



# An *Epichloë* endophyte improves photosynthesis ability and dry matter production of its host *Achnatherum inebrians* infected by *Blumeria graminis* under various soil water conditions

Chao Xia,<sup>a</sup> Nana Li,<sup>a</sup> Xingxu Zhang,<sup>a,\*</sup> Yao Feng,<sup>a</sup> Michael J. Christensen,<sup>b</sup> Zhibiao Nan<sup>a</sup>

<sup>a</sup> State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agricultural Science and Technology, Lanzhou University, Lanzhou 730020, PR China

<sup>b</sup> Retired from AgResearch, Grasslands Research Centre, Private Bag 11-008, Palmerston North 4442, New Zealand

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## ABSTRACT

The interaction between an *Epichloë* endophyte, *Blumeria graminis* and *Achnatherum inebrians*, was studied at four soil water contents. Lesion length and disease index were recorded dynamically, and the chlorophyll content, photosynthetic parameters and final dry matter accumulation were measured after a 4-week period of disease. Infection by *B. graminis* significantly ( $P < 0.05$ ) decreased the chlorophyll content, net photosynthetic rate, intercellular carbon dioxide concentration and dry matter at some soil water contents. Presence of the endophyte lowered the disease index while significantly ( $P < 0.05$ ) increasing the chlorophyll content and net photosynthetic rate. The endophyte also positively affected the intercellular carbon dioxide concentration. In addition, it increased the dry matter accumulation per host plant under pathogen stress. The study demonstrated that while powdery mildew depresses the photosynthetic parameters of *A. inebrians*, the presence of the *Epichloë* endophyte can reduce the damage caused by the pathogen.

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## 1. Introduction

Symbioses of plants and animals with microorganisms have led to major transitions in the evolution of life (Margulis, 1996). Many cool-season grasses are hosts of the endophyte genus *Epichloë*, whose asexual stages had previously been classified as *Neotyphodium* spp. (Leuchtman et al., 2014). The associations between grasses and *Epichloë* endophytes are considered in general to be mutualistic (Müller and Krauss, 2005). Mutualism plays important roles in influencing species coexistence and determining community composition (Umbanhowar and McCann, 2005). These endophytic fungi colonize asymptotically the plant shoot meristems where they grow systemically in the apoplast of developing leaves and culms, obtaining nutrients (Kuldau and Bacon, 2008), their growth being synchronized with that of their host plants (Christensen et al., 2008). Host plants provide photosynthates and shelter for the endophytes and the presence of endophytes can improve the ability of the host grass to persist when exposed to abiotic (Kuldau and Bacon, 2008; Oberhofer et al., 2014; Song et al., 2015) and biotic stresses (Matsukura et al., 2014; Ma et al., 2015).

*Achnatherum inebrians*, colloquially known as drunken horse grass (DHG), is a perennial bunchgrass of China. This grass species is a host for two related taxa of seed-transmissible fungal symbionts *Epichloë gansuensis* (Li et al., 2004) and *E. gansuensis* var. *inebrians* (Moon et al., 2007), the latter of which has been proposed to be raised to species rank as *E. inebrians* (Chen et al., 2015). These two

*Epichloë* endophytes are reported to produce alkaloids including ergonovine and ergine (i.e. lysergic acid amides) (Zhang et al., 2011, 2014a), which are associated with livestock toxicosis (Zhang et al., 2014a). *Achnatherum inebrians* is mainly found in north and north-west China in the alpine and subalpine grasslands, where natural conditions are usually harsh (Zhang et al., 2014a). A survey of 20 populations of *A. inebrians* from north and northwest China grasslands found that 19 populations were 100% infected with *Epichloë* spp. endophytes, while the remaining population was 80% infected (Nan and Li, 2000). In addition to producing toxic alkaloids, the presence of an *Epichloë* spp. endophyte also increases the tolerance of *A. inebrians* to drought (Li et al., 2008), salt (Li et al., 2008), heavy metals (Zhang et al., 2010), pests (Zhang et al., 2012), grazing by livestock (Li et al., 2009) and pathogens (Zhang et al., 2014b, 2015; Xia et al., 2015).

Powdery mildew is a disease caused by the fungal pathogen *Blumeria graminis*. This pathogen is biotrophic (Brown and Hovmøller, 2002; Ridout et al., 2006) and colonizes the host epidermal cells by penetrating through the cell wall to form haustoria inside cells where it obtains the nutrients required for survival (Bruggmann et al., 2005). This fungus can infect all aerial parts of a plant, but is mainly found on the upper surface of leaves (Rossi and Giosuè, 2004). Powdery mildew is a common fungal disease and has a broad host range, including 634 hosts belonging to the Poaceae, causing serious and widespread damage to a variety of the world's major cereal food and forage crop plants (Inuma et al., 2007), including wheat, barley, rye and oats. Pathogenicity is closely related to soil moisture and air humidity, high soil moisture and humid climate can increase the susceptibility of plant to this pathogen (Huo et al., 2002; Panstruga and Schulze-Lefert, 2002). *Blumeria graminis*

\* Corresponding author.

Email address: xxzhang@lzu.edu.cn (X. Zhang)

causes a decline in photosynthesis and loss of chlorophyll in the tissue as infection progresses (Farrar and Lewis, 1987), and further causes a negative impact on agricultural yields (Liu et al., 2015a,b). Its importance as a pathogen of important food crops has led to the disease being widely studied, although this fungus is difficult to investigate away from a host because it has not yet been successfully grown *in vitro*. Li et al. (2003) in their field survey in August found the powdery mildew pathogen occurred on *A. inebrians*. Observations over several years have provided evidence that *A. inebrians* plants (both endophyte-infected, E+ and endophyte-free, E-) will become naturally infected by powdery mildew in greenhouses from spring through to summer. This predictable natural infection has laid the foundation for the present trial and other studies involving interaction among powdery mildew, the *Epichloë* spp. endophyte and *A. inebrians*. A recent study that we conducted showed that E + *A. inebrians* seedlings have an advantage over E-seedlings in their ability to resist and tolerate powdery mildew disease, as reflected by improved morphological parameters and reduced disease injury (Xia et al., 2015).

