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Effect of the endophyte *Neotyphodium lolii* on susceptibility and host physiological response of perennial ryegrass to fungal pathogens

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Abstract The effect of the endophyte *Neotyphodium lolii* on susceptibility of perennial ryegrass (*Lolium perenne*) to ten fungal pathogens in detached leaves was studied. The pathogens were *Alternaria alternata*, *Ascochyta leptospora*, *Bipolaris sorokiniana*, *Curvularia lunata, Fusarium acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. solani*, *F. oxysporum*, and *Gliocladium roseum*. In addition, the effect of the endophyte on four pathogens (*A. alternata*, *B. sorokiniana, Curvularia lunata* and *F. avenaceum*) in living plants was studied, and changes in host superoxide dismutase (SOD) or peroxidases (POD) activity were examined. The total lengths of lesions on detached leaves were greater ($P<0.05$) on E- plants than on E+ plants except for *A. leptospora* although differences between E+ and E- were not consistently significant at all sample times (days after inoculation).The numbers of lesions were greater ($P<0.05$) and the lesions were larger ($P<0.05$) on intact E- plants than on intact E+ plants for all of the four pathogens. SOD enzyme activity was significantly greater ($P<0.05$) in E+ plants than in E- plants only for *A. alternata*, *C. lunata*, and *F. avenaceum*. POD enzyme activity was significantly greater ($P<0.05$) in E+ plants than in E- plants only for *C. lunata*, *B. sorokiniana* and the uninoculated control.

Keywords *Lolium perenne* · Endophyte · Pathogen · Superoxide dismutase · Peroxidases · Host-pathogen interaction

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Introduction

Perennial ryegrass (*Lolium perenne*) is one of the most important pasture and turfgrass species in temperate regions of the world (Reed 1996). It is the host of the endophyte *Neotyphodium lolii*. Endophytes that form mutualistic associations with perennial ryegrass have been the most widely studied (Siegel et al. 1987). The mutualism can enhance ecological fitness of both host and microbe, including greater host tolerance to abiotic and biotic stresses (Latch 1993). However, there are reports that the effect of a symbiont on a host may be positive or negative, depending on environmental conditions and host genetics (Faeth and Sullivan 2003) and that the ecological benefits of grass endophytes are complex and labile (Saikkonen et al. 2004).

The influence of endophytes on plant disease resistance has not been extensively investigated. Latch (1993) reported that the first record of an endophyte affecting plant disease was that by Shimanuki and Sato (1984), who demonstrated that timothy (*Phleum pratense*) plants infected with the choke fungus *Epichloë typhina* were resistant to the fungus *Cladosporium phlei*, Gwinn and Gavin (1992) found that in a soilless medium amended with *Rhizoctonia zeae*, survival of tall fescue (*Festuca arundinacea*) seedlings increased with an increasing percentage seeds with the endophyte. However, endophytes do not always improve, and may even reduce disease resistance in a host. Welty et al. (1991) showed that endophyte infection did not influence stem rust (*Puccinia graminis*) infection type in seedlings of tall fescue grown in a greenhouse. Wäli et al. (2006) showed that *N. uncinatum* infection increased the winter damage of meadow ryegrass (*L. pretense*). In addition, although endophyte-infected plants may have greater disease resistance under controlled environmental conditions, this does not imply greater disease resistance under field conditions (Schmidt 1993). The inconsistent and inconclusive results may be one reason why rare reports on endophyte and pathogen interactions exist.

There are few reports concerning the mechanisms of how endophytes enhance disease resistance. Christensen (1996) found that plants infected with some fungal endophytes contained diffusible substances that inhibited growth of one or more pathogens. White and Cole (1985, 1986) found endophytes that produce antifungal substances inhibiting growth of some pathogens on PDA. Siegel and Latch (1991) reported inhibition of pathogens by endophytic fungi *in vitro*, although the occurrence of the inhibition varied, depending on pathogen and endophyte isolates. The inhibition was not associated with alkaloids produced by the endophytic fungi. Additional studies are needed to understand the effects of endophytes on disease resistance.

Superoxide dismutase (SOD) and peroxidases (POD) are key enzymes that scavenge active oxygen species to protect plant cells. There are reports that plants adjust enzyme activities under stressful conditions including drought, salinity, extreme temperatures or atmospheric pollution (Zaka et al. 2002). However, effects of endophyte infection on enzyme activity of their host plants when under disease stress have not been reported in the literature.

The objectives of this study were to (1) evaluate resistance to a range of pathogens on detached leaves and on living plants of endophyte-infected (E+) and endophyte-free (E−) perennial ryegrass, and (2) determine whether there are any changes in host enzyme levels (SOD or POD) in response to the fungal pathogens.

