Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants

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Summary

1. Plants can host microbial communities whose integrated functions are often responsible for their success. Understanding mechanisms regulating such functions is thus a major goal in ecology.

2. Fungal endophytes of grasses, particularly of the genus *Neotyphodium*, have been reported to reduce colonization of their host plant by arbuscular mycorrhizal (AM) fungi. However, it is unclear which mechanism(s) may explain the effects produced by the endophyte and whether these effects are present in nature.

3. We used *Schedonorus phoenix* (syn. *Lolium arundinaceum*) plants that were endophyte-free or infected with one of two strains of *Neotyphodium coenophialum* known to produce different putative allelochemicals to test the hypotheses that (i) allelopathic effects of the endophyte reduce AM fungal spore germination; and (ii) the allelochemical compound(s) are leached into the soil even after the death of *S. phoenix*, where they reduce AM fungal colonization of other plants.

4. In a first experiment, aqueous extracts from the shoots of *S. phoenix* were applied onto spores of the AM fungus *Glomus intraradices* to test germination effects. Both endophyte strains reduced spore germination by approximately 10% relative to endophyte-free controls.

5. In a second experiment, we placed dried shoot material ('thatch') on the soil surface of pots containing *Bromus inermis*, which were either inoculated with *G. intraradices* or not. We watered the plants through the thatch, relying upon leaching to translocate potential allelochemicals to the soil. AM fungal colonization of *B. inermis* was significantly reduced when thatch was infected with the common strain, but not with AR542, compared to the endophyte-free thatch. Furthermore, the arbuscule : vesicle ratio was 11-fold smaller when thatch was infected with the common strain compared to endophyte-free thatch, suggesting that *G. intraradices* was stressed by the presence of common strain-leachate.

6. We observed situations whereby two ecologically widespread plant-microbe symbioses interact. Potential mechanisms may include allelopathic effects, although other factors are also possible, and leaching is a mode of entry of putative endophyte-induced AM fungal inhibitors in soil. Understanding these processes is important as they affect AM fungal communities which contribute to plant success and, consequentially, grassland ecosystem dynamics.

Key-words: arbuscular mycorrhizal fungi, allelopathy, AMF, *Schedonorus phoenix*, *Neotyphodium*, endophyte, spore germination

Introduction

Endophytic fungi from the genus *Neotyphodium*, formerly *Acremonium* (Glenn *et al*. 1996), infect a variety of cool season grasses (Schardl *et al*. 1997). Although derived from the sexually reproducing pathogenic *Epichloë* (Clay 1990), *Neotyphodium* species lack a sexual phase, and do not produce spores (however, see White, Martin & Cabral 1996). They have no free-living stage and are thus 'trapped' in their host plant, never moving to uninfected hosts (i.e. there is no horizontal transmission). It has been argued that such an intimate relationship should select for evolution away from

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parasitism and toward mutualism (Ewald 1987). Nevertheless, the exact nature of the relationship between the grass and its endophytic fungus has been hotly debated in the literature (Faeth 2002; Saikkonen *et al.* 2004; Faeth & Hamilton 2006).

Endophytic fungal hyphae occur in all above ground plant tissues, although hyphal density tends to be greatest in the stem and crown (White *et al.* 2003). Until recently, it was thought that the endophyte never occurs in the plant's roots (e.g. Schardl, Leuchtmann & Spiering 2004). However, Christensen & Voisey (2007) using GFP-expressing endophytes, showed that hyphae are often present in the tips of roots that commence development within the true stem (see also Azevedo & Welty 1995).

Two commonly studied *Neotyphodium* species are *N. lolii* (Latch, Christensen & Samuels) Glenn, Bacon & Hanlin, which infects *Lolium perenne* L. (perennial ryegrass), and *Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon & Hanlin, which infects *Schedonorus phoenix* (Scop.) Holub (tall fescue), formerly known as *Lolium arundinaceum* (Schreb.) Darbysh. The relationship between these pairs of interacting species has frequently been described as a mutualism. The fungi benefit by obtaining nutrients from the host plant. The grasses benefit in several ways. Faeth *et al.* (2002) summarize the benefits as follows: (i) enhanced resistance of seeds and seedlings to predators and pathogens; (ii) reduced herbivory of mature plants; (iii) increased drought tolerance; (iv) increased resistance to other abiotic factors such as fire; and (v) increased intra- and interspecific competitive abilities of the host plant. While the mechanisms are not all known for each of these benefits, certainly some of them derive, at least in part, from the production of secondary metabolites, *in planta*, by the grass and fungus together (but see Rasmussen *et al.* 2007, 2008 for additional details on the biochemical implications of the grass-endophyte relationship).