However, the effect of the endophyte on *A. inebrians* susceptibility and host physiological response to powdery mildew infection has not yet been investigated. The aim of this study is to determine the effects of the endophyte on the susceptibility to powdery mildew of *A. inebrians* plants, looking mainly at the effects on host physiological parameters. Different soil water conditions were employed, in view of the close relationship between humidity and susceptibility of plants to this pathogen.

## 2. Materials and methods

### 2.1. Plant material collection and grass management

We collected *Epichloë* E+ and E- *A. inebrians* plants that were grown in the experimental field of the College of Pasture Agriculture Science and Technology, Yuzhong campus of Lanzhou University (104°39' E, 35°89' N, Altitude 1653 m) in 2011. Seeds from one E+ plant and one E-plant were each sown separately in 2012 at the same site, and the respective resulting seeds, collected in September 2013 for the present study.

A controlled-environment pot experiment was performed from 25 March to 13 June 2015 in the greenhouse of the College of Pastoral Agriculture Science and Pastoral Agriculture Science and Technology, Lanzhou University. On March 25, healthy-looking and well-filled seeds were sown into 200 pots (100 pots for E+ and 100 pots for E-plants), with 3 seeds per pot (diameter: 75 mm; height: 100 mm), filled with vermiculite (70 g) that had been sterilized in an oven at 120 °C for 5 h. After germination, plants were thinned to one uniform seedling per pot. These were assigned at random to a position within a constant temperature greenhouse (temperature: 26 ± 2 °C, moisture: 42 ± 2%) and watered sufficiently to keep the surface of the vermiculite moist. Following the appearance of the second fully expanded leaf, Hoagland's solution was used to quantitatively water the pots every other day (Xia et al., 2015).

### 2.2. Experimental design

On May 13, a small number of disease lesions of powdery mildew were observed. One hundred and sixty pots (80 with E+ and 80 with E-plants), each of which contained an equal sized seedling, were selected for the following trial. Before selection, the infection status of each plant was determined by microscopic (Olympus, Japan) exam-

ination of leaf sheath pieces stained with aniline blue. These plants were cut to 15 cm above the vermiculite surface to ensure the height of all plants was equal prior to imposing the trial conditions. On the same day, watering of the pots ceased so that the water-holding capacity (WHC) of each pot was reduced to 15% relative saturation moisture content (RSMC), a ratio of actual soil moisture content relative to potential maximum soil moisture saturation. Then four different water-holding capacities were established, including strong drought (15% RSMC), light drought (30% RSMC), normal moisture (45% RSMC) and abundant moisture (60% RSMC) conditions. Each soil water-holding capacity condition for either E+ or E-seedlings were assigned 20 pots, ten experimental replicates exposed directly to the air and 10 control replicates covered by transparent cylindrical plexiglass (Seebold et al., 2001). The top of each cylinder was covered by gauze (to avoid infection by powdery mildew spores) (Liu, 2002). Before these ten control pots were covered, every leaf of each plant was wiped with 75% alcohol to kill any powdery mildew propagules on the leaf. The plexiglass cylinder topped with gauze was used to prevent pathogen spores from reaching plants. Therefore, plants in cylinders were not as colonized by the powdery mildew pathogen during the period of this experiment. Every evening of the trial at 6 o'clock, each pot was weighed and water was added to maintain soil moisture content at 15%, 30%, 45% and 60% RSMC. After watering, the position of each pot was changed randomly.

### 2.3. Measurement protocols

#### 2.3.1. Disease investigation

The pathogen was detected and identified using the same method as Xia et al. (2015), and was confirmed as *B. graminis*. From May 13 to June 13 the number and length of lesions were noted each week to assess disease severity. Determination of the disease index (DI) was done at the same time. The disease severity was classified into nine different levels according to the percentage leaf area affected by disease on individual leaves. DI severity values of 1, 5, 10, 20, 30, 40, 60, 80, and 100 percent, were calculated using the following formula:

$$DI = \frac{\sum (x \times t)}{s \times \sum t} \times 100$$

where x is the disease severity degree, t is the total number of leaves of each degree of disease severity and s is the highest degree of disease severity observed.

#### 2.3.2. Collection and measurement of photosynthetic data

The measurement of photosynthetic parameters included photosynthesis rate (Photo), intercellular carbon dioxide concentration (Ci), stomatal conductance (Cond) and transpiration rate (Trmmol). These parameters were measured on 3 June, 3 weeks after powdery mildew pathogens had been observed colonizing the *A. inebrians* seedlings. At that time, the differences both between powdery mildew-infected (PM+) vs. uninfected (PM-) and E+ vs. E-plants were obvious by casual observation. Photosynthesis in the top leaf of five plants in each treatment was measured using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) from 9:00 to 11:00 a.m. The chamber was equipped with red/blue LED light source (LI6400-02B), with the PAR set at 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the detesting conditions as T = 28 ± 1 °C, air carbon dioxide concentration = 410 ± 10  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ .

