Effects of Endophyte Infection on the Performance of Fall Armyworm Feeding on Meadow Fescue under a Range of Water Stress Levels

Siow Yan Jennifer Angoh

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Abstract

Endophytes have been shown to provide protection against herbivory to their host via the synthesis of alkaloids. Under drought stress, some photosynthetic organisms do benefit from their symbiotic relationship with certain fungus. In fact, endophytes facilitate changes in their host morphology, osmotic properties, resource allocation, and regrowth dynamics, which subsequently could provide the latter with enhanced drought resistance. Changes in the morphology and physiology of fodder species can also affect the herbivores feeding on them. In this study, cloned daughter endophyte-infected and endophyte-uninfected meadow fescue (*Schedonorus pratensis*) plants were assigned to two greenhouse experiments in which water levels needed to cause drought stress in the grass was determine. Also, water stressed plants utilised for a bioassay with fall armyworms (*Spodoptera frugiperda*) larvae were generated. Percentage water content of meadow fescue leaves decreased over a period of 6 days. Larvae fed with endophyte-infected grass maintained under a low water regime had the lowest relative growth rates (RGR) (0.19±0.05 mg/mg/day) which was significantly different from the RGR of larvae fed with grasses maintained under higher water regimes.

Résumé

Les endophytes fournissent une protection à leur hôte contre les herbivores via la synthèse d'alcaloïdes. Sous le stress de la sécheresse, certains organismes photosynthétiques profitent de leurs relations symbiotiques avec quelques champignons. En effet, certains endophytes facilitent des changements dans la morphologie, les propriétés osmotiques, l'allocation des ressources et la dynamique de la repousse de leur hôte, ce qui pourrait par la suite fournir à ce dernier une meilleure résistance contre la sécheresse. Les changements dans la morphologie et la physiologie des espèces fourragères peuvent également affecter les herbivores qui s'en nourrissent. Dans cette étude, des clones de fétuque des prés (*Schedonorus pratensis*) infectés d'endophytes et non infectés ont été attribués à deux essais en serre où les niveaux d'eau nécessaires pour causer un stress sur l'herbe furent déterminés. Aussi, les plantes générées ont été utilisées pour un essai biologique avec des laves de légionnaires d'automne (*Spodoptera frugiperda*). Le pourcentage de la teneur en eau des feuilles de la fétuque des prés a diminué durant une période de 6 jours. Les larves nourries avec de l'herbe infectée d'endophytes et maintenues sous le régime d'eau le plus bas avaient le taux de croissance relatifs le plus faibles ('RGR') (0,19 ± 0,05 mg / mg / jour) qui était sensiblement différent de la 'RGR' des larves nourries avec de l'herbe maintenu sous les régimes d'eau plus élevés.

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Introduction

1.1. Implications of Climate Change on Plants

Today, it is widely agreed upon by the scientific community that the Earth's climate is changing (IPCC, 2007). Despite the uncertainties about how and to what extent climate change will affect different regions of the world at various spatio-temporal scales, changes in weather conditions are likely to be omnipresent (Chakraborty et al., 2008). By 2100, the global average temperature is expected to rise by 1.4°C to 5.8°C due to increasing carbon dioxide and other greenhouse gas concentrations in the atmosphere (IPCC, 2007).

Regional climate warming and uneven distribution of rainfall are already causing stress in many natural and agricultural plant communities (Taiz and Reiger, 2002). Environmental factors that produce stress in plants can do so at different rates and levels depending on the severity of the change in the factor itself or the physiological and morphological nature of particular plant species (Taiz and Reiger, 2002). For instance, effects of air temperature can be experienced by plant tissues in a matter of minutes, whereas it may take the plant days to weeks to react to soil water content (Taiz and Zeiger, 2002). Accordingly, abiotic stress, usually caused by a combination of different environmental factors (e.g. temperature, water and nutrient availability), greatly influences how the distribution of different plants species is limited (Taiz and Zeiger, 2002).

Drought stress, often associated with climate change, includes aspects of water, temperature and nutrient stress (William and Haack, 1987). It is also one of the major abiotic stresses limiting plant productivity worldwide (Yue et al., 2006). Hence, the ability of plants to resist drought via drought escape (i.e. completion of life cycle during wet season to avoid drought), desiccation postponement (i.e. enhanced water uptake and reduced water loss to

maintain hydration), or drought tolerance (i.e. the ability to function while dehydrated via physiological mechanisms like osmotic adjustment and increased antioxidant capacity) strategies is crucial (Taiz and Zeiger, 2002; Yue et al., 2006).

Furthermore, drought stress has been shown to render some photosynthetic organisms more susceptible to phytophagous fungi and insects (Mattson and Haack, 1987, Pautasso et al., 2012). In 1987, Mattson and Haack found a positive correlation between outbreaks of insects like bark beetles and leaf feeders, and warmer, dryer weather. Therefore, more frequent extreme weather events, like droughts and heat waves may further exacerbate the impacts of already existing and impending biotic and abiotic environmental disruptions on plant health (Pautasso et al., 2012).

1.2. Benefits of Plant-Endophyte Symbioses

There are many ways in which plants have evolved strategies to escape, avoid or tolerate drought conditions (Yue et al., 2006). One mean by which temperate grass species in the subfamily Pooideae have been found to acquire drought tolerance (physiological and biochemical adaptations), drought avoidance (morphological adaptations), drought recovery mechanisms or a combination of some of these mechanisms, and live under hostile environmental conditions, is through mutualistic symbiosis with *Neotyphodium* fungal endophytes (Malinowski and Belesky, 2000; Taiz and Zeiger, 2002; Cheplick et al., 2009). Pooid grasses include some of the most important crop, forage and turf species (e.g. *Hordeum* and *Lolium*) (Febrer et al, 2010). These grasses appear to be benefiting from an exchange of "goods and services" when growing in hostile environmental conditions, due to their association with fungal microorganisms present in their above-ground parts (Leuchtmann, 1992; Clay, 1990).

Endophytes, being either bacterial or fungal, are obligate biotrophs present in most, if not all, herbaceous and woody angiosperms, including grasses and trees (Cheplick et al., 2009). Endophytic fungi grow in between plant cells and derive their nutrients (sugars and nitrogen compounds diffused from the cytoplasm to intercellular spaces) from their living host's apoplasm (Clay, 1990; Bacon, 1993; Cheplick et al., 2009). According to Clay, 1989, at least 80 genera and 259 species of graminoids contain elongated and sparsely branched hyphae of endophytic fungi (family Clavicipitaceae; tribe Balansieae) in the intercellular space of their leaves and stems (Fig. 1.1). In grasses, depending on the species of fungal endophytes, transmission of the endosymbionts to future generations of plants can either be asexual, sexual or occur by both means (Scharld et al., 2004, Cheplick et al., 2009). *Neotyphodium* endophytes are usually transmitted vertically from the mother to daughter plants via mature seeds (Moon et al., 2002) (Fig. 1.2). For endophytes with a sexual life cycle (e.g. *Epichloë* and *Atkinsonella*), horizontal transmission requires transfer of spermatia (male gamete) between fruiting bodies (stroma) of opposing mating types before ascospores, which mediate the infection of new host plants, are produced (Chung and Schardl, 1997, Scharld et al., 2004). Mature ascospores are then transferred by vectors like wind or insects from the inflorescences of endophyte-infected host plants to those of new plants (Fig. 1.2) (Clay, 1990; Cheplick et al., 2009).

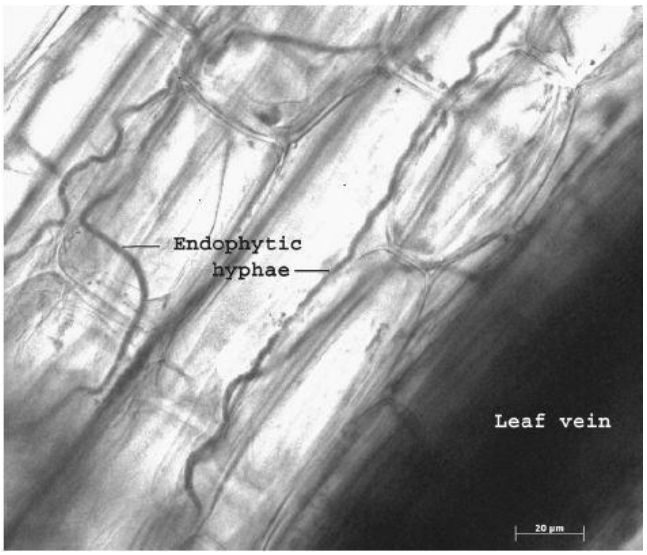

Figure 1.1. Endophytic hyphae of *Neotyphodium lolii* in the leaf sheath of perennial ryegrass (*Lolium perenne*) at 400× (Cheplick et al., 2009).