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with the roots of a wide variety of plant species, including a variety of grass species (Smith & Read 1997). In exchange for photosynthates, AM fungi can provide their host plants with increased access to nutrients and water and enhanced protection against pathogens (Newsham, Fitter & Watkinson 1995). Similarly to *Neotyphodium* species, AM fungi are obligate symbionts, fully reliant on their plant hosts for fixed carbon.

It has been known since the early 1990s that endophytes can affect AM fungi in the soil. These studies can be divided into two types: those that study the effects of the endophyte on AM fungal colonization of the endophyte's host plant; and those that study the effect of the endophyte on AM fungal colonization of non-endophytic plants or of AM fungal abundance in the soil in general. We will refer to the former studies as 'direct effects' of the endophyte, and the latter as 'indirect effects' of the endophyte.

A number of studies have examined the direct effects of endophyte-infection on AM fungal colonization. Several host plant – endophyte combinations were investigated using a variety of AM fungal species, primarily from the *Glomus*

genus. In general, these studies show that AM fungal root colonization is reduced when the host plant is infected with the endophyte (e.g. Chu-Chou *et al.* 1992; Guo *et al.* 1992; Omacini *et al.* 2006; Mack & Rudgers 2008). Nevertheless, Novas, Cabral & Godeas (2005) observed an increase in colonization in their study. While the possible mechanism(s) by which the endophyte reduces colonization have not been investigated, the general assumption seems to be that endophyte-produced allelochemicals are likely responsible for the effect. Studies showing negative effects are from agronomic cultivars and the only study involving a native grass in its native habitat showed positive effects (Novas *et al.* 2005).

Indirect effects have been hypothesized based on the results from experiments on the direct effects of endophytes on AM fungi, and on the basis of the few studies that have specifically investigated indirect effects (Chu-Chou *et al.* 1992; Guo *et al.* 1993; Matthews & Clay 2001). In general, the idea is that endophytes alter the AM fungal community, either via allelopathic effects of the endophyte, or via the effect of the endophyte on the plant community and hence on the AM fungal community that might subsequently develop. In addition to studying the direct effects of *N. coenophialum* on AM fungal colonization, Chu-Chou *et al.* (1992) also reported that propagule densities of total *Glomus* spp. (i.e. all species combined), and specifically of *G. fecundisporum* and *G. monosporum*, were smaller in soil from endophyte-infected *S. phoenix* plots than from endophyte-free plots. In contrast, even though Matthews & Clay (2001) did not look at AM fungi directly, their data provide no evidence that endophyte induced changes in AM fungal communities directly affect the growth of subsequent plants over a large spatial scale.

Allelopathy is one possible mechanism by which endophyte-infected plants may exhibit reduced AM fungal colonization. Such potential effects have been reported several times on plant species (Preece *et al.* 1991; Malinowski, Belesky & Fedders 1999; Bertin *et al.* 2003; Orr, Rudgers & Clay 2005), but only rarely on AM fungal species (see above). While these previous studies demonstrate the potential for endophytes to produce allelopathic effects, they do not isolate the putative compounds involved, nor do they have high external validity. That is, these experiments show the potential for allelopathic effects, but are largely divorced from an appropriate ecological context, so it is unclear whether such effects are present in nature. Furthermore, there is a natural tendency to assume that alkaloids are the putative allelochemicals, for both plants and AM fungi. However, as Rasmussen *et al.* (2007, 2008) recently showed, endophyte-infected and endophyte-free grasses differ in the concentrations of many biochemicals, not just alkaloids. It is entirely possible that other compounds could be involved, in addition to, or instead of alkaloids.