### 2.3.3. Chlorophyll content

Along with the measurement of photosynthetic parameters on the same day, the same 5 plants from each treatment were measured with a chlorophyll meter (SPAD-502Plus, Konica Minolta Sensing, INC, Japan), as the average of 3 measurements of each leaf, close to the spot where photosynthesis measurements were performed. The average of values measured at 3 points along each leaf was used in subsequent data analysis, the relative value for the chlorophyll concentration being called a SPAD value.

### 2.3.4. Biomass production and growth

When disease investigations and the determinations of photosynthetic parameters and chlorophyll content were completed, all plants, from all the treatments, were carefully removed from the vermiculite on the 13th June, and cleaned with distilled water before being separated into root and foliage components. The water adhering to the foliage surface was removed by blotting with filter paper, the tiller number of each plant from each treatment was counted, and the length of leaves of the E+ or E-plants was also measured. All samples were oven-dried at 80 °C until a constant weight was reached, and then each dry foliage part from each treatment was weighed to determine total dry matter per plant.

### 2.3.5. Statistical analysis

Data analyses were performed with the SPSS software, Version 17.0 (SPSS, Inc., Chicago, IL, USA). Effect of endophyte on the weekly disease index was evaluated through repeated-measures ANOVA. Effects of powdery mildew disease, endophyte and differing soil water contents on cohort physiological parameters were analyzed by three-way ANOVA. Fishers Least Significant Differences (LSD) test was used to determine whether differences between means were statistically significant. If this indicated significant differences of experimental parameters between E+ and E-plants, then an independent-sample T-tests was employed to determine the significance of difference between E+ and E-plants at the same water content, in order to assess the effects of the presence of endophyte. Statistical significance was defined at the 95% confidence level. Means are reported with their standard error.

## 3. Results

### 3.1. Dynamic change of disease index

The disease index was calculated after every survey for the length of lesions (Supplementary Fig. 1) and disease severity. During the entire progress of this trial, the presence of the endophyte demonstrated an improvement of the ability of E+ hosts to resist infection by powdery mildew. Repeated-measures ANOVA revealed that the endophyte significantly decreased the disease index of host plants at 15% ( $P = 0.003$ ), 30% ( $P = 0.021$ ) and 45% ( $P < 0.001$ ) RSMC, but the effect at 60% RSMC was not significant ( $P = 0.135$ ). Differences in disease index of E+ and E-plants markedly changed through time ( $P < 0.001$ ) at four various water contents. An endophyte  $\times$  time effect indicated that differences in disease index between E+ and E-plants were present at 15% ( $P = 0.002$ ), 30% ( $P = 0.030$ ) and 45% ( $P = 0.047$ ) RSMC, but this difference was not significant ( $P = 0.541$ ) at 60% RSMC (Fig. 1).

### 3.2. Chlorophyll content

Highly significant ( $P < 0.001$ ) interaction effects for powdery mildew, endophyte and soil water content were detected on chlorophyll content of *A. inebrians*. In addition, there were disease  $\times$  endophyte, disease  $\times$  soil water content and disease  $\times$  endophyte  $\times$  soil water content interactions for chlorophyll content (Table 2). The chlorophyll content of both powdery mildew-infected (PM+) and uninfected (PM-) *A. inebrians* increased with an increase of soil water content. This infection caused a significant ( $P < 0.05$ ) reduction of chlorophyll content at 15%–45% RSMC (Fig. 2A). Comparing E+ vs. E-plants infected by the pathogen showed that the presence of the endophyte significantly ( $P < 0.05$ ) increased the chlorophyll content at each soil water content, or at least prevented the loss of chlorophyll content, and this content also increased with the increase of soil water content (Fig. 2B).

