The Endophyte Neotyphodium coenophialum Affects Root Morphology of Tall Fescue Grown under Phosphorus Deficiency

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Abstract

Mechanisms involved in mineral stress tolerance of cool-season grasses infected by Neotyphodium spp. endophytes are not known. In a controlled-environment experiment, two genotypes (DN2 and DN4) of tall fescue (Festuca arundinacea Schreb.) infected (E+) with their naturally occurring strains of N. coenophialum (Morgan-Jones and Gams) Glenn, Bacon and Hanlin, and their non-infected (E−) isolines were cultivated in nutrient solution at two phosphorus (P) levels of 31 mg P dm−3 (P+) and 0.31 mg P dm−3 (P−) for 3 weeks. Diameters of lateral roots, root hair length, and distance between root hairs were recorded using a digital image analysis system (Dage 72S CCD camera controlled by a Power MacIntosh 7200/120PC compatible computer equipped with an AG-5 frame grabber board and NIH-Image). Irrespective of tall fescue genotype and P level in nutrient solution, E+ plants had roots with a smaller diameter (16 %) than E− plants. In response to P deficiency, root diameter of E+ plants declined by 11 % and root hair length increased by 17 % when compared to E− plants. Altered root diameter and root hair length might be one of the mineral stress tolerance mechanisms in endophyte-infected tall fescue.

Key words: Endophyte — Festuca arundinacea — Neotyphodium coenophialum — phosphorus — root development — tall fescue

Introduction

Cool-season grasses infected with Neotyphodium spp. endophytes often have enhanced resistance to drought and mineral stress (Bacon 1993), greater competitive ability (Marks et al. 1991, Clay 1994, Malinowski et al. 1997b), and altered dry matter partitioning (Belesky and Fedders 1995). Endophyte-infected (E+) grasses also are more resistant than endophyte-free (E−) grasses to biotic stresses (Siegel and Bush 1994); however, the alkaloids that occur in endophyte–grass associations may create problems for livestock (Cross et al. 1995). Development of new endophyte–grass associations designed with low production of alkaloids and sustained high resistance to stresses (Agee and Hill 1994, Adcock et al. 1997) could minimize fescue toxicity to livestock. Expanded understanding of mechanisms involved in abiotic stress physiology of endophyte–grass associations is essential for continued improvement of symbiotic grasses for a range of applications.

Tall fescue (Festuca arundinacea Schreb.) is one of the most important cool-season pasture grasses in the US. About 95 % of tall fescue pastures contain plants infected with the fungal endophyte N. coenophialum (Morgan-Jones and Gams) Glenn, Bacon and Hanlin. This has important ecological and economic consequences for pasture management and livestock production systems. Infection of tall fescue by Neotyphodium spp. endophytes often results in increased root biomass (De Battista et al. 1990b), altered root morphology (Malinowski et al. 1997a,b, 1998c), and altered root–shoot ratio (Knox and Karnok 1992). Endophyte infection also enhances mineral uptake and as a consequence can confer a competitive advantage to infected plants in nutrient-deficient environments (Malinowski et al. 1998c). Responses in root morphology and physiology occur, although the N. coenophialum mycelium colonizes only shoot and not the roots of the tall fescue host (Hinton and Bacon 1985). Reports on endophyte effects on grass root morphology are scarce, partly because of difficulties in collecting and quantifying root response data.

A range of factors, including plant growth regulating substances (Pilet 1996), influence root morphology and growth. The Neotyphodium spp. endo-
phytes produce growth-regulating substances in vitro in the host plant (De Battista et al. 1990a). Mineral stress also modifies root growth and physiology (Marschner 1986). Specific root length of E+ tall fescue was greater than that of E− isolines, and had greater concentrations of P, other minerals, and more herbage mass (DM) when grown with low levels of P (Malinowski et al. 1998c). Roots of E+ tall fescue release greater amounts of reductants, which are probably phenolic compounds, than roots of E− plants (Malinowski et al. 1998a). Phenolic compounds can influence the availability of P in the rhizosphere (Kaškafi et al. 1988). Unlike the physical involvement of fungal hyphae in roots of mycorrhizal plants (Wilcox 1996), root functions in Neotyphodium–grass associations are probably regulated through chemical signals sent or induced by the fungal component of the association.

