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Summer survival of arbuscular mycorrhiza extraradical mycelium and the potential for its management through tillage options in Mediterranean cropping systems

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Abstract

The potential to manage arbuscular mycorrhizal colonization within Mediterranean agricultural systems depends on the summer survival of extraradical mycelium. To investigate this further a three-stage experiment was undertaken. The first stage was the creation of two contrasting levels of extraradical mycelium development, achieved by two contrasting levels of soil disturbance (typifying full tillage and no-till). In the second stage, this differential mycelial inoculum was subjected to Mediterranean summer temperature and soil water regimes representing the post-harvest fallow. During the third stage, corresponding to the next growing season, survival was evaluated without further soil disturbance (typifying no-till conditions) using wheat as host crop. The results clearly indicate that the extraradical mycelium survived the prevailing summer conditions. The knowledge that extraradical mycelium can survive the Mediterranean summer encourages the use of tillage systems that minimize mechanical disturbance of the soil, such as no-till. The results from this study suggest that by making the appropriate choice of crops to establish a mycorrhizal-supportive rotation there can be opportunities for agro-ecosystem management to benefit from the symbiotic relationship.

Keywords: Arbuscular mycorrhiza, wheat, mycelium survival, tillage, Mediterranean conditions

Introduction

The obligate nature of the arbuscular mycorrhiza (AM) symbiosis requires that a plant propagation system be established for inoculum production. This takes place either under greenhouse conditions or through *in vitro* laboratory propagation. These techniques make inoculum production expensive, so that large-scale inoculation with arbuscular mycorrhizal fungi (AMF) is generally impractical in most regions except, perhaps, in the case of some high value crops (Saito & Marumoto, 2002). In addition, we have little understanding of the consequences of using such inoculation (Schwartz *et al*., 2006), and there is a clear need to identify the possibilities for mycorrhizal technology in support of 'sustainable plant production and soil conservation' (Gianinazzi & Vosátka, 2004). Importantly, the ability to take full advantage of indigenous AMF for sustainable production needs to be developed within cropping systems.

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AM colonization can be initiated by three different types of propagules: spores, extraradical hyphae and hyphae from colonized roots fragments. Runner hyphae from a well-developed extraradical mycelium are quicker to initiate colonization in a new host than other sources of inoculum (Martins & Read, 1997), particularly when the number of viable spores is limited (Read *et al*., 1976) or soil temperature is not optimal (Entry *et al*., 2002). In Mediterranean regions, the temperature over the typical period for seeding small grain cereals (Table 1) is much cooler than the range (18–40, optimum 30 °C), usually identified for spore germination (Entry *et al*., 2002). However, the ability to produce new mycorrhiza (infectivity) is not the same for all AMF, but depends on their life-cycle strategy under specific environmental conditions. The different strategies adopted by AMF to affect colonization appear to be defined at the family level, consistent with the view that in addition to the morphological and developmental bases of taxonomy there is also a functional component (Hart & Reader, 2002). It has been established that isolates of *Glomus* and *Acaulospora* colonize from all inoculum types, whereas those of *Gigaspora*

Table 1 Seeding and harvesting periods for small grain cereals in the Mediterranean region and average air temperatures (30 yr mean, 1971–2000)

Month	September	October	November	December	January	February	March	April	May	June	July	August
Crop cycle			Seeding period							Harvesting period		
Maximum air temperature (°C)	27.4	21.7	16.8	13.7	12.9	14.1	16.9	18.1	21.4	26.6	30.6	30.5
Minimum air temperature (°C)	15.5	12.4	9.2	7.1	5.7	6.6	7.8	8.8	11.0	13.9	16.1	16.3

Source: Évora meteorological station.

and *Scutellospora* colonize mainly from spores and to a limited extent from root fragments (Abbott *et al*., 1992; Brundrett *et al*., 1999; Klironomos & Hart, 2002).

Augé (2001) cite more than 150 studies into the effects of drought (including the duration), aridity and soil moisture gradients on the behaviour of AMF in several host–fungus combinations. In most studies, the evaluation was based on the colonization rate of AMF, and results varied widely depending on the experimental parameters. Only a few of these studies were concerned specifically with the survival of propagules and their infectivity.

