Mycorrhiza (1997) 7:197–200

ORIGINAL PAPER

Z. Kabir · I. P. O'Halloran · C. Hamel

Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance

Accepted: 9 August 1997

Abstract We investigated the overwinter survival in the field of indigenous arbuscular mycorrhizal (AM) hyphae either connected to corn roots or detached from them, and either intact or disrupted. We buried soil-filled pouches which either allowed root entry or excluded roots in the root zone of a field-grown corn (*Zea mays*) crop in eastern Canada. Following crop harvest in the fall, pouches either remained undisturbed, were disturbed outside the pouch, or were disturbed both inside and outside the pouch. Total and metabolically active AM hyphae in undisturbed pouches declined 20% and 33% (average of coarse- and fine-mesh treatments), respectively, from fall to spring, presumably because of death overwinter. In the spring, living hyphae were more abundant in the presence of roots than in their absence, suggesting that attachment or proximity to roots favored overwinter survival. Total hyphal density, metabolically active hyphal density, and the proportion of total living hyphae progressively diminished with increased disturbance.

Key words Arbuscular mycorrhizal fungi · Soil disturbance · Overwintering survival · Extraradical hyphae · *Zea mays*

Z. Kabir (✉)
Department of Horticulture, College of Agricultural Sciences, The Pennsylvania State University, 102 Tyson Building, University Park, PA 16802-4200, USA
Fax: +1-814-863-6139; e-mail: MZK5@PSU.edu

I. P. O'Halloran
Department of Land Resource Science, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

C. Hamel
Department of Natural Resource Sciences, McGill University, Macdonald Campus, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X 3V9 Canada

Introduction

Arbuscular mycorrhizal (AM) fungi form mutualistic associations with plants, thereby facilitating plant uptake of phosphorus and other nutrients (Rhodes and Gerdemann 1975; Evans and Miller 1988; Manjunath and Habte 1988). The extraradical hyphae of AM are commonly regarded as an efficient aid for nutrient uptake, but their role as a source of inoculum is often overlooked. Extraradical hyphae probably are the principal source of inoculum in soil (Read et al. 1976; Brundrett et al. 1985). Hyphae can continue growth after the death of their host plant (Warner and Mosse 1980; Tommerup and Abbott 1981; Bierman and Linderman 1983; Hepper and Warner 1983; St. John et al. 1983). Moreover, mycorrhizal colonization of crops proceeds quickly in undisturbed soil (Jasper at al. 1989a,b), but may be delayed if the soil is disturbed (Jasper et al. 1989a,b; Evans and Miller 1990; Jasper et al. 1991; Douds et al. 1995). Such a delay in colonization is often accompanied by reduction in both P uptake by plants and plant growth (McGonigle et al. 1990).

Soil disturbance-induced reduction of mycorrhizal colonization has been linked to fragmentation of the AM hyphal network in the soil (O'Halloran et al. 1986; Evans and Miller 1988; Fairchild and Miller 1988). However, a recent study (Addy et al. 1994) showed that AM hyphae detached from plant roots survived and retained infectivity even after exposure to winter conditions with a minimal temperature of –3.5 °C. Thus, it is not clear if soil disturbance affects soil mycorrhizal potential primarily through detachment of AM fungi from roots or through disruption of AM hyphae. In either case, plowing fields before winter may adversely affect AM fungal survival.

We studied the effects of the separation of extraradical hyphae from roots and of the mechanical disruption of AM hyphae on their overwinter survival.

Materials and methods

Experimental treatments

The experiment was conducted in 1993 on a St. Benoit sandy loam soil (Eutric Cambisol) at the E. Lods field research station of McGill University, Québec, Canada. The soil had a pH (water) of 5.9, contained 2.44% organic carbon and was composed of 55% sand, 28% silt and 17% clay. The extractable nutrient levels (Mehlich 1984) were 87 mg P, 114 mg K, 1565 mg Ca, 91 mg Mg, 3.3 mg Zn, and 1.3 mg Cu per kg of soil. No P fertilizers had been applied to the experimental site since 1988. *Glomus mosseae* Gerdemann & Trappe, *G. aggregatum* Schenck & Smith, *G. macrocarpum* Tul. & Tul. and *G. rubiforme* Almeida & Schenck (Gerdemann & Trappe) made up the mycorrhizal community of this soil.