Both Orr *et al.* (2005) and Preece *et al.* (1991) point out that the method of extraction of allelopathic compounds strongly influences the reported effects and interpretations of endophyte effects. Some of these methods are clearly not ecologically relevant. However, there are at least three ecologically relevant mechanisms by which putative allelopathic compounds

might enter the soil and influence AM fungi. Koulman *et al.* (2007) demonstrated that alkaloids are at least sometimes mobilized and translocated in *S. phoenix* and *L. perenne*. They were able to detect alkaloids in both the guttation fluid and 'cut leaf fluid' (i.e. the fluid that flows out of tissue damaged by, for example, grazing mammals) of endophyte-infected *L. perenne*. Another possible pathway is via root exudation. Although these exudates have not, to our knowledge, been tested for alkaloid presence, Koulman *et al.*'s demonstration that alkaloids can be mobilized and translocated makes this route a viable possibility. Finally, potentially allelopathic compounds can enter the soil via the processes of leaching and decomposition, but little is known about these pathways' potential role in the reported allelopathic effects of endophyte infection.

In this study, we use two strains of *N. coenophialum* (common and AR542) infecting the same cultivar of *S. phoenix* and known to produce different profiles of putative allelochemicals (i.e. alkaloids) to test the hypotheses that (i) allelopathic effects of the endophyte reduce AM fungal spore germination; and (ii) the allelochemical compound(s) are leached into the soil even after the death of *S. phoenix*, where they reduce AM fungal colonization of other plants.

Methods

AM FUNGAL SPORE GERMINATION EXPERIMENT

To test allelopathic effects of the endophyte *N. coenophialum* on spore germination, we created aqueous extracts from the shoot material of three lines of cv. Georgia 5 *S. phoenix*. These lines were either endophyte-free (E–), infected with the commonly occurring strain of the endophyte (E+), or infected with the AR542 strain (AR542; see Hunt & Newman 2005 for details). The endophyte-status of these plants was confirmed by microscopy (Hignight, Muilenburg & Van Wijk 1993), prior to the start of shoot collection. For each endophyte-infection line, 12 g of fresh shoot material was combined with 75 mL of de-ionized water and macerated vigorously for 30 s in a Black & Decker Diecast Blender. The resulting mixture was vacuum filtered through a Whatman #1 filter and stored in a –20 °C freezer for 2 weeks prior to use.

Glomus intraradices Schenck & Smith spores (Premier Tech, Biotechnologies, Rivière-du-Loup, Quebec, Canada, IRBV'95) of identical age were removed from a Ri T-DNA carrot root organ culture (Becard & Fortin 1988) by dissolving the medium with sterilized 0·01 M citrate buffer (see Cranenbrouck *et al.* 2005). Spore clusters were transferred to sterilized water, separated with a scalpel and were then stored at 4 °C 1 day prior to the start of the experiment.

Fifty millimetre Petri dishes were half-filled with silica sand, which was then saturated with the aqueous extracts from one of the three endophyte treatments (E–, E+ and AR542) or with de-ionized water as a control (H_2O). We cut small discs of nitrous cellulose using a single hole paper punch. Each Petri dish received 20 discs evenly distributed across the sandy surface, and a single *G. intraradices* spore was placed on each disc. Ten replicates were prepared for each of the three endophyte-conditions and seven replicates for the water control treatment. The Petri dishes were sealed with parafilm and incubated at 26 °C for 5 days. Following the 5 days, the spores were stained with 0·05% (w/v) trypan blue and examined under a 40×

microscope for evidence of hyphal growth. Previous trials indicated that a spore is dead if germination does not occur within 48 h under the conditions provided.

BROMUS INERMIS COLONIZATION EXPERIMENT

To test the effects of *S. phoenix* thatch on AM fungal colonization of *Bromus inermis* Leyss. (smooth brome), an experiment was conducted in a glasshouse between August and December of 2006 under ambient light conditions, 24·7 : 18·2 °C mean day: night temperatures. The choice of *B. inermis* was based on previous reports suggesting that this plant is mycorrhizal dependent (e.g. Klironomos, McCune & Moutoglis 2004; Klironomos *et al.* 2005). Furthermore, *B. inermis* is a common grass in pastures and grasslands where it often co-occurs with *S. phoenix* (e.g. Riesterer *et al.* 2000).

