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ORIGINAL PAPER

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Colonization of nonmycorrhizal plants by mycorrhizal neighbours as influenced by the collembolan, *Folsomia candida*

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Abstract Soil microarthropods have been shown to stimulate or be detrimental to arbuscular mycorrhizal function by their grazing actions, but their role as dispersal agents has not been assessed. The ability of three species of arbuscular mycorrhizal (AM) fungi (*Glomus etunicatum*, *Acaulospora denticulata*, *Scutellospora calospora*) infecting *Plantago lanceolata* roots to further colonize neighbouring plants was measured in response to the distance between root systems and the presence of the collembolan, *Folsomia candida*. In the absence of collembola, all three fungal species infected neighbouring plants in two weeks or less (at short distances), but were not successful when neighbouring plants were placed 45 cm away or further. Colonization by *G. etunicatum* was the quickest at short distances, but *S. calospora* showed greatest ability to colonize at increasing distance,whereas *A. denticulata* was intermediate. In the presence of the collembolan, *G. etunicatum* took longer to colonize neighbouring plants, but was able to infect at least 30 cm further, illustrating the arthropod's ability to disperse the AM inoculum. *A. denticulata* increased its range by 10 cm in the presence of *F. candida*, but unlike *G. etunicatum*, there was no delay in the colonization. In contrast, colonization of neighbouring plants by *S. calospora* was negatively affected both in terms of overall distance and time. These data support the hypothesis that soil arthropods can act as dispersal agents for AM inoculum, but the extent of this is fungal species-specific.

Key words Microarthropods · Arbuscular mycorrhizae · Grazing · Collembola

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Introduction

Microarthropods are very abundant in soils and may directly influence fungal communities through grazing and dispersal of hyphal fragments and spores (Visser 1985). They feed on fungi growing in the rhizosphere and on decaying root and leaf litter (Klironomos and Kendrick 1995). Fungi are very abundant in these zones, and play important roles in soil fertility and primary production through their activities in organic matter decomposition and nutrient cycling, as root pathogens, and as participants in symbiotic associations with plant roots (Kendrick 1992). Knowledge on the ways in which animals and fungi interact is important if we are to understand better how soil ecosystems function.

Grazing can significantly influence fungal activity and community structure in soil (Parkinson et al. 1979; Newell 1984a,b; Bardgett et al. 1993). Saprobic and parasitic fungi are particularly palatable (McMillan 1975; Moore et al. 1987; Shaw 1988; Klironomos et al. 1992; Kaneko et al. 1995), especially darkly pigmented taxa such as *Alternaria*, *Cladosporium* and *Epicoccum*, and fungal activities either increase or decrease in response to grazing depending on edaphic conditions and animal densities (Klironomos and Kendrick 1995). However, fungi belonging to the order Glomales, which form arbuscular mycorrhizae (AM), are less palatable (Klironomos and Kendrick 1996; Klironomos and Ursic 1998). They are rarely grazed upon when presented with other, more palatable, taxa. Regardless, gut content analyses of microarthropods have revealed the presence of AM fungal hyphae and microarthropods have been shown to negatively impact the functioning of the symbiosis (Finlay 1985; Harris and Boerner 1990; Klironomos and Ursic 1998; McGonigle and Fitter 1988; Warnock et al. 1982), mainly by grazing on the external hyphal network and subsequently reducing the transport of mineral nutrients to roots.

The influence of microarthropods on fungal dispersal has received far less attention. Many conidial fungal species have been isolated from the body surface as

well as gut contents of collembolans and mites extracted from soil (Visser et al. 1987). Often, spores can survive ingestion and remain viable in the faeces. Also, many conidial fungal species produce a slimy spore mass and can adhere to animals' exoskeleton (Kendrick 1992).