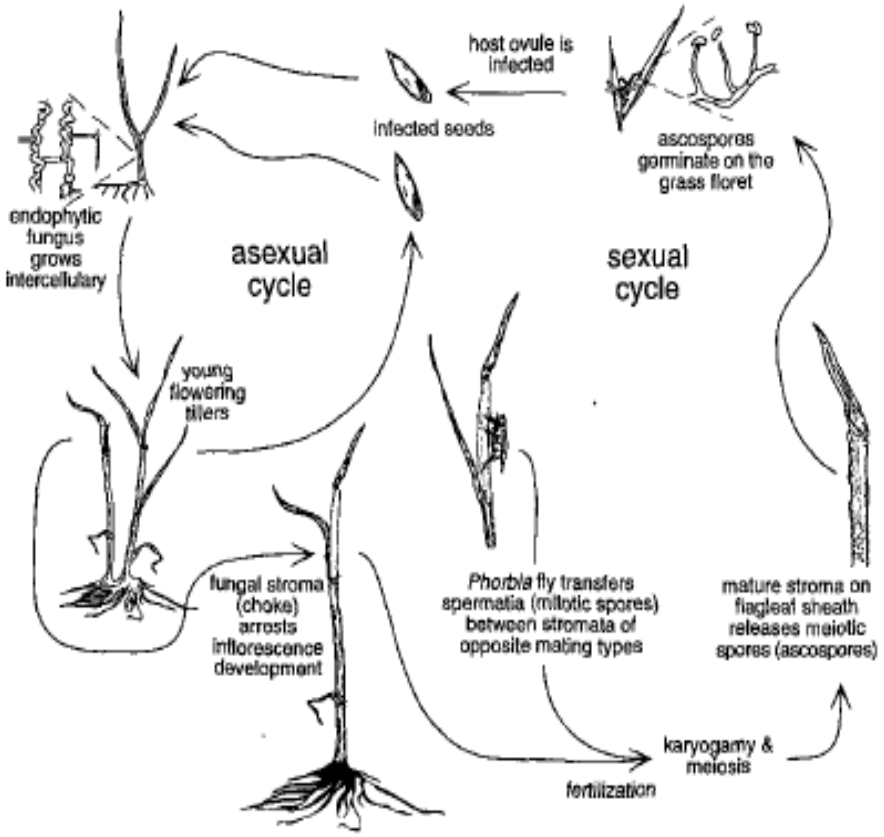

Figure 1.2. Schematic illustration of asexual and sexual cycles of *Epichloë festucae* in symbiosis with a cool-season grass species. Some endophytes (e.g., *Neotyphodium*) are exclusively asexual and only vertically transmitted within host seeds. Other endophytes (e.g., *Epichloë*) may show asexual and sexual stages (Bush et al., 1997).

Many globally important forage crops and turf grasses may have developed effective strategies for counteracting some of the effects of global warming through mutualistic relationships with systemic *Neotyphodium* species (Bayat et al., 2009). The latter endosymbionts have been shown to stimulate physiological and morphological changes in their hosts, which ultimately confer drought resistance on the infected individuals for abiotic stresses like high heat and water limitation (Brosi et al. 2005; Cheplick, 2004; Bayat et al., 2009), and improve their resistance against herbivory pressure (Vicari and Bazely, 1993; Bultman and Bell, 2003). Morphologically, endophytes contribute to drought avoidance by increasing root depth and density (increase water intake via roots), and encouraging early shedding of older leaves and rolling of younger leaves (reduce water loss by evaporation) (Bacon, 1993; Hesse et al., 2003). Plant physiological mechanisms, mediated by fungal endophyte-infection, that can increase drought stress tolerance include decreased electrolyte conductivity, increased osmotic adjustment (accumulation of cell solutes like K^+, Ca^{2+} and Mg^{2+}, independent of cell volume changes), increased tillering during stress recovery, and reduced water desiccation from leaves and shoot meristems (Eerens et al., 1998; Secks. et al., 1999; Bayat et al., 2009; Sabzalian and Mirlohi, 2010).

Bayat et al., 2009, found that endophyte-infected tall fescue, *Schedonorus phoenix* (Scop.) Holub, had lower electrolyte conductivity (i.e. a more stable cell membrane). Higher cell membrane stability in endophyte-infected tall fescue as compared to endophyte-free specimens may have been due to an accumulation of the disaccharide trehalose, which stabilises phospholipid bilayers and proteins of plant cell membrane (Secks. et al., 1999). The increased ability of endophyte-infected plants to recover after drought stress was also observed by Bayat et al. (2009). Additionally, the enhanced tillering that followed stress relief, may have been attributable to the production of auxin (mobilising hormone) by endophytes, which favoured

rapid carbohydrate translocation necessary for higher regrowth rates after stress removal (Bayat et al., 2009).

Forage species like *Kochia* have been shown to suffer under high levels of water stress. In this species, reduced water availability can alter membrane permeability and trigger the formation of reactive oxygen species (ROS), substantially damaging the plant via a decrease in leaf relative water content (RWC) and an increase in electrolyte leakage within the plant cells (Masoumi et al., 2010). More recently, Hamilton and Bauerle (2012) have reported that endophytes could further increase stress tolerance by means of antioxidant synthesis in spite of increased ROS activity. Under abiotic stress (e.g. drought and high UV exposure), absorption of light exceeds photosynthetic utilisation, thus resulting in the release of electrons in plant tissues and triggering the formation of cell damaging reactive oxygen species (ROS) like superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (as cited by Zhang and Nan, 2007). Thus, the additional production of antioxidant by *Neotyphodium* could help "mop up" the excess ROS and provide protection to infected plants during stress periods (Gill and Tuteja, 2010).

Genotypes of endophyte-infected perennial ryegrass (*Lolium perenne*) that originated from dry sites have been shown to regenerate more rapidly after drought than their endophyte-free counterparts (Hesse et al., 2003). However, when grown under normal water availability, endophyte-infection was detrimental to these genotypes of *Lolium perenne* compared with plants without endophytes (Hesse et al., 2003). Also, Bacon, 1993, found that in another species of pooid grass, tall fescue, endophyte-infected seeds require more moisture to germinate than uninfected ones and endophyte-infected seedlings require more nutrients than uninfected seedlings. Thus, depending on the degree to which a region is affected by drought, plant communities may vary according to the recruitment and survivorship of infected (E+) plants,

which may be higher in those communities experiencing harsher ecological conditions (Bacon, 1993; Brosi et al., 2011).

1.3. Endophyte Infection and Herbivory

Neotyphodium endophytes benefit their photosynthetic host via the production of alkaloids and other secondary metabolites (Tan and Zou, 2001). In many grass species infected with fungal symbionts (endophytes) of the genus *Neotyphodium*, loline alkaloids (saturated 1-aminopyrrolizidines with an oxygen bridge), peramine (a pyrrolopyrazine), ergot alkaloids (amine and amide), and indolediterpenes are generally metabolised and are essential in protecting the host plants against certain herbivores, parasites and drought (Vicari and Bazely 1993; Wilkinson et al., 2000). Rare alkaloids like lolines occur in grasses associated with *Neotyphodium* spp (Wilkinson et al., 200). These types of alkaloids can be toxic when ingested by insects (Bush et al. 1993). Furthermore, Wilkinson et al. (2000) reported that when two aphid species, *Schizaphis graminum* and *Rhopalosiphum padi*, were fed endophyte-infected grass containing high levels of lolines, their survival rates decreased significantly. The insecticidal properties of alkaloids also apply to sporadic crop pests such as fall armyworms (*Spodoptera frugiperda*) which every year migrate northward, towards the United States and Canada (Braman et al., 2002). When fed endophyte-infected pooid grass, fall armyworm larval survival and biomass were significantly lower than when fed uninfected grasses (Clay et al., 1985). High concentrations of some ergot alkaloids (e.g., ergonovine) appeared to have antibiotic effects on these insects, while others (e.g. ergotamine and agroclavine) had antifeedant effects (Clay et al., 1985).

While many previous research findings support the hypothesis that fungal endophytes provide the plant with a defence against herbivory (Clay et al., 1985, Cheplick and Clay, 1998), not all researchers agree that the primary benefit of endophytes is in providing an anti-herbivore

defence (Bultman and Bell, 2003). Some authors argue that endophytes are not always mutualists, and sometimes are parasites in their host grass (Saikkonen et al., 1998). The extent to which endophytes provide protection for their host against herbivores, including invertebrates and vertebrates, depends on several factors including specific species interactions and a combination of different abiotic factors (Saikkonen et al., 1998; Saona et al., 2010).