The survival and persistence of less resistant forms of inoculum, such as extraradical mycelium, is known to depend on edaphic conditions, particularly soil temperature and moisture content (Entry *et al*., 2002). Mediterranean climates are noted for maximum air temperatures above 30 °C and very limited water availability in summer. Both of these hamper plant growth (crops and weeds) under rain fed conditions. Knowledge of the ability of extraradical mycelium to remain infective for the next crop under these conditions is important information if we aim to optimize the potential benefits from mycorrhiza. Jasper *et al*. (1989) give the first direct demonstration of the ability of external hyphal networks of AMF to maintain infectivity in dry soil. They established that the hyphal network of *Acaulospora leavis* maintained its infectivity as the soil dried, with the matric potential reaching −21 MPa which is much drier than the permanent wilting point (−1.5 MPa) for at least 36 days. The capacity of extraradical hyphae to remain infective under drought is variable depending on whether sporulation occurred at the onset of drying, the AMF taxonomic group, the associated host plant and any soil disturbance (Jasper *et al*., 1993; McGee *et al*., 1997; Klironomos *et al*., 2001). The survival of extraradical mycelium over summer will enhance AM potential within the cropping system. Therefore, the mycotrophic level of the crops to be included in the rotation, and the impact of soil disturbance induced by tillage on the disruption of the extraradical mycelium (Kabir, 2005) have to be more carefully considered.

This study tested the hypothesis that extraradical mycelium of native AMF can survive the dry and hot summer in Alentejo Province (Portugal), a typical Mediterranean region, when plants are absent, and will initiate colonization of wheat plants at the onset of the growing season. The answer to this question may provide opportunities for agro-ecosystem management systems that could benefit from the symbiotic relationship between AMF and crops. Wheat (*Triticum aestivum* L., cv. Coa) was chosen as host plant because it is the most representative cash crop in Mediterranean regions and in Alentejo Province it requires a high level of agrochemical input.

Material and methods

Use was made of a modified version of the cycles of disturbance technique developed by Fairchild & Miller (1988) and Experiment 1 aimed to determine the conditions necessary to establish differential AM infection by extraradical mycelium. This involved growing successive cycles of a mycotrophic crop (each cycle lasting 3 weeks), with and without soil disturbance. The disturbed soil treatment corresponded to sowing into a fully prepared seedbed created by several tillage operations, while the undisturbed treatment was comparable to no-till, where sowing would occur into soil with no modification below the depth of seed placement. The contrasting levels of soil disturbance were expected to induce a differential extraradical mycelium inoculum development. Using this technique after three or four cycles of plant establishment relative to plants from disturbed soil, greater AM colonization rates have consistently been observed in maize and soybean plants from undisturbed soil (Goss & de Varennes, 2002; McGonigle *et al*., 2003; Antunes *et al*., 2006). This was reflected in better growth of the host crop in undisturbed soil after the same number of cycles. However, there was no previous information on the suitability of wheat as an AM host plant to achieve the desired differential AM inoculum potential. Therefore, the focus of Experiment 1 was to determine the number of growing cycles required by wheat to induce significant AMF inoculum potential difference (Figure 1). The experiment was conducted under greenhouse conditions with 6 L plastic pots. Four pre-germinated wheat seeds were sown