### 3.3. Net photosynthetic rate

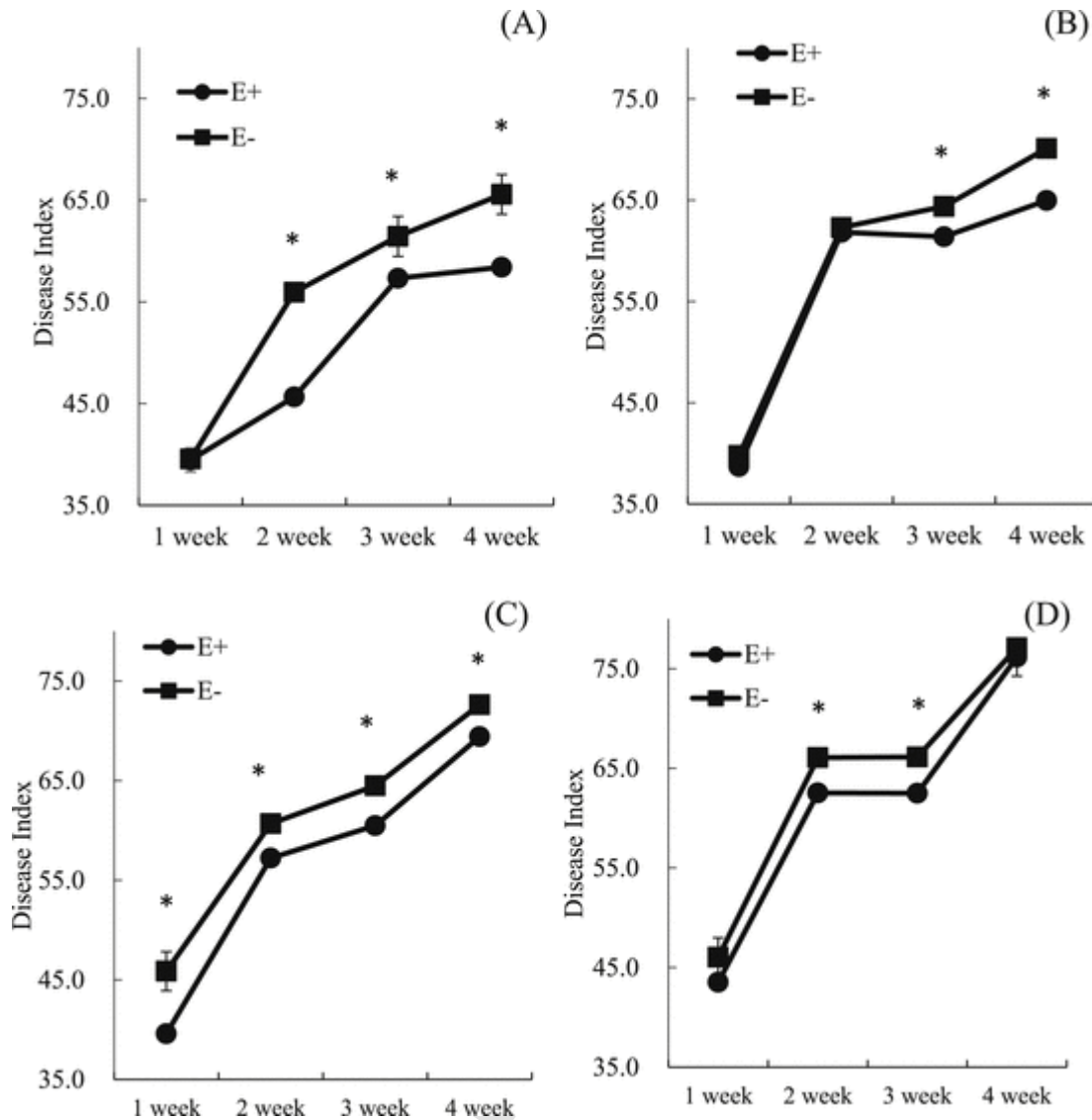
Highly significant ( $P < 0.001$ ) effects of powdery mildew disease, endophyte and soil water content were detected for the net photosynthetic rate of *A. inebrians*. In addition, there were disease  $\times$  water, endophyte  $\times$  soil water content and disease  $\times$  endophyte  $\times$  soil water content interactions for net photosynthetic rate (Table 1). The net photosynthetic rate for both PM+ and PM- *A. inebrians* increased with an increase of soil water content. Infection by powdery mildew significantly ( $P < 0.05$ ) reduced the net photosynthetic rate of *A. inebrians* at each soil water content (Fig. 3A). Comparing E+ vs. E-plants infected by powdery mildew pathogens, the presence of endophyte significantly ( $P < 0.05$ ) enhanced the net photosynthetic rate under the powdery mildew pathogen stress at each soil water content, and the net photosynthetic rate of both E+ and E-plants also increased with the increase of soil water content (Fig. 3B).

### 3.4. Intercellular carbon dioxide concentration

Disease  $\times$  soil water content interaction for intercellular carbon dioxide concentration was evident (Table 1). The intercellular carbon dioxide concentration of PM+ plants decreased from 15% to 30% RSMC and then remained steady from 45% to 60% RSMC. The intercellular carbon dioxide concentration of PM-plants decreased firstly from 15% to 30% RSMC, then increased from 30% to 45% RSMC then remained steady. Infection by powdery mildew significantly ( $P < 0.05$ ) increased the intercellular carbon dioxide concentration of plants at 15% and 30% RSMC. At the other soil water contents, differences of intercellular carbon dioxide concentrations between infected and uninfected plants was not significant ( $P > 0.05$ ) (Fig. 3C). Comparing the E+ and E-plants infected by powdery mildew demonstrated that the presence of endophyte only significantly ( $P < 0.05$ ) increased intercellular carbon dioxide concentration at 15% RSMC, the differences between E+ vs. E-not being significant ( $P > 0.05$ ) at 30%–60% RSMC (Fig. 3D).

### 3.5. Transpiration rate

The only significant ( $P < 0.001$ ) effect of soil water content was detected for transpiration rate of *A. inebrians*. In addition, there were disease  $\times$  endophyte and endophyte  $\times$  soil water content interactions for transpiration rate (Table 1). Comparing the E+ vs. E-plants infected by powdery mildew, the presence of endophyte significantly ( $P < 0.05$ ) increased the transpiration rate of host plants at 30% and



**Fig. 1.** The dynamic change of disease index of both E+ and E-plant at (A) 15%, (B) 30%, (C) 45% and (D) 60% RSMC. Values are mean  $\pm$  standard error (SE), with bars indicating SE. The asterisk (\*) means significant difference at  $P < 0.05$  (independent  $t$ -test) between E+ and E-plants at corresponding water content.

45% RSMC, but was not effective for host plants under the strong drought (15% RSMC) and abundant moisture (60% RSMC) conditions. In addition, the transpiration rate of both E+ and E-plants increased with increases of soil water content (Fig. 4A).