To our knowledge, the potential for soil microarthropods to act as dispersal agents for AM fungi has not been investigated. This is likely because AM fungal spores are larger than those from any other fungal group, and are unlikely to be ingested whole. All attention has been focused on larger animals, such as mammals (Allen 1987; Warner et al. 1987; Allen and MacMahon 1988) and macroinvertebrates (McIlveen and Cole 1976; Ponder 1980; Harinikumar and Bagyaraj 1994). However, microarthropods may also influence the dispersion of AM fungal inoculum in soil. In a preliminary laboratory study we found that AM hyphae of *Glomus etunicatum* were ingested by the collembolan, *Folsomia candida*, and were still viable after excretion in the feces. The majority of hyphal fragments in the faecal pellets were greater than 25 μm in length and they still contained cytoplasm. Furthermore, the temporal and spatial occurrences of these two groups of organism in the field are similar (McGonigle and Fitter 1988; Klironomos and Kendrick 1995). They are frequently found travelling along root surfaces at a rate exceeding 1 m per day (personal observation). At this scale, mycelium (runner hyphae and hyphal bridges) from one mycorrhizal root system can potentially extend and colonize neighbouring plants, but little is known regarding the distances over which this can occur, and the influence of microarthropods on this process.

The objective of this study was to test the ability of three AM fungi colonizing one plant to extend into the soil and colonize a neighbouring plant. This was done under varying distances between plants and in the presence/absence of the collembolan, *F. candida*.

Materials and methods

The experiment was conducted in glass microcosms (1 m long × 0.1 m wide × 0.5 m tall) containing silica sand (Grade 15, W.R.Barnes Inc, Waterdown, Ontario, Canada). Each microcosm was considered a single experimental unit. Two *Plantago lanceolata* L. seedlings were planted in each microcosm, and separated by two 30-μm meshes. One plant was pre-grown from seed in a 4-inch pot containing silica sand and 1 g finely chopped mycorrhizal leek roots for 1 month. It was then transferred to the microcosm. The second plant was pre-grown under similar conditions before transplantation, but with finely chopped nonmycorrhizal leek roots. A preliminary study revealed that percent colonization of the 1-month old seedlings ranged from 10% to 39%, with no significant difference among fungal species. Plants grown with nonmycorrhizal leek roots remained nonmycorrhizal. In the microcosms, the fine meshes were placed at varying distances from each other. Plants were fertilized as needed with half-strength Hoagland's solution.

A total of 2490 experimental units were used in the study [(3 fungal species × 10 distances × 8 sequential harvests × 2 animal levels × 5 replicates) plus 90 units used as controls (see below)]. The study was conducted in a single growth-room maintained at 15 or 25 °C with 14-h photoperiod of 600 μmol m^{-2} s^{-1}. It was logistically impossible to run all experimental units at the same time, so we divided them into four blocks, each containing 600 microcosms. Treatments (fungal species × distance × harvest time × animal level) were randomly assigned to the experimental units. A new block was set up after previous one was completely harvested. Within each microcosm, distances between meshes were 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 cm. Harvests were performed at 1, 2, 3, 4, 5, 6, 10, and 14 weeks. The animal treatments were either 0 or 50 *F. candida* individuals. This animal species was isolated from the Long-Term Mycorrhiza Research Site (LTMRS) (http://www.uoguelph.ca/botany/fasel/index.htm) at Guelph, Ontario, Canada, where it is the third most abundant collembolan species in the soil. It was chosen for this study because it was successfully cultured in the laboratory, and has been shown to feed on AM fungi (Klironomos and Kendrick 1996). The animals were added between the two meshes in each microcosm. Only adults were used, since young animals rarely move from their release site. The three arbuscular mycorrhizal fungal species used were *G. etunicatum* Becker & Gerdemann, *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders, and *Acaulospora denticulata* Sieverding & Toro, all three also isolated from the LTMRS, and maintained in leek dual pot cultures. Two controls were also performed, one without fungi or animals, and the second without the fungi. For each control there were 45 experimental units (5 replicates × 3 distances × 3 × harvests). Distances between meshes were 5, 25, and 50 cm, and harvests were 1, 5, and 14 weeks. In both controls, all plants remained nonmycorrhizal throughout the 14 week period and at all distances.

At harvest, the non-mycorrhizal plants were harvested, all roots were collected, stained with Chlorazol Black E (Brundrett et al. 1984) and the presence/absence of mycorrhizal structures (arbuscules or vesicles) was assessed for each entire root system. If a minimum of one plant (from the five replicates) was found to be mycorrhizal, then that time × distance treatment was scored positive.