Materials and methods

Plant materials

Commercially available cvs Barball and Pinnacle of perennial ryegrass seeds were obtained from the seed bank of Gansu Grasslands Ecological Research Institute, China. The seeds were assessed for endophyte infections by staining and microscopic examination (Nan 1996a). The cv. Pinnacle was selected, based on its high percentage of endophyte-infected seed (63% compared to the 20% infection rate of Barball). Plastic trays (30 cm×25 cm×8 cm) were filled with quartz sand (1 kg/pot) which had been sterilized in an oven at 130°C for 30 min. Only well-filled, healthy-looking seeds were used for planting. Five rows of 10 seeds each were planted per tray at a depth of 5 mm. Prior to planting, 1000 ml water was added to each tray. Trays were placed in a temperature-controlled greenhouse (18°C–24°C) with 10 h of illumination per day. They were watered with Knop nutrient solution (Johnston and Booth 1983) twice a week with supplementary watering as required. One month after sowing, the infection status of seedlings was determined by microscopic examination of host leafsheath pieces stained with aniline blue (Nan 1996b). The E+ and E- seedlings were marked and transplanted into individual pots (150 mm diam, height=100 mm) filled with soil (commercial fine sandy soil) and placed on a veranda (night temperature=18°C day temperature=25°C, light=10 h). This provided 15 lines of E+ and E− plants, respectively. The plants were watered daily. Three months after transplantation, plants were divided into individual tillers with attached roots, and four tillers, taken as a ramet, and planted in one plastic pot (150 mm diam, height=100 mm) filled with soil (commercial fine sandy soil). On average, one plant yielded five ramets resulting in a total of 70 pots of E+ and E− that were then used for the experiment. The plants were also

returned to the veranda (18–25°C, 10 h light day^{-1}). One month later when the ramets grew well, the plants were inoculated with spore suspensions as described below.

Inoculation of detached leaves and measurement of disease development

Fungal pathogens isolated from perennial ryegrass growing in Lanzhou, China, by Li et al. (2007) were obtained from the Gansu Grasslands Ecological Research Institute, China. The pathogens were *Alternaria alternate*, *Ascochyta leptospora*, *Bipolaris sorokiniana*, *Curvularia lunata*, *Fusarium acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. solani*, *F. oxysporum*, and *Gliocladium roseum*. For inoculum production, cultures were grown on PDA (five plates per pathogen) at 20°C for two weeks. Each plate was examined microscopically for contamination before preparing spore suspensions. Spores were washed from each plate with 10 ml sterile distilled water (SDW) and filtered through sterile gauze. Spore concentrations were determined with a haemocytometer and concentrations obtained were used undiluted (Table 1). For the inoculation the second leaves from the base of tillers of endophyte-infected (E+) and endophyte-free (E−) plants were collected at random from all pots. Detached, fully expanded leaves were placed on moistened filter paper in Petri dishes (150 mm diam), with four leaves placed in each of four replicate dishes. Leaves were inoculated by spraying the spore suspensions using a pressure sprayer (King spray No 6, Toyo Manufacturing Co., Japan) until small droplets were seen on the leaves. The control was sprayed with SDW. Dishes were kept in a greenhouse (18°C–24°C) with 14 h day of illumination. SDW was added to plates daily to maintain the moist filter paper.

The number of lesions was visually counted daily for one week. Following the visual count, the length of each lesion was measured with a Vernier caliper (Mitutoyo Japan) (Nan and Li 2000). Total length of lesions were calculated by the number of lesions multiplied the mean length of each lesion. Seven days after inoculation, a 1.5 cm long leaf segment was cut from the base of each detached leaf and each segment was placed in 1 ml sterilized water in 1.5 ml Eppendorf tubes. The tubes were vibrated for 5 min on a swirl vibrator (SK-1, Hengfeng Instrument Factory, Jintan, China) to dislodge and suspend spores. Spore concentrations were assessed using a haemocytometer, and expressed as number of spores ml^{-1}.

Inoculation of intact plants and measurement of disease development

Fifty pots of E+ and of E− plants with at least seven tillers were chosen for the experiment and were placed on the same veranda (18–25°C, 10 h light day^{-1}). Each pot was a replicate and there were ten replicates of E+ and of E− per pathogen. Four fungal pathogen species (*A. alternata*, *B. sorokiniana*, *C. lunata* and *F. avenaceum*) and an uninoculated control were used in the experiment. The plants were inoculated as described for detached leaves. The spore concentrations are listed in the Table 1. The control treatment was inoculated with the same amount of SDW. After inoculation, plants were

Table 1 Pathogens and spore concentrations ($\times 10^8$ ml^{-1}) used in the inoculation experiments

Pathogen	Concentration used on detached leaves	Concentration used on intact plants
Alternaria alternata	1.02	2.65
Ascochyta leptospora	6.8	n.t.
Bipolaris sorokiniana	3.5	0.8
Curvularia lunata	0.64	1.6
Fusarium acuminatum	0.36	n.t.
F. avenaceum	4.27	2.39
F. chlamydosporum	1.31	n.t.
F. oxysporum	0.47	n.t.
F. solani	2.6	n.t.
Gliocladium roseum	5.78	n.t.

n.t. not tested

immediately covered with a plastic bag for 24 h to maintain humidity. The number of total leaves and diseased leaves per pot were counted and diseased leaves expressed as percentage of the total number of leaves. The five largest lesions developed on the tillers of each pot were selected and the lesion length measured during the experiment by a Vernier caliper (Mitutoyo Japan). On day 14, the tillers were cut near the base of the plants using scissors and three pots bulked as one replicate, with three replicates per treatment for the determination of enzyme activities. Superoxide dismutase (SOD) and peroxidases (POD) were extracted and measured using the methods described by Šimonovičová et al. (2004).

