infected plants tended to have lower midday P_n and g_l in both water availability regimes. Lower P_n in E+ plants appeared primarily due to stomatal, rather than biochemical, limitations to photosynthesis. When a more severe water stress was imposed in the low water availability treatment over the last 61 days of the experiment, E+ plants tended to have higher midday P_n and g_l. Infected plants also tended to have less negative leaf Ψ regardless of water availability regime. Lower g_l and transpirational losses of E+ plants probably conserved soil moisture, such that when a more severe water stress was subsequently imposed, higher soil moisture availability allowed E+ plants to maintain higher P_n and g_l. *Neotyphodium* infection appears beneficial to Arizona fescue performance under low water availability and detrimental under ample water availability.

Keywords: Drought; *Festuca arizonica*; Fungal endophyte; Growth rate; Photosynthesis; Conductance

1. Introduction

Fungal endophytes have been defined as fungi that live for a significant part of their life cycle

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internally and asymptomatically (without causing any apparent tissue damage) in plants (Saikkonen et al., 1998). They are ubiquitous in vascular plants, usually occurring in aboveground organs, but occasionally in roots, where they differ from mycorrhizae in that they lack external hyphae or mantels (Wilson, 1995; Saikkonen et al., 1998).

Endophytes of the genus *Neotyphodium* (Morgan-Jones and Gams) are obligate seed-born fungi that commonly form intercellular infections in leaves, culms and inflorescences in many cool-season grasses in the subfamily Pooidaceae (Schardl et al., 1997). The influence of *Neotyphodium spp*. infection has been most extensively studied in *Festuca arundinacea* Schreb. (tall fescue), a selectively bred, agronomic grass from Eurasia that has been widely planted for pasture and turf in North America. This *Neotyphodium*-grass interaction is generally considered mutualistic (Clay, 1988, 1990), with infection generally enhancing host plant herbivore and pest resistance, and vegetative growth, reproduction, and drought tolerance (Clay, 1987; Arachevaleta et al., 1989; Cheplick, et al., 1989; Clay, 1990; Hill et al., 1991; West et al., 1993, 1995; Elmi and West, 1995; Hill et al., 1996). The suite of benefits conferred by *Neotyphodium* infections often result in high frequencies in pastures within a few years (Clay, 1988). As the endophyte is strictly seed borne, but infections can be lost from seeds (Siegel et al., 1984), high frequencies can only be maintained if the interaction is mutualistic (Clay, 1988; but see Faeth, 2002). However, exceptions to this generalization have been found in this *Neotyphodium*–grass system (White et al., 1992; Elbersen and West, 1996; Hill et al., 1996). In the case of another reasonably well studied *Neotyphodium*-infected pasture and turf grass, *Lolium perenne* L. (perennial ryegrass), the effects of infection on the host plant appear more variable (Cheplick et al., 1989; Clay, et al., 1993; Barker et al., 1997; Cheplick, et al., 2000). The endophyte does not appear to confer drought resistance (Cheplick et al., 2000). Increasingly evidence suggests that *Neotyphodium*–grass interactions depend on environmental conditions (Siegel, 1993; West, 1994; Marks and Clay, 1996; Saikkonen et al., 1998; Faeth and Fagan, 2002), as well as plant genotype (Elbersen and West, 1996; Hill et al., 1996; Marks and Clay, 1996; Cheplick, 1997; Cheplick et al., 2000).

Far less is known about the influence of *Neotyphodium* infections on wild populations of native grasses, especially in terms of drought resistance (e.g. Faeth, 2002). *Festuca arizonica* Vasey (Arizona fescue) is a native perennial bunchgrass, that is widespread in semi-arid ponderosa pine (*Pinus ponderosa*)/grassland communities above 2000 m elevation in the southwest USA (Kearney and Peebles, 1960). *Neotyphodium* infection frequencies in wild populations of Arizona fescue are usually high. Schulthess and Faeth (1998) sampled five populations of Arizona fescue in north-central Arizona and found that, on average, 90% of plants were infected with *Neotyphodium*. Other surveys show the frequency of infected plants in Arizona fescue populations ranges from 50 to 100% (Schulthess and Faeth, 1998; Saikkonen et al., 1999). Despite high frequencies, however, the infection by asexual *Neotyphodium* does not appear to benefit the host, as predicted for asexual symbionts (Wilkinson and Schardl, 1997; Law, 1985; Ewald, 1994). For example, infected Arizona fescue is not more resistant to invertebrate (Lopez et al., 1995; Saikkonen et al., 1999; Tibbets and Faeth, 1999) or vertebrate herbivores (Saikkonen et al., 1999). Therefore, we tested the alternative hypothesis that *Neotyphodium* affects Arizona fescue growth differently depending on water availability. Water availability should be a particularly important factor for Arizona fescue because it inhabits semi-arid regions subjected to prolonged seasonal and yearly droughts.