Bultman and Bell (2003) found that endophyte-infected grasses affected aphids and fall armyworms differently. While aphid reproduction was negatively affected by endophyte-infected grass diets under normal water levels, the armyworm response to these conditions was the opposite. On the other hand, under drought stress, aphid performance was enhanced by endophytes, whereas caterpillar performance was reduced (Bultman and Bell, 2003). Thus, more research into the impact of a range of different stresses on plant-endophyte interactions, and the subsequent responses of herbivores, is essential.

1.4. Grass and Herbivore Species under investigation

Meadow fescue, *Schedonorus pratensis* (Huds.) P.Beauv, is one of the most widely used forage grasses in the Nordic area due to its superior combination of fodder quality and winter hardiness (Fjellheim et al., 2006). The present-day native distribution of meadow fescue is considered to cover most of Europe and large areas eastwards into Central Asia, with some more isolated occurrences such as in the Caucasus and the Fertile Crescent region in western Asia (Hultén & Fries, 1986; Fjellheim et al., 2006). In addition, it has been introduced to North America, Japan, Australia and New Zealand (Hultén & Fries, 1986). This species of grass is shade-intolerant and prefers deep, rich soils, but can also grow on sandy soils provided that they are moist (Fjellheim et al., 2006).

The fall armyworm (*Spodoptera frugiperda*) is a serious pest to forage grasses, rice, sorghum, maize, soybean, and peanuts in tropical areas of South and Central America, Mexico

and subtropical regions of south Florida and Texas in the United States (Ashley et al., 1989; Mitchell et al., 1990). The insect has no diapause mechanism and usually overwinters in mild climate of south Florida and Texas (temperature > 9.9^{o}C, year-round) and seasonally invades continental United States and southern Canada during the growing season (Sparks, 1979). Given their occurrence in southern Canada (Sparks, 1979), fall armyworms were a relevant study organism for my project which used meadow fescue grass that has been naturalized in Canada (Dore and McNeil, 1980).

1.5. Hypothesis and Study Objectives

The overall objective of this experiment was to investigate the potential benefits and/or costs of harbouring *Neotyphodium uncinatum* endophytes by meadow fescue *Schedonorus pratensis*, under a range of water stress conditions. More specifically, the water status of meadow fescue leaves was manipulated, under three different watering regimes (low, medium and high water availabilities), and the impact that these had on the host grass-endophyte system was assessed with an insect herbivore (fall armyworm, *Spodoptera frugiperda*), in a feeding bioassay. Thus, water status was used as an indicator of drought intensity (see Medrano et al., 2002) on endophyte-infected (E+) and endophyte-free (E-) grass plants in this study. The novelty of this research is widespread as I assessed the effect of water stress on the plant-endophyte symbiosis.

These objectives were broken down into several sub-objectives and a number of preliminary experiments were carried out prior to the feeding bioassay with the fall armyworm larvae.

1. A study was carried out with the objective of manipulating the water status of the grass leaves of endophyte-infected and endophyte-free meadow fescue plants. I asked how different watering levels (low, medium and high) would cause a change in water status (indicated by

relative water content and percentage water content in leaf blade) of E+ grass compared to E- grass.

2. A second objective was to determine how variation in plant water status affects endophyte-mediated resistance of the host grass to herbivory by the fall armyworm, as indicated by amount of grass consumed.
3. A third objective was to determine whether the performance (relative growth rates, RGR) of the fall armyworm larvae would differ when feeding on E+ or E- grass subjected to the different water treatments.

The results from the preliminary experiment, associated with objective 1, in which watering regimes were manipulated and plant water status measured, were used to determine appropriate watering regimes in a second water stress experiment that provided the forage grasses used in the bioassay feeding experiment. It should be noted that paired daughter plants for each of the three water stress treatments (12 pairs of E+ and E- per water treatment = 72 plants) used to feed the fall armyworms were controlled for genotypic variation by obtaining E+ and E- ramets from the same genotypes (Saona et al., 2010).

Based on the literature (Bacon, 1993; Cheplick and Clay, 1998; Bultman and Bell, 2003), I predicted that herbivory pressure by the fall armyworm (measured by relative consumption rates-RCR) would be greater on the E- plants under lower water regimes than higher water regimes, since fall armyworm larvae feeding on the E- plants would eat more of the grass to compensate (increase RGR) for the lack of water, and E- grass does not have the ability to deter herbivory via alkaloid accumulation (Bultman and Bell, 2003; Bayat et al., 2009). I also hypothesised that there would be no change in RCR, RER and RGR of armyworms when either E+ or E- plants are fed to them under higher water regimes since the meadow fescue leaves of both strains would have similar water status under no stress (Table 1.1)

Table 1.1. Outcome predictions for 4 treatments (low/E-, low/E+, high/ E-, high/ E+).

	E+	**E-**
Low Water	-No water decrease in leaves -No increase in herbivory	-Decrease in water in leaves -Increase herbivory (compensation mechanism)
High Water	-No decrease in water in leaves -No increase in herbivory	-No decrease in water in leaves -No increase in herbivory

Methods

In order to determine the impact of varying (1) the water stress and (2) the endophyte-status of meadow fescue grass on the forage consumption and growth of the invertebrate herbivore, fall armyworm (*Spodoptera frugiperda*), a series of trials and experiments were performed between March 2012 (began during a Biology research practicum with Dr. Mark Vicari) and March 2013. The timeline is given in Table 2.1.

The first stage was preliminary research aimed at clearing the fungal endophyte from meadow fescue grass plants to generate a number of plant clones in which the same grass host genotype had both and endophtye-infected and uninfected type. The second stage was also preliminary research determining how best to vary and measure the water status of the grass plant leaves. The third stage of the research resulted in the growth of plant material with varying water and endophyte status that was fed to the insects, so that their response and growth could be measured (Table 2.1).

Table 2.1 Timeline of experiments investigating the effects of the *Neotyphodium uncinatum* endophyte of the grass, meadow fescue, under a range of water stress levels.

Timeline		Experiment	Brief Description
Start Date	End Date		
Mar-12	Apr-12	Generation of E+ and E- daughter clonal genotypes from source plants (E+)	Treated E+ tillers with fungicide to obtain E- plants. From this 16 E+ and 16 E- clones
Tillers were left to grow and propagate in pots for seven to eight months			
Screening for endophyte infection in all daughter plants to confirm E+ and E- plants (Sept. 2012)			
25-Sep-12	25-Oct-12	Further separation and propagation of E+ and E- plants used in water stress experiments and in insect bioassay	150 tillers transplanted (90 E- and 60 E+) in individual pots with sand
Tillers were left to grow for a minimum of 90 days in growth chamber and greenhouse			
01-Feb-13	06-Feb-13	Preliminary water stress experiment	Sample plants were not watered for 6 days to determine three watering regimes to be used in further water stress experiment (RWC, % water content and pot weight obtained) based on % water content of leaves
08-Feb-13	10-Feb-13	Random assignment of plants to low, medium and high water treatments. Watering ceased for low and medium watering groups (high and medium water stress)	
11-Feb-13	03-Mar-13	Pre-bioassay water stress experiment – generated plant material for bioassay	Meadow fescue subjected to three different water stress levels
04-Mar-13	08-Mar-13	Bioassay: with fall armyworm	Larvae fed with E+ and E- (3 water levels)

Generating E+ and E- Plant Materials for Water Stress Experiments and Feeding Trial

The meadow fescue grass plants used in this study were grown in the York University greenhouse and obtained from the collection of Dr. Mark Vicari. Dr. Vicari collected the source plants in 2008 and 2009 from 7 different locations in Ontario: McGregor Point Provincial Park Bruce Addition (42°36'N, 80°57'W), Ramsden Park (43°67'N, 79°39'W), Halstead Bay (44°54'N, 93°41'W), Long Point (44°24'N, 81°27'W), Awenda Provincial Park (44°51'N, 80°0W), Nordheimer Ravine (43°68'N, 79°40'W) and St. Thomas (42°77'N, 81°18'W). The plants, collected from naturalized populations, were all infected by a *Neotyphodium* endophyte as determined by ELISA assay (Phytoscreen Field Tiller kit, Agrinostics, Watkinsvlle, GA) and confirmed by microscopic observation.