Statistical analysis

In all experiments, data were analyzed and mean differences determined using the independent-sample *T* test in SPSS (version 13.0, SPSS, Inc., Chicago, USA). The correlations of lesion length between detached and intact leaves taken from both E+ and E− were further analyzed in SPSS and the regression equations calculated for each significantly correlated pair using Excel. The data obtained at 3 to 7 days were used for correlation analyses to make the data comparable between the detached and intact leaves.

Results

Lesion development on detached leaves

All fungal inocula tested were able to cause disease lesions on the leaves. Lesions increased both in size and number as time progressed after inoculation, indicated by the total length of the lesions except for *A. leptospora* which caused minor damage only at day 7 after inoculation (Fig. 1). The endophyte caused inhibitory effects on lesion development. The total lesion lengths on detached E+ leaves caused by each of ten fungal inoculations were all smaller than those on the E− leaves (Fig. 1). However, the endophyte inhibition varied with the pathogens tested. Seven out of the 10 pathogens tested, for example, responded to endophyte infection a few days after inoculation. These fungi included *A. alternata*, *B. sorokiniana*, *F. acuminatum*, *F. avenaceum*, *F. chlamydosporum*, *F. solani* and *Gliocladium roseum*. However the endophyte inhibited lesion development, caused by *C. lunata* and *F. oxysporum* only at two of six sampling days. On the other hand, no significant difference between E+ and E− leaves was observed in length of lesions caused by *A. leptospora* (Fig. 1).

Pathogen spore production on detached leaves of E− plants was greater ($P<0.05$) than on the corresponding E+ plants for eight of the ten fungal pathogens (*A. alternata*, *A. leptospora*, *B. sorokiniana*, *F. acuminatum*, *F. chlamydosporum*, *F. solani*, *F. oxysporum* and *G. roseum*) tested (Fig. 2).

Disease development on intact plants

There were 40–50 leaves in total on the plants in each pot at the end of the trial. Leaves obtained from E+ plants developed fewer diseased leaves than those from E− plants, indicated by the lower percentage of diseased leaves. The inhibitory effects were especially prominent when the plants were inoculated by *B. sorokiniana* and *F. avenaceum*. Significant differences ($P<0.05$) in the percentage of diseased leaves between E+ and E− plants existed at all measuring dates during the experimental period (Fig. 3). The leaves inoculated with *A. alternata* or *C. lunata*, showed significant differences between the two treatments only at certain measurements (Fig. 3).

In general, E+ leaves developed smaller lesions than E− leaves after inoculation although the effects varied with the inocula. Similarly, lesions incited by *B. sorokiniana* and *F. avenaceum*, respectively, on the E+ leaves were significantly smaller ($P<0.05$) than those on the E− leaves after inoculation through the entire experiments. The lesion sizes caused by *A. alternata* and *C. lunata* on E+ leaves were only significantly smaller at some measurements (Fig. 4).

Correlation of lesion length between detached and intact leaves

The length of lesions recorded from detached leaves correlated well with those from intact leaves in some treatments. For the leaves from E+ plants, lesion lengths incited by all four pathogens correlated significantly between the detached and intact leaves except for *F. avenaceum*. However, for E− plants, the correlations in lesion length between the two treatments were significant only for *B. sorokiniana* and *F. avenaceum*. The relationships between the lesion