Results

At short distances (5–15 cm) *G. etunicatum* was able to infect neighbouring plants within the first week (Fig. 1a). Time of infection was, however, delayed in the presence of *F. candida* by at least one extra week (Fig. 1b). In the absence of the animals, *G. etunicatum* was not able to infect plants placed further than 20 cm within the entire 14-week time frame. However, successful infection was detected at all distances tested in the presence of animals.

It took at least 1 week longer for both *A. denticulata* and *S. calospora* to infect neighbouring plants at short distances, compared to *G. etunicatum* (Figs. 2a, 3a). In the absence of animals, the maximum successful distances differed between these two fungal species. *A. denticulata* was able to reach plants up to 30 cm away by week 10, whereas *S. calospora* was successful at 40 cm by week 5. The distance by which *A. denticulata* was able to infect neighbouring plants was also increased from a maximum of 30 cm to 40 cm over a period of 14 weeks in the presence of animals. In contrast, *S. calospora* was negatively affected by the animals, as they increased the time required at most distances, and decreased the maximum distance by 10 cm.

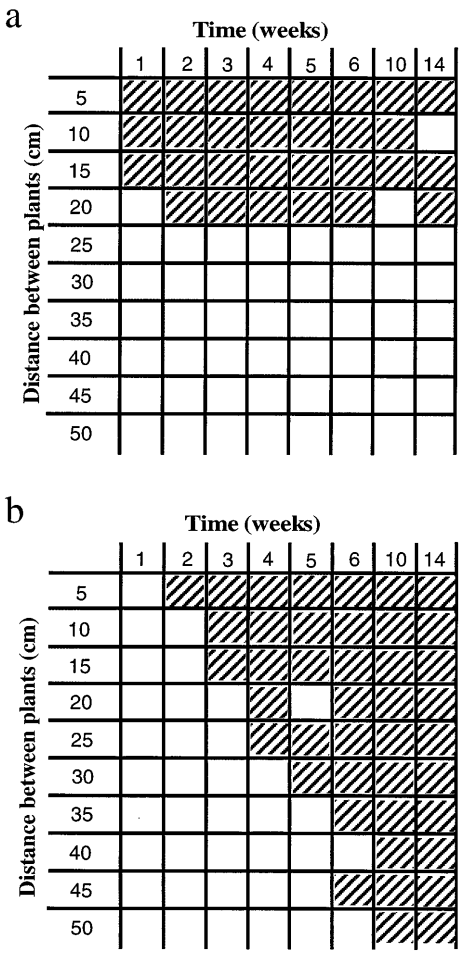

Fig. 1 Colonization of non-mycorrhizal neighbouring plants by *Glomus etunicatum* as a function of time and distance between mycorrhizal and non-mycorrhizal plants, **a** in the absence of *Folsomia candida* and **b** in the presence of *F. candida*. ▨ Neighbouring plants colonized; □ Neighbouring plants not colonized

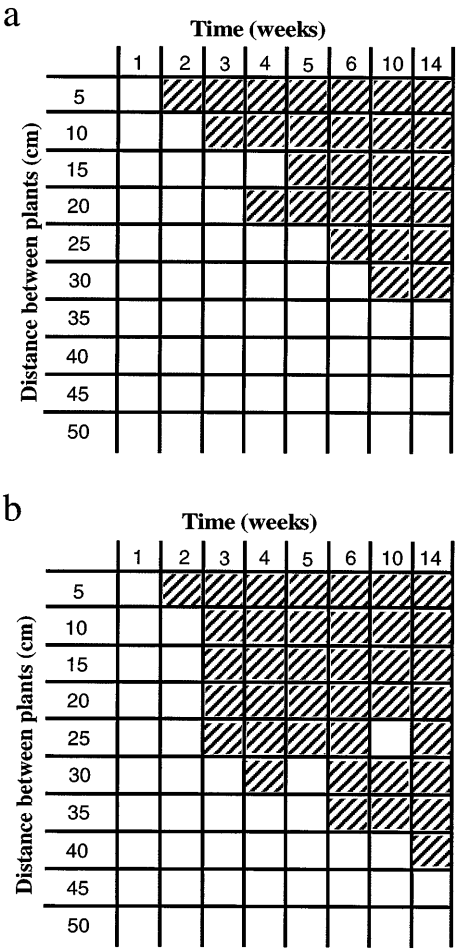

Fig. 2 Colonization of non-mycorrhizal neighbouring plants by *Acaulospora denticulata* as a function of time and distance between mycorrhizal and non-mycorrhizal plants, **a** in the absence of *F. candida* and **b** in the presence of *F. candida*. ▨ Neighbouring plants colonized; □ Neighbouring plants not colonized