In this study we assessed how *Neotyphodium* infection influenced the biomass production and growth of Arizona fescue under two contrasting water availability regimes. We measured traditional growth analysis parameters, in combination with leaf net photosynthesis, chlorophyll fluorescence parameters, conductance to water vapor and water potential, to provide explanations for differences in biomass production and relative growth rates. Based on previous findings with the congeneric tall fescue, we predicted that *Neotyphodium*-infected (E+) Arizona fescue should

produce more biomass and have faster relative growth rates than uninfected (E−) plants, and this effect would be most pronounced when water was more limiting.

2. Materials and methods

2.1. Plant material

Several naturally growing Arizona fescue plants were collected from Merritt Draw (34°49′N, 111°17′W; 2500 m elevation) in the Coconino National Forest of north-central Arizona in fall 1999, potted and transported to Arizona State University. *Neotyphodium* infection status of plants was determined with a modified tissue print immunoblotting (Gwinn et al., 1991; Schulthess and Faeth, 1998). At least two species of *Neotyphodium* have been described from Arizona fescue (An et al., 1992; White et al., 1993). Based on anatomical features and microsatellite DNA (Sullivan and Faeth, 2001), the *Neotyphodium* in our plants most closely resembled *Neotyphodium starrii* Morgan-Jones and Gams. One randomly chosen E+ and one E− plant were each split into 24 plants of two to four 4 tillers on 12 November 1999 and replanted in individual square pots (14 cm wide × 13 cm tall) in native soil (Brolliar stony clay loam). Plants were fertilized with Stern's Miracle Grow (Port Washington, NY, USA), and allowed to establish in a greenhouse, where the temperatures were maintained at 22 °C/18 °C (day/night), and supplemental light was provided for 18 h each day. Soils were watered to field capacity three times a week.

2.2. Treatments

When we began our experiment, on 29 January 2000, pots were transferred to an unshaded area outdoors. Prior to beginning the treatments, eight randomly chosen E+ and E− plants were harvested to assess initial biomass. Another eight randomly chosen E+ and E− plants were each randomly assigned to one of two water treatments: high water availability (HW; watered three times a week to field capacity; ≈300 ml), and low water availability (LW; watered once a week to field capacity). We recognize that our experimental design does not control for plant or endophyte genotype, which may influence growth etc. (e.g. Meijer and Leuchtmann, 2001; Cheplick et al., 2000). However our experiment is the first test, to our knowledge, of the effect of *Neotyphodium* influence on growth and detailed physiological parameters in a native grass. We monitored leaf gas-exchange, Ψ, and plant growth over 87 days. On the 26th day of the experiment (24 February 2000), the LW treatment, for all plants, was modified from watering once a week to once every other week to impose more severe water stress.

2.3. Biomass production and growth

The effect of endophyte infection and water stress on biomass production and growth parameters was determined over the 87-day period. At the end of the period, plants were divided into roots and aboveground parts, and soil was washed from roots by hand. Specific leaf mass (SLM) was determined on a subsample of each plant (containing about 25% of the total leaves) using a leaf area meter (Decagon Devices, Pullman, WA, USA). Biomasses of E+ or E− plants at the initial harvest were randomly paired with respective E+ or E− plants at the final harvest, and the relative growth rate (RGR; rate of biomass gain per biomass) and net assimilation rate (NAR; rate of biomass gain per leaf area) of each plant was estimated using the equations in Xiong et al. (2000), following Hunt (1990). (LAR; leaf area per total plant biomass), leaf mass ratio (LMR; leaf biomass per total plant biomass), and root:-shoot biomass ratio (R:S) were also calculated.

2.4. Leaf gas exchange, chlorophyll fluorescence and water potential

Rates of net photosynthesis (P_n) and transpiration (E) of one group of leaves on each plant were measured at midday (1100–1400 h) on 11 dates during the experiment. These measurements were made at midday because we suspected that water limitation treatment effects should be greatest at