Our objective was to determine whether P nutrition influenced selected attributes of tall fescue–endophyte association root morphology (Schubert and Mengel 1989), i.e. root diameter, root hair length, and distance between root hairs. Our results suggest endophyte-induced mechanisms that may function to enhance host plant survival under abiotic stresses.

Materials and Methods

Two genotypes of tall fescue (DN2 and DN4), infected with their naturally occurring fungal endophyte N. coenophialum and corresponding non-infected isolines, were selected for this experiment. In their E− status, the two tall fescue genotypes differed in morphological features. Genotype DN2 was an upright-growing type and genotype DN4 was a short-leaf (grazing) type. The origin of both genotypes and differences in chemical features in E+ status are described by Belesky and Fedders (1995) and Malinowski et al. (1998c). Non-infected plants were obtained by elimination of the endophyte with benzyl fungicide (Lath and Christensen 1982) with at least five vegetatively propagated generations between treatment and experimentation. Endophyte status was checked in each plant before the experiment. No endophyte mycelium was detected in roots of E+ plants before or at the end of the experiment. Controlled-environment conditions were maintained at 23/18 °C day/night temperatures, air humidity 75/60 % day/night, with a 14-h photoperiod providing 550–650 μmol photons m−2 s−1 at pot height. Cloned experimental plants were generated from plantlets with 3–4 tillers and similar mass (0.45–0.48 g DM). Plantlets were grown for 2 weeks to ensure adequate production of roots in containers (10 dm3) with full nutrient solution. The nutrient solution consisted of: Ca(NO3)2 1.5 mm; K2HPO4 0.5 mm and MgSO4 0.5 mm. Micronutrients were supplied according to the Long Ashton formula (Hewitt 1966). After the pre-culture period, one group of plants was grown in nutrient solution for 3 weeks at 0.31 mg P dm−2 (P−) and the other group at 31 mg P dm−2 (P+) in a continuous-flow system (Alloush and Sanders 1990). This system provided aeration of nutrient solution under ambient oxygen conditions. Recommended P levels for grasses grown in hydroponics are in the range from 1.55 mg P dm−3 (Lewis et al. 1996) to 15.5 mg P dm−3 (Lewis and Vaughan 1995). Nutrient solutions were renewed every day during preliminary as well as experimental periods. The pH values of both nutrient solutions were adjusted to 6.0 at the beginning of each 24-h growth cycle.

Basal roots were teased from the root mass of a clump of one tall fescue plant per treatment in two replications, arranged using a small paint brush in a glass tray partially filled with distilled water and placed on top of a light box (Aristo model DA-10, Roslyn NY, USA1). A digital image of the entire root was recorded using a Dage 72S CCD (charge coupled device) camera (Dage-MTI Inc., Michigan City, IN, USA) fitted with a Minolta 50/1.7 lens. Image capture was controlled by a Power Macintosh 7200/120PC compatible computer equipped with an AG-5 frame grabber board (Scion Inc., Frederick, MD, USA) and NIH-Image (version 1.60 adapted by Scion, Inc. from public domain software). Primary lateral roots were excised from two zones (arbitrary lengths of 0–15 and 15–30 cm from the root cap of the basal root) to investigate possible effects of endophyte infection and P status in nutrient solution on lateral roots differing in age. Roots were placed in a Petri dish, covered first with a few drops of distilled water and then covered with glass slips. Roots were examined using bright field optics and 4× objective on an inverted microscope (Olympus model IMT-2, Lake Success, NY, USA). A Dage-MTI 72S CCD camera was attached to the side port of the microscope. Images were focused onto the CCD camera using a 2.5× NFK lens. The operation of the CCD camera was controlled using the NIH-Image and the hardware set-up described above. Consecutive digital images, each representing ≈ 2 mm of root length, were captured starting at the root cap. From stored computer images, the length of each root hair in pixels in the focal plane of either the top or bottom edge of each root segment was determined using the freehand line selection tool in the NIH-Image software. Root diameter measurements were made in a similar manner. The number of pixels in 1 mm was determined from images of a micrometer using comparable hardware configurations.