In March 2012, eight genotypes of meadow fescue were selected (Table 2.1). Four infected tillers (ramets) with roots were removed from each genotype and transplanted into new 10 cm^2 individual pots (12 cm deep) containing greenhouse soil mix (Promix, sand and garden soil). Two of the transplanted ramets of each genotype were treated with 20 mL of 1.25g/L Benomyl systemic fungicide (Wilson, Dundas, Ontario) to kill off the fungal endophyte in the plant, while allowing the host plant to survive. All ramets were fertilized once per week with with 20 ml of 30 g/l 20 N-20 P-20 K all-purpose fertiliser and watered abundantly for the next eight months (between March 2012 and October 2012) (Table 2.1) under greenhouse conditions (approx. 20.2°C and 66 % humidity) with natural light.

In September, 2012, a tiller sample was taken from each ramet, stained with lactophenol cotton blue (10 g phenol: 10 mL 85% lactic acid: 10 mL glycerol: 10 mL water: 0.02 g aniline blue) for 48 hours and screened for endophyte infection using a compound microscope at 400x magnification. Benomyl-treated ramets of two of the genotypes were found to still be endophyte-

infected. These two genotypes were discarded (Halstead Bay genotype and Bruce Addition 2) from the study. The remaining six of the initial eight meadow fescue genotypes were used to generate grass plants for subsequent water stress and bioassay experiments. Fifteen E- and 10 E+ ramets of each genotype (90 E- and 60 E+ in total) were transferred to individual pots (10 cm in diameter, 12 cm deep) filled with approximately 1200 g of wet sand. They were immediately fertilised with 20 mL of 30 g/L 20 N-20 P-20 K all-purpose fertiliser, and well-watered. On September 25 2012, all plants were transferred to a growth chamber (14 hours light: 10 hours dark, 18^{o}C, humidity of 70 %) where they grew for 52 days. They were then transferred back to the greenhouse (20.2°C and humidity of 66%) for another 38 days (Table 2.1). Throughout this period of growth, each plant was amply watered daily. Once a week all plants were fertilized (40 mL of 30 g/L 20 N-20 P-20 K all-purpose fertiliser per plant).

Water Stress Experiments

Preliminary Experiment

I conducted a preliminary experiment to determine the level of watering needed to exert water stress on meadow fescue. The aim of the experiment was to determine the lowest watering level under which meadow fescue plants would survive, and levels range under which the water content of leaf blade tissue would vary, so that a spectrum of water-stress treatments could be devised for the grass plants (infected and uninfected).

This part of the study was divided into two components:

1. Assessing the water status in the grass plant pots under increasing water stress.
2. Assessing the response of the grass leaf tissue to increasing lack of water by means of two measures of plant leaf water content: relative water content (RWC) and percentage water content as a function of fresh weight.

Twelve meadow fescue plants (one E- and one E+ plant of each of the six selected genotypes) were selected at random from the previously generated plants, for the preliminary experiment. The pots were lined with landscaping fabric (discs diameter: 11 cm) that allowed water to drain from pots, but contained the sand and other particles, and prevented its loss from the pots. The amount of sand in each pot was equalized before the beginning of the preliminary experiment, by filling the pots with sand up to a mark 2 cm below the pots' rim. The weights of the 12 pots were recorded each day for a period of six days. On the first day (1 February 2013) the 12 plants were watered to saturation and their weight noted 30 minutes later, allowing excess water to drain from the pots.

For the next five days (2 February 2013 to 6 February 2013) the plants were not watered. Each day the weight of the 12 pots, each containing one plant, was measured and used to determine water loss (in grams) through further drainage and evapotranspiration. On day 6, the sand from three randomly selected pots (including the whole plant as dry mass of plant is relative small compared to amount of sand in pot) was dried at 60 °C in a drying oven for 48 hours, and weighed to estimate the dry weight of sand in each pot. The amount of water in the 12 pots at the beginning and at the end of the experiment was then estimated (Table 2.2). All weights recorded exclude the weight of the plastic pot used (25g).

Table 2.2. Approximate Fresh weight (12 pots) and mean dry weight, (mean ± SEM of 3 sample pots) of sand and plant in pots and approximate amount of water in 12 experimental pots on day 1 and amount of water left in pots on day 6.

Day 1 fresh weight of sand + plant (g)	Day 6 Dry weight of sand + plant (g)	Amount of water in pots day 1 (g)	Amount of water in pots day 6 (g)
1229	957±7.3	272	148

From day 1 to day 6, one fully expanded leaf from each of the 12 sample plants was collected (cut made nearest to pseudostem) and the fresh weights (FW), turgidity weights (TW) and dry weights (DW) of the 12 leaves were recorded. The FW of each tiller was recorded using

an analytical balance (AE 100, Mettler). The leaves were then submerged in 50 mL plastic tubes containing distilled water and stored at room temperature of 25°C for a minimum of 24 hours to achieve full turgidity of leaf. The TW was obtained when leaves were removed from the tubes, quickly blotted and weighed. Finally, the leaves were oven dried for 48 hours at 70°C and the dry weight (DW) was measured and recorded. The leaves RWC was determined using the equation; RWC = [(FW-DW)/ (TW-DW)] (Yamasaki and Dillenburg, 1999). The experiment was terminated on day 6, because most tillers had dried out and were beginning to senesce.

Experiment Subjecting Meadow Fescue to a Range of Water Stress (Pre-bioassay Water Stress Experiment)

Some of the results from the preliminary watering experiment are presented in this Methods section, as they were used to determine the watering regime in the final and main experiment. The daily water loss from all pots was plotted on a graph (Fig. 2.1a) and could be associated with the RWC and percentage water content of the leaves (Fig. 2.1b; Fig. 2.2).

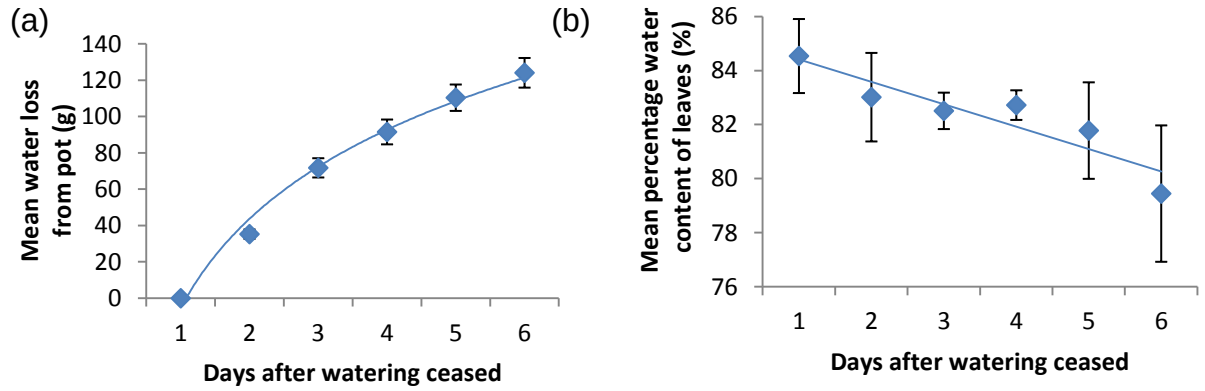

Figure 2.1. Change in the water status of grass leaves and pots of grass plants during the six day period. Plants were watered on the first day only. (a) cumulative water loss from pots (mean +/- SEM, n=12) pooled for all pots and (b) Percent water content (mean +/- SEM, n=12). Raw data and calculations for percent water content and water loss from pots are provided in the Appendix.

The cumulative water loss from the pots increased rapidly over the first three days, but slowed in the second half of the six-day period (Fig. 2.1a). The mean total volume of water lost from pots by the end of six days was 124 ± 8.15 g. During the six day-period following cessation

of saturated watering, the overall mean percentage water content of leaves collected daily (n = 12) decreased ($y = -0.8266x + 85.23$) significantly ($p < 0.05$) from 84.5 ± 1.4 % to 79.5 ± 2.5 % (Fig. 2.1b).

The two datasets presented in figure 2.1, for percent water content of the grass leaf and the water loss from the plant pot, were plotted against each other (Fig. 2.2) to generate a relationship between the water content of a pot (based on its weight) and the leaf water content of the plant in that pot. This was used to determine the weight at which pots could be maintained in order to maintain three specific leaf water contents (high, medium and low; see Table 2.3). Accurate turgidity weights needed for the relative water content calculations could not be obtained from this experimental procedure because leaves may not have been left to absorb water long enough to attain turgidity weight; therefore, only leaf water content (measured as a percentage of fresh weight) could be used to assess the plant leaf water status at this stage of the research.