A fine sandy loam soil was collected on 3 May 2006, from the top 20 cm of an arable field on a farm near Belwood (43°45′N, 80°15′W), Ontario, Canada. The soil was broken up mechanically and passed through a 4 mm sieve before use. To eliminate the native AM fungal community, the soil was pasteurized by gradually raising its temperature to 90 °C over a period of 60 min in an electric unit, and then cooling it gradually (McGonigle & Miller 1996). Soil pasteurization was repeated twice. Soil samples (*n* = 3) analysed before and after pasteurization contained, respectively: $12 ± 0·6$ and $0·7 ± 0·20$ mg NO_3^--N kg^{-1}; $2 ± 0·3$ and $24 ± 0·4$ mg NH_4^--N kg^{-1}; $35 ± 1·2$ and $28 ± 0·9$ mg $NaHCO_3^-$ extractable P kg^{-1}; $220 ± 4·9$ and $199 ± 1·2$ mg $CH_3COONH_4^-$ extractable K kg^{-1}; $255 ± 5·2$ and $244 ± 4·1$ mg $CH_3COONH_4^-$ extractable mg kg^{-1}; 7 and 7·3 pH (1 : 1 in water).

Experimental units were arranged in a fully randomized manner using a $5 × 2$ factorial design where one factor was *S. phoenix* 'thatch' (E–, no endophyte; AR542-infected; E+, common strain endophyte-infected; LT, leached 'thatch'; and NT, no thatch), the second factor was AM inoculation (inoculated, and AM fungal-free). Each treatment combination was repeated 10 times (i.e. for overall total of 100 experimental units). AM treatments were prepared by packing 1·5-L pots with substrate to a bulk density of approximately 1·3 g cm^{-3}, which is consistent with the bulk densities commonly observed in agricultural soils (Dam *et al.* 2005). The substrate in each pot was arranged from the bottom-up with 580 g of pasteurized soil, 10 g of AM inoculant, which was sterilized by autoclaving at 121 °C for 30 min for the AM fungal-free treatment, and a top layer of 910 g of pasteurized soil. The amount of AM inoculant (MYKE® PRO SG2; Premier Tech) added to each pot was calculated based on a rate of approximately 7·5 kg ha^{-1}, as recommended by the producer. To correct for differences in non-mycorrhizal microbial communities natural to the soil and eventually present in the inoculant, each experimental unit received a 5 mL filtered washing comprised of extract from a mixture of non-pasteurized soil and AM inoculum (Ames, Mihara & Bethlenfalvay 1987).

Five *B. inermis* seeds were surface-sterilized (50% ethanol for 5 min), rinsed with de-ionized water, and sown in each pot. Plants were thinned to two plants per pot after germination. *Schedonorus phoenix* dried 'thatch', cut to a 4 cm length, was placed onto the surface of each appropriate pot. Thatch was prepared by bulking, within an endophyte treatment, the shoot material, which was harvested after approximately 6 weeks of re-growth from approximately 40 plants of each endophyte status. The endophyte-status of these plants was confirmed as described earlier. The bulked samples were dried at 70 °C for 72 h. For the leached treatment, a 1 : 1 mixture of common strain-infected and AR542-infected dried material was placed in 1000 mL Erlenmeyer flasks, saturated with tap water and

the flasks placed in a shaker bath at 22 °C. The water was removed from the flasks every 24 h and replaced with fresh water for a total of 96 h of leaching. This leached thatch was again dried for 3 days at 70 °C. Thatch was replaced by new thatch after 2 months (half way through the experiment). The pots were watered to 'field capacity', and then maintained at that moisture content by watering with de-ionized water every 2 days.

Four months after the experimental set-up, all shoots were excised, dried at 65 °C for 48 h, and weighed. Root systems were carefully washed to remove the soil, and a 2 cm mid-segment of the root system stained (Brundrett, Piche & Peterson 1984), before being examined for AM colonization (Mcgonigle *et al.* 1990). This technique enables the quantification of arbuscular, vesicular and hyphal colonization separately. The arbuscular : vesicular colonization ratio may be useful to assess stress responses because arbuscules are considered the primary sites of nutrient exchange between symbionts (i.e. a sign of vitality), while vesicles are energy reserve structures, whose production may be enhanced in response to unfavourable conditions (see Smith & Read 1997).