The experiment had a 2 × 3 factorial design with two mesh sizes for buried soil-containing pouches and three levels of disturbance. Treatment combinations were randomized in five complete blocks. Mesh allowed the penetration and inclusion of roots and hyphae (2 mm) or hyphae alone (37 μm) in pouches. The pouches (15 × 6 cm) were buried such that their tops were 3 cm below the soil surface and bottoms 18 cm below the surface in the root zone of corn at the 10–12 leaf stage. They contained 490 g of the field soil which had been passed through a 2-mm sieve to remove debris. One pouch of each mesh per block was collected just after the corn harvest to measure hyphal density (fall value). Disturbance treatments imposed in the fall were: (1) surrounding and soil pouch contents were left undisturbed (UU), (2) pouches were removed, the surrounding soil was disturbed and the pouches were replaced intact (DU), in order to detach the pouch contents from external roots and hyphae, (3) soil was disturbed both inside and outside the pouch (DD) in order to both detach and disrupt the pouch contents. In this case, pouches were removed from the soil, their contents emptied and roots were carefully chopped into 2- to -3-cm lengths. All pouch contents were put back into the pouch. Soil surrounding the pouches was disturbed as in the DU treatment and pouches were replaced. In the spring, directly following the thaw, all pouches were removed from the soil and stored in a cold room at 4 °C. Hyphae contained in the pouches were extracted and their abundance and metabolic activity measured.

Hyphal extraction and measurement

A membrane filter technique modified from Abbott et al. (1984) was used to extract extraradical hyphae from soil samples taken from pouches. Soil from each pouch was thoroughly mixed and four 5-g subsamples were taken. Each subsample was placed into a blender with 300 ml deionized water, blended for 30 s, and then poured through a 250-μm sieve. Hyphae were collected on a 45-μm sieve placed below the 250-μm sieve, which was rinsed with water. Recovered material was resuspended in water, transferred to a beaker, shaken for several seconds, and allowed to settle for 1 min. The supernatant was then filtered (pore size 11 μm) under vacuum. Shaking, settling and filtration were repeated three times on each subsample to obtain a thorough extraction of soil hyphae. Although some hyphae passed through the 45-μm sieve (measurements obtained using a 30-μm sieve were about 10% higher), soil parameters dictated the use of a 45-μm sieve.

Hyphae recovered from two of the four subsamples taken from each pouch were stained by flooding filters with 0.2% aqueous acid fuchsin. Excess stain was removed by deionized water wash and vacuum filtration. Hyphae recovered from the two other subsamples were stained by flooding filters with a solution containing equal parts of iodonitrotetrazolium (1 mg ml^{-1}), NADH (3 mg ml^{-1}), and 0.2 M Tris buffer pH 7.4 (Sylvia 1988). These filters were incubated for 12–16 h at room temperature. This stain detects dehydrogenase activity of living hyphae. Length of AM hyphae and of living, metabolically active hyphae was determined by the modified grid-line intersect method (Tennant 1975).

Statistical analysis

Statistical analysis of variance was performed using the general linear models procedure of SAS (SAS Institute 1988). The Proc Univariate procedure indicated that the data were normally distributed. The protected least significant difference test was used to detect differences between treatment means at the 5% level of significance. T-tests were used to compare fall and spring UU values and both total and metabolically active hyphal density, in both root-included (coarse mesh) and root-excluded (fine mesh) treatments.