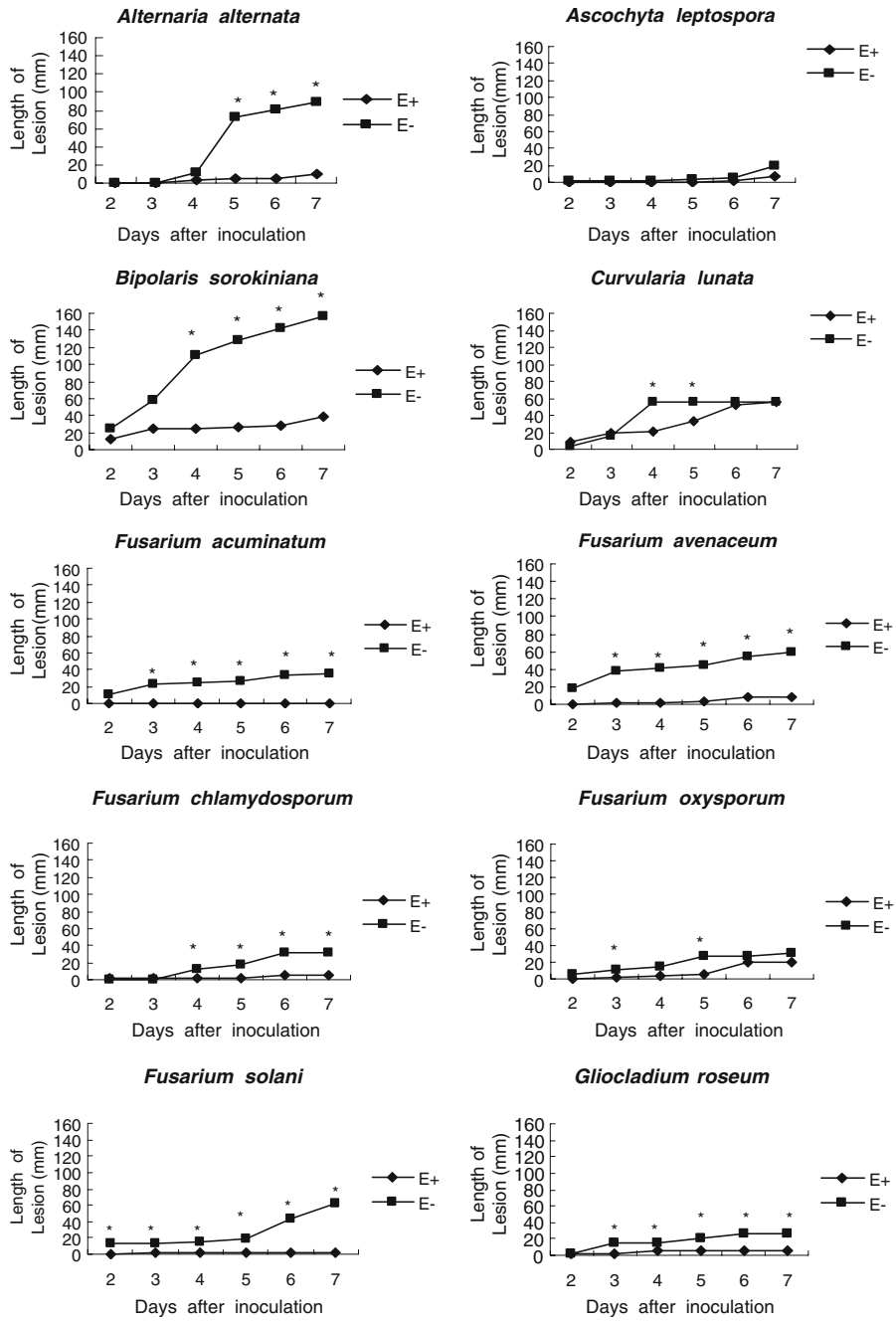

Fig. 1 Total length of lesion developed on detached leaves of E− (solid squares) and E+ (solid diamonds) perennial ryegrass plants inoculated with a range of pathogens, incubated at 18–24°C over 7 days. *significant difference ($P<0.05$) compared to the E− plant using the same data

length obtained from detached and intact leaves were further expressed by regression equation $y = ax + b$ for those that correlated significantly (Table 2).

Activities of SOD and POD enzyme

Leaves obtained from E+ plants had higher SOD enzyme activity than those from E− plants in all treatments. Significant differences ($P<0.05$) were found between the leaves inoculated with *A. alternata*, *C. lunata*, and *F. avenaceum* and the uninoculated control (Fig. 5). A similar trend for POD enzyme activity was observed between E+ and E− plants. However, a significant difference ($P<0.05$) was found only between the leaves inoculated with *C. lunata*, *B. sorokiniana* and the uninoculated control (Fig. 6).

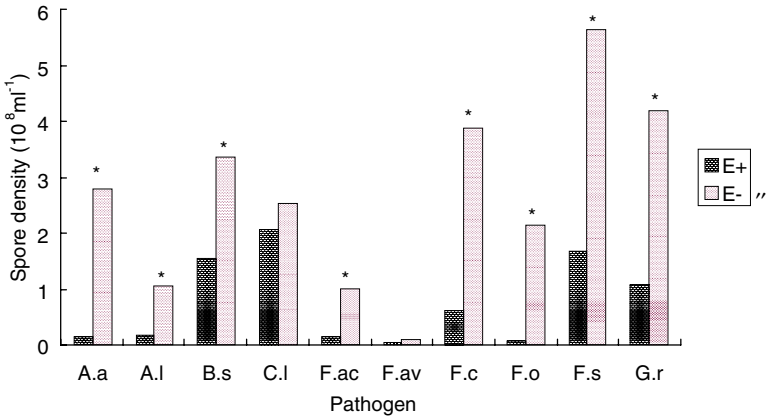

Fig. 2 Number of spores produced on detached leaves of E+ and E− perennial ryegrass plants 7 days after inoculation with a range of pathogens and incubated at 18–24°C. Bars with * were significantly different at $P<0.05$ for the same pathogen. Key: A.a = *Alternaria alternata*, A.l = *Ascochyta leptospora*, B.s = *Bipolaris sorokiniana*, C.l = *Curvularia lunata*, F.ac = *F. acuminatum*, F.av = *Fusarium avenaceum*, F.c = *Fusarium chlamydosporum*, F.o = *Fusarium oxysporum*, F.s = *Fusarium solani*, G.r = *Gliocladium roseum*