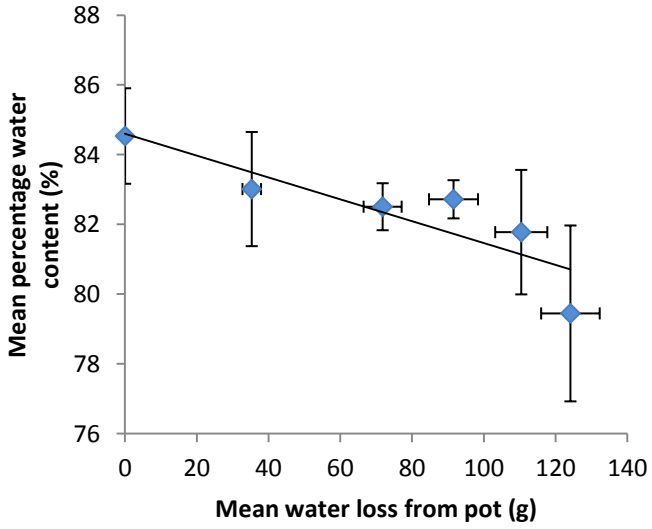

Figure 2.2. Mean percent water content of the grass leaf plotted against mean water loss from pot, for each day (1-6) following cessation of watering on day 1 (mean +/- SEM, n=6).

Table 2.3. Weights to be maintained in pots chosen for low, medium and high water treatments.

Water Treatment	Percentage Water Content in leaf (%)	Water loss from pot (g)	Weight maintained in pot (g)
Low	80.3	124	1105
Medium	82.00	81	1148
High	84.50	0	(watered to saturation) 1229

During the three weeks prior to the feeding trial and during the bioassay, three groups of randomly chosen E+ and E- meadow fescue plants from six different genotypes (12 E+ and 12 E- for each group, n=72, plants in total) were subjected to the three different watering treatments.

On 6 and 7 February 2013, I lined the bottoms of the 72 pots with landscape filter fabric (discs diameter: 11 cm). Water saturated sand was also removed or added to all the 72 pots to bring all pots to a similar weight (approx. 1229 g), 30 minutes after watering. On the 8 February 2013, 72 of the surviving 123 plants generated in Fall 2012 (originally 150 plants) were randomly assigned to either a high, medium or low water treatment group. Twelve E+ and 12 E- plants (two of each per genotype) were used for each water treatment group in this experiment. The final sample size was 24 plants in each of the six endophyte/watering treatment combinations were E+ low water = 12; E- low water = 12; E+ medium water = 12; E- medium water =12; E+ high water = 12; E- high water = 12. On that same day, 8 February 2013, watering was stopped for plants in the low and medium water groups until the 11 February 2013 to allow water to evapotranspirate from the plants and evaporate from the pots.

Watering was resumed on the 11 February 2013. The sand and plant in pots in the low water treatment were watered to a weight of 1105 g; those in the medium water treatment, to 1148 g; those in the high water treatment, to 1229 g (Table 2.3). The top-loading balance (Mettler 2000) was set for tare by 25 g (to exclude weight of pot) and pots were weighed, and

maintained at the above weights by watering , every day for 21 days (11 February 2013 to 3 March 2013) prior to the bioassay. Every day the pots were rotated so that each plant would be under the influence of similar microclimate within their respective treatment. The same water regimes and pot arrangement patterns were also maintained throughout the bioassay experiment.

Bioassay to determine the response of an insect herbivore to meadow fescue diets generated under different watering stresses

One hundred 2nd-instar armyworm larvae were obtained from French Agricultural Research Inc. laboratory in Minnesota, USA. Forty-eight larvae of approximately equal size (24 mg) were selected, weighed with an analytical balance (AE 100, Mettler), and transferred to separate labelled Petri dishes lined with filter paper on 4 March 2013. The Petri dish lids were sprayed every day during the bioassay with distilled water to reduce desiccation of plant leaves provided to the larvae (until 8 March 2013).

The different assays were as follows: six larvae were fed low water treatment E+ grass, six were fed low water treatment E- grass, six were fed medium water treatment E+ grass, six were fed medium water treatment E- grass, 12 were fed high water treatment E+ grass (two replicates) and 12 were high water treatment E- grass (two replicates). Each larva was assigned to a genotype and fed one leaf at beginning of the bioassay and two leaves from day 3 to day 5. The second and third newest leaves (both for E+ and E-) were used.

Leaves collected were put in small flasks containing distilled water and left to absorb water for at least one hour to reduce the effect of low water content in some grass which could have deterred caterpillars from feeding. The leaves were then blotted dry. Before being fed to caterpillars, leaf ends were cut and the FW and DW of the cut pieces were obtained using the same analytical scale used to weight the caterpillars. This data collected were used to calculate the DW of food fed to caterpillars during the bioassay. FW and DW of cut pieces of leaves were

used a substitute of the actual leaf fed to the armyworm to find the percentage water content in the leaves on particular days.

Formulas used for this calculation were:

1. % Water Content of cut leaf = [(FW-DW)/ (FW)] x 100

2. DW leaf fed to armyworms = [(% Water Content of respective cut leaf / 100) x FW]-FW] x -1

During the experiment, larvae were kept at 25°C, 50–60% relative humidity with a photoperiod of 2 hours Light: 22 hours Dark. On day 5, last day of the bioassay, the FW of the caterpillars were recorded before the larvae were put in a freezer (-23°C) for 48 hours. The dead larvae were then transferred to a drying oven (70°C for 48 hours) and the DW of larvae was obtained. The feces of each armyworm were separated from the grass left in the Petri dish, both were put in paper envelope and kept in the drying oven for 48 hours, and weighed to obtain DW of grass egested (contain minute amount of excretion products) and grass ingested respectively. Also, the DW of a sample of six 2^{nd} instar armyworms was recorded, following the same procedure as with the other bioassay armyworms, to obtain the DW of armyworms at the beginning of the bioassay.

DW of grass ingested = DW of grass fed – DW of grass left in Petri Dish after insect removed.

The total DW of leaves fed to armyworms for the 5-day feeding trial, DW of larvae, DW of egested food (feces) and food ingested (grass consumed) were used to calculate relative growth rate (RGR), relative consumption rate (RCR) and relative egestion rate (RER) according to Vicari et al., 2002. Efficiency of conversion of ingested food (ECI), approximate digestibility (AD) and efficiency of conversion of digested food (ECD) were also calculated according to Kogan (1986).

Relative growth rate (RGR) was calculated as follows:

RGR (mg/mg/d) = Dry mass gain / (Mean body mass x Feeding period)

(Vicari et al., 2002), where mean body mass was calculated as Mean body mass = Dry mass gain/ln (Final dry mass/ Initial dry mass).

Relative consumption rate (RCR) and relative egestion rate (RER) were calculated in an analogous manner, using dry matter consumed or egested instead of dry mass gain. Efficiency of conversion of ingested food (ECI), approximate digestibility (AD) and efficiency of conversion of digested food (ECD) were calculated according to Kogan, 1989.

ECI = [(DW of caterpillar at the end of the bioassay – mean DW of six sample 2^{nd} instar armyworms) / DW of total grass ingested]

AD = [(DW of total grass ingested - DW of total grass egested)/ DW of total grass ingested]

ECD = [(DW of caterpillar at the end of the bioassay – mean DW of six sample 2^{nd} instar armyworms) / (DW of total grass ingested - DW of total grass egested)]

Statistical Analysis

For the preliminary water stress experiment, I used a regression analysis in order to know how well the amount of time the plants were maintained under water stress (explanatory variable) explains two response variables: a) mean water loss from pot and b) percentage water content in leaves (Fig.2.1). For the bioassay, analysis of variance (unbalanced factorial ANOVA) was done with the RCR, RGR and RER in order to determine the variation between the three water treatments on the armyworm performance.

Results

Water Stress Experiments

Preliminary Experiment

Following cessation of watering, the water content of grass leaves measured as a percentage of fresh leaf weight, declined over a 6 day period, for all plants – both endophyte-infected and uninfected, as explained in the Methods. This was related to pot weight and used to determine water stress treatments for the grass plants used in the feeding trial with fall armyworm. However, the results of this preliminary experiment, which were pooled to determine the overall watering levels, were further analysed to determine whether the presence of the fungal endophyte affected the water status of leaves.

When the water status expresses as percent water content was compared for the leaves of endophyte-infected and uninfected plants (Fig. 2.3), there was a difference in response, with the water content of E+ leaves declining significantly from 82.66 ± 0.97% to 75.81 ± 4.45% from day 1 to day 6 following cessation of watering. In contrast, the water content of the leaves of uninfected did not decline significantly over the same period: the slope of the line was not significant when a linear regression was fitted (Fig. 2.3). Since the slope in one case was significant (E+ plants) and the other case was not significant (E- plants), it was not possible to analyze the data further to determine whether there was a significant impact of endophyte status on water content, with a regression analysis approach. However, a paired t-test controlling for genotype, in which water content on infected and uninfected leaves was compared on day 6 after watering ceased, indicated that the water content measured as a percentage of leaf fresh weight, was significantly different (lower) in E+ genotypes. Paired t-test, t=16.8, df = 5, $p<0.001$).