this time. Measurements were made 1–2 days after watering the HW treatment on a sunny day using a closed infra-red gas analyzer (IRGA) system (LI-6200, Li-COR, Lincoln, NE, USA). The three to five longest, completely green leaves on a plant were held parallel in the chamber for measurements. The CO_2 concentration of air entering the chamber (c_a) ranged from 391 to 420 ppm over the experiment, but was maintained within 25 ppm over a given sampling date. Air temperature during measurements ranged from 22–26 °C in January and February to 28–30 °C in March and April. Leaf temperature in the cuvette during measurements ranged from 22–27 °C in January and February to 27–31 °C in March and April. Net photosynthesis, leaf conductance to water vapor (g_l), and the intercellular CO_2 concentration (c_i) were calculated using the equation of von Caemmerer and Farquhar (1981), and instantaneous water use efficiency (WUE) was calculated as P_n/E in the chamber. Leaf water potential (Ψ) was measured after each gas-exchange measurement using a pressure chamber (Model 1003, PMS, Corvallis, OR, USA) on one of the leaves used in the gas-exchange measurements. The leaf was cut 6 cm from its tip. We also measured the gravimetric moisture content of soil in each pot during the final plant harvest. A soil core (2.2-cm diameter × 10-cm deep) was extracted from along the edge of each pot, dried at 100 °C for 72 h, and weighed.

To assess the relative importance of stomatal and biochemical limitations to P_n under our treatments, we measured the P_n–c_i response curves of four plants from each treatment during the 37th–41st days of the experiment. Response curves were assessed on two randomly chosen plants (one E+ and one E−) from the HW treatment on 6 March and again on 8 March, and on two randomly chosen plants (one E+ and one E−) from the LW treatment on 9 March and again on 10 March. Curves were generated by measuring P_n of three to five leaves in a chamber over a series of ambient CO_2 concentrations (45–1600 ppm) using an open IRGA system (LI-6400, Li-COR) and a CO_2-injector system (6400-01, Li-COR). Air temperature in the chamber was maintained at 29 °C, and a metal-halide lamp (1000 W, Crawfordsville, IN, USA) provided 1200 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR; 400–700 nm) at the plant surface, as measured with a quantum sensor (LI-190SA, Li-COR). Leaf temperatures ranged from 29 to 30 °C during measurements. This temperature was near optimal and this PAR was saturating for photosynthesis based on preliminary temperature and light response curve results. The equation $(P_a - P_n)/P_o$, where P_o is the photosynthetic rate at a c_i of 360 ppm and P_a is the rate at a c_a of 360 ppm, was used to estimate the relative stomatal limitation (l_s) to photosytnesis (Farquhar and Sharkey, 1982). The apparent carboxylation efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was estimated from the initial slope of the linear portion of the P_n–c_i response using the program in Photosyn Assistant (Dundee Scientific, Dundee, Scotland, UK).

To further assess biochemical limitations to P_n, we also measured chlorophyll *a* fluorescence parameters using a pulse amplitude modulated fluorometer (OS-500, OPTI-Sciences, Haverhill, MA, USA). On three sunny days in March (1, 4 and 22 March), we measured midday effective or light-adapted quantum yield of PSII (photosystem II) electron transfer (Φ_{PSII}), along with the potential quantum yield or ratio of variable to maximal fluorescence (F_v/F_m), on eight plants in each treatment. For measurements, the end of the fiber-optic probe was held at a 40° angle about 0.5 cm from a group of fully expanded leaves that were held parallel with a leaf clip. Weak modulated light (0.2 μmol m^{-2} s^{-1} PAR) and a saturating pulse (11 000 μmol m^{-2} s^{-1} PAR) were used to induce F'_o and F'_m, respectively. We averaged four measurements from each plant to estimate Φ_{PSII}, which was calculated according to Genty et al. (1989), and measured eight plants within each treatment combination. Thereafter, we shaded one group of leaves on each plant for 20 min, using the leaf clip, and measured F_v/F_m.

2.5. Statistical analyses

Analysis of variance (ANOVA) was used to examine endophyte and water availability treatment effects on biomass production and growth

parameters, and least significant difference tests were used to compare among individual treatment means. Repeated-measures multivariate analysis of variance (MANOVA) tests were used to examine treatment and sampling date effects on P_n, g_l, c_i, WUE, and Ψ over the experiment (SYSTAT, 2000). All data sets satisfied the assumptions of ANOVA based on homogeneity of variances, normality of errors, and independence of errors.

3. Results

3.1. Biomass production and growth

To account for differences in initial size of the plants, we assessed biomass production by calculating the percentage change in biomass over the 87-days treatment period, relative to that of plants at our initial harvest. Biomass at the initial harvest averaged 10.04 g (±1.6924 S.D.). Not surprisingly, water availability had a significant effect on total (above- and belowground) relative biomass production, with plants under LW producing less biomass (Fig. 1A). Although endophyte infection did not have a significant overall effect on total biomass, E+ plants produced less total biomass than E− plants under high water availability.