The data for root diameter, root hair length, and distance between the root hairs were subjected to analyses of variance with P level in nutrient solution, genotype, endophyte status and replicate as class variables. Analyses of variance were performed using programs of the SAS statistical package (SAS Institute, Cary, NC, USA). In each treatment, roots were collected from a randomly

1 Trade or proprietary names are used for the convenience of the reader and do not imply endorsement by USDA over comparable products.
Table 1: Analysis of variance summary of phosphorus level in nutrient solution, genotype and endophyte effects on root diameter, root hair length, and distance between root hairs of tall fescue

<table>
<thead>
<tr>
<th>Source</th>
<th>Root diameter</th>
<th>Root hair length</th>
<th>Distance between root hairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Endophyte (E)</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>Distance from root tip (D)</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>G*E</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>G*P</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>E*P</td>
<td>*</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>G*D</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td>E*D</td>
<td>ns</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>P*D</td>
<td>ns</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>G<em>E</em>P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
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<td>G<em>E</em>D</td>
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<td>E<em>P</em>E*D</td>
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</tr>
<tr>
<td>G<em>E</em>P*D</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
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</table>

* Significant at P < 0.05; ns, non-significant.

Fig. 1: Distribution of root diameter of tall fescue roots in response to endophyte infection (E−, non-infected; E+, endophyte-infected) and phosphorus (P) level in nutrient solution (P−, 0.31 mg P dm−1; P+, 31 mg P dm−1), averaged over two genotypes.

Tall fescue genotype, P level in nutrient solution, endophyte status, age of lateral roots, and their interactions determined root hair length (Table 1). Longer root hairs were more frequent in response to P deficiency in nutrient solution and endophyte infection (Fig. 3). Generally, genotype DN2 had longer root hairs (587 μm) than genotype DN4 (365 μm) and longer root hairs (494 μm) were formed by E+ plants when compared to E− plants (454 μm). Also, root hairs formed on older lateral roots were longer (504 μm) than those on younger lateral roots (448 μm). Regardless of lateral root age, genotype DN2 produced longer root hairs when grown in P− compared to P+ nutrient solution, and when endophyte-infected (Table 3). Similarly, lateral roots of E+ DN4 plants formed longer root hairs...
Table 2: Average root diameter of two tall fescue genotypes (DN2 and DN4) in response to endophyte infection (E−, non-infected; E+, infected) and phosphorus nutrition (P−, 0.31 mg P dm−3; P+, 31 mg P dm−3). n = 613

<table>
<thead>
<tr>
<th>Distance from the root tip (cm)</th>
<th>P level</th>
<th>DN2</th>
<th></th>
<th>DN4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E−</td>
<td>E+</td>
<td>E−</td>
<td>E+</td>
</tr>
<tr>
<td>0–15</td>
<td></td>
<td>144.8±1</td>
<td>119.6b</td>
<td>147.4±a</td>
<td>122.1±b</td>
</tr>
<tr>
<td></td>
<td>P−</td>
<td>150.3±a</td>
<td>134.1b</td>
<td>153.2±a</td>
<td>135.1±b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ns2</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>15–30</td>
<td>P−</td>
<td>161.1±a</td>
<td>128.4b</td>
<td>146.0±a</td>
<td>116.7b</td>
</tr>
<tr>
<td></td>
<td>P+</td>
<td>147.8±a</td>
<td>127.6b</td>
<td>152.7±a</td>
<td>149.6a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ns</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

1 Means for endophyte status within a tall fescue genotype. Within rows, means followed by the same letter are not significantly different (P > 0.05).
2 Means for P level within an endophyte status (columns). *, significant at P < 0.05; ns, non-significant.

Fig. 2: Averaged root diameter of E− and E+ tall fescue (pooled for two genotypes) in response to P level in nutrient solution. Bars indicate standard errors (n = 613)

than roots of E− plant. The influence of P was not as clear. A significant interaction between endophyte status and P level solution showed that endophyte did not affect root hair length of plants grown in P+ nutrient solution but caused a 10% increase in root hair length when plants were grown in P− nutrient solution (Fig. 4).