Discussion

In our studies, all ten fungi caused lesions on ryegrass detached leaves regardless of endophyte status. The pathogens used are all known to cause disease of ryegrass and some of them are ryegrass pathogens globally (Smith et al. 1989). The current work demonstrated that although endophyte-infected plants remained susceptible to infection, fewer or smaller lesions were more likely to occur than on endophyte-free plants. Pathogenic fungal species tested is one of the main factors affecting the outcome of endophyte–pathogen-host interactions. *Ascochyta leptospora*, for instance, causes diseases of many grasses including ryegrass worldwide (Smith et al. 1989). It has been reported that endophyte infection inhibited development of disease caused by *A. leptospora* on drunk horse grass (*Achnatherium inebrians*) (Li et al. 2007). However, in the current experiments, disease incited by *A. leptospora* on detached leaves of ryegrass developed very slowly and only a few tiny lesions were observed at the end of the trial; this may be the reason why it did not show the endophyte inhibitory effects. These results provide further evidence to support the conclusion that endophyte effects vary with pathogen and host. A previous study with *N. coenophialum* showed effective inhibition of growth of *A. alternata* (White and Cole, 1985); *N. lolii* effectively inhibited *Drechslera andersenii*, *D. siccans* and *D. teres* (Holzmann-Wirth et al. 2000). Nan and Li (2000) showed that detached leaves of E+ *Elymus cylindricus* had fewer and smaller lesions than

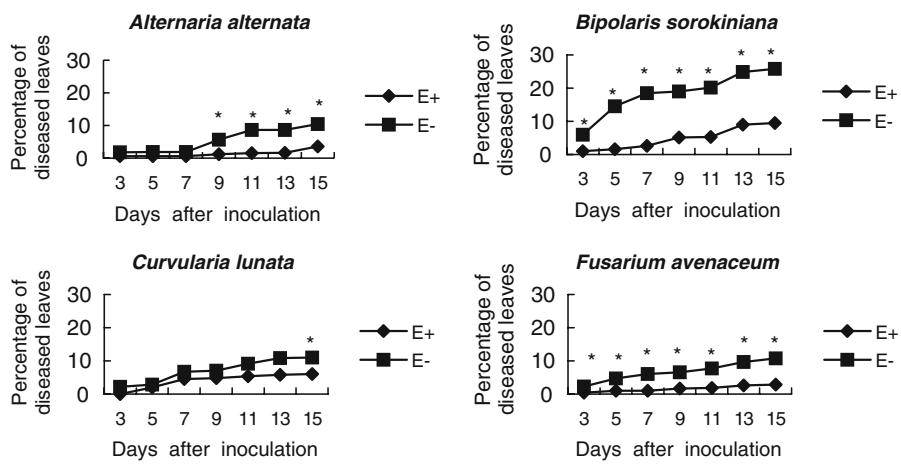

Fig. 3 Percentage diseased leaves on intact E− (solid squares) and E+ (solid diamonds) perennial ryegrass plants 14 days after inoculation with a range of pathogens. *significant difference ($P<0.05$) compared to the E− plant using the same data

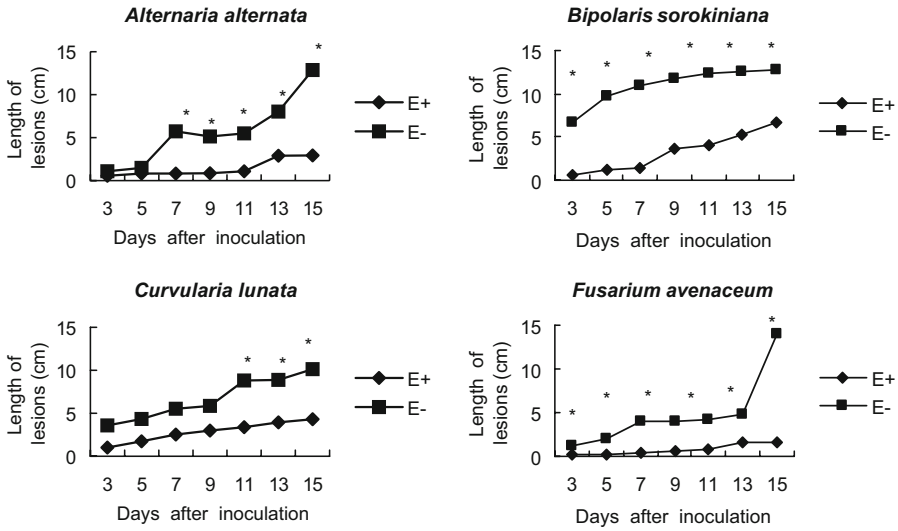

Fig. 4 Mean length of lesions developed on leaves of intact E− (solid squares) and E+ (solid diamonds) perennial ryegrass plants 14 days after inoculation with a range of pathogens. *significant difference ($P<0.05$) compared to the E− plant using the same data

those on E− plants inoculed with *A. alternata*, *F. avenaceum*, *F. culmorum*, *F. equiseti* or *F. oxysporum*. These studies addressed antifungal activities of endophyte *in vitro* or on detached leaves of other grass species.