STATISTICAL ANALYSES

All data were Box–Cox transformed to stabilize the residual variance (Box & Cox 1964). An examination of the residuals after each transformation suggested that all the assumptions of the ANOVA were met. Unless stated otherwise, we used Tukey's honest significant difference test to assist in the interpretation of significant effects (Miller 1981). Although the statistics refer to the transformed data, the untransformed data were used to construct plots of the treatment effects. In all cases, standard error of the mean was used as a measure of data dispersion. Statistical analyses were performed using JMP version 7·0 (2007, SAS Institute Inc., Cary, NC, USA).

Results

AMF SPORE GERMINATION EXPERIMENT

The germination of *G. intraradices* spores in Petri dishes was affected by the aqueous extracts ($F_{3,33} = 3·38$, $P < 0·03$) (Fig. 1). The presence of an endophyte reduced spore germination
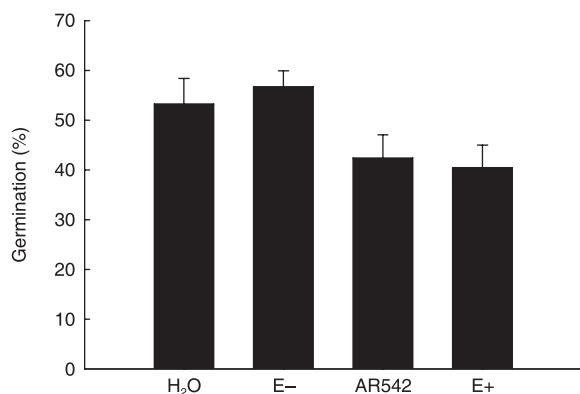
Fig. 1. Effect of aqueous extracts prepared from water only (H₂O) endophyte-free (E−), AR542 or common endophyte (E+) infected *S. phoenix* plants on *G. intraradices* spore germination. Bars represent the mean ($n = 7$ for H₂O and $n = 10$ per each of the other treatments) ± 1 SE; bars followed by the same letter are not significantly different ($P < 0.05$).

success by approximately 10% relative to endophyte-free treatments, as supported by a Least Square Means contrast within the endophyte effect ($F_{1,33} = 9·23$, $P < 0·005$).

BROMUS INERMIS COLONIZATION EXPERIMENT

Bromus inermis biomass was unaffected by the presence of AM fungi ($F_{1,90} = 0·86$, $P < 0·36$). However, *B. inermis* produced more biomass when thatch was present, regardless of the thatch's endophyte status ($F_{4,90} = 84·2$, $P < 0·0001$). Thatch had a 'fertilization effect' on the growth of the host plant (Fig. 2a). The thatch by AM fungal treatment interaction was not significant ($F_{4,90} = 1·18$, $P < 0·33$).

Arbuscular AM colonization was strongly influenced by the thatch treatment ($F_{4,45} = 9·68$, $P < 0·0001$) (total AM fungal colonization data showed identical trends, ($F_{4,45} = 4·44$,
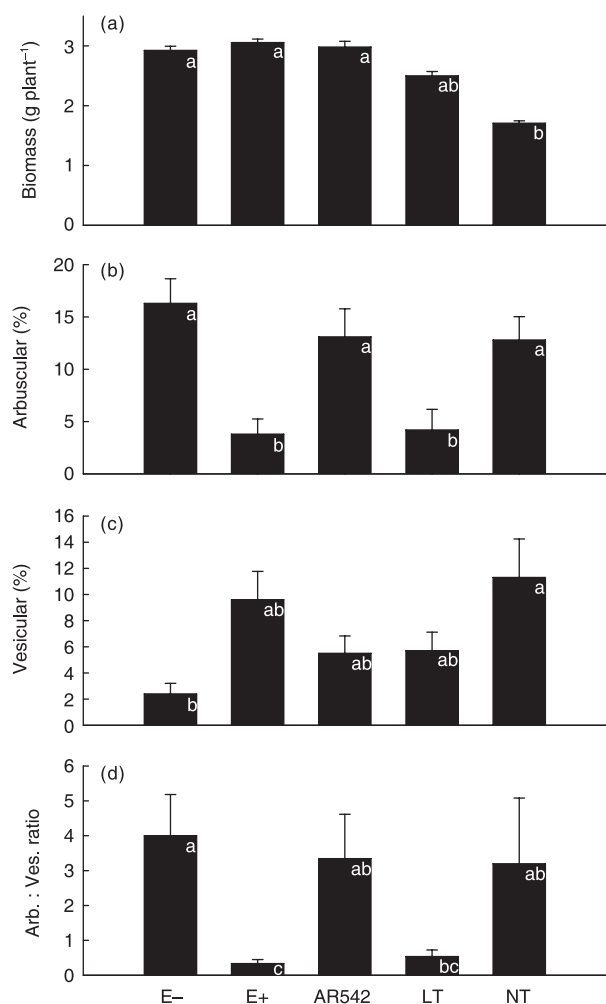
Fig. 2. Effect of *S. phoenix* thatch treatment (endophyte-free, E−, infected by the common endophyte, E+, or AR542 strains, leached thatch, LT, and no thatch, NT) on a) shoot biomass, b) arbuscular and c) vesicular AM fungal root colonization by *G. intraradices* and d) arbucular to vesicular colonization ratio of *B. inermis*. Bars represent the mean ($n = 20$ per treatment for plot a) and $n = 10$ for all other plots) ± 1 SE; bars in each plot followed by the same letter are not significantly different ($P < 0.05$).