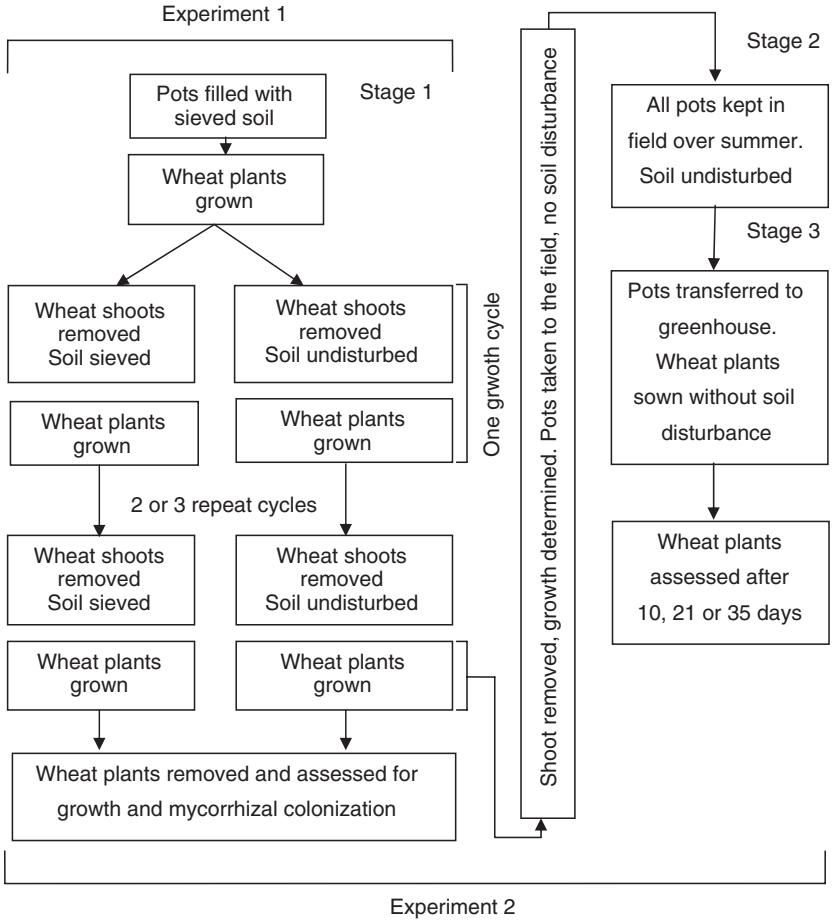

Figure 1 Schematic of experimental procedures for the two experiments.

in each of the 26 pots (two soil disturbance treatments with 13 replicates) and 5 days later were thinned to two plants per pot. The wheat seeds were germinated at room temperature on absorbent paper saturated with water. Three weeks after emergence shoot height and dry weight were evaluated (Figure 1). In half of the pots (the disturbed soil treatment), the soil was removed as two 10 cm layers and passed separately through a 4-mm sieve. All root material separated on the sieve was cut into 2-cm-long segments and mixed into the soil of the appropriate layer. Soil was repacked in the pots and arranged in the same two layers. In the other 13 pots, the soil remained undisturbed (Figure 1). More pre-germinated seeds were then added to each of the 26 pots and a new cycle initiated (Figure 1). In each cycle, except the last, 1 week after transplant, ammonium nitrate (50 μg N/g dry soil) was applied (10.7 mL of 1 M solution of NH_4NO_3 diluted in 100 mL of distilled water). Shoot growth became significantly greater in the undisturbed treatment by the end of the fourth cycle. At the end of this cycle, roots from all pots were stained with Trypan Blue and AM colonization rate assessed by the magnified intersections method (McGonigle *et al.*, 1990). In addition, a 100 g subsample of soil was collected from each pot and the AMF spores present were removed by the method described by Gerdemann & Nicolson (1963) and counted.

The information gained from Experiment 1 on conditions for the development of a differential AM fungal inoculum allowed the effect of summer heat and drought to be considered in Experiment 2. This study used clay pots to ensure better control of the factors involved and consisted of three stages: Stage 1, representing the growth of the crop under different tillage systems to develop a differential extraradical mycelium potential; Stage 2, corresponding to the summer fallow of the Mediterranean region was when climatic stressing of the mycorrhizal hyphal network took place; and in Stage 3, we investigated new AM fungal colonization of a wheat test crop, equivalent to the next crop growing season under undisturbed conditions. Stage 1 of Experiment 2 was in a greenhouse at the Mitra Campus of the University of Évora (38°32′26″N; 08°00′01″W) from February to July. The soil used was a Luvisol, collected from the top 20 cm of an arable field containing 28 mg P_2O_5/kg, 94 mg K_2O/kg (Egner-Riehm method for P and K), and 710 mg total N/kg (Kjeldhal method) of which 20 mg/kg was as NO_3^-–N (nitrate-specific electrode method), with 15 mg organic matter/g and a pH (in water) of 6.4. AMF diversity of this soil, based on nested PCR, found 11 different Operational Taxonomic Units, almost all belonging to the Glomus group (Brito *et al.*, 2006). This result is consistent

with those found by Oehl *et al.* (2003) for different agro-ecosystems and land use intensities.

The experimental procedure of this stage was similar to that of Experiment 1 with the necessary adaptations (Figure 1). Forty-two clay pots were used, corresponding to two soil disturbance treatments, with three sampling dates for wheat in Stage 3 and seven replicates ($2 \times 3 \times 7 = 42$). This number of pots was the minimum required to ensure that significant differences in root colonization would be detected during Stage 3 of the experiment according to the results from Experiment 1. With the space available, it was not possible to include enough additional pots to determine the different levels of colonization of roots at the end of Stage 1. Six pre-germinated wheat seeds were planted in each pot. Five days later they were thinned to four plants per pot to give a similar plant density to that used for commercial field crops. The pots were watered to weight with distilled water, and after the first week the water was applied from the base to avoid soil consolidation. Every 3 weeks a new cycle was initiated according to the methodology already described for the Experiment 1 (Figure 1). During the 4th cycle of Stage 1 in Experiment 2, the leaves of the wheat unexpectedly showed Zn and S deficiency symptoms. As this could have reduced photosynthesis, it was also likely to have impaired development of the extraradical mycelium. Consequently, a fifth wheat growth cycle was added after the soil received 3.4 mg Zn/kg (320 μL of 1 M solution of $ZnSO_4 \cdot 7H_2O$ per pot corresponding to 8 kg of Zn/ha and 4 kg of S/ha) as well as 50 mg N/kg.