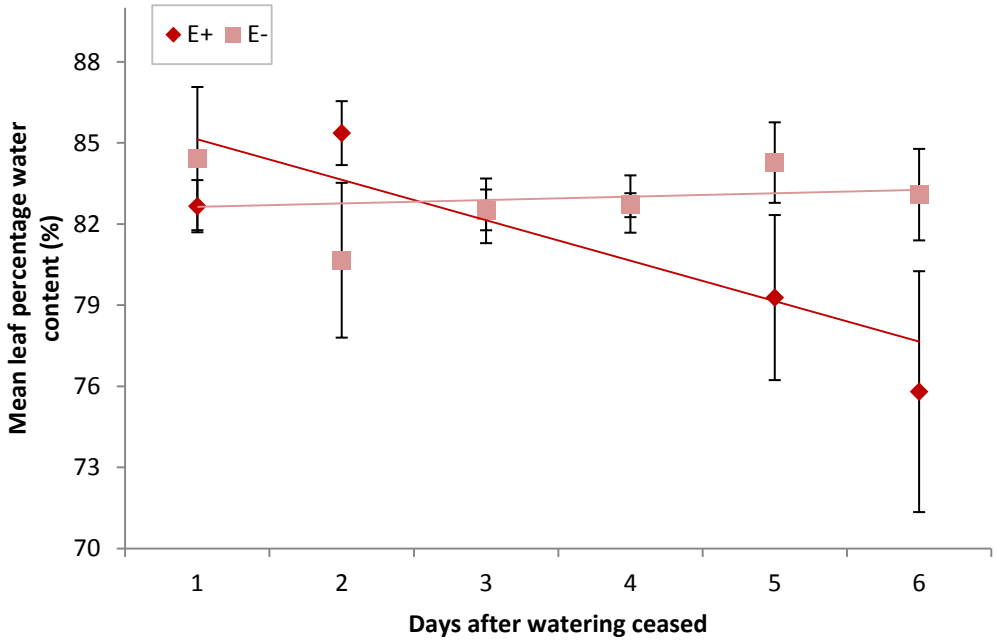

Figure 2.3. Effect of water stress on percent water content of endophyte-infected (diamond) and uninfected (square) meadow fescue leaves over a 6-day period following cessation of watering. Sample plants (n=12) were watered to saturation on day 1 only and not watered for the rest of this experiment. Points are mean +/- SEM. E+ line: y = -1.5 x + 85.1, $F_{1,34}$ = 7.5, p<0.01, E- line: y = 0.12 x + 82.6, $F_{1,34}$ = 0.08, p=0.78

Although the determination of the Relative Water Content of the leaves was not considered to be successful, because the leaves did not increase their fresh weight when immersed in tubes of water, the results are presented here to illustrate the findings. The RWC of six E+ leaves increased from 0.85±0.05% to 0.96±0.02% from day 1 to day 3. A decrease in RWC was then observed from day 3 (0.96±0.02%) to day 6 (0.75±0.13%) (Fig. 2.4). For the RWC of E- leaves, no clear overall trend could be observed over the six days under water stress. The higher RWC obtained with E- leaves was on the last day (0.93±0.06%) (Fig. 2.4).

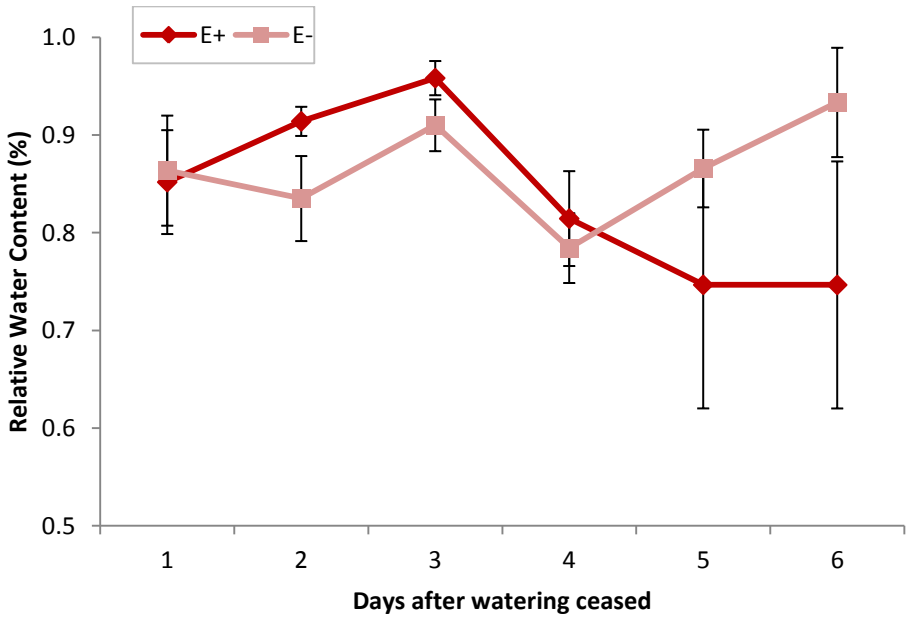

Figure 2.4. Effect of water stress on relative water content of meadow fescue over a 6-day period. Sample plants (n=12) were watered to saturation on day 1 only, no water was added to the pots for the remaining days. Vertical lines show standard error.

Experiment Subjecting Meadow Fescue to a Range of Water Stress (Pre-bioassay Water Stress Experiment)

After leaving the base of the cut grass leaves for at least one hour in distilled water, to allow an equilibration, the percentage water content of E+ (74.37 ± 1.44 %) and E- (70.30 ± 2.26%) leaves from plants under low water treatment were found to have a lower water content than the leaves from plants under medium and high water treatments (max. 80.17± 0.85%; high water level/ E-). The mean percent water content of the E- leaves (70.30 ± 2.26%) were lower than the E+ leaves (74.37 ± 1.44 %) under the low water treatment (Fig. 2.5).

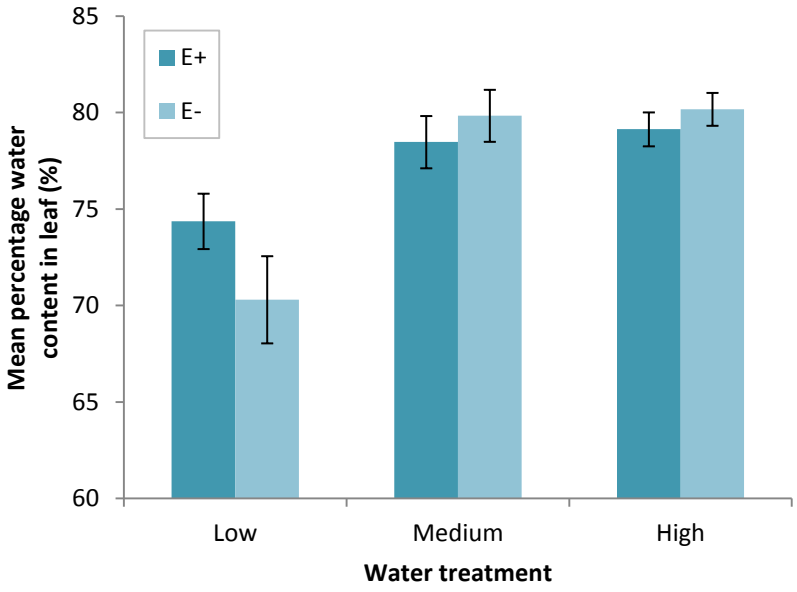

Figure 2.5. Mean percentage water content in low, medium and high water treatments fed to fall armyworms during bioassay.Vertical lines show standard error.

Bioassay

Various parameters were calculated from the feeding trial: food ingested, food egested, biomass gained, approximate digestibility (AD), efficiency of ingestion (ECI) and efficiency of digestion (ECD), for each of the two low and two medium treatments, using 12 caterpillars (Table 3.1). The same calculations were carried out for the 24 caterpillars feed the high watering treatment E+ and E- plants. The mean total dry weights of grass consumed, dry weight of feaces (including minute amount of excreted waste) varied widely across plant diet groups, although there were clear trends in biomass gained by caterpillars at the end of the 5 day bioassay, with caterpillars in the infected grass diet, under low water availability (highest water stress), having the lowest biomass (Table 3.1).