Treatment effects on aboveground biomass production followed similar trends, but were more pronounced than those on total biomass production. Water availability interacted with infection; E− plants produced more aboveground biomass than E+ plants under high water availability, whereas at LW E+ plants produced more aboveground biomass (Fig. 1C). In contrast, there were no significant effects on belowground biomass production (Fig. 1E) or on R:S ratio ($P > 0.05$; data not shown). Trends in RGR (calculated using total biomass values) paralleled those of total biomass production, and results are not shown. Water availability affected RGR (ANOVA; $P < 0.05$), with plants under LW having lower RGR (LSD, $P < 0.05$). Although infection did not have an overall effect on RGR (ANOVA, $P = 0.12$), E− plants had a greater RGR under high water availability (LSD, $P < 0.05$; data not shown). Water availability interacted with infection on NAR, although at both water availabilities, E+ plants had a greater NAR than E− plants (Fig. 1B). Water availability also interacted with infection on LAR, with E− plants having greater LAR than E+ plants under high water availability, whereas under LW the E+ plants had greater LAR (Fig. 1D). We further assessed allocation to leaves by examining LMR; water availability did not affect LMR ($P > 0.05$; data not shown). Water availability interacted with infection to affect SLM (Fig. 1F). Under high water availability, the E+ plants had greater SLM, whereas under LW, the E− plants had greater SLM.

3.2. Leaf gas exchange, chlorophyll fluorescence and water potential

Infection affected midday P_n. Comparing means on individual sampling dates, E− plants had higher P_n on seven of 11 dates under high water availability, and on 6 of 11 dates under LW (Fig. 2A and B). The exception to this trend was the last two sampling dates, after a more severe LW treatment was imposed, then the E+ plants had higher P_n rates than E− plants under LW (Fig. 2B).

Consistent with trends of P_n, infection affected midday g_l. Non-infected plants had greater g_l than E+ plants on eight of 11 sampling dates under high water availability, and five of 11 dates under LW (Fig. 2C and D). As was the case with P_n, on the last two sampling dates, after a more severe LW treatment had been imposed, E+ plants had a higher g_l than E− plants (Fig. 2D).

Water availability interacted with infection for c_i (Fig. 2E and F). The most apparent trend was in the LW treatment, where E+ plants tended to have a higher c_i than E− plants, although these differences were significant on only four of 11 sampling dates.

Water use efficiency followed a trend similar to that of P_n and g_l. Uninfected plants tended to have a higher WUE than E+ plants, although these differences were significant on only a few sampling dates (Fig. 3A and B). Infected plants had a higher