Daytime temperatures inside the greenhouse during Stage 1 ranged between 17 and 30 °C and after March the cooling system was kept on. Photosynthetic active radiation during last plant growth cycle was 350 μmol/m^2.s, and sunshades were opened in the greenhouse roof to maintain temperatures at or below 30 °C.

Stage 2 started immediately after the last (5th) wheat growth cycle (Figure 1). On 28 July 2005, the pots were buried in the field with their tops level with the soil surface. This coincided with the typical period for wheat harvest (Table 1). When the pots were buried, their clay construction allowed water and temperature in the experimental soil material to equilibrate with the ambient field soil. For the duration that the pots were buried, there was no rain. Screen temperatures recorded over the period showed that the mean monthly temperature in August was 24.8 and 21.6 °C in September. Maximum temperatures ranged between 26.9 and 40.8 °C in August and between 24.7 and 35.2 °C in September. The minimum temperature recorded was 11.1 °C.

Stage 3 started in the first week of October when the pots were removed from the field, taken back to the greenhouse and slowly watered to field capacity 0.17 g H_2O/g dw (Figure 1). Eight pre-germinated wheat seeds were sown into each of the 42 pots and five days later thinned to four plants per pot. There was no disturbance of the soil at this stage because the aim of this experiment was to evaluate the survival of the extraradical mycelium differentially developed by the two soil disturbance treatments in Stage 1 of the experiment. Pots were watered to weight from the top with distilled water and thereafter from the base of the pots, according to plant needs. Application of N, Zn and S was made at the second week after planting and at the same rates as reported above for Stage 1.

To follow the evolution of AM colonization over time, the three sampling dates were 10, 21 and 35 days after planting (Figure 1). At each sampling date plants from seven pots per treatment were excised, the height and node length measured and shoots dried at 70 °C for 48 h and weighed. Roots were removed from the soil and a random sample per pot was excised. Roots were stained with Trypan Blue and AM colonization rate assessed by the magnified intersections method (McGonigle *et al.*, 1990). Three slides per pot were prepared as a representative root sample, and a minimum of 200 intersections per sample were evaluated. Data were analysed by ANOVA with the MSTAT-C (version 1.42; Michigan State University) statistical package in all the stages of the experiment. The observations followed a normal distribution confirmed by Shapiro–Wilk's W-test (Shapiro *et al.*, 1968) and homogeneity of variances was confirmed by Levene's test (Conover *et al.*, 1981).

Stage 1 was analysed as a randomized complete block experimental design with two factors: soil disturbance (two levels: disturbed and undisturbed soil) and wheat growth cycles (the five cycles of wheat growth). Stage 3 of the experiment was also analysed as a randomized complete block design with two factors: soil disturbance (two levels: disturbed and undisturbed soil) and wheat growth periods (10, 21 and 35 days). When the *F*-test indicated that there were significant differences between treatments, means were compared using the Student–Newman–Keul's test ($P = 0.05$).

Results

In Experiment 1, differences in plant dry weight between disturbed and undisturbed treatment were significant ($P \leq 0.05$) only after the fourth cycle (Figure 2). These differences were consistent with a differential AM colonization rate being measured between the two treatments (Figure 3), with greater colonization taking place in the undisturbed soil treatment, despite the total number of fungal spores present in the soil at the end of fourth cycle being larger in the disturbed treatment (Figure 4).