The distance between root hairs on lateral roots was affected by interactions of genotype, endophyte status and P level in nutrient solution with root age (Table 1). Generally, the distance between root hairs of genotype DN2 (117 μm) was less than that for genotype DN4 (130 μm) on younger lateral roots. The distance between root hairs on younger lateral roots of plants grown in P− conditions was 10% less when compared to roots grown in P+ nutrient solution. In contrast, the distance between root hairs on older lateral roots was 11% greater in P− than in P+ nutrient solution. The distance between root hairs was also influenced by a significant interaction between endophyte status and the age of lateral roots (Table 1). The distance between root hairs of E+ plants was 7% greater than that of E− plants on younger lateral roots (Fig. 5). On the older lateral roots, in contrast, the distance between root hairs of E+ plants was 7% less than that of E− plants.
Table 2: Average root hair length of two tall fescue genotypes (DN1 and DN3) in response to endophyte infection (E−, non-infected; E+, infected) and phosphorus nutrition (P−, 0.31 mg P dm⁻¹; P+, 31 mg P dm⁻¹). n = 6308

<table>
<thead>
<tr>
<th>Distance from the root tip (cm)</th>
<th>P level</th>
<th>DN2</th>
<th>DN4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E−</td>
<td>E+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E−</td>
<td>E+</td>
</tr>
<tr>
<td>0–15</td>
<td>P−</td>
<td>563.1b¹</td>
<td>621.7a</td>
</tr>
<tr>
<td></td>
<td>P+</td>
<td>463.2b</td>
<td>548.6a</td>
</tr>
<tr>
<td>15–30</td>
<td>P−</td>
<td>509.7b</td>
<td>568.7a</td>
</tr>
<tr>
<td></td>
<td>P+</td>
<td>352.1b</td>
<td>437.5a</td>
</tr>
</tbody>
</table>

¹Means for endophyte status within a tall fescue genotype. Within rows, means followed by the same letter are not significantly different (P > 0.05).

²Means for P level within an endophyte status (columns). *, significant at P < 0.05; ns, non-significant.

Fig. 3: Averaged root hair length of E− and E+ tall fescue (pooled for two genotypes) in response to P level in nutrient solution. Bars indicate standard errors (n = 2001)

Discussion

Endophyte-infected tall fescue plants, in contrast to their E− isolines, responded to P deficiency in nutrient solution with reduced root diameter and considerable increase in root hair length. To our knowledge, this is the first report of *N. coenophialum* endophyte-induced changes in root morphology of tall fescue related to P deficiency. In many plant species, root diameter, root hair length, and the density of root hair formation are strongly affected by mineral nutrient supply, especially phosphorus (Bhat and Nye 1973, Itoh and Barber 1983, Marschner 1986). Root hairs are often absent or rudimentary when plants are grown at high P concentrations, whereas at low P concentrations long root hairs are formed (Foehse and Jungk 1983).

These adaptive mechanisms lead to an increased surface area of roots in response to mineral stress (Foehse and Jungk 1983). Apparently, decreasing root diameter and increasing root hair length would increase root surface area of tall fescue under P deficiency conditions. Morphological adaptations were more pronounced in E+ plants than in E− plants, suggesting an endophyte-related response.

