Disease and pest resistance of endophyte infected and non-infected drunken horse grass

CHUNJIE LI[1,2], XINGXU ZHANG[1], FEI LI[1], ZHIBIAO NAN[1*] and C. L. SCHARDL[2]
[1]*College of Pastoral Agriculture Science and Technology, Lanzhou University; Key Laboratory of Grassland Argo-Ecosystem, Ministry of Agriculture; Gansu Grassland Ecological Research Institute; 730020 Lanzhou, China;*
[2]*Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA*
chunjie@lzu.edu.cn

Abstract

The naturally occurring mutualistic symbiosis of *Neotyphodium gansuense* and drunken horse grass (*Achnatherum inebrians*) was studied previously in China. In this paper, new data on the interactions of endophyte, host and pathogenic fungi, mite and insect pests are presented. Fungal diseases and pests were examined when test plants were grown in pots in a climate chamber or in the field. There were usually no significant ($P>0.05$) differences in the levels of powdery mildew infection (caused by *Blumeria graminis*) under climate chamber conditions; the only exception was that E+ plants had significantly less powdery mildew infection at 50% soil water holding capacity (WHC) than at 30% WHC. There was no significant difference ($P<0.05$) in the levels of infection of E- and E+ plants with leaf spot disease, caused by *Bipolaris sorokiniana,* under field conditions. Regardless of whether grown under growth chamber or field conditions, densities of the mite *Tetranychus cinnabarinus* on E+ grass were significantly ($P<0.05$) lower than that on E- grass. Densities of the bird-cherry-oat aphid (*Rhopalosiphum padi*) on E+ grass were significantly ($P<0.05$) lower than that on E- grass.

Keywords: *Neotyphodium gansuense*, *Achnatherum inebrians*, diseases, pests, resistance, endophytes

Introduction

Neotyphodium and *Epichloë* endophytes have been shown to confer enhanced fitness by increasing drought tolerance, and protecting the host plant against certain nematodes, fungal pathogens, insect herbivores and mammalian herbivores (Siegel *et al*. 1987; Latch 1993; Schardl & Phillips 1997). There is some evidence that colony growth of plant-pathogenic fungi is inhibited by *Neotyphodium* endophytes (White & Cole 1985; Christensen *et al*. 1991; Christensen & Latch 1991; Christensen 1996; Holzmann-Wirth *et al.* 2000), and that disease tolerance or resistance can be imparted by some but not all *Neotyphodium* species (West *et al.* 1989; Gwinn & Gavin 1992; Welty *et al.* 1991; Nan & Li 2000; Wheatley *et al*. 2000; Trevathan 1996; Blank *et al.* 1993; Funk *et al*. 1994; Hume *et al.* 1997).

Deterring herbivores from feeding on the host grass is one of the most beneficial features of endophytes (Siegel *et al.* 1987; Latch 1993; Nan & Li 2004; Popay & Bonos 2005). So far, at least 50 species in 35 genera have been shown to be deterred from feeding on endophyte-infected grasses (Siegel *et al*. 1987; Latch 1993; Popay *et al*. 2000; Nan & Li 2004; Popay & Bonos 2005).

The symbiotic association of *Neotyphodium gansuense* and *Achnatherum inebrians* is distributed widely in China, and can be found under harsh conditions such as arid or semi-arid and alpine or subalpine grasslands (Li *et al*. 2004a,b). Endophyte infected *A. inebrians* has been shown to contain high levels of the ergot alkaloids, ergonovine and lysergic acid amide (Miles *et al.* 1996; Li *et al*. 2006). Dual-culture testing and inoculation of detached leaves have shown that *N. gansuense* can inhibit growth and disease lesion development by some fungal pathogens (Li 2005). Our previous investigation included six diseases of *A. inebrians*: seedling rot (*Alternaria alternata*), rust (*Puccinia stipae-sibiricae*), powdery mildew (*Blumeria graminis*), smut (*Ustilago hypodytes*), leaf spot (*Bipolaris sorokiniana*) and ergot (*Sphacelia* sp.) (Li *et al*. 2003). To explore possibilities of utilising *N. gansuense* for bio-control, this research aimed to determine whether or not endophyte infection reduces the activity of naturally occurring diseases and pests of *A. inebrians*.

Materials and Methods

Seeds of *Achnatherum inebrians* were collected from Sangke grassland, Gansu province, China, and kept under constant 5 °C storage at the Official Forage Seed Testing Center of Ministry of Agriculture, Lanzhou, China.

Endophyte infected seeds were heat treated at a high moisture content to obtain E- seeds (Li 2005). Treated seeds were sown in plastic baskets. Endophyte status was detected by microscopy of samples from 4-week-old seedlings stained with 0.8% aniline blue.