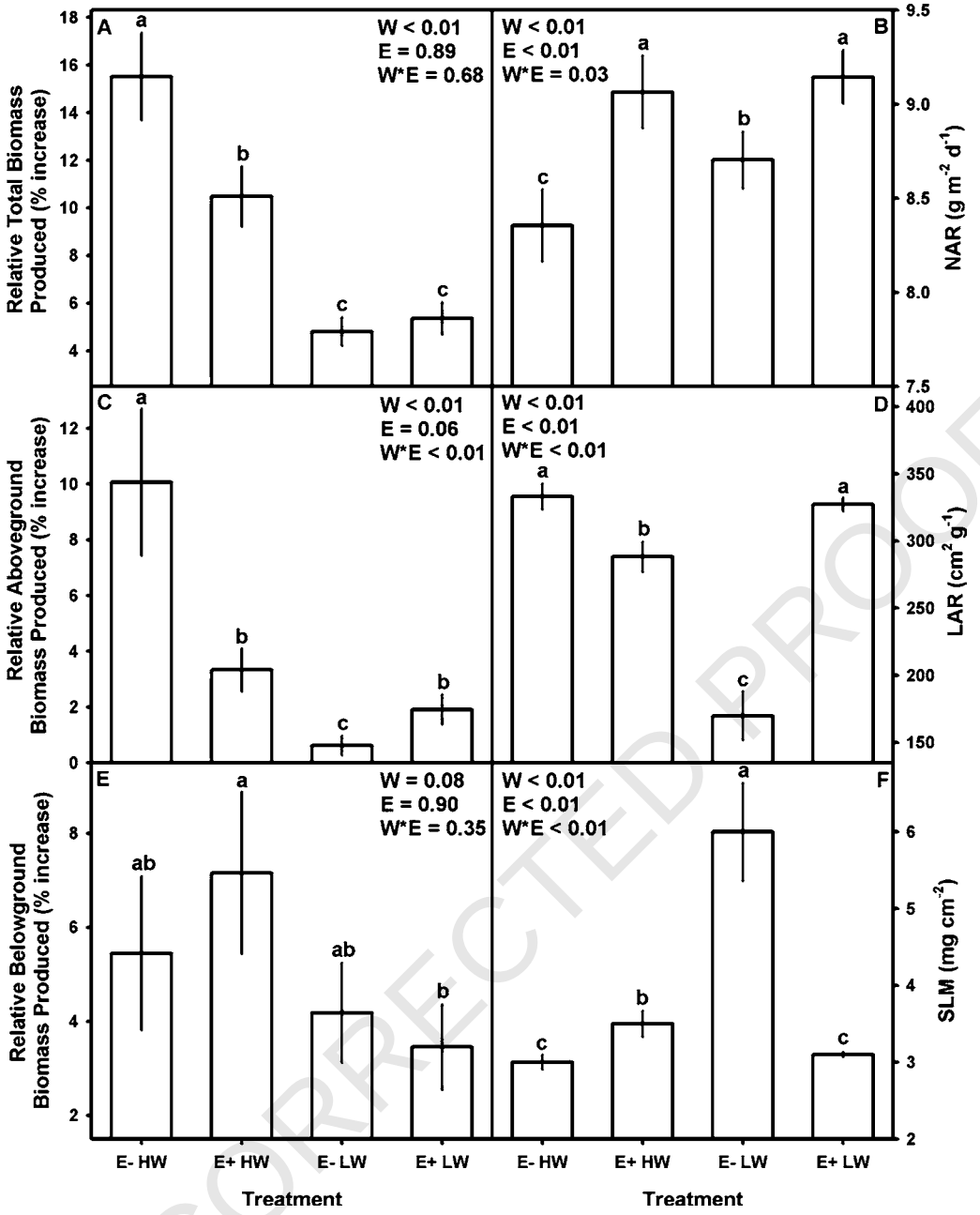

Fig. 1. Relative total (A), aboveground (C), and belowground (E) biomass, (NAR; B), (LAR; D), and (SLM; F) of uninfected (E−) and infected (E+) Arizona fescue grown at high (HW) or LW. The P-values for water availability (W), infection (E), and water availability x infection effects, based on two-way ANOVA, are shown in the upper right or left of each panel. Values are means (±S.E.) of eight replicates. Different letters indicate means are significantly different ($P < 0.05$).

WUE than E− plants in the LW treatment on one of the two sample dates, after the low water treatment had been made more severe (Fig. 3B).

Water availability and infection affected leaf Ψ (Fig. 3C and D). As expected, leaf Ψ tended to be more negative under the LW treatment, especially