$P < 0.004$); therefore, not shown) as was the vesicular colonization ($F_{4,45} = 3.16$, $P < 0.023$) and the arbuscular : vesicular colonization ratio ($F_{4,45} = 5.26$, $P < 0.002$) (Fig. 2b,c and d). Thatch infected with the common endophyte strain resulted in a reduction in arbuscular colonization and an increase in vesicular colonization, greatly reducing the arbuscular : vesicular colonization ratio compared to plants grown with thatch that was either uninfected, or infected with AR542. In general AM fungal colonization for plants grown with the leached thatch was similar to that of plants grown with thatch infected with the common strain of the endophyte.

Discussion

We identified a mode of entry into soil of chemicals possibly associated with reductions in AM fungal colonization. This was done in an ecological context, using a plant that co-exists with *S. phoenix* and soil that was supplied with its native microbial community. Moreover, given that the common strain of *N. coenophialum* is known to produce different putative allelochemicals (i.e. alkaloids) than AR542, specific compounds may potentially be responsible for those reductions. Ergovaline, peramine, *N*-formyl loline, *N*-acetyl loline and *N*-acetyl norloline are produced by the common endophyte, while AR542-infected plants produce only peramine and *N*-acetyl norloline (see Hunt & Newman 2005 for more details). Therefore, the fact that AR542-infected thatch did not affect the symbiosis suggests that ergovaline, *N*-formyl loline, *N*-acetyl loline, or some combination of these alkaloids could potentially be the cause of allelopathy.

Although the current results are consistent with the hypothesis that one or more of the alkaloids that are absent from AR542 are responsible for the reduced AM colonization, reasonable alternative hypotheses exist. First, Rasmussen *et al.* (2007) have shown that different strains of endophyte achieve different fungal densities when grown in hosts from the same genetic background (cultivar). They also showed that alkaloid concentration is linearly related to fungal concentration. Mack & Rudgers (2008) showed that AM fungal colonization was linearly correlated with endophyte hyphal density. Taken together, these results suggest the hypothesis that AR542 does not produce as dense a population of hyphae as the common strain of the endophyte, and as a result does not produce sufficient *quantities* of alkaloids to impact the AM fungal colonization. A second reasonable alternative hypothesis is that alkaloids have nothing to do with the reduction in AM colonization. Rasmussen *et al.* (2007, 2008) have shown that, within the same cultivar of ryegrass, plants infected with different strains of the endophyte were biochemically very different from each other, beyond differences in alkaloid production. This suggests that the difference between AR542 and common strain infected thatch, in terms of the AM colonization, might be due to compounds other than alkaloids. Until someone does a full metabolomics screening of these grass-endophyte combinations (cf. Rasmussen *et al.* 2008) little can be said about the likelihood of this hypothesis. Thus, differences in AM fungal response cannot be unequivocally attributed either to specific alkaloids, or even specifically to alkaloids. Nevertheless, differences in AM fungal responses to these two strains of endophyte offer a tool, albeit crude, for the preliminary investigation of mechanisms and compounds that may have adverse effects on AM fungi.