Results

The *t*–tests of fall and spring UU values yielded no significant difference between coarse- (presence of roots) and fine-mesh (absence of roots) treatments. The averaged values show that, in the absence of disturbance (fall versus spring UU values), both total hyphal density and metabolically active hyphal density significantly declined from fall to spring by 20% and 33%, respectively (Figs. 1, 2), although metabolically active hyphae as a proportion of total hyphae did not (Fig. 3). Analysis of variance results for all response variables in the spring are shown in Table 1. There was no significant effect of presence or absence of roots in the pouches on total hyphal density (Fig. 1) or on proportion of viable hyphae (Fig. 3), but significantly more metabolically active hyphae occurred in the presence than in the absence of roots within pouches (Fig. 2). Disturbance had a significant effect on all variables, with fall disturbance both within and outside of pouches (DD) causing greater over winter decline (67% and 77% of total hy-

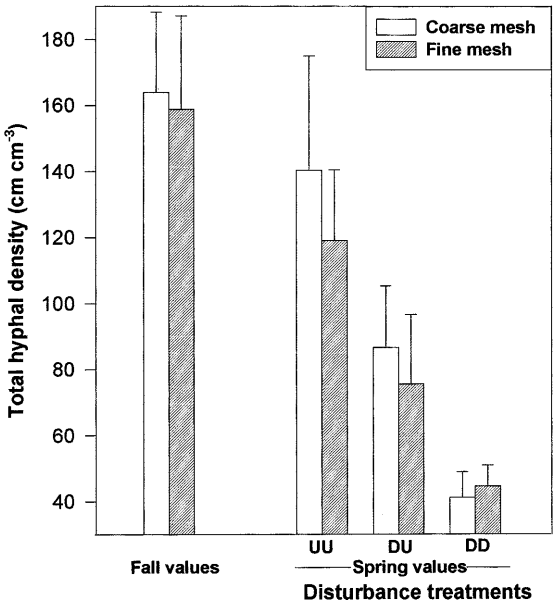

Fig. 1 Total hyphal density in soil within coarse-mesh (presence of roots) and fine-mesh (absence of roots) pouches in fall and spring after various disturbance treatments prior to overwintering (*UU* no disturbance, *DU* soil disturbed outside of the pouch but not inside, *DD* soil disturbed both inside and outside the pouch); *error bars* represent standard deviation for $n = 5$

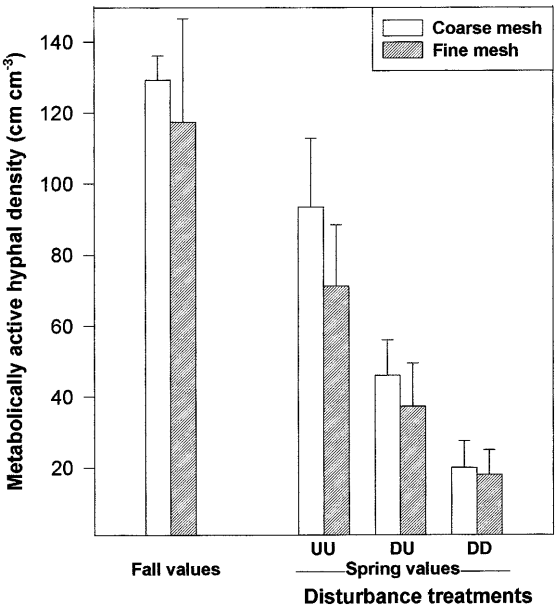

Fig. 2 Densitiy of metabolically active hyphae within coarse-mesh (presence of roots) and fine-mesh (absence of roots) pouches in fall and spring after various disturbance treatments prior to overwintering. Symbols as in Fig. 1