The highest and lowest approximate digestibility values were obtained from caterpillars fed with E+ grass under low water level (0.705±0.062) and with larva fed with medium water stressed E+ grass (0.496± 0.057) respectively (Table 3.1). The caterpillars fed with intermediate

water stessed E- grass had the highest ECI (0.276±0.015) (Table 3.1). The ones fed with E+ grass under low water treatment had the lowest ECI (0.101±0.036). When ECD values were derived, the larvae fed with E- grass under medium water treatment was found to have the highest ECD (0.632±0.211) . The lowest ECD was obtained with caterpillars fed with E+ grass watered with low water level (0.183±0.092) (Table 3.1).

Fall armyworm larvae in all plant diet groups consumed material made available: for example, and growth rates varied widely across and among groups. For example, at an individual level, the highest mean RCR (2.21±0.29), RER (0.87±0.08) and RGR (0.37±0.04) were obtained from six fall armyworms fed with low water/ E-, six fed with medium water/ E+ grass and 12 fed with high water/ E- respectively (Fig. 2.6 and Table 3.1).

Table 3.1. Nutritional indices of fall armyworm larvae fed with grass grown under 6 different treatments (mean±SE). n = 12 in Low and Medium groups and n = 24 in High groups.

Treatment	Parameter (mean ± SE)					
	Food Ingested (mg)	Food Egested (mg)	Biomass Gained (mg)	AD	ECI	ECD
Low/ E+	72.715 ± 9.584	21.883 ± 5.036	7.672 ± 2.446	0.705 ± 0.062	0.101 ± 0.036	0.183 ± 0.092
Low/ E-	99.552 ± 8.839	31.6 ± 3.342	14.053 ± 2.175	0.665 ± 0.047	0.147 ± 0.024	0.238 ± 0.049
Medium/ E+	81.767 ± 14.328	38.45 ± 5.577	15.365 ± 2.042	0.496 ± 0.057	0.204 ± 0.025	0.472 ± 0.11
Medium/ E-	53.093 ± 12.994	25.65 ± 7.241	12.201 ± 3.188	0.532 ± 0.087	0.267 ± 0.073	0.632 ± 0.211
High/ E+	69.21 ± 6.425	34.167 ± 3.475	14.175 ± 1.385	0.512 ± 0.045	0.203 ± 0.015	0.452 ± 0.067
High/ E-	89.067 ± 12.56	37.017 ± 2.535	15.975 ± 1.432	0.538 ± 0.036	0.199 ± 0.02	0.409 ± 0.063

Unbalanced factorial ANOVAs comparing the effects of endophyte-infection status and watering treatment, on relative consumption and relative egestion rates were not significant for relative consumption rate, but the watering treatments did have a significant effect on relative egestion rate (ANOVA $F_{2,42} = 3.36$, $p = 0.044$) and relative growth rate (ANOVA $F_{2,42} = 4.69$, $p = 0.014$) (Fig.2.6), as reflected in the low watering treatment which resulted in lower egestion and growth. While none of the interaction terms (endophyte status-watering treatment) from these ANOVAs were significant, visual inspection of data indicated that there may have been an additional effect of the endophyte on relative growth rate in the low water treatment. In a simple one-way ANOVA, carried out to explore differences among the 6 treatment groups of fall armyworm larvae, in terms of relative growth rate, the group fed infected leaves grown under the lowest water treatment, had significantly lower growth rates than any other group of larvae, and none of the other groups varied significantly from each other (one-way ANOVA: $F_{5,42} = 2.87$, $p < 0.05$, LSD pairwise comparisons). In the case of relative egestion rates, the one-way ANOVA was not significant, although, again, the larvae fed endophyte-infected, low water leaves, did show significantly lower egestion rates compared with 2 other groups.

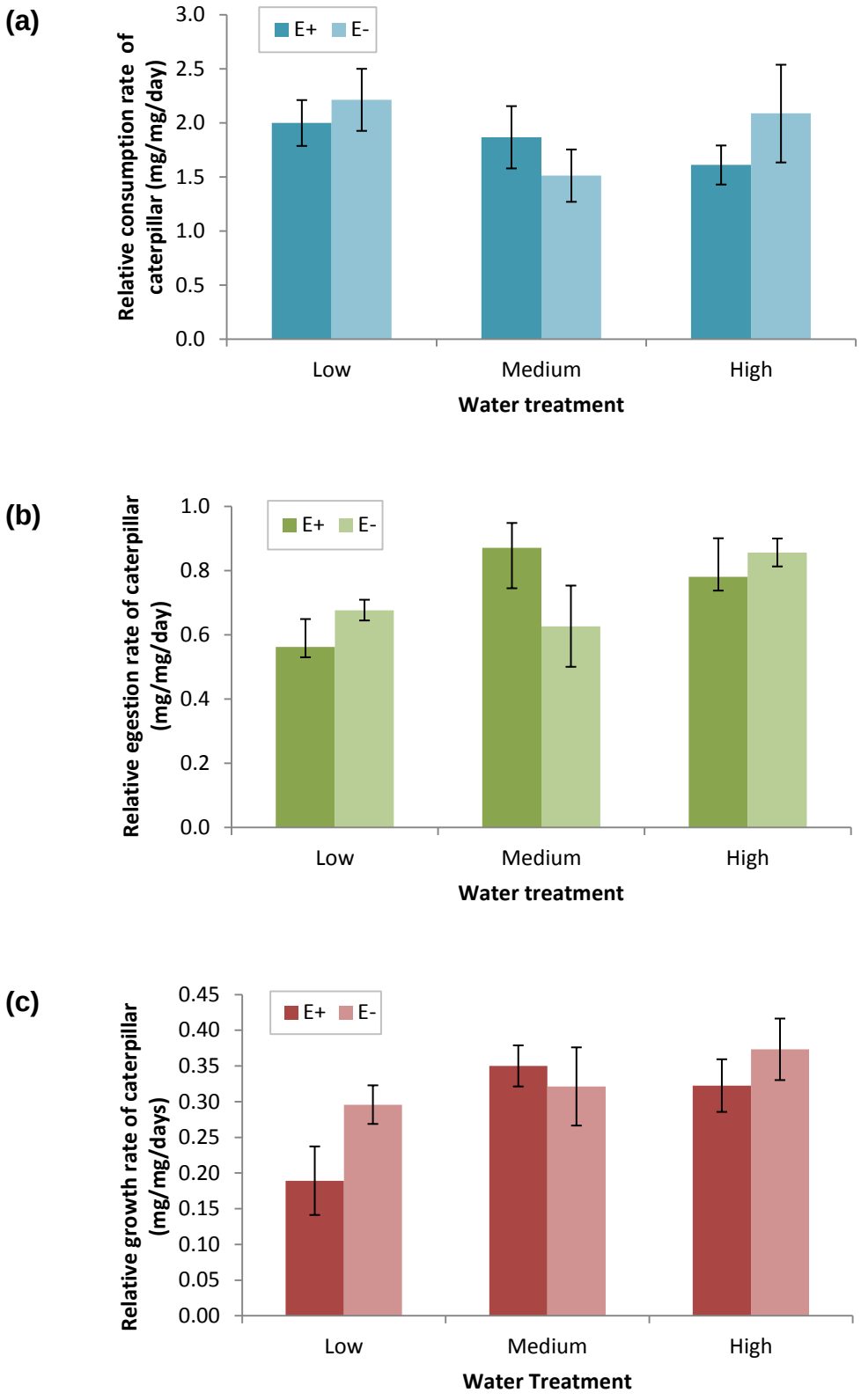

Figure 2.6. Nutritional indices; (a) relative consumption rates (RCR), (b) relative egestion rates (RER), and (c) relative growth rates (RGR) of fall armyworms fed with meadow fescue maintained under three different water treatments and having E+ or E- status. Vertical lines show standard error.

Discussion

Drought stress and precipitation changes due to climate change is expected to have a negative impact on the growth of many plant species (Grzegorz, 2011; Pautasso et al., 2012), and mechanisms that may allow plants to tolerate drought, such as benefits that *Neotyphodium* endophytes may provide for their host grasses are of interest to researchers (Eerens et al., 1998; Salminen et al., 2005; Panka et al., 2011). The relationship between the plant-endophyte symbiotic relationship under environmental stress and the production of secondary metabolites and alkaloids by the endophyte, that also play a major role in plant defence mechanisms against herbivory (Bacon, 1993; Bultman and Bell, 2003; Salminen et al., 2005), is a topic on which there is little research. Hence, the different experiments in this study aimed at exploring the connection between increasing water stress and the effect on the plant–endophytes association and the plant-herbivory response are at the forefront of this field.