In Stage 1 of Experiment 2, wheat shoots from the undisturbed treatment achieved a significantly greater dry matter production than observed in plants from the disturbed treatment only at the end of the fifth cycle after Zn deficiency had been rectified (Figure 5). Based on the results from Experiment 1, it was reasonable to anticipate that a differential mycorrhizal inoculum potential had been

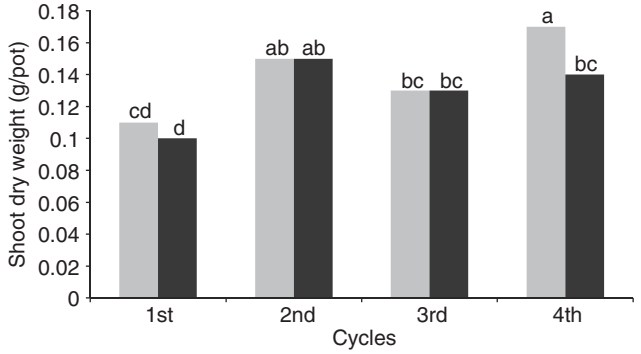

Figure 2 Effect of soil disturbance on shoot dry weight (g/pot) of wheat over four growth cycles in Experiment 1. Bars in each pair with the same letter are not significantly different at $P = 0.05$. Grey bars – undisturbed treatment; black bars – disturbed treatment.

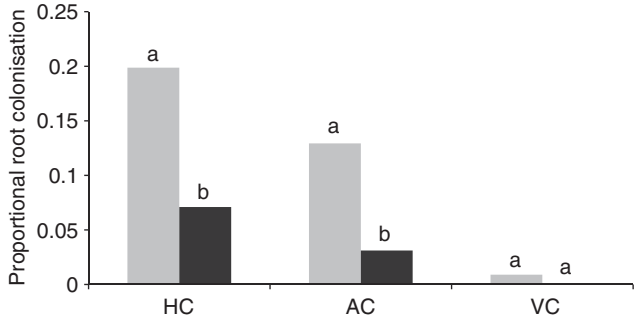

Figure 3 Effect of soil disturbance on AM colonization parameters for roots of wheat after four growth cycles in Experiment 1. HC, hyphal colonisation; AC, arbuscular colonisation; VC, vesicular colonisation. Bars in each pair with the same letter are not significantly different at $P = 0.05$. Grey bars – undisturbed treatment; black bars – disturbed treatment.

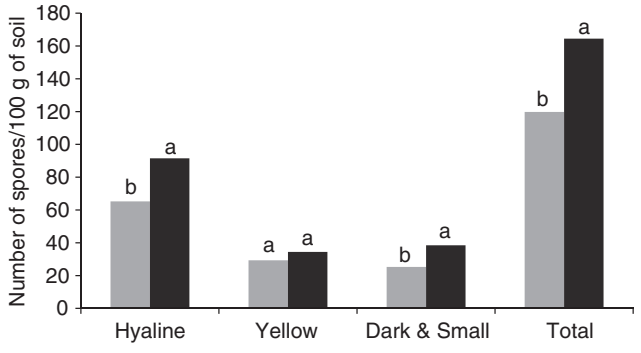

Figure 4 Effect of soil disturbance on the number of spores present in 100 g soil after four growth cycles in Experiment 1. Bars in each pair with the same letter are not significantly different at $P = 0.05$. Grey bars – undisturbed treatment; black bars – disturbed treatment.

established in Experiment 2 between the disturbed and undisturbed treatment with more being present in the undisturbed soil.

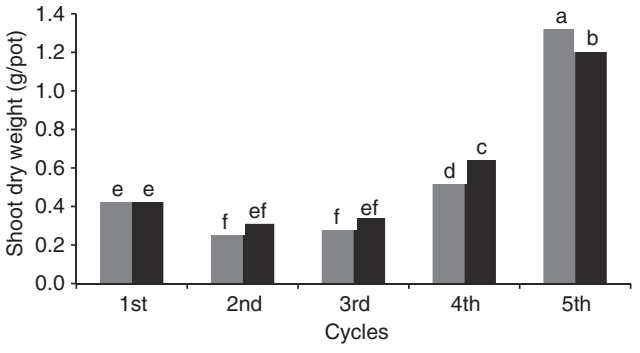

Figure 5 Shoot dry weight at the end of each cropping cycle in Stage 1 of Experiment 2. Bars with the same letter are not significantly different at $P = 0.05$. Grey bars – undisturbed treatment; black bars – disturbed treatment.

For the wheat grown after summer (Stage 3), the values for AM colonization parameters after 10 days were very small showing that colonization was still incipient. Differences between soil disturbance treatments in hyphal and arbuscular colonization were not significant at $P \leq 0.05$ (Table 2). However, by 21 days after planting, hyphal and arbuscular colonization in plants from undisturbed soil treatment were significantly greater that those from the disturbed soil and even 35 days after planting they were still significantly different. However, there was no significant difference in shoot dry matter at any of the three dates when the wheat was sampled during Stage 3 of the experiment.