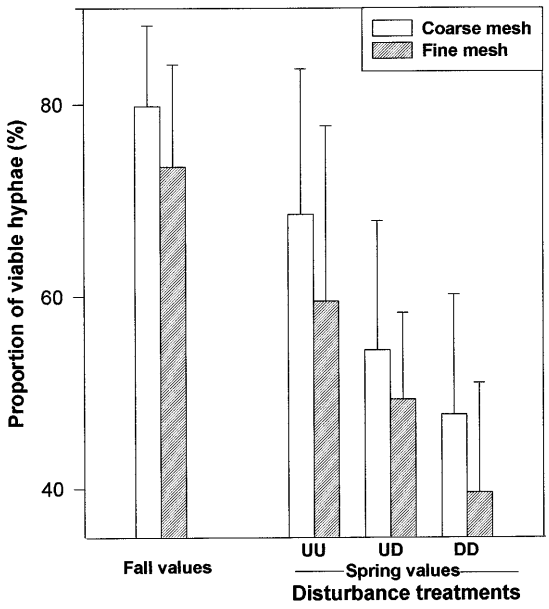

Fig. 3 Proportion of viable hyphae in soil within coarse-mesh (presence of roots) and fine-mesh (absence of roots) pouches in fall and spring after various disturbance treatments prior to overwintering. Symbols as in Fig. 1

Table 1 Analysis of variance for the abundances of total and metabolically active hyphae and the proportion of viable hyphae in the spring

Sources	Degrees of freedom	Probabilities of significance P-values[a]		
		Total hyphae	Metabolically active hyphae	Proportion of viable hyphae
Mesh size	1	0.19	0.035	0.106
Disturbance treatments	2	0.0001	0.0001	0.004
Mesh size × disturbance trt.	2	0.38	0.24	0.93

[a] Significance was assumed when $P < 0.05$

phae and metabolically active hyphae, respectively) than external disturbance alone (DU; 34% and 49% of total hyphae and metabolically active hyphae, respectively). Similarly, with increased disturbance, metabolically active hyphae as a proportion of total hyphae diminished (Fig. 3). There was no significant interaction of presence (coarse mesh) and absence of roots (fine mesh) with disturbance.

Discussion

This experiment was conducted to study the winter survival of AM hyphae under field conditions, especially after disturbance. Our results indicate that more hyphae survived the winter, when near or attached to dead plant (corn) roots than when detached or remote from them. Diminution of spring total hyphal density by fall disturbance treatments suggests that dead hyphae were quickly degraded and, consequently, that total hyphal density provides a good indication of the mycorrhizal status of the soil.

To explain our results, we hypothesize that plant assimilate concentrations are highest close to roots and decrease with distance as assimilates are taken up by proximal hyphae; the larger food reserves in hyphae near roots may facilitate hyphal survival. Addy et al. (1994) showed that AM hyphae can survive and maintain infectivity over winter in the absence of roots and suggested that hyphae survive freezing by forming "resting hyphae" containing shrunken beads of cytoplasm. If high concentrations of host assimilates act as cytoplasm antifreeze or are needed to sustain spring recovery of metabolic activity, then our hypothesis accounts for death of hyphae distal to roots. Nicolson (1959) suggested that oil globules within the cytoplasm of thick-walled AM hyphae might be a nutrient store allowing extraradical hyphae to survive in the absence of living plants. Lipid-rich AM vesicles in roots might also supply energy to hyphae attached to roots (Bier-

man and Linderman 1983; Millner 1991) and in this way improve winter survival.

Disturbance markedly reduced total hyphal density, metabolically active hyphal density and the proportion of hyphae viable in the spring. Disruption of extraradical hyphae far outweighed the effect of host root presence on survival. Our soil disturbance findings are consistent with those of Evans and Miller (1990) who found, in a pot experiment with corn, that disturbance of extraradical hyphae in a root-free zone reduced corn root infection and shoot P and Zn concentrations. Survival of AM extraradical hyphae over the winter is important in cool climates where field crop production is restricted to only a few warm months. Soil disturbance by fall plowing probably physically disrupts roots and hyphal networks and thus reduces the mycorrhizal potential of fall-plowed fields (O'Halloran et al. 1986; McGonigle et al. 1990; McGonigle and Miller 1993), by reducing the ability of the mycorrhizae to survive over the winter.