### 3.6. Stomatal conductance

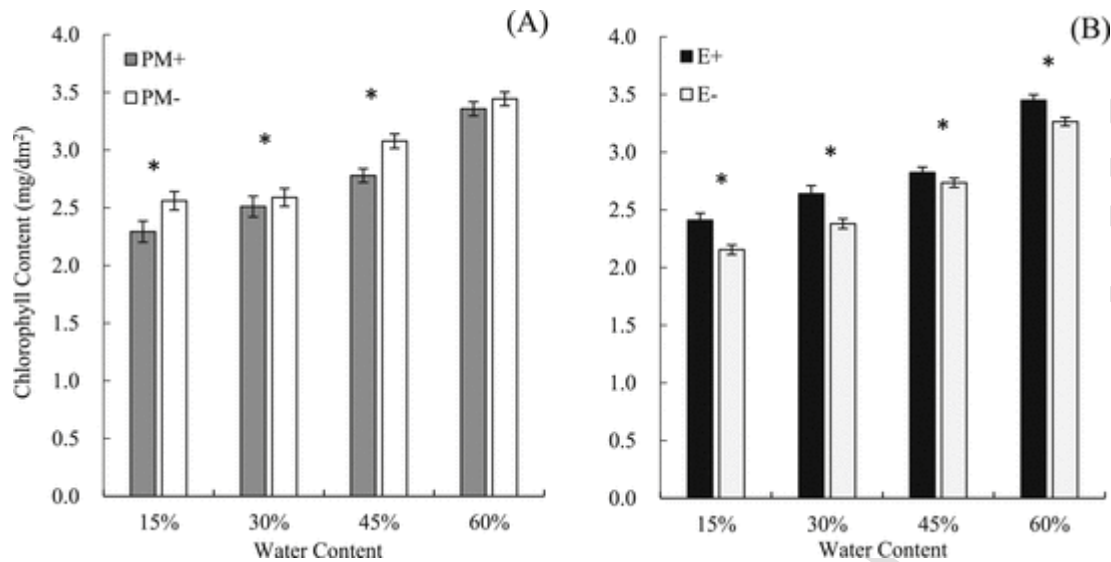
A significant ( $P < 0.001$ ) effect of soil water content was detected for the stomatal conductance of *A. inebrians*. In addition, there were disease  $\times$  endophyte and endophyte  $\times$  soil water content interactions for the stomatal conductance (Table 1). Comparing the E+ vs. E-plants infected by powdery mildew, the presence of endophyte increased the stomatal conductance at each soil water content, but the stomatal conductance was only significantly ( $P < 0.05$ ) affected by the endophyte at 30% RSMC. In addition, with the increase of soil water content, the stomatal conductance of both E+ and E-increased (Fig. 4B).

### 3.7. Dry matter per plant

Although highly significant effects of powdery mildew disease ( $P = 0.001$ ), endophyte ( $P = 0.007$ ) and soil water content ( $P < 0.001$ ) were detected for the net photosynthetic rate for *A. inebrians*, no interaction for dry weight per plant was observed (Table 2). The infection of powdery mildew significantly ( $P < 0.05$ ) reduced the dry matter per plant at each soil water content except 15% RSMC. With the increase of soil water content, dry matter of both PM+ and PM-plants increased (Fig. 5A). Comparing E+ vs. E-plants infected by powdery mildew showed that the presence of endophyte increased the dry matter per plant at each soil water content, but the significant ( $P < 0.05$ ) effect of endophyte on dry matter was only observed under strong (15% RSMC) and light (30% RSMC) drought conditions. In addition, with an increase of soil water contents, the dry weight of both E+ and E-plants increased (Fig. 5B).

**Table 1**  
 Three-way ANOVA for the effects of powdery mildew disease (D), endophyte (E) and water content (W) on photosynthetic rate, stomatal conductance intercellular Co concentration and transpiration rate of *A. inebrians*.

Treatments	df	Photosynthetic rate		Stomatal conductance		Intercellular Co concentration		Transpiration rate	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
D	1	234.043	0.000	0.976	0.325	156.460	0.000	3.588	0.060
E	1	113.623	0.000	0.103	0.749	11.965	0.001	0.011	0.915
W	3	398.966	0.000	56.550	0.000	38.514	0.000	80.545	0.000
D × E	1	3.448	0.065	4.078	0.045	2.988	0.086	5.740	0.018
D × W	3	66.087	0.000	2.196	0.090	55.107	0.000	1.359	0.257
E × W	3	5.049	0.002	3.166	0.026	1.455	0.228	3.429	0.018
D × E × W	3	2.677	0.049	1.700	0.169	1.538	0.206	1.561	0.200



**Fig. 2.** Chlorophyll content of (A) plants infected (PM+) and uninfected (PM-) with powdery mildew pathogens and (B) powdery mildew-infected plants with (E+) and without (E-) endophyte under different water contents. Values are mean  $\pm$  standard error (SE), with bars indicating SE. The asterisk (\*) means significant difference at  $P < 0.05$  (independent  $t$ -test) between PM+ and PM- or E+ and E- plants at corresponding water content.

#### 4. Discussion

The present work is one of the few studies on the interaction between the *Epichloë* spp. endophytes, the powdery mildew pathogen (*B. graminis*) and *A. inebrians*. The key finding of this study was that the infection of powdery mildew significantly reduced the chlorophyll content and photosynthetic rate. In addition, the infection also negatively affected intercellular carbon dioxide concentration, transpiration rate and stomatal conductance. These effects led to a reduction of dry matter per plant. The study also revealed that the presence of the endophyte enhanced the performance of host plants under pathogen stress, or at least ameliorated host plant damage to some degree.

