

Dynamics of arbuscular mycorrhizal fungi spore populations and their viability under contrasting tillage systems in wheat at different phenological stages

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Arbuscular mycorrhizal fungi (AMF) are influenced by soil management. The use of different tillage systems affects AMF activity. AMF spores are formed in soils or roots and provide a long-term reservoir of inoculum in the field. As spores can persist in soils, they reflect the accumulated sporulation history of the respective soil and not necessarily the current symbiosis of the crop. The aim of this study was to evaluate the AMF spore population dynamics and spore viability in conventional tilled and non-tilled soils throughout the wheat growing cycle and its fallow in the warm temperate Argentine Pampas. It was found that the differences in spore abundance between both tillage systems depend on the phenological stage of the crop. In both years, at the early phenological stages of the wheat crop, spore counts were about two or three times higher in no-tillage (NT) than in conventional tillage (CT). The lower spore counts in CT at the end of the fallow and at the early crop stages could be explained by the dilution of the AMF propagules in the zone of seedling establishment by ploughing. The percentages of viable spores varied with the treatments and the sampling periods, with values ranging from 10.5% to 58.8%, and were higher in CT in all the phenological stages and significantly higher at tillering stage. Assuming that a viable spore could be newly formed, then the lower percentages of viable spores in NT may suggest a higher accumulation of old residual spores.

Keywords: conventional tillage; Glomeromycota; no-tillage; number of spores; vital stain

Introduction

The Argentine Pampas region is one of the most important cropping regions in the world and accounts for more than 90% of the national grain production, with soyabean, wheat, corn and sunflower as the main crops (Magrin et al. 2005). Limited tillage systems, especially no-till, have spread widely in recent years, currently occupying around 70% of the surface devoted to annual crops (Alvarez & Steinbach 2009; Derpsch et al. 2010).

Arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Schüßler et al. 2001), are heavily influenced by soil management. The use of different tillage systems affects AMF activity (Sieverding 1991; Curaqueo et al. 2011), AMF community structure (Jansa et al. 2003; Schalamuk et al. 2006; Schnoor et al. 2011) and other soil properties and microbial abundance (Aon, Cabello, et al. 2001; Aon, Sarena, et al. 2001). AMF are able to colonize host plants by three inoculum sources: spores, mycorrhizal roots and extraradical mycelia (Smith & Read 2008), which comprise the so-called

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propagule bank (Schalamuk & Cabello 2010). It is well known that tillage negatively affects AMF colonization (McGonigle & Miller 1996; Schalamuk et al. 2004; Kabir 2005). The most studied mechanisms by which tillage reduces AMF colonization are the destruction of the mycelial network (Alvaro-Fuentes et al. 2008) and the dilution of the AMF propagules in the zone of seedling establishment by ploughing below this depth (Kabir 2005).

AMF spores are formed by differentiation of vegetative hyphae in the soil or roots and provide a long-term reservoir of inoculum in soils. As spores can persist in field soils, they reflect the accumulated sporulation history of the respective soil and not necessarily the current symbiosis of the crop (Hijri et al. 2006). From all mycorrhizal characteristics, the number of spores has been mentioned as an early and useful indicator of the effect of tillage systems on mycorrhizal propagules in short-term experiments (Castillo et al. 2006). As in field soils, spore populations are heterogeneous in age, taxa and viability, thus assessing the viability of spores allows determining their potential as propagules (An et al. 1998). Viability can also be potentially evaluated as an indicator of changes in mycorrhizal activity. To the knowledge of the authors there are no studies on the influence of tillage on spore viability.

Some studies have shown higher spore counts in no-tilled soils than in tilled soils (Jansa et al. 2002; Schalamuk et al. 2003). However, few studies have compared the influence of tillage on spore counts at different phenological stages of a crop, including the fallow (Troeh & Loynachan 2003; Castillo et al. 2006). Most of the studies comparing AMF population and/or activity in conventional tillage (CT) and no-tillage (NT) have carried out only one sampling in the growing season (Jansa et al. 2002; Alguacil et al. 2008). However, under field conditions, sporulation patterns have been found to fluctuate with the season or host phenology (An et al. 1993). Hence, further studies on the dynamics of the number of spores throughout a crop cycle and on how it is influenced by tillage are required.

Wheat is one of the most important crops in the Argentine Pampas (Covacevich et al. 2007) and the most cultivated in winter. Under the warm temperate conditions of the Pampas region, there is one short fallow between two consecutive winter crops, which occurs from summer to autumn, with a variable presence of weeds (Poggio et al. 2004). The conditions of the fallows in Argentina notably differ from those of other agricultural regions where mycorrhizal seasonal changes have been studied (Thompson 1987; Troeh & Loynachan 2003). Understanding the effect of the fallows on the number of AMF spores and its viability is relevant for managing mycorrhizal populations (Smith & Read 2008). To the knowledge of the authors, there is no information on spore counts in the fallows under different tillage systems in the Argentine Pampas.