Acknowledgements We thank Canada (McGill) International Fellowship for financial support. Our work was also supported by the Natural Science and Engineering Research Council of Canada. Thanks go to Dr. Yolande Dalpé for identification of the AM fungal species, Dr David P. Janos for help in revising the manuscript and anonymous reviewers for their valuable suggestions and criticism.

References

Abott LK, Robson AD, De Boer G (1984) The effects of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. New Phytol 97:437–446

Addy HD, Schaffer GF, Miller MH, Peterson RL (1994) Survival of the external mycelium of a VAM fungus in frozen soil over winter. Mycorrhiza 5:1–5

Bierman B, Linderman RB (1983) Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. New Phytol 95:97–105

Brundrett MC, Piché Y, Peterson RL (1985) A development study of the early stages in vesicular-arbuscular mycorrhiza formation. Can J Bot 63:184–194

Douds DD Jr, Galvez L, Janke RR, Wagoner P (1995) Effects of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric Ecosyst Environ 52:111–118

Evans DG, Miller MH (1988) Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrients absorption in maize. 1. Causal relations. New Phytol 110:67–74

Evans DG, Miller MH (1990) The role of the external mycelial network in the effect of soil disturbance upon vesicular-arbuscular mycorrhizal colonization of maize. New Phytol 114:65–71

Fairchild GL, Miller MH (1988) Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize. II. Development of the effect. New Phytol 110:75–84

Hepper CM, Warner NA (1983) Organic matter in the growth of a vesicular-arbuscular mycorrhizal fungi in soil. Trans Br Mycol Soc 81:155–156

Jasper DA, Abbott LK, Robson AD (1989a) Soil disturbance reduces the infectivity of external hyphae of VA mycorrhizal fungi. New Phytol 112:93–99

Jasper DA, Abbott LK, Robson AD (1989b) Hyphae of a vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. New Phytol 112:101–107

Jasper DA, Abbott LK, Robson AD (1991) The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soil from different vegetation types. New Phytol 118:471–476

Manjunath A, Habte M (1988) Development of vesicular-arbuscular mycorrhizal infection and uptake of immobile nutrients in *Leucaena leurocephala*. Plant Soil 106:97–103

McGonigle TP, Miller MH (1993) Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. Soil Sci Soc Am J 57:1002–1006

McGonigle TP, Evans DG, Miller MH (1990) Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. New Phytol 116:629–636

Mehlich A (1984) Mehlich-III soil test extractant: a modification of Mehlich-II extractant. Comm Soil Sci Plant Anal 15:1409–1416

Millner PD (1991) Characterization and use of VAM in agricultural production systems. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer, Dordrecht, The Netherlands, pp 335–342

Nicolson TH (1959) Mycorrhizae in the Gramineae. 1. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans Br Mycol Soc 42:421–438

O'Halloran IP, Miller MH, Arnold G (1986) Absorption of P by corn (*Zea mays* L.) as influenced by soil disturbance. Can J Soil Sci 66:287–302

Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhizae in natural vegetation systems. I. The occurrence of infection. New Phytol 77:641–653

Rhodes LH, Gerdeman JW (1975) Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol 75:555–561

SAS Institute (1988) SAS user's guide, release 6.03. SAS Institute Inc. Cary, NC

St. John TV, Coleman DC, Reid CPP (1983) Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. Ecology 64:957–959

Sylvia DM (1988) Activity of external hyphae of vesicular-arbuscular mycorrhizal fungi. Soil Biol Biochem 20:39–43

Tenant D (1975) A test of a modified line intersect method of estimating root length. J Ecol 63:995–1001

Tommerup IC, Abbott LK (1981) Prolonged survival and viability of VA mycorrhizal hyphae after root death. Soil Biol Biochem 13:431–433

Warner A, Mosse B (1980) Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. Trans Br Mycol Soc 74:407–410