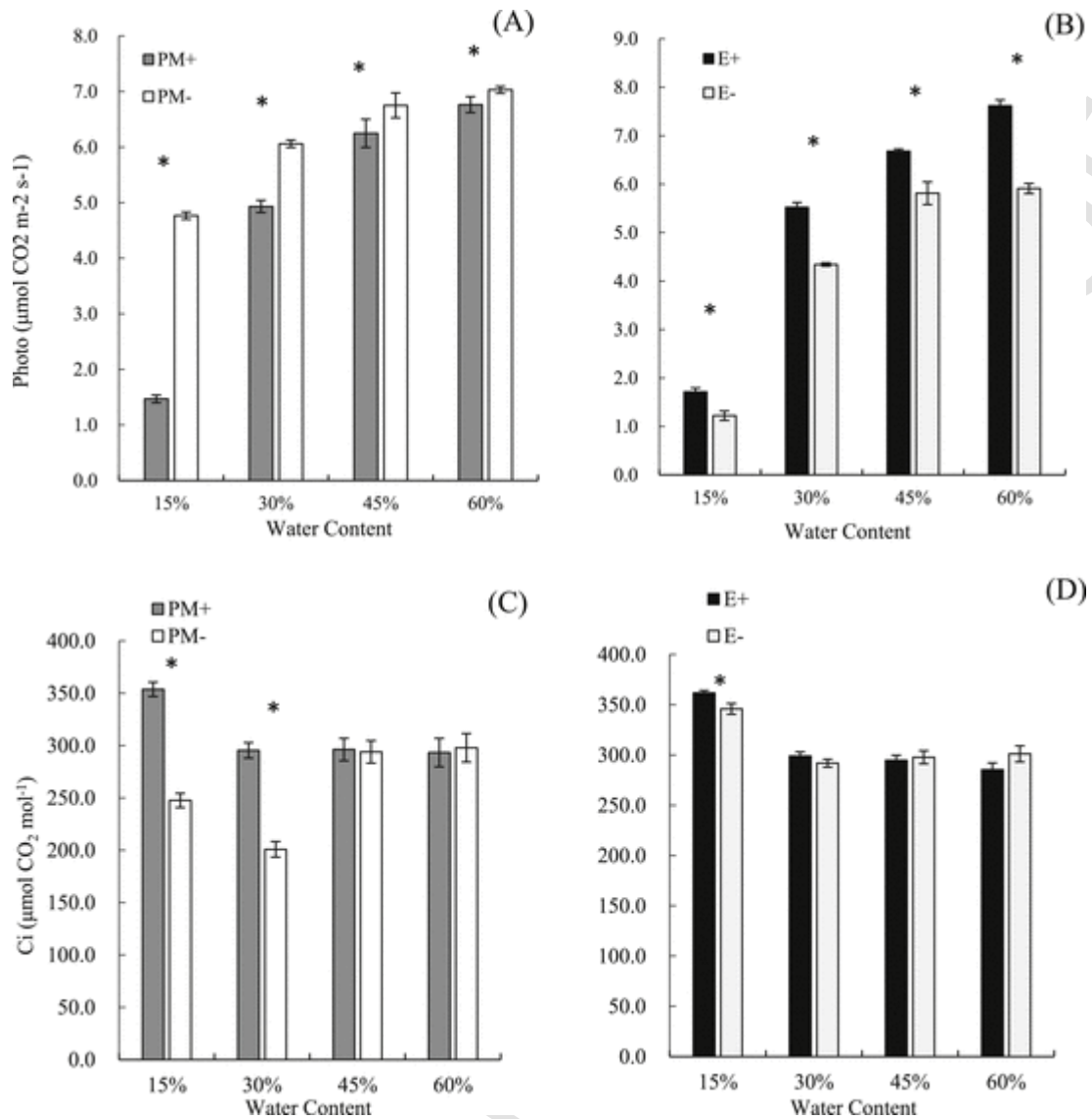
The effects of temperature and moisture on the susceptibility of plants to powdery mildew are large (Brown and Hovmøller, 2002; Huo et al., 2002; Panstruga and Schulze-Lefert, 2002). High soil moisture increases the susceptibility of wheat (*Triticum aestivum*) to powdery mildew pathogens (Huo et al., 2002), and humid climates induce severe yield losses in a wide range of cereals, especially barley crops (Panstruga and Schulze-Lefert, 2002). Similarly, the present trial demonstrated that severity of powdery mildew disease of *A. inebrians* is influenced by environmental conditions, with increases of soil water content resulting in longer lesions and a higher disease index.

A great deal of research has shown that foliar pathogens not only reduce the photosynthetic parameters in infected leaves by reducing green leaf area, but also affect photosynthesis in asymptomatic areas of diseased leaves (Bassanezi et al., 2001; Robert et al., 2005). Powdery mildew disease also has the same effects on photosynthesis of affected leaves. Carbon dioxide assimilation by host leaves was depressed both around lesions and on remaining green tissues (Moriondo et al., 2005; Swarbrick et al., 2006). This depression of photosynthesis may be due to the alteration of stomatal conductance and intercellular carbon dioxide concentration with powdery mildew disease affecting CO<sub>2</sub> diffusion to the carboxylation sites via effects on stomatal functions (Ayres, 1976; Moriondo et al., 2005). These negative alterations of photosynthetic parameters ultimately lead to the reduction of carbon assimilation by host plants and this negatively

affects crop yield (Liu et al., 2015a,b), seed yield (Cao et al., 2014) and plants biomass (Xia et al., 2015). The present trial produced results similar to previous studies, as the presence of powdery mildew reduced intercellular carbon dioxide concentrations and further negatively affected the photosynthetic rate. The dry matter per plant was also significantly decreased for PM+ plants at every soil water content, except 15% RSMC.