Discussion

The results clearly show that AM fungal species have different abilities of infecting neighbouring plants at varying distances and, furthermore, that this ability is modified by soil microarthropods. It is clear from this study that AM fungi are structurally and functionally diverse. The three fungi studied here differed markedly on their colonizing range. *G. etunicatum* was aggressive at short ranges but was dependent on the presence of microarthropods for long-range dispersal. *S. calospora* was capable of colonizing plants over longer distances but was very sensitive to microarthropod grazing, which rendered this species less effective. *A. denticulata* displayed an intermediate activity.

Mechanisms for the different fungal strategies are not clear, since there is a lack of research on the extraradical phase of AM symbioses. There is evidence that different fungi produce different densities of mycelium (Abbott et al. 1992; Jakobsen et al. 1992). *Glomus* species have been found to develop high intra- to extraradical hyphal growth, whereas in *Acaulospora* and *Scutellospora* species the ratio is much lower (Klironomos et al. 1998). Some *Glomus* species produce more extensive intraradical colonizations, whereas the species in the other genera produce more extensive extraradical hyphal systems (Klironomos et al. 1998). This implies that microarthropods affect external mycorrhizal hyphal systems as a function of their architecture, and thus could explain why *Acaulospora* and *Scutellospora* were capable of colonizing plants that were placed further away, whereas *Glomus* was successful only at relatively short distances. In a study by Warner and Mosse (1983) none of the fungi tested could infect neighbouring roots positioned further than 20 cm away, even though isolates representing different genera were used (i.e., *G. fasciculatum*, *Gigaspora margarita* and *A. laevis*). It is obvious from such inconsistent results that we desperately need to characterize the external hyphal network for a range of AM fungi in more detail. This

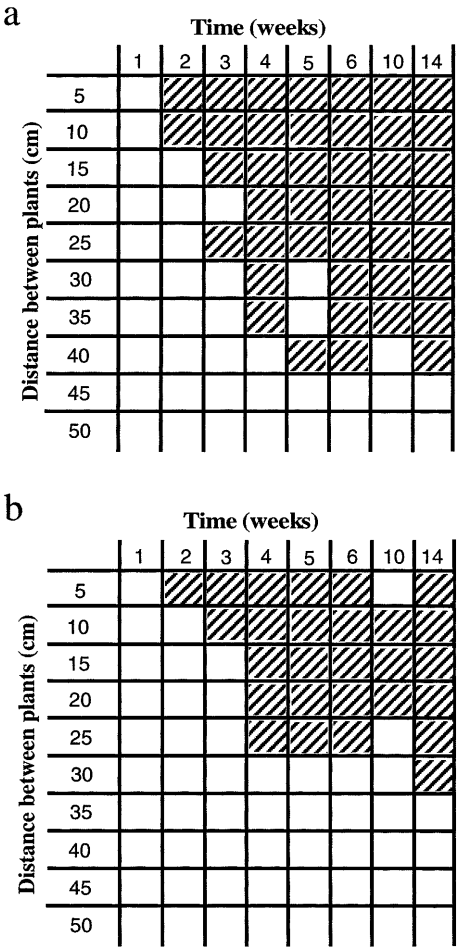

Fig. 3 Colonization of nonmycorrhizal neighbouring plants by *Scutellospora calospora* as a function of time and distance between mycorrhizal and non-mycorrhizal plants, **a** in the absence of *F. candida* and **b** in the presence of *F. candida*. ▨ Neighbouring plants colonized; □ Neighbouring plants not colonized

was initiated by Friese and Allen (1991), who described the development of runner hyphae and the absorptive hyphal network. This was done with a general soil inoculum, so inter-specific components cannot be separated.