The aims of this study were: (i) to evaluate the AMF spore population dynamics in tilled and non-tilled soils throughout the wheat growing cycle and its fallow in the warm temperate Argentine Pampas over 2 years and (ii) to determine the viability of indigenous mycorrhizal fungal spores present in different tillage systems and a non-disturbed control (NDC) over the course of 1 year.

It was hypothesized that sporulation in field soils is linked to crop phenology and that the accumulation of spores from previous periods can influence spore count and viability determinations. Therefore, total spore counts will not reflect crop phenological changes.

Materials and methods

The field experiment was carried out at the agricultural experimental station Ingeniero Agrónomo Hirshhorn, Faculty of Agriculture, University of La Plata (Buenos Aires, Argentina). The climate is temperate and the mean annual temperature is 15.8°C, with the highest monthly mean temperature in January (24.2°C) and the lowest in July (10.5°C).

Rainfall is mainly concentrated in autumn and spring, and the mean annual rainfall is 1000 mm.

The samples were collected from a long-term field tillage experiment initiated in 1993, composed of two tillage treatments: NT and CT, autumn ploughed and disked before planting. The previous crops had been: 1993, soyabean; 1994, corn; and since 1995, wheat. AMF spore counts were carried out from 2002 to 2004, when the tillage experiment was sown with wheat (*Triticum aestivum* L.), a mycorrhizal crop plant. The wheat cultivar used was Buck Pronto. The CT plots were tilled to a depth of 25 cm. The treatments were arranged in randomized, split-plot design and replicated three times and the size of each plot was 3.8 m × 12 m. Samples were taken from plots without N and P fertilization. In the fallow, the plots were invaded by weeds, mostly *Cynodon dactylon* (L.) Pers. Details of the experimental design are described in Schalamuk et al. (2006).

From 2003, another field treatment was added called the 'NDC' on the edges of the field experiment, which had not been cultivated for more than 20 years. These plots had the following plant species: *Ammi visnaga* (L.) Lam., *Avena fatua* L., *Briza minor* L., *Cynara cardunculus* L., *C. dactylon*, *Deyeuxia viridiflavescens* (Poir.) Kunth, *Ipomea purpurea* (L.) Roth, *Lolium perenne* L., *Plantago lanceolata* L., *Vicia* sp. and *Xanthium cavanillesii* Schouw ex Didr.

The rhizospheric soil was sampled at three phenological stages of wheat, tillering, flowering and grain filling, stages Z25, Z65 and Z95, respectively, using the scale proposed by Zadoks et al. (1974), and at the end of the following fallow of both growing cycles (2002–2003 and 2003–2004), after primary tillage operations in CT treatments. Composite random (Dick et al. 1996) soil samples were collected at a depth of 5–20 cm from all phenological stages. In places where each sample was collected, five to six subsamples (250 g) from square areas of approx. 3 m² were pooled. Such composite samples were collected and stored at 4°C until processed. AMF spores were isolated from 100 g (fresh weight) soil samples using the wet-sieving and decanting method (Gerdemann & Nicolson 1963) and the supernatant was centrifuged in a sucrose gradient (Walker et al. 1982). Only apparently healthy spores were counted in a 9-cm Petri dish by direct observation under a stereomicroscope. Spore counts were reported as the number of spores per 100 g dry soil. Soil moisture content was calculated for each soil sample as per cent oven-dry weight of soil by drying at 80°C for 48 h.

The viability of AMF spores was evaluated by vital staining in samples from 2003 to 2004, following the procedure by An and Hendrix (1988). Spore suspensions were diluted 1:1 with a stock solution of 0.5 mg ml⁻¹ 3-(4,5-dimethyliazol-yl)-2,5-diphenyl-2H-tetrazolium (MTT, Merck®, Germany) and incubated for 40 h. By this method, AMF viable spores develop a red colour with the tetrazolium bromide vital stain. The viability was expressed as a percentage of viable spores. When dark-coloured spores were encountered, which are difficult to stain, viability was confirmed by breaking these spores under stereomicroscope because the content of viable spores is purple coloured, whereas that of non-viable spores is hyaline. In the case of the values given as percentages, data were subjected to an arcsine square-root transformation to ensure homogeneity of variances. Then, the differences in total spore counts and percentage of viable spores between field treatments were compared with ANOVA and Least Significant Difference (LSD) tests.

Results

The number of spores strongly depended on the phenological stage of the wheat crop and the season, with values ranging from 80 to 390 spores in 100 g of soil in CT, from 300 to 560 in NT (Figures 1 and 2) and from 280 to 600 in NDC (Figure 2).