The results from our pot trial suggest that endophytes can improve the disease resistance of the host. Gwinn and Gavin (1992) showed that E+ seedlings of tall fescue were more resistant to *R. zeae* than E− seedlings in greenhouse tests and Blank et al. (1993) found the endophyte can significantly improve the seedling numbers with the *R. solani* treatment; this was different from results reported by Wäli et al. (2006) and Welty et al. (1991). These different reports show that the mutualistic interactions are complex and labile and influenced by environmental and genetic factors (Faeth and Sullivan 2003).

Results obtained in the field are also inconsistent; Wheatley et al. (2000) found that under field conditions some cultivars of perennial ryegrass free from endophyte had a greater disease incidence than the same cultivars infected with the endophyte. Guy (1992), however, reported that endophyte infection did not influence disease resistance to pathogens in ryegrass. These field experiments showed that the resistance mechanisms of the host and any interactions with endophytes could also be affected by environmental factors such as temperature, moisture and the soil microbiota (Bacon et al. 1997). In the current experiments only laboratory and greenhouse trials have been undertaken. There is a need to carry out similar types of experiments under field conditions so that the role of an endophyte in the disease resistance of ryegrass can be better understood.

The length of lesions recorded from detached leaves correlated well with those from attached leaves in some treatments. For *C. lunata* and *A. alternata* of E−, the correlation was not significant maybe because the lesions on detached leaves developed very fast; the lesions were as long as the leaf 4 days after inoculation, so lesion length did not change from that day and this is the likely reason the attached leaves had no significant correlation with detached leaves. For *F. avenaceum* of E+, the correlation was not significant because there were no lesions on attached leaves 3 to 5 days after inoculation. However, these results show that lesion development was similar on detached leaves and attached leaves and suggest that use of detached leaves could be a quicker and more efficient way to study the grass-microbe interaction under certain situations.

Plants with endophytes appear to have higher levels of defence response (DR) enzymes than those without endophytes, although some treatments were not significantly different. These results show that the endophyte could induce the plant to produce more enzymes under disease pressure. Previous studies reported that perennial ryegrass infected with an endophyte had higher DR enzyme activities (Chen et al. 2001). This paper reports the interaction of endophyte–pathogen-enzyme activities. A common consequence of most abiotic and biotic stresses is that they result in an increased production of reactive oxygen species (ROS) (Polle and Rennenberg 1993).

Table 2 Correlation coefficients and regression equations of lesion length between detached and intact leaves taken from both E+ and E− of perennial ryegrass (N=5)

Pathogen	E+				E−			
	Correlation coefficients	P value	Regression equation	R^2	Correlation coefficients	P value	Regression equation	R^2
Alternaria alternata	0.94	0.017	y=0.0507x+0.5815	0.884	0.803	0.102		
Bipolaris sorokiniana	0.897	0.039	y=0.038x − 0.1045	0.7376	0.918	0.028	y=0.3545x − 10.148	0.8107
Curvularia lunata	0.951	0.013	y=0.0335x+0.5854	0.9037	0.657	0.228		
Fusarium avenaceum	0.860	0.061			0.966	0.008	y=0.2444x − 4.5623	0.9325

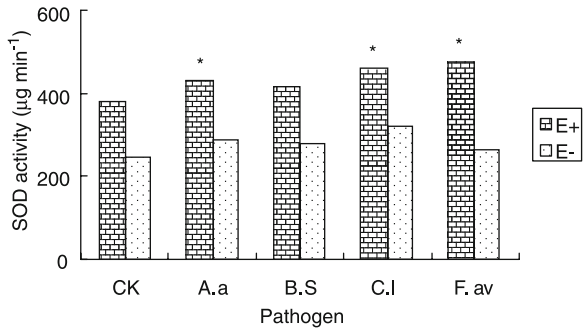

Fig. 5 SOD enzyme activity in E+ and E− perennial ryegrass plants 14 days after inoculation with a range of plant pathogens. Bars with * were difference significantly different at P<0.05 for the same treatment. Key: A.a = *Alternaria alternata*, B.S = *Bipolaris sorokiniana*, C.K = comparison, C.l = *Curvularia lunata*, F.av = *Fusarium avenaceum*

ROS may lead to the unspecific oxidation of proteins and membrane lipids or may cause DNA injury. The control of oxidant levels is achieved by antioxidative systems. These defence systems are composed of metabolites such as ascorbate, glutathione, tocopherol, etc., and enzymatic scavengers of activated oxygen such as superoxide dismutase, peroxidases and catalases (Asada 1999). There are reports of plants adjusting enzyme activities under stress conditions (Zaka et al. 2002). Similar reactions could occur in plants when inoculated with pathogens (Shetty et al. 2001). In the present study, we documented increased ROS-regulating enzymes in endophyte-infected ryegrass. Additional studies are needed to better understand the role of an endophyte in mediating host enzymatic activity in response to fungal pathogens.