Although *Neotyphodium* spp. effects on root DM and root:shoot ratio of symbiotic *Festuca* spp. and *Lolium perenne* L. are known (Latch et al. 1985, De Battista et al. 1990b, Malinowski et al. 1997a,b), the influence of endophytes on the root morphology...
of cool-season grasses remains obscure. Endophyte effects on DM production in tall fescue genotypes DN2 and DN4 were previously investigated by Belesky and Fedders (1995). Under a high level of readily available P in the soil (one-time P fertilization of 800 mg P kg$^{-1}$ soil as KH$_2$PO$_4$), root and shoot DM production were significantly increased in response to endophyte infection (11 and 13 %, respectively), whereas root–shoot ratio was not. Malinowski et al. (1998b) re-examined growth of the same tall fescue genotypes under sparingly available P in the soil (one-time P application of 100 mg P kg$^{-1}$ soil as phosphate rock). Endophyte-infected plants responded with increasing root DM (10 %), but P uptake rate was greater (24 %) only in DN2 E+ plants when compared to E− plants. Shoot DM and root–shoot ratio were not significantly influenced by endophyte infection. Malinowski et al. (1998c) analysed growth of tall fescue genotypes DN2 and DN4 in relation to endophyte infection at different levels of readily available soil P. They found that specific root length (SRL) of E+ plants was increased considerably when compared to that of E− plants at low (17 mg P kg$^{-1}$ soil) and medium (50 mg P kg$^{-1}$ soil) P levels (16 and 28 %, respectively). Endophyte infection was inconsequential in terms of SRL at high (96 mg P kg$^{-1}$ soil) P level. Although root diameter was not measured in that experiment, a greater SRL suggested that roots of E+ plants were finer (i.e. had a smaller diameter) than roots of E− plants under lower soil P levels. Apparently, the presence of the endophyte *N. coenophialum* in shoots can induce a mechanism that causes an increased allocation of photosynthate to roots and might affect mineral, and quite possibly water, acquisition by increasing root surface area. Our results may, in part, explain enhanced growth and mineral acquisition in roots of soil-grown E+ tall fescue under P-limiting conditions.

In P− nutrient solution, root hair elongation was greater on older lateral roots of E− plants when compared to younger lateral roots. In contrast, E+ plants formed longer root hairs than E− plants on both younger and older lateral roots. This suggests that, under limited P supply, root hair elongation may somehow be promoted in E+ plants to a greater extent than in E− plants, especially on younger lateral roots. Formation and growth of root hairs are highly influenced by auxins (Thien et al. 1979). *Neotyphodium* spp. endophytes and the closely related endophyte *Balansia epichloë* Weese can produce auxin *in vitro* (Porter et al. 1985, De Battista et al. 1990a). This may influence production and balance of growth-regulating substances in the host plant. There are no reports of endophyte influence on auxin production in grasses *in vivo*.

Our results suggest that infection of tall fescue with the endophyte *N. coenophialum* can alter root morphology. Modifications in root morphology are a possible mechanism for greater tolerance to P deficiency or related mineral stresses in E+ tall fescue. Modifications occur as a reduction in root diameter and an increase in root hair length in E+ plants when compared to E− plants at low P concentration in nutrient solution. These changes in root morphology may affect surface area for mineral acquisition. Altered root morphology of tall fescue caused by endophytes, however, might not be the only mechanism available to overcome P deficiency stress. Malinowski et al. (1998a) showed increased reducing activity of root exudates of E+ plants when compared to E− plants grown under P-deficient conditions. Thus, the influence of *N. coenophialum* on tall fescue under mineral stress conditions may be multifaceted, involving not only morphological modifications but physiological changes as well. Since *Neotyphodium* spp. endophytes are not present in roots, the changes might be caused by a chemical signalling system in the endophyte–grass association. This hypothesis, however, requires further study and warrants examination under field conditions at the population level.

Zusammenfassung

*Neotyphodium coenophialum*-Endophyten beeinträchtigen die Wurzelmorphologie von Rohrschwingel unter Phosphormangel

Rohrschwingelgenotyp und der P-Konzentration in der Nährösung wiesen E-+P-Pflanzen Wurzeln mit einem geringeren Durchmesser (16%) als E-+P-Pflanzen auf. Als Reaktion auf P-Mangel nahm der Wurzeldurchmesser von E-+P-Pflanzen um 11% ab und die Länge der Wurzelaare um 17% im Vergleich zu E--+P-Pflanzen zu. Eine Änderung der Wurzeldurchmesser und der Länge der Wurzelhaare könnte ein Mechanismus größerer Toleranz gegenüber Mineralstoff bei von Endophyten infizierten Rohrschwingel-Pflanzen sein.

Acknowledgements
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