In a field survey, Li et al. (2003) reported that powdery mildew was observed on *A. inebrians* in August. Further study under controlled environmental conditions demonstrated that a mutualistic symbiosis of the *Epichloë* spp. endophyte with *A. inebrians* may enhance the host's ability to resist this pathogen. Other research has demonstrated that endophytes seem to protect their host against specific fungal pathogens (Nan and Li, 2000; Ma et al., 2015). The *Epichloë* spp. endophyte colonizes intercellularly (Christensen et al., 2008), after the endophyte has successfully colonized the host tissue, that ecological niche is occupied. In the endophytic niche, the limited source of nutrition from plant, including fragment, exudates and leachates are obtained by endophytes, this may prohibit other microorganisms from obtaining nutrition from the plant and this may provide protection for the host from other microorganisms, including plant pathogens. Furthermore, the endophyte-infected plants would produce lignin and other cell-wall deposits to limit the growth of endophytes (Harman et al., 2004). Therefore, the cell-wall would be re-reinforced, and difficult to infest for pathogens. However, it is worth mentioning that the effect of endophytes on host resistance to stresses may be different under different environmental conditions or different genotypes of host species (Faeth et al., 2004). Recently, Xia et al. (2015) performed a further study on the resistance of *A. inebrians* to powdery mildew, demonstrating that infection by *E. gansuensis* can reduce the ability of powdery mildew to colonize *A. inebrians*. The present trial provided a similar result in that the presence of the *Epichloë* sp. endophyte inhibited the expansion of disease lesions and so reduced the powdery mildew disease index. A significant mitigating effect of the endophyte on lesion size and disease index always occurred under normal or abundant soil water content conditions, conditions that result in the most severe disease development of this fungal pathogen (Huo et al., 2002; Panstruga and Schulze-Lefert, 2002). The benefits associated with endophytes





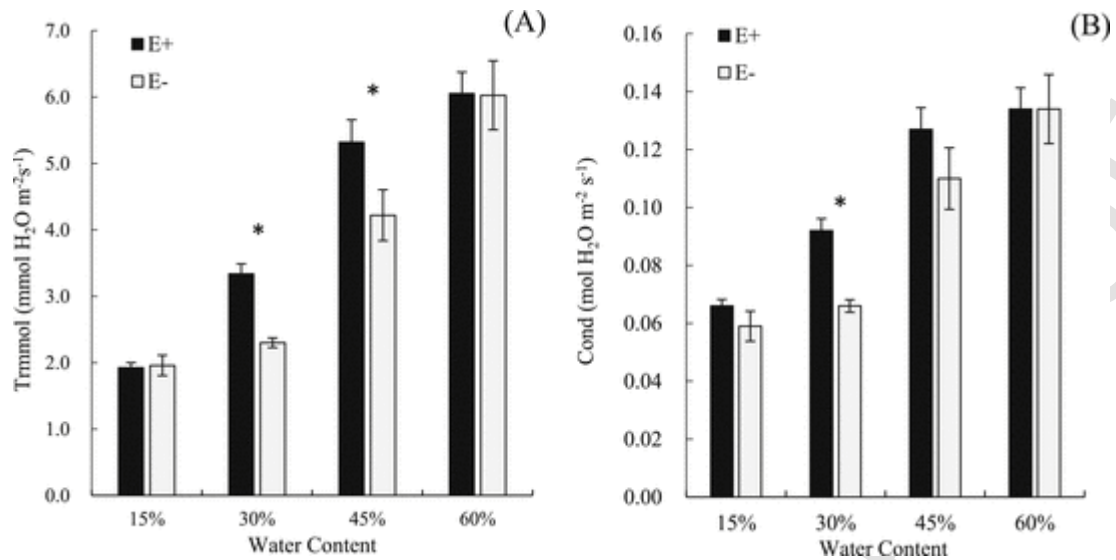
**Fig. 3.** Net photosynthetic rate and intercellular carbon dioxide concentration of (A, C) plants infected (PM+) and uninfected (PM-) with powdery mildew pathogens and (B, D) powdery mildew-infected plants with (E+) and without (E-) endophyte under different water contents. Values are mean  $\pm$  standard error (SE), with bars indicating SE. The asterisk (\*) means significant difference at  $P < 0.05$  (independent  $t$ -test) between PM+ and PM- or E+ and E- plants at corresponding water content.

within host plants are frequently observed only when the host plants have suffered from stresses (Zhang et al., 2010; Song et al., 2015). As green leaf area becomes smaller with increased lesion area, the alleviating effects of the endophyte upon chlorophyll content and photosynthesis may become more important.

Other studies of endophyte effects on photosynthesis have often been inconclusive. The research of Newman et al. (2003) on tall fescue showed that under high nitrogen conditions, plants infected with an *Epichloë* endophyte had higher photosynthetic rates than under low nitrogen conditions. This was similar to research performed on ryegrass by Amalric et al. (1999), which showed that endophyte-infected plants have higher stomatal conductance, transpiration rate, and net photosynthetic rate than endophyte-free plants. However, a study on maize showed that the presence of the *Fusarium* sp. endophyte, *F. moniliforme*, significantly reduced chlorophyll content, and therefore decreased photosynthesis (Pinto et al., 2000). Some research has indicated that there is no endophyte effect of consequence on photosynthesis (Bacon, 1993). Photosynthesis is limited

by biophysical and biochemical barriers such as carbon assimilation and stomatal conductance (Rozpadek et al., 2015). Carbon assimilation is strongly related to  $\text{CO}_2$  accessibility which, in turn, depends on intercellular carbon dioxide concentration and limitations in its diffusion. Stomatal conductance affects gas exchange and diffusion rates resulting in a decline of chloroplastic  $\text{CO}_2$  concentration that subsequently limits photosynthesis (Centritto et al., 2009). Considerable other research has demonstrated that the presence of an *Epichloë* endophyte can increase the biomass of host plants under stress conditions, such as drought (Li et al., 2008), salt (Song et al., 2015), heavy metal (Zhang et al., 2010) and disease pathogens (Xia et al., 2015). The present results parallel those studies, in that endophyte presence affected host chlorophyll content and photosynthetic parameters, increasing dry matter yields of diseased plants significantly at 15% and 30% RSMC.