The dispersal of AM inoculum to neighbouring plants by collembola is difficult to explain. There are two possibilities: either (1) the animals, via their grazing activities, stimulated hyphal growth and extension or (2) hyphae and spores were carried by the animals closer to the neighbouring plants. The former is not likely since we could not detect hyphal bridges connecting the two plants that were placed further than 20 cm apart. As for carrying the inoculum, spores are too large for the animals to ingest whole, and we did not detect any spores attached to animals' cuticle. The most likely explanation is that hyphal fragments were ingested and were later expelled with the faeces without digestion. Subsequent analysis of animal gut contents (data not shown) revealed that hyphae were ingested. However, we do not have data to show that these hyphae are still infective after passing through the animal's gut. The fact that these coenocytic hyphae are capable of "plugging up" quickly and do not readily leak their cytoplasmic contents once cut (Y. Piche, personal communication), supports this hypothesis. This needs further study. Even though microarthropods can ingest AM hyphae, they are not very palatable and very little nutrition is gained from this food source (Klironomos and Kendrick 1996). They prefer to feed on conidial fungi on which they grow faster and bigger, and have higher fecundity rates (Klironomos and Kendrick 1996; Klironomos and Ursic 1998). The present study needs to be repeated using other animal, fungal and plant taxa to make any further inferences regarding AM hyphal extension and interactions with microarthropods.

The stimulative effect of the animals on the colonizing capacity of AM fungi over longer distances is a novel finding. Until now, microarthropods have typically been considered detrimental to the AM symbiosis primarily because they can dislocate external hyphal networks from the root, and thus can interfere with nutrient translocation (Fitter and Sanders 1992). We did not measure nutrient uptake in this study, so this cannot be addressed here. However, these results suggest that any costs associated with severed hyphal networks may be offset by enhanced dispersal ability. Thus, factors other than nutrient uptake should be taken into account when assessing the impact of soil animals on the functioning of AM symbioses or, more generally, on mycorrhizal "fitness". In mature terrestrial ecosystems mycorrhizal inoculum is rarely spatially distributed in a uniform manner, and even more so in disturbed ecosystems (Reeves et al. 1979; Gibson and Hetrick 1988; Klironomos and Kendrick 1995). With such patchy distributions, the extent to which fungi can colonize plants in the field will depend, in part, on their abilities to extend from the source and to make contact with the growing root, as well as with how they interact with microarthropods.

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References

Abbott LK, Robson AD, Jasper D, Gazey C (1992) What is the role of VA mycorrhizal hyphae in soil? In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 37–41

Allen MF (1987) Re-establishment of mycorrhizae on Mount St. Helens: migration vectors. Trans Br Mycol Soc 88:413–417

Allen MF, MacMahon JA (1988) Direct VA mycorrhizal inoculation of colonizing plants by pocket gophers (*Thomomys talpoides*) on Mount St. Helens. Mycologia 80:754–756

Bardgett RD, Whittaker JB, Frankland JC (1993) The effect of collembolan grazing on fungal activity in differently managed upland pastures: a microcosm study. Biol Fertil Soils 16:155–262

Brundrett MC, Piche Y, Peterson RL (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can J Bot 62:2128–2134

Finlay RD (1985) Interactions between soil micro-arthropods and endomycorrhizal associations of higher plants. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Spec Publ no 4, Br Ecol Soc. Blackwell, Oxford, pp 319–331

Fitter AH, Sanders IR (1992) Interactions with the soil fauna. In: Allen MF (ed) Mycorrhizal functioning, an integrative plant-fungal process. Chapman and Hall, New York, pp 333–354

Friese CF, Allen MF (1991) The spread of VA mycorrhizal fungal hyphae in soil: inoculum types and external hyphal architecture. Mycologia 83:409–418

Gibson DJ, Hetrick BAD (1988) Topographic and fire effects on the composition and abundance of VA-mycorrhizal fungi in tallgrass prairie. Mycologia 80:433–441