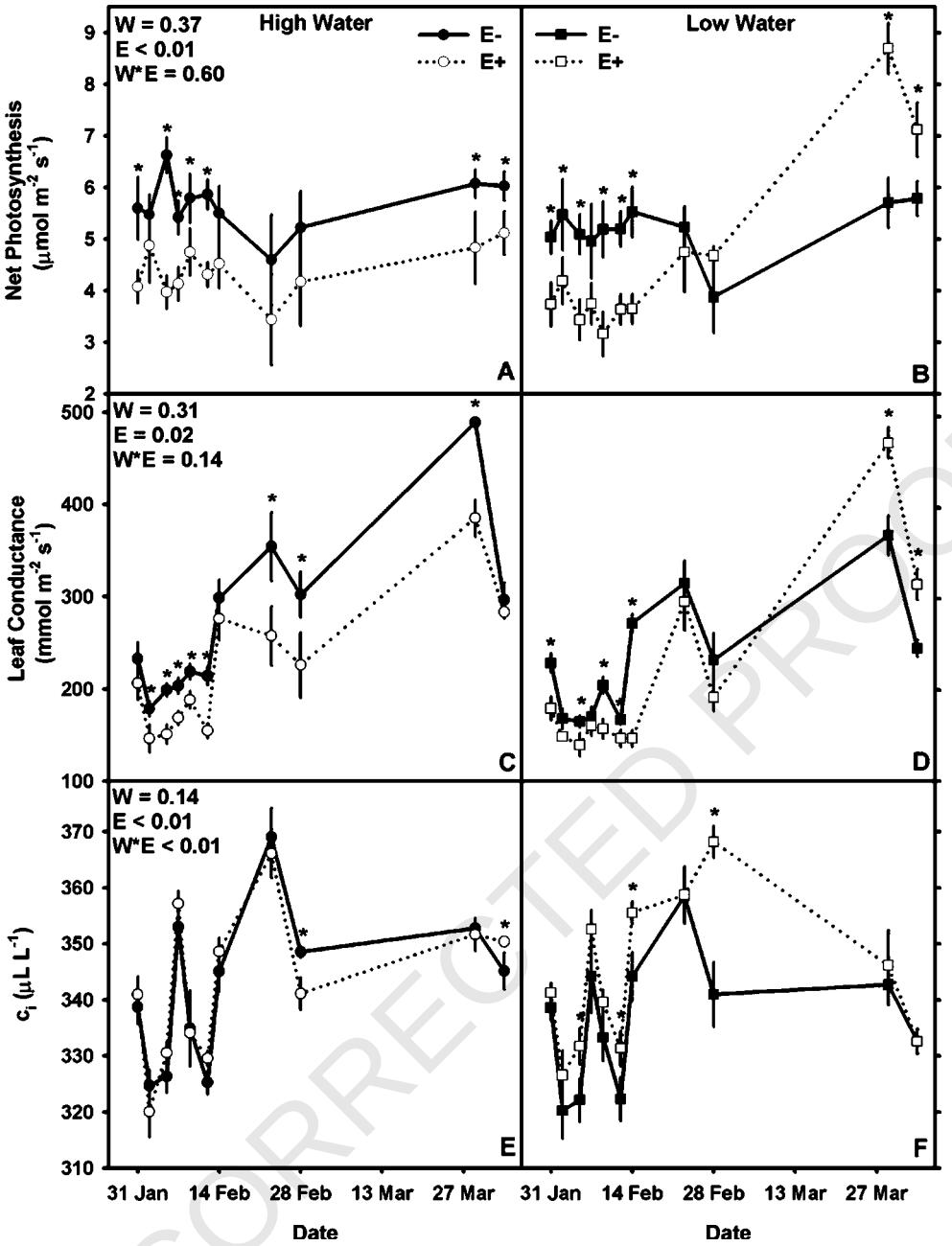

Fig. 2. Net photosynthesis, leaf conductance to water vapor, and intercellular CO_2 concentration (c_i) of uninfected (E−) and infected (E+) Arizona fescue grown at high (HW; left panels A, C, E, respectively) or low water availability (LW; right panels B, D, F, respectively). On 24 February the HS treatment was modified from watering once a week to once every other week in order to impose more severe water stress. The *P*-values for water availability (S), infection (E), and water availability x infection effects, based on two-way ANOVA, are shown in the upper left of the left panels. Values are means (±S.E.) of eight replicates. Asterisks indicate means are significantly different ($P < 0.05$).

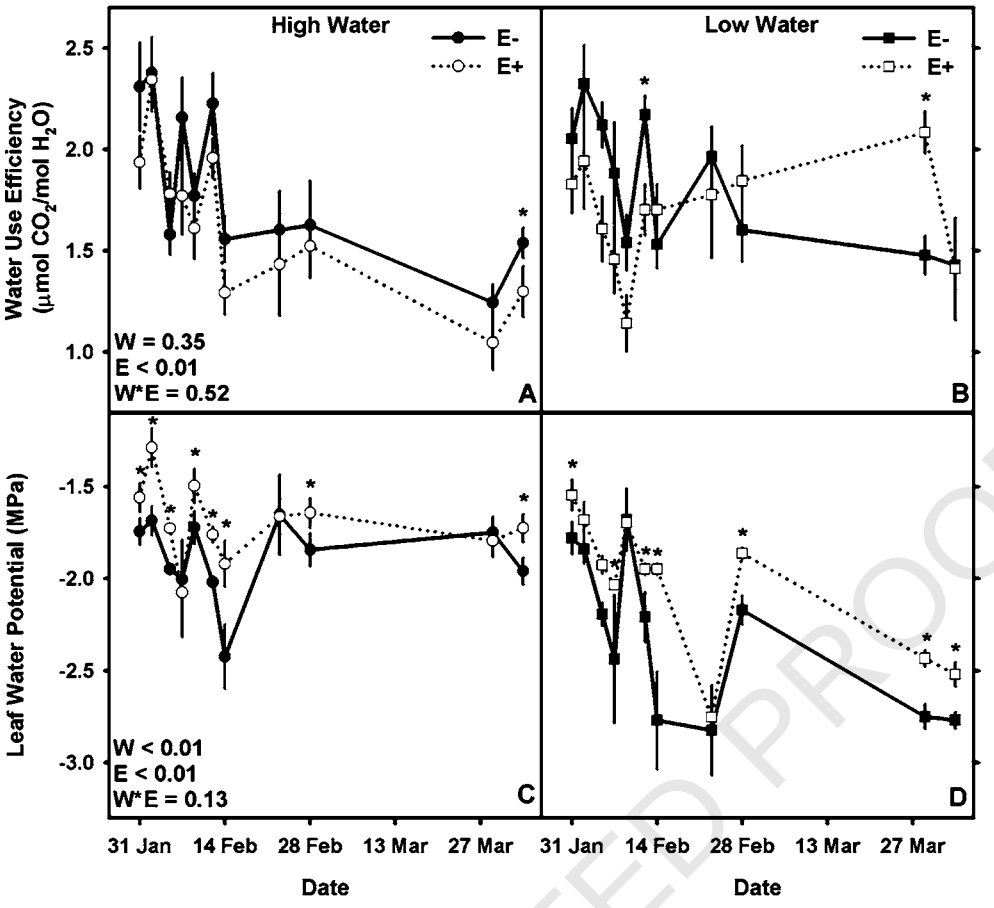

Fig. 3. Water use efficiency and leaf water potential of uninfected (E−) and infected (E+) Arizona fescue grown at high water availability (HW; left panels A, C, respectively) or low water availability (LW; right panels B, D, respectively). On 24 February the HS treatment was modified from watering once a week to once every other week in order to impose more severe water stress. The P-values for water availability (W), infection (E), and water availability × infection effects, based on two-way ANOVA, are shown in the lower left of the left panels. Values are means (±S.E.) of eight replicates. Asterisks indicate means are significantly different ($P < 0.05$).