Discussion

For wheat, four cycles of plant growth in disturbed and undisturbed soil should normally be sufficient to establish a differential AM inoculum primarily through the development of extraradical mycelium (Figure 3, Experiment 1). This is consistent with the number of cycles required for maize (Fairchild & Miller, 1988; Goss & de Varennes, 2002). Credibility for the role of the extraradical mycelium in the formation of the differential AM inoculum potential was obtained from the observation that the total number of fungal spores present in the soil at the end of the fourth cycle was greater in the disturbed treatment (Figure 4). Root colonization by hyphae was only 20% in the higher of the two levels of inoculum potential, and the largest value of arbuscular colonization was only 12%. Nevertheless, despite the rather small values for colonization there was a significant impact on plant growth (Figure 2). At the end of Stage 1 of Experiment 2, a significant difference in wheat growth was already established between disturbance treatments (Figure 5), indicating that a differential AM colonization of the wheat had been established at this time. From both experiments (1 and 2), there were clear indications that when AM colonization rate was enhanced (undisturbed/no-till treatment), the wheat growth was greater.

Table 2 Effect of soil disturbance during Stage 1 of Experiment 2 on shoot dry weight, height, and node length, and arbuscular and hyphal colonisation of roots in wheat, between 10 and 35 days after planting in Stage 3

		Plant parameters			Proportional AM colonisation	
Previous soil treatment	Days after emergence	Shoot height (cm/plant)	Node length (cm/plant)	Shoot dry weight (g/pot)	Hyphal colonization	Arbuscular colonization
Undisturbed	10	20.92c	4.25c	0.11b	0.10c	0.04d
Disturbed		20.53c	4.08c	0.09b	0.07c	0.02d
Undisturbed	21	32.85b	6.22b	0.29b	0.19b	0.11c
Disturbed		31.17b	5.75b	0.26b	0.10c	0.06d
Undisturbed	35	38.41a	8.57a	1.55a	0.29a	0.21a
Disturbed		40.12a	8.98a	1.63a	0.21b	0.15b
SE		0.715	0.224	0.065	0.019	0.011
CV (%)		6.17	9.39	26.03	32.72	30.26

Wheat was grown after the soil was exposed to summer weather conditions. Values with the same letter within columns are not significantly different from each other ($P = 0.05$).

After the soil had been through the Mediterranean hot and dry summer condition, when maximum temperatures ranged from 24.7 to 40.8 °C and matric potential can fall below −28 MPa (Santos *et al*., 2007), the extraradical mycelium was obviously able to maintain infectivity. The AM colonization reflected the differences in inoculum potential established in Stage 1 of the Experiment 2 being favoured (faster and greater) where plants were grown in previously undisturbed soil. These results were consistent with the hypothesis that greater extraradical mycelium development would survive better and be more effective in initiating colonization after summer. Otherwise, the AM colonization rate in disturbed soil after Stage 3 would have been expected to equal or exceed that in the undisturbed treatment given the greater number of spores found in the disturbed treatment at the end of the fourth cycle of the Experiment 1 (Figure 4). According to results shown in Figures 2 and 5, wheat growth benefitted from greater AM colonization during the crop cycle and the extraradical mycelium developed can also benefit the next crop if no-tillage is used once the AM colonization rate in the next crop is also enhanced.

The soil used in this study showed a level of AMF diversity normally found in agricultural soils with species of the *Glomus* genus being the most numerous. Therefore, the results allow generalization of the report by Jasper *et al*. (1989) which was specific to *Acaulospora leavis* Gerd. and Trap in demonstrating that AMF can retain the ability to start new colonizations through the extraradical mycelium between one crop and the next in the following season despite considerable drying of the soil.

Inoculation is expensive and thus is likely to be limited to high value crops (Saito & Marumoto, 2002) and success in many locations is also uncertain (Schwartz *et al*., 2006). The lack of registration procedures impairs the development of reliable commercial inoculum (Gianinazzi & Vosátka, 2004) and other approaches to enhance AM activity are required. Soil disturbance associated with tillage systems promotes the disruption of extraradical mycelium and the AM colonization of new crops is mainly dependent on spore germination. According to our results, no-till is an important management technique as it keeps the extraradical mycelium intact and allows the next crop to benefit from the mycelium developed by the previous crop in the rotation. This is especially important given that the extraradical mycelium is a better source of inoculum than spores in promoting the initial AM colonization rate (Martins & Read, 1997; Goss & de Varennes, 2002).

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