It is interesting that the 'leached thatch' treatment produced results similar to those seen with the common strain endophyte-infection. Even tough we cannot provide a definite explanation for this result, since the leached treatment consisted of a 1 : 1 mix of common strain-infected and AR542-infected thatch, and this mixture was leached by soaking it in water for 96 h, the fact that it did not differ substantially from the common strain treatment suggests that: (i) at least 50% less common strain-infected thatch is needed to produce the same result; and (ii) perhaps the compound(s) responsible is sparingly water soluble and effective at low concentrations. Many secondary metabolites, including alkaloids, are typically extracted by organic solvents under basic conditions (Schardl *et al.* 2007). So it would seem that our protocol based on leaching is unlikely to have freed much of the alkaloid content of the thatch, if indeed these were to be the chemicals responsible for the observed effects.

While there was undoubtedly some decomposition of the grass thatch, it would have been relatively low over the time course of this experiment (120 days). Moreover, the endophyte-infected thatch would decompose more slowly than the endophyte-free thatch (Omacini *et al.* 2004). Therefore, since watering was done through thatch laid on the soil surface of the pots, we presume that the mode of action was long-term leaching. A 96 h of leaching and half the biomass were insufficient to reduce the effectiveness of the common strain-endophyte in interfering with the *B. inermis*–*G. intraradices* relationship hence the long-term.

In contrast to AM fungal root colonization, the endophyte-effect on spore germination was comparable between the common and AR542 strains. It is possible that allelopathy produced by the common strain endophyte on the AM fungal symbiosis, is stronger on AM fungal growth or colonization, than on spore germination end points. A potential explanation for this is that starting with the germination of AM fungal spores, hyphal growth through the soil, host recognition and root colonization, each step may be influenced by different signalling compounds (Douds, Nagahashi & Podila 2000). Alternatively, it is possible that the differential patterns between endophytes observed in the second experiment are due to interactions between edaphic factors, biotic and/or abiotic, and the different varieties of thatch.

Certainly further work is needed to explain the phenomenon of reduced colonization by AM fungi in the presence of endophytes. We need to know whether the effects are indeed the result of alkaloid allelopathy, and if so what compound(s) is responsible, what concentrations are necessary and the mode of action on AM fungi. To further clarify these questions, we may be able to follow the same simple and low-cost approach used in this study to exploit other endophyte strains that produce even different profiles of secondary metabolites.

For example, Lp19, AR1 and AR37 have been used previously with *L. perenne*. Lp19 produces ergovaline, lolitrem B and peramine. AR1 produces only peramine, and AR37 produces only a class of alkaloids called janthitrems (Rasmussen *et al.* 2007). From our study, we would predict that *L. perenne* infected with AR1 would not show decreased AM fungal colonization. However, we can say nothing about the potential of janthitrems to influence colonization.

Further work also needs to be conducted on the ecological implications of endophyte-mediated changes on the AM fungal symbiosis. This is a component that has been largely ignored in previous studies. If endophyte-allelochemicals inhibit AM colonization of competing plants, does this increase competitive abilities of *S. phoenix*? Alternatively, direct endophyte effects may negate any beneficial effects of inhibiting AM fungal colonization on competing plants. On one hand, this is supported by studies showing endophyte-AM fungal interactions which result in reductions of the beneficial effect of the endophyte on the host plant, in terms of insect resistance (Vicari, Hatcher & Ayres 2002). On the other hand, absence and sometimes even reduction of growth responses in plants colonized by AM fungi have been shown to be a common phenomenon, although the mechanisms responsible are not clear (Klironomos 2003; Jones & Smith 2004). This is consistent for example with our results for *B. inermis* and also with those of Mack & Rudgers (2008) for *S. phoenix*. Nevertheless, caution should be taken in generalizing that certain endophyte strains have negative impacts on the AM fungal symbiosis. As mentioned earlier, there is only a reasonable degree of support for this in agronomic cultivars.

In conclusion, specific characteristics of different *Neotyphodium* endophytes of *S. phoenix* may be responsible for negative effects on the AM fungal colonization of roots of other neighboring plant species, even upon the death of *S. phoenix*, through long-term leaching into soil. Such possibility may have important implications for natural ecosystem functioning, the management of pastures or forage crops and conservation efforts.

Acknowledgements

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