when more severe water stress was imposed later in the experiment. Infected plants had less negative leaf Ψ than E− plants on eight of 11 sampling dates under high water availability, and seven of 11 dates under LW (Fig. 3C and D). Under high water availability, soil moisture content at the final harvest tended to be higher in pots of E+ (mean = 24.4%) than E− plants (mean = 17.9%; $P < 0.10$; data not shown). Under LW, soil moisture content tended to be higher in pots of E+ (mean = 9.4%) than E− plants (mean = 7.1%; $P = 0.10$). The high water availability treatments had higher soil moisture content than LW treatments.

We investigated whether differences in P_n between treatments might be attributable to differences in stomatal and biochemical limitations by assessing P_n–c_i responses. Water availability and infection affected stomatal limitations to P_n (Fig. 4A). Stomatal limitation was greater in E+ plants in both water availability treatments, and was greater at LW. Under high water availability the E− plants had higher carboxylation efficiencies than the E+ plants, while under LW, efficiencies of E+ and E− plants were not different (Fig. 4B).

Midday F_v/F_m was not affected by treatment on any sampling date (data not shown). The Φ_{PSII} was significantly lower in plants under LW on one

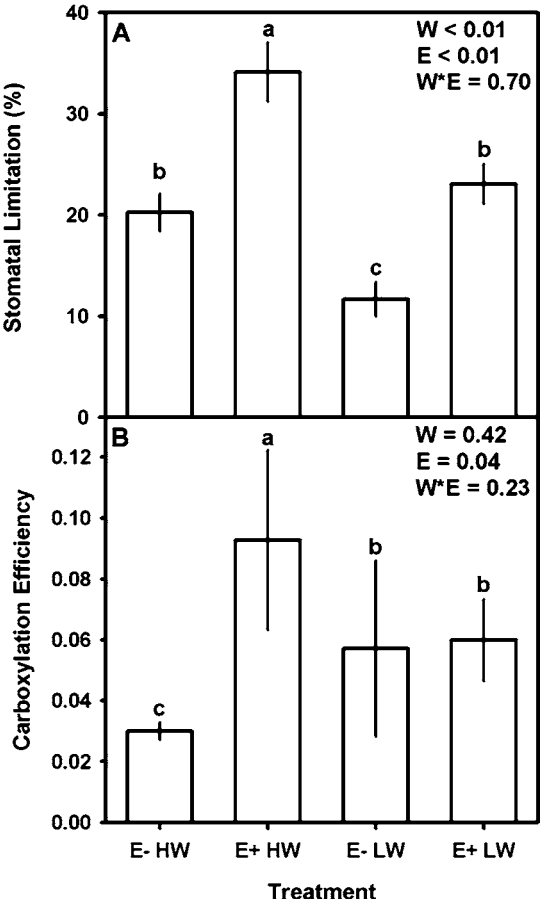

Fig. 4. Stomatal limitation to photosynthesis (A) and carboxylation efficiency of Rubisco (B) in uninfected (E−) and infected (E+) Arizona fescue grown at high (HW) or low water availability (LW), sampled in early March. The *P*-values for water availability (W), infection (E), and water availability × infection effects, based on two-way ANOVA, are shown in the upper right of each panel. Values are means (±S.E.) of four replicates. Different letters indicate means are significantly different ($P < 0.05$).

sampling date (4 March; LSD, $P < 0.05$; data not shown).

4. Discussion

Neotyphodium infected Arizona fescue plants produced more aboveground biomass than E− plants, but only under LW. Similarly, E+ tall fescue plants typically produce more biomass than E− plants under limiting water availability (West et al., 1988; Arachevaleta et al., 1989; West et al., 1993). Under high water availability, however, E− plants produced more total biomass than E+ plants. In contrast, E+ tall fescue typically produce similar amounts of biomass, or more biomass, than E− plants (Arachevaleta et al., 1989; Assuero et al., 2000). Hence, the effects of *Neotyphodium* infection on Arizona fescue growth tends to differ from that on tall fescue, at least under adequate water availability conditions.