This study highlights the challenge of manipulating and linking water soil availability to plant water status. Because of the difficulties in obtaining Relative Water Content of leaf tissue, and obtaining the turgidity weight, I had to rely on the less desirable metric of percent water content based on tissue fresh weight, as a measure of the water status in E+ and E- plant. The development of water stress treatments based on this measure did result in decreased water content of both E+ and E- leaves under low water regimes compares to the high and medium regimes. However, the relationship between endophyte-infection status and watering treatment was variable and not clear cut, and within the low water treatment, the E+ leaves may have contained more water due to the water tolerance mechanisms facilitated by endophytes (Eerens et al, 1998).

Effects of water stress on plant-endophyte interactions

Research in the field of plant-water relations indicates that water stress is one of the main components of drought stress, temperature being another factor (Taiz and Zeiger, 202). The use of RWC as an indicator of water stress was inconclusive. While I found no distinct change in RWC in either E+ or E- meadow fescue leaves, the percentage water content recorded with E+ leaves over a 6-day water stress period gradually decreased, which suggests that stress may be increasing over time as water left the pot via evapotranspiration and drainage . However, the E- leaves water content stayed constant throughout the six days of water stress. This was a surprising result that was not consistent with the findings of other research (Bacon, 2003). Bacon (2003) found that the additional acquired water-stress tolerance by grasses via their endophytic symbionts could have been the result of an increased cellular turgor pressure in their leaves due to lower stomatal conductance (e.g. decreased rates of water loss from stomata in the event of water stress) and enhanced osmoregulatory system (e.g. osmotic adjustment mechanisms) mediated and/ or facilitated by the endophytes.

Several confounding variables may have contributed to the decrease in water content of E+ grass compared with E- grasses. First, in the preliminary experiment, fully expanded leaves were used, regardless of their age, to find percentage water content at a particular point in time after watering had been stopped. In endophyte-infected plants, not all tissues are equally sensitive to water stress, e.g.,, percentage osmotic adjustment in mature leaf blades (5.8%) has been shown to be lower than in immature leaf blades (26.4%) (West et al., 1990). Hence, because of a higher osmotic adjustment in immature leaf blades, these younger tissues could maintain higher water content compared to mature ones (West et al., 1990). Not controlling for leaf and/ or plant developmental stages when collecting leaves for water content measurement could have led to the our results being different from previous findings as state of interactions

can change from commensalistic to mutualistic or antagonistic depending on the age of the leaf or plant (West et al., 1990; Saona, 2011)

Using the percentage water content of the leaves, I was not able to make accurate deductions about the ability of the plant cells to absorb and retain water. An increased amount of osmoregulatory metabolites and ions (e.g. proline, polyols and potassium) may have accumulated in the endophyte-infected leaves and could have eventually affected plant survival by decreasing water stress (Bacon, 1993; Bayat et al., 2009). However, these solutes could also have contributed to an increase in dry mass of leaves (Bacon, 1993; Bayat et al., 2009). Lower percentage water content (relative to solutes dry mass) was obtained in my results with endophyte-infected plants compared to the water content in endophyte-uninfected plants. When the leaves were dried to obtain DW, which were used in the calculation for percentage water content, the additional solutes in E+ could have contributed to the DW of the leaves and decreased the percentage (Bayat et al., 2009).

To measure the water status of plants, RWC in leaf tissues is often used (Yamasaki and Dillenburg, 1999). RWC of either endophyte-infected or uninfected plants obtained in the preliminary experiment did not show any apparent change when subjected to a water stress treatment for six days. This result contrasts with previous work reported with tall fescue and grove bluegrass (*Poa alsodes*) Kannadan and Rudgers, 2008, found a significant influence of endophyte symbiosis on relative water content. Under lower watering regimes, researchers found that, endophyte-free tall fescue and grove bluegrass had a significantly higher RWC than their corresponding endophyte-infected plants (Eerens et al., 1998; Kannadan and Rudgers, 2008). These results were consistent with my hypothesis that *Neotyphodium* endophytes cause plants to up regulate water conservation mechanisms faster by mobilising more soluble solutes to the leaves shoot cells in response to drought.

Technique used to determine RWC must be adjusted for each type of plant material under investigation (Yamasaki and Dillenburg, 1999). Adjustments mainly involve the length of time grass is left to absorb water which is needed to obtain accurate turgidity weights (Yamasaki and Dillenburg, 1999). The procedure to obtain RWC of meadow fescue leaves that I followed in the preliminary experiment was based on an experiment using tall fescue by Bayat et al., (2009). However, my study grass was the meadow fescue; this difference in plant species used may have hindered any positive effects of endophyte-infection on the meadow fescue under higher water stress (Yamasaki and Dillenburg, 1999). Furthermore, despite controlling for possible genotypic effects on the endophyte-infected and endophyte-uninfected by using daughter plant pairs (E+ and E-) obtained from same parent plant, six different genotypes from different populations (to represent wild population more accurately) were used as parent plants initially. This may have contributed to some genotype response being masked by other genotype response (Saona, 2011). Saona et al. (2010) found that different interactions between different genotypes and endophytes in different parts of the population, further adding to the complexity of endophyte–grass interactions.

When the plants were maintained for a longer period of time under the water stress treatment (pre-bioassay water stress experiment), the percentage water content of both endophyte-infected and endophyte-free plants were lower under the lowest water treatment. These results could signify that even after leaves were allowed to absorb water for at least 1 hour before fresh weights were measured, less water could be absorbed within the leaves (E+ and E-) under the low water treatment, in comparison to the medium and high water treatment. Thus, when given the time to acclimatise to the various water stresses, meadow fescue grass, irrespective of endophyte-infection status, indicated developmental responses to water stress (e.g. increase deposition of wax on inner and outer surfaces of leaf cuticle that reduce water movement through the cuticle) (Taiz and Zeiger, 2002). Another explanation for this lower water

content in the leaves of plants grown under low water levels could be that more time was needed for these leaves to absorb as much water as the ones under medium and high watering regimes (Yamasaki and Dillenburg, 1999). The E+ grass had higher percentage water content (74.37 ± 1.44 %) than E- grass (70.30 ± 2.26%) under the low water treatment. This result could have reflected drought tolerance strategies in the grass when subjected to water stress for a longer period (Eerens et al., 1998; Saona, 2011).

Consequence of water stress on plant-herbivory response (via endophytes)

Effects of water stress on phytophagous insects can be altered by endophytes due to the influence of the endophytes on their food source's response to abiotic stress (Bultman and Bell, 2003). *Neotyphodium* are thought to increase drought tolerance by, for instance, changing stomatal conductance and increasing osmotic adjustments (West et al., 1990). These mechanisms in E+ plants are mediated by the production of additional osmoregulatory products (e.g. loline alkaloids) that may not be metabolised by the plants on their own (Bacon, 1993, Salminen et al., 2005; Bryant et al., 2010). Since the E+ leaves may have higher water content than E- leaves (Eerens et al, 1998), they could be more appealing to the some phytophagous insects that depend on the water content of grass for hydration. The accumulation of endophyte-produced alkaloids in E+ plants during high drought stress would deter consumption by insects, such as the model insects in this study, armyworms (Salminen et al., 2005). My results for relative growth rates of fall armyworms obtained with the feeding trial experiment were consistent with those of Bultman and Bell (2003) in that, the growth rates of caterpillars were the lowest when fed with endophyte infected grass grown under low watering regimes (higher stress). Bultman and Bell (2003) attributed this observation to loline alkaloids and lower total protein nitrogen content of the E+ grass under the drought stress. Therefore, my hypothesis that there would have been no change in RCR, RER and RGR of armyworms when either E+ or E- plants grown under medium

and high water regimes (used to feed the insects) was supported by my results. I found that under no water stress, leaves of both E- and E+ plants had high water content thus less/ no apparent effect on herbivores could be seen under non stressful conditions.

Conclusions

This study showed that teasing apart the interactions between fungal endophytes, their host grass plants and herbivores is extremely challenging and complex, and therefore, that determining how this relationship will respond to climate change, in which multiple factors that affect water availability, e.g., warming temperatures or less precipitation (Pautasso et al., 2012), will change, will make this a fertile research area in the future. Limiting water availability and leaf water content could negatively affect the performance of armyworms feeding on E+ grass (Fig. 2.6), but the underlying mechanisms remain unclear. Further experiments must be conducted to investigate the toxicity of different alkaloids produced by endophytes in the meadow fescue, under different water availabilities in order to gain a better understanding of the mechanisms mediated by *Neotyphodium* endophytes in the pooid grass. To date, little research has been done on the effect of water stress on endophyte-plant symbiosis and its subsequent implications on herbivory. It still needs to be established how combinations of different environmental stresses in nature would affect these relationships. Taking into account the extent to which endophytes benefit plant under different climate change scenarios, this study along with others alike, could be valuable in predicting more accurately how well insect pests can disperse and thrive under current and future environmental change.

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