Although the present study has demonstrated that the *Epichloë* sp. positively affected diseased *A. inebrians*, this endophyte may not have directly promoted the aforementioned host parameters. The im-



**Fig. 4.** Transpiration rate (A) and stomatal conductance (B) of plants with (E+) and without (E-) endophyte under different water contents. Values are mean  $\pm$  standard error (SE), with bars indicating SE. The asterisk (\*) means significant difference at  $P < 0.05$  (independent  $t$ -test) between E+ and E- plants at corresponding water content.

proved plant performance may have been induced only by indirect effects of the endophyte (e.g., inhibition of powdery mildew infection and consequent damage). Differentiating the mechanisms by which the endophyte plays a role in improving host performance under diverse stresses, especially in the presence of pathogens such as powdery mildew, need to be clarified by further studies.

Powdery mildew is a widespread fungal disease of cereals, especially on wheat, and causes great losses in crop yield (Liu et al., 2015a,b). The importance of cereals for the growing world population and increasingly severe soil and water resource pollution, caused by the extensive use of chemical pesticides, means that there is a demand for novel, effective and environmentally-friendly pathogen control strategies (O'Hanlon et al., 2012). Development of cultivars of cereals with powdery mildew resistance is crucially important for global cereal production. The finding of the present study that the presence of an *Epichloë* endophyte improved the photosynthetic parameters and the dry matter accumulation of *A. inebrians* under powdery mildew stress raises the question of whether it is possible that the same process could be applied to cereal species. At this stage there are no *Epichloë* species recorded in cereal species but these plants are in the same sub-family as the grass species that host *Epichloë* endophytes and close relatives have natural associations with an *Epichloë* species (Leuchtman et al., 2014). There is thus real

potential to introduce an *Epichloë* endophytes into cereals and possibly emulate the suppression of powdery mildew and the ability to better tolerate water stress as present in *E. gansuensis*/*A. inebrians* associations.

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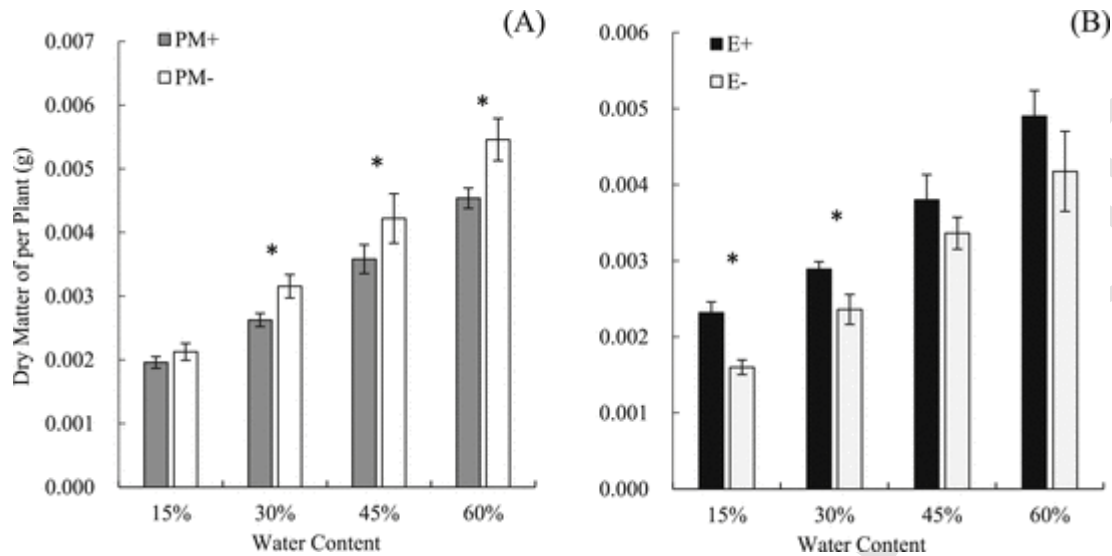
#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2016.04.002>.



**Table 2**  
 Three-way ANOVA for the effects of powdery mildew disease (D), endophyte (E) and water content (W) on leaf water content, Chlorophyll content, plant height and tiller number of *A. inebrians*.

Treatments	df	Leaf water content		Chlorophyll content		Dry weight of per plant		Plant height		Tiller number	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
D	1	152.402	0.000	36.082	0.000	11.689	0.001	0.094	0.760	1.365	0.244
E	1	10.848	0.002	140.984	0.000	7.802	0.007	4.773	0.030	36.913	0.000
W	3	20.749	0.000	45.074	0.000	59.996	0.000	63.845	0.000	8.918	0.000
D × E	1	0.179	0.674	22.663	0.000	0.740	0.393	0.698	0.405	0.882	0.349
D × W	3	3.855	0.015	10.063	0.000	0.894	0.449	1.115	0.345	1.849	0.141
E × W	3	0.455	0.715	1.706	0.166	0.490	0.690	4.251	0.006	0.732	0.543
D × E × W	3	2.503	0.070	3.521	0.016	0.215	0.886	1.1041	0.376	0.715	0.545



**Fig. 5.** Dry matter of per plant (A) infected (PM+) and uninfected (PM-) with powdery mildew pathogens and (B) powdery mildew-infected plants with (E+) and without (E-) endophyte under different water contents. Values are mean  $\pm$  standard error (SE), with bars indicating SE. The asterisk (\*) means significant difference at  $P < 0.05$  (independent  $t$ -test) between PM+ and PM- or E+ and E-plants at corresponding water content.

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