Harinikumar KM, Bagyaraj DJ (1994) Potential of earthworms, ants, millipedes, and termites for dissemination of vesicular-arbuscular mycorrhizal fungi in soil. Biol Fertil Soils 18:115–118

Harris KK, Boerner REJ (1990) Effects of belowground grazing by collembola on growth, mycorrhizal infection and P uptake of *Geranium robertianum*. Plant Soil 129:203–210

Jakobsen, I Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. New Phytol 120:371–380

Kaneko N, McLean MA, Parkinson D (1995) Grazing preference of Onychiurus subtenuis (Collembola) and Oppiella nova (Oribatei) for fungal species inoculated on pine needles. Pedobiologia 39:538–546

Kendrick B (1992) The fifth kingdom, 2nd edn. Mycologue, Waterloo, Ontario

Klironomos JN, Kendrick B (1995) Relationships among microarthropods, fungi, and their environment. Plant Soil 170:183–197

Klironomos JN, Kendrick WB (1996) Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. Biol Fertil Soils 21:43–52

Klironomos JN, Ursic M (1998) Density-dependent grazing on the extraradical hyphal network of the arbuscular mycorrhizal fungus, *Glomus intraradices*, by the collembolan, *Folsomia candida*. Biol Fertil Soils 26:250–253

Klironomos JN, Widden P, Deslandes I (1992) Feeding preferences of the collembolan *Folsomia candida* in relation to microfungal successions on decaying litter. Soil Biol Biochem 24:685–692

Klironomos JN, Ursic M, Rillig M, Allen MF (1998) Interspecific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO_2. New Phytol 138:599–605

McGonigle TP, Fitter AH (1988) Ecological consequences of arthropod grazing on VA mycorrhizal fungi. Proc R Soc Edin 94B: 25–32

McIlveen WD, Cole H Jr (1976) Spore dispersal of Endogonaceae by worms, ants, wasps and birds. Can J Bot 54:1486–1489

McMillan JH (1975) Interspecific and seasonal analyses of the gut contents of three Collembola (family Onychiuridae). Rev Ecol Biol Sol 12:449–457

Moore JC, Ingham ER, Coleman DC (1987) Inter- and intraspecific feeding selectivity of Folsomia candida (Willem) (Collembola, Isotomidae) on fungi. Biol Fertil Soils 5:6–12

Newell K (1984a) Interactions between two decomposer basidiomycetes and a collembolan under Sitka spruce: distribution, abundance and selective grazing. Soil Biol Biochem 16:227–233

Newell K (1984b) Interactions between two decomposer basidiomycetes and a collembolan under Sitka spruce: grazing potential effects on fungal distribution and litter decomposition. Soil Biol Biochem 16:235–239

Parkinson D, Visser S, Whittaker JB (1979) Feeding preferences for certain litter fungi by Onychiurus subtenuis. Soil Biol Biochem 11:529–535

Ponder F Jr (1980) Rabbits and grasshoppers: vectors of endomycorrhizal fungi on new coal mine spoil. North Central Forest Exp Stn Res Notes 250:1–2

Reeves FB, Wagner DW, Moorman T, Kiel J (1979) The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs natural environments. Am J Bot 66:1–13

Shaw PJA (1988) A consistent hierarchy in the fungal feeding preferences of the collembola *Onychiurus armatus*. Pedobiologia 31:179–187

Visser S (1985) Role of the soil invertebrates in determining the composition of soil microbial communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil. Spec Publ No 4, Br Ecol Soc. Blackwell, Oxford, pp 297–317

Visser S, Parkinson D, Hassall M (1987) Fungi associated with *Onychiurus subtenuis* (Collembola) in an aspen woodland. Can J Bot 65:635–642

Warner A, Mosse B (1983) Spread of vesicular-arbuscular mycorrhizal fungi between separate root systems. Trans Br Mycol Soc 80:353–380

Warner NJ, Allen MF, MacMahon JA (1987) Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79:721–730

Warnock AJ, Fitter AH, Usher MB (1982) The influence of a springtail, *Folsomia candida,* (Insecta: Collembola) on the mycorrhizal association of leek, *Allium porrum*, and the vesicular-arbuscular mycorrhizal endophyte, *Glomus fasciculatus*. New Phytol 90:285–292