The mechanisms by which endophytes improve fungal disease resistance are not clearly defined but

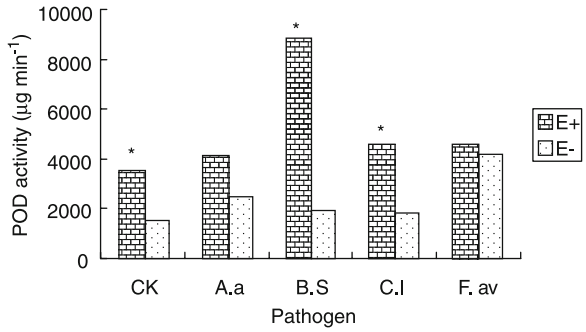

Fig. 6 POD enzyme activity in E+ and E− perennial ryegrass plants inoculated with a range of plant pathogens. Bars with * were significantly different at P<0.05 for the same treatment. Key as in Fig. 5

could be related to the production of antifungal substances (Yue et al. 2000), alterations of host chemistry (West and Gwinn 1993) or induction of plant defence reactions (Schulz and Boyle 2005). The alkaloids produced in endophyte-infected plants, confer benefits such as herbivore resistance and a greater ability to withstand environmental stresses (Bush et al. 1997), and are likely not to be directly associated with fungal pathogen resistance. Holzmann-Wirth et al. (2000) found that the anti-fungal substances were not alkaloids, and Siegel and Latch (1991) found that alkaloids had no anti-fungal effect *in vitro*.

Further investigations are required to clarify the mechanisms by which endophytes can induce resistance to plant pathogens and whether alkaloids have a role in this process.

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References

Asada, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annual Reviews of Plant Physiology and Plant Molecular Biology*, *50*, 601–639.

Bacon, C. W., Richardson, M. D., & White, J. F. (1997). Modification and uses of endophyte-enhanced turfgrasses: a role for molecular technology. *Crop Science*, *37*(5), 1415–1425.

Blank, C. A., Gwinn, K. D., & Gavin, A. M. (1993). Tolerance of tall fescue to soilborne pathogens is influenced by *Acremonium coenophialum*. In D. E. Hume, G. C. M. Latch, & H. S. Easton (Eds.), *Proceedings of the 2nd international symposium on acremonium/grass interactions* (pp. 145–150). New Zealand: AgResearch Grasslands Research Centre, Palmerston North.

Bush, L. P., Wilkinson, H. H., & Schardl, C. L. (1997). Bioprotective alkaloids of grass-fungal endophyte symbioses. *Plant Physiology*, *114*, 1–7.

Chen, S. P., Gao, Y. B., Liang, Y., & Ren, A. Z. (2001). Effects of endophyte infection protective enzyme activities in leaves of *Lolium perenne* under water stress. *Chinese Journal of Applied Environment Biology*, *7*(4), 348–354 (in Chinese with English abstract)..

Christensen, M. J. (1996). Antifungal activity in grasses infected with *Acremonium* and *Epichloë* endophytes. *Australasian Plant Pathology*, *25*(3), 186–191.

Faeth, S. H., & Sullivan, T. J. (2003). Mutualistic asexual endophytes in a native grass are usually parasitic. *The American Naturalist*, *161*, 310–325.

Gwinn, K. D., & Gavin, A. M. (1992). Relation between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. *Plant Disease*, *76*(9), 911–914.

Guy, P. L. (1992). Incidence of *Acreamnium lolii* and lack of correlation with *Barley yellow dwarf virus* in Tasmanian perennial ryegrass pastures. *Plant Pathology*, *41*, 29–34.

Holzmann-Wirth, A., Dapprich, P., Eierdanz, S., Heerz, D., & Paul, V. H. (2000). Anti-fungal substances extracted from *Neothyphodium* endophytes. Proceedings of The 3rd International Conference on Harmful and Beneficial Microorganisms in Grassland, Pasture and Turf. Soest, Germany. pp. 65–69.

Johnston, A., & Booth, C. (1983). *Plant pathologist's pocketbook*, 2nd ed. England: CAB.

Latch, G. C. M. (1993). Physiological interactions of endophytic fungi and their hosts. Biotic stress tolerance imparted to grasses by endophytes. *Agriculture, Ecosystems and Environment*, *44*, 143–156.

Li, C. J., Gao, J. H., & Nan, Z. B. (2007). Interactions of *Neotyphodium gansuense*, *Achnatherum inebrians* and plant-pathogenic fungi. *Mycological Research*, *111*, 1220–1227.