In a climate chamber, E+ or E- plants were transplanted individually into plastic pots, and were watered regularly and weighed to maintain soil moisture content at 30 or 50% WHC; i.e. drought and normal growth conditions, respectively. E+ and E- seedlings were also transplanted into the field to establish trial plots under rain-fed conditions. Incidence of the naturally

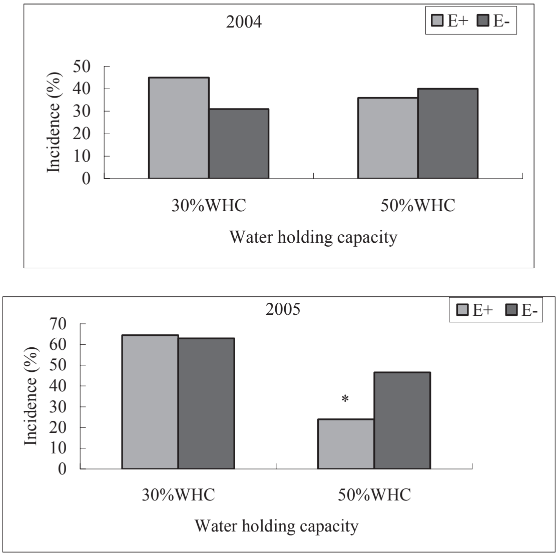

Figure 1 Effects of powdery mildew (*B. graminis*) on E+ and E- *A. inebrians* at 30% and 50% WHC soil moisture content in pot conditions. *Differences are significant between E+ and E- plants ($P<0.05$).

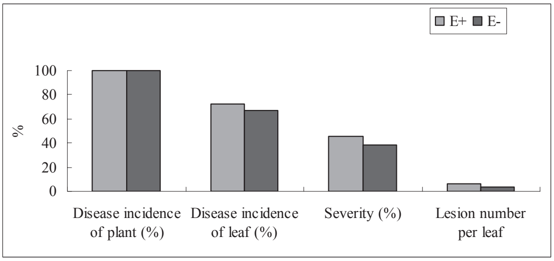

Figure 2 Incidence of E+ and E- *A. inebrians* leaf spot (*B. sorokiniana*) under field conditions.

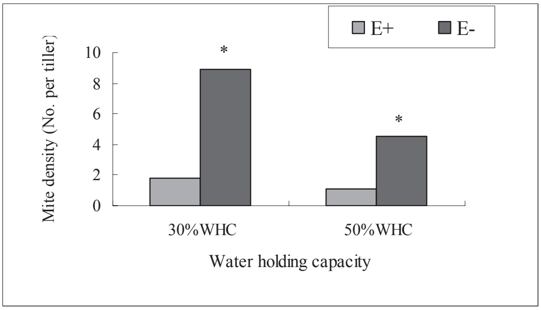

Figure 3 Density of *T. cinnabarinus* on *A. inebrians* under soil moisture content of 30% and 50% water holding capacity in greenhouse pot conditions. *Differences are significant between E+ and E- plants (P<0.05).

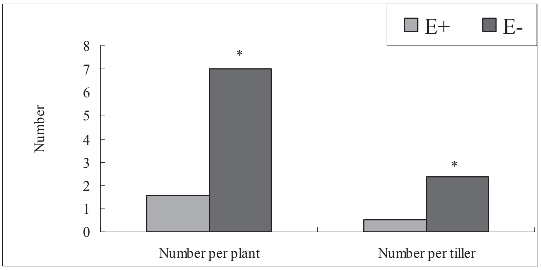

Figure 4 Density of *R. padi* on E+ and E- *A. inebrians* under field conditions. *Differences are significant between E+ and E- plants (P<0.05).

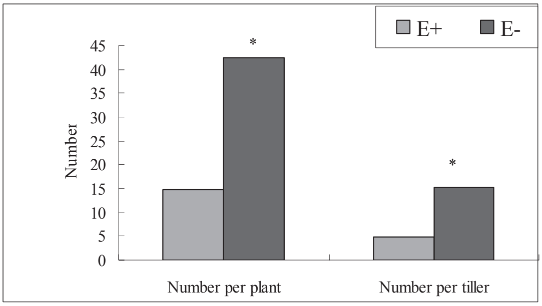

Figure 5 Density of *T. cinnabarinus* on E+ and E- *A. inebrians* under field conditions. *Differences are significant between E+ and E- plants (P<0.05).

occurring diseases powdery mildew (*Blumeria graminis*) and leaf spot (*Bipolaris sorokiniana*), and numbers of mites (*Tetranychus cinnabarinus*) and bird-cherry-oat aphids (*Rhopalosiphum padi*) damage were investigated.

Results

Disease resistance of E+ and E- plants in the climate chamber

In 2004, powdery mildew (*B. graminis*) incidence on E+ and E- *A. inebrians* was similar under a soil moisture content of both 30 and 50% WHC. In 2005, incidence was again similar at 30% WHC but at 50% WHC disease incidence on E+ declined by 49% and was significantly (P<0.05) lower than on E- plants (Fig. 1).

Disease resistance of E+ and E- plants in the field

In the field, the existence of *N. gansuense* did not significantly affect the incidence, severity and leaf lesion number of leaf spot (*B. sorokiniana*) on *A. inebrians* (P>0.05) (Fig. 2).