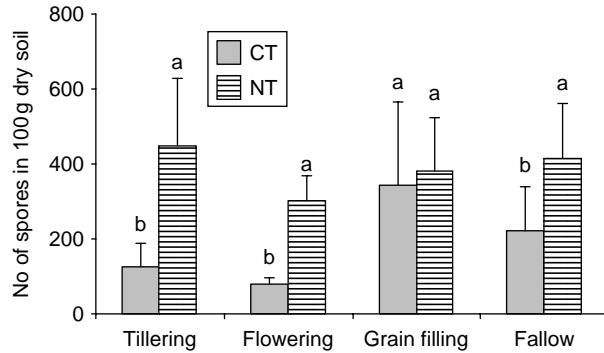


Figure 1. Number of spores in 100 g of soil as influenced by tillage at tillering, flowering, grain filling and fallow in the growing season 2002–2003 in CT and NT. Data are means of three replicates. Error bars: Standard Error. The same letter above bars indicates that the values did not differ significantly within each sampling period between management treatments as determined by LSD ($p < 0.05$).

In 2002–2003 (Figure 1) and 2003–2004 (Figure 2), there were higher spore counts ($p < 0.05$) in NT than in CT at tillering, flowering and in the fallows following the wheat crop. In 2003–2004, the NDC treatment was included. In NDC, which was not cultivated with wheat (Figure 2), similar spore counts were observed over the sampling period and an increase in autumn, when the cultivated plots were in fallow.

The percentages of viable spores in different treatments and phenological stages in the growing season 2003–2004 are shown in Table 1. At tillering, significant differences among the three treatments were observed, with the lowest values in NT and the highest in NDC. In the following phenological stages, within the cultivated plots, the percentages of viable spores were higher in CT than in NT, although differences were not significant. However, at grain filling and fallow, the percentages of viable spores in CT were significantly higher than those in NDC.

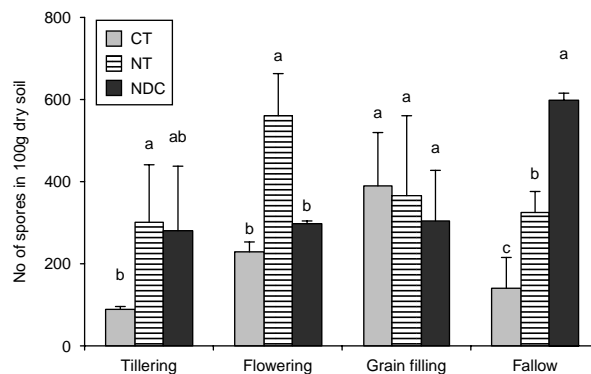


Figure 2. Number of spores in 100 g of soil as influenced by tillage at tillering, flowering, grain filling and fallow in the growing season 2003–2004, in CT, NT and in the NDC. Data are means of three replicates. Error bars: Standard Error. The same letter above bars indicates that the values did not differ significantly within each sampling period among management treatments as determined by LSD ($p < 0.05$).

Table 1. Percentage of viable spores in the different wheat phenological stages (tillering, flowering, grain filling) and fallow in the treatments: CT, NT and NDC, in the growing season 2003–2004.

| Treatments | Tillering | Flowering | Grain filling | Fallow |
|------------|-----------|-----------|---------------|---------|
| CT | 30.0 b | 52.0 a | 58.8 a | 32.9 a |
| NT | 24.3 c | 46.7 a | 40.1 ab | 17.6 ab |
| NDC | 42.1 a | 36.7 a | 32.8 b | 10.5 b |

Notes: Data are means of three replicates. Values within a column followed by the same letter are not statistically significant within each sampling period among management treatments as determined by LSD ($p < 0.05$).

Discussion

Several researchers have found higher number of spores in NT than in CT in different crops (Kabir et al. 1998; Jansa et al. 2002). Most of these studies were carried out at only one sampling in the crop growing season. In contrast, in this study, spores were sampled at different phenological stages of the wheat crop and in the following fallow and it was found that the differences in spore abundance between tillage systems depend on the phenological stage of the crop. In both years, at tillering and flowering the spore counts were higher in NT than in CT. In the fallows of both growing seasons, which were sampled after primary tillage operations, higher spore counts were found in NT than in CT.

The higher spore counts in NT than in CT in the fallows of both growing seasons can be explained by the dilution of the AMF propagules in the zone of seedling establishment by ploughing (Kabir 2005). Therefore, this could also explain the lower spore counts in CT at the end of the fallow and at the early crop stages. On the other hand, tillage enhances aeration of the soil, speeding degradation of organic matter (Aon, Sarena, et al. 2001), and hence, disappearance of old spores. The NDC treatment, which was covered with spontaneous vegetation and was not cultivated, had similar spore counts throughout the evaluation period, but spore counts increased when the cultivated treatments were in fallow (end of autumn). Agricultural systems with annual crops show two periods with marked differences: a part of the year when there is a high density of host plants, belonging to the same species and growing simultaneously, and a fallow period after harvesting, with no hosts or, in some cases, with the presence of weeds. In contrast, non-agricultural soils, which are not mechanically disturbed (similarly to NT soils), show a given diversity of host plant species of varying age and seasonality hosting AMF, and spore counts reflect the phenological stage of the diverse plant community (Vandenkoornhuyse et al. 2002). In NDC plots, plant species were found which had been mentioned as mycorrhizal (see Wang & Qiu 2006). Differences between cultivated and non-cultivated treatments could be explained by