Nan, Z. B. (1996a). Incidence and distribution of endophytic fungi in seeds of some native and introduced grasses in China . *Acta Prataculturae Sinica*, *5*(2), 1–8 (in Chinese with English abstract).

Nan, Z. B. (1996b). Incidence and distribution of endophytic fungi in seeds and plants of some native and introduced grasses in China. *Acta Prataculturae Sinica*, *5*(3), 13–17 (in Chinese with English abstract).

Nan, Z. B., & Li, C. J. (2000). *Neotyphodium* in native grasses in China and observations on endophyte/host interactions. Proceedings of the 4th International *Neotyphodium*/Grass Interactions Symposium, Soest (Germany). pp. 41–50.

Polle, A., & Rennenberg, H. (1993). Significance of antioxidants in plant adaptation to environmental stress. In T. Mansfield, L. Fowden, & F. Stoddard (Eds.), *Plant Adaptation to Environmental Stress* (pp. 263–273). London: Chapman & Hall.

Reed, K. F. M. (1996). Improving the adaptation of perennial ryegrass, tall fescue, phalaris, and cocksfoot for Australia. *New Zealand Journal of Agricultural Research*, *39*, 457–464.

Saikkonen, K., Wäli, P., Helander, M., & Faeth, S. H. (2004). Evolution of endophyte–plant symbioses. *Trends in Plant Science*, *9*, 275–280.

Schmidt, D. (1993). Effects of *Acremonium uncinatum* and a *Phialophora*-like endophyte on vigor, insect and disease resistance of meadow fescue. Proceedings of the Second International Symposium on *Acremonium*/Grass Interactions pp. 185–188.

Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research*, *109*(6), 661–686.

Shetty, H. S., Vasanthi, N. S., Sarosh, B. R., & Kini, K. R. (2001). Inheritance of downy mildew resistance, β-1,3-glucanases and peroxidases in pearl millet [*Pennisetum glaucum* (L.) R. Br.] crosses. *Theoretical and Applied Genetic*, *102*, 1221–1226.

Shimanuki, T., & Sato, T. (1984). Occurrence of the choke disease on timothy caused by *Epichloee typhina* in Hokkaido and location of the endophytic mycelia with

plant tissue. Abstract of Paper. *Phytopathological Society of Japan*, 224.

Siegel, M. R., & Latch, G. C. M. (1991). Expression of antifungal activity in agar culture by isolates of grass endophytes. *Mycologia*, *83*(4), 529–537.

Siegel, M. R., Latch, G. C. M., & Johnson, M. C. (1987). Fungal endophytes of grasses. *Annual Review of Phytopathology*, *25*, 293–315.

Šimonovičová, M., Tamás, L., Huttová, J., & Mistrík, I. (2004). Effect of aluminium on oxidative stress related enzymes activities in barley roots. *Biologia Plantarum*, *48*(2), 261–266.

Smith, J. D., Jackson, N., & Woolhluse, A. R. (1989). *Fungal disease of Turf grasses*. London New York: E & F.N. Spon.

Wäli, P. R., Helander, M., Nissinen, O., & Saikkonen, K. (2006). Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). *Canadian Journal of Botany*, *84*, 1043–1051.

Welty, R. E., Barker, R. E., & Azevedo, M. D. (1991). Reaction of tall fescue infected and noninfected by *Acremonium coenophialum* to *Puccinia graminis* subsp. *graminicola*. *Plant Disease*, *75*, 883–886.

West, C. P., & Gwinn, K. D. (1993). Role of *Acremonium* in drought, pest, and disease tolerances of grasses. Proceedings of the Second International Symposium on *Acremonium*/Grass Interactions pp. 131–140.

Wheatley, W. M., Nicol, H. I., Hunt, E. R., Nikandrow, A., & Cother, N. (2000). An association between a perennial ryegrass endophyte, a leafspot caused by (*Pyrenophora semeniperda*) and preferential grazing by sheep. Proceedings of The 3rd International Conference on Harmful and Beneficial Microorganisams in Grassland, Pasture and Turf. Soest, Germany. pp. 71–74.

White, J. F., & Cole, G. T. (1985). Endophyte–host association in forage grasses: III. *In vitro* inhibition of fungi by *Acreamnium coenophialum*. *Mycologia*, *77*(3), 487–489.

White, J. F., & Cole, G. T. (1986). Endophyte–host association in forage grasses: IV. The endophyte of *Festuca versuta*. *Mycologia*, *78*(1), 102–107.

Yue, Q., Miller, C. J., White, J. F., & Richardson, M. D. (2000). Isolation and characterization of fungal inhibitors from *Epichloë* festucae. *Journal of Agricultural and Food Chemistry*, *48*, 4687–4692.

Zaka, R., Vandecasteele, C. M., & Misset, M. T. (2002). Effects of low chronic doses of ionizing radiation on antioxidant enzymes and G_6PDH activities in *Stipa capillata* (Poaceae). *Journal of Experimental Botany*, *53*(376), 1979–1987.