Pest resistance of E+ and E- plants

In the climate chamber, regardless of drought (30% WHC) or normal (50% WHC) soil conditions, the density of *T. cinnabarinus* on E+ plants was significantly (P<0.05) lower than that on E- plants. Mite levels were highest under drought conditions (Fig. 3).

In the field, the densities of *R. padi* on E+ plants were significantly (P<0.05) lower than that on E- plants (Fig. 4), as was the case for *T. cinnabarinus* (Fig. 5).

Discussion

Epichloë endophytes (*Neotyphodium* and *Epichloë* spp.) may enhance the abilities of grasses to resist pathogens by inhibiting sporulation (Li 2005) and spore germination (Holzmann-Wirth *et al*. 2000), preventing invasion and colonisation, delaying or reducing subsequent lesion development (Nan & Li 2000 2004), and deterring virus-vector insects as well (West *et al*. 1990; Latch 1993). Many studies have shown that pure cultures of many epichloë endophytes inhibit growth of plant-pathogenic fungi (White & Cole 1985; Christensen & Latch 1991; Holzmann-Wirth *et al*. 2000; Siegel & Latch 1989).

In the climate chamber pot trial, effects of endophytes of *A. inebrians* on powdery mildew varied according to the environment (Fig. 1), but in the field neither incidence nor severity of Bipolaris leaf spot was affected by endophyte infection (Fig. 2). Other researchers have reported some instances of enhanced disease resistance with *Neotyphodium* or *Epichlo*ë infection, but not always (Siegel & Latch 1991; Latch 1993; Nan & Li 2004; Popay & Bonos 2005). For example, tall fescue (*Festuca arundinacea*) seedling stem rust (*Puccinia graminis* subsp. *graminicola*) infection was unaffected by *N. coenophialum* infection under glasshouse conditions (Welty *et al.* 1991). Also, *N. coenophialum* did not reduce tall fescue root rot by *Cochliobolus sativus* (Trevathan 1996), root disease by *Pythium aphanidermatum* (Blank *et al.* 1993) or foot rot by *Fusarium oxysporum* and *F. equiseti* (Hume *et al.* 1997). A few studies indicated that endophytes could improve resistance to diseases such as turfgrass blight caused by *Pythium* (Funk *et al.* 1994), purple spot on timothy grass caused by *Cladosporium phlei* (Shimanuki 1987), seedling disease of tall fescue caused by *Rhizoctonia zeae* (Gwinn & Gavin 1992), and dollar spot caused by *Sclerotinia homeocarpa* (Clarke *et al*. 2006). Also, detached leaves of E+ *Elymus cylindricus* had fewer and smaller lesions than those on E- plants 3 days after inoculation with *A. alternata*, *F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. oxysporum* (Nan

& Li 2000). E+ tall fescue plants were more resistant to crown rust (*P. coronata*) under field conditions (West *et al.* 1989) and *N. lolii* infected perennial ryegrass plants were observed to be more resistant to leaf spot (*Pyrenophora semeniperda*) than uninfected plants (Wheatley *et al.* 2000).

Grass disease resistance is influenced by many biotic and non-biotic factors, including variety, pathogen type, environmental conditions, endophyte status and so on (Siegel & Latch 1991; Latch 1993). Our research showed variation of powdery mildew incidence between 30% and 50% WHC soil conditions, and between E+ and E- plants (Figs. 1 & 2). Moreover, different isolates may produce different quantities and types of antifungal substances and some probably confer different agronomic characters on the host plant (Christensen & Latch 1991). Two isolates of *N. uncinatum* and three of four isolates of *Epichloë festucae* gave no inhibition of *D. erythrospila* and *R. zeae* (Christensen 1996).

This is the first report of an evaluation of endophytic grass resistance to pests in China. Our results showed that *Neotyphodium* infection could significantly ($P<0.05$) reduce densities of *R. padi* and *T. cinnabarinus* on E+ *A. inebrians* compared to E- plants (Fig. 3-5). At least four species of aphids are deterred by grass endophytes: *Diuraphis noxia*, *R. padi*, *Schizaphis graminum* and *Sipha flava* (Siegel *et al*. 1990; Latch 1993; Schardl & Phillips 1997), but only one species of Acarina mites, *Abacarus hystrix* (Li & Nan 2004). This trial shows another mite, *T. cinnabarinus* is deterred by endophytic *A. inebrians*. High levels of two main ergot alkaloids, ergonovine and ergine, are produced in endophytic *A. inebrians* (Miles *et al*. 1996; Li *et al*. 2006). Of the more than 10 known alkaloids associated with grass endophytes, peramine and loline have proved more toxic to insects than have ergot alkaloids and indolediterpenes (Bacon 1995; Schardl & Phillips 1997; Faeth & Bultman 2002). It is possible the very high levels of ergot alkaloids or other substances produced in the interactions between *A. inebrians* and *N. gansuense,* deterred *R. padi* and *T. cinnabarinus*. Future studies to elucidate the chemical basis for invertebrate deterrence would be of considerable practical importance for the use or manipulation of endophytes as bioprotective agents.

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