We investigated which growth parameters best explained greater aboveground biomass production and RGR in Arizona fescue. Higher RGR is often associated with (1) assimilating more biomass (NAR) or carbon per unit leaf area (the latter could be reflected in higher P_n), and/or (2) producing more leaf area per unit of total plant biomass (i.e. higher LAR) (Hunt, 1990; Lambers et al., 1998). Greater LAR could be accomplished by allocating relatively more biomass to leaves (LMR), or producing less dense leaves that would increase leaf area per unit leaf mass (i.e. lower SLM).

Greater aboveground biomass production by E+ plants under LW appears attributable to greater NAR, as well as the production of less dense, leaves that provided more surface area and therefore greater light harvesting capability per unit of investment leaf mass. In contrast, under high water availability, E− plants produced greater aboveground biomass than E+ plants. In this case, the greater growth of E− plants appeared to involve higher P_n rates per unit leaf area, as well as producing less dense leaves, which provided more surface area.

With the exception of the severe water stress imposed later in the experiment, E+ plants had consistently lower P_n, as well as g_l. (Fig. 2). The greater l_s found in E+ plants under each treatment (Fig. 4A) suggests that the lower g_l of E+ plants was at least partly responsible for lower P_n in these plants. Furthermore, the lack of infection effects on chlorophyll fluorescence parameters suggests that infection had little effect on biochemical limitations to P_n. *Neotyphodium*-infected tall fescue plants sometimes have lower g_l than uninfected plants (Elmi and West, 1995). It is unclear why

infection leads to lower g_l in Arizona and tall fescue; infection may lead to anatomical or morphological changes such as lower stomatal densities or more prevalent leaf folding (Arachevaleta et al., 1989; but see West et al., 1993; West, 1994). Infection may also alter physiological mechanisms, via hormonal signals such as abscisic acid (Joost et al., 1993) that promote guard cell closure.

After we imposed a more severe water limitation in late February, E+ plants, however, had higher P_n and g_l. Similarly, E+ tall fescue plants often have higher g_l than E− plants under water limitations (Elbersen et al., 1994; Elbersen and West, 1996). We suspect that the higher gas-exchange rates we observed in E+ plants after severe water stress resulted from greater soil moisture availability in pots containing E+ plants. Leaf Ψ of E+ plants were consistently higher than those of E− plants (Fig. 3D), and lower g_l and transpiration rates of E+ plants during the first month of the experiment may have conserved soil moisture, under severe water limitation. At the final harvest, soil in pots of E+ plants tended to have higher moisture content within each water availability treatment compared with E− plants. It appears that infection may lead to lower g_l and transpiration rates in Arizona fescue under high or moderate water availability, and that this may conserve soil moisture, so that if a severe water limitation subsequently occurs, the conserved soil water allows maintenance of higher P_n and g_l, as well as WUE. Our results suggest that *Neotyphodium* can have antagonistic as well as mutualistic effects on the performance of Arizona fescue, depending in part on water availability. Our findings support the idea that fungal endophyte–plant interactions vary in direction depending on environmental conditions (Siegel, 1993; West, 1994; Saikkonen et al., 1998; Faeth and Fagan, 2002). Our findings also provide one explanation as to how E+ and E− plants are maintained in wild populations of Arizona fescue. A typical growing season for semi-arid ponderosa pine/Arizona fescue communities in north-central Arizona begins in early May with relatively high soil moisture availability ($\sim$40%) due to snow melt, followed by an extreme dry-down period extending into late June, and then a partial recharge period from July through August due to summer rains. This is followed by another dry-down period in the fall until winter rains begin in December. In these communities, soil moisture content in the upper 10-cm around the edge of Arizona fescue canopies typically drops to 5–25% during June (Morse, unpublished data), and then rises to 60–100% during the summer thunderstorm period during July and August. Hence, the soil moisture content in the pots of our LW treatment (9.4 and 7.1% for E+ and E− plants, respectively), and our high water availability treatment (24.4 and 17.9% for E+ and E− plants, respectively), are comparable to those found in natural Arizona fescue populations during the dry June period and the wetter July–August period, respectively. During the dry periods, E+ plants may be favored because of their ability to maintain higher P_n, g_l, growth rates and biomass production in the face of severe water limitations, whereas E− plants would be favored during the subsequent wet season, because of their higher P_n, g_l, growth rates and biomass production under high available soil moisture. During the 10–20 year life span of an Arizona fescue plant, several exceptionally wet and dry years also occur (based on 50 year precipitation averages). Thus, E+ and E− plants also experience long-term changes in water availability that may promote their recovery and coexistence.

5. Uncited references

West and Gwinn, 1993; Bacon, 1993.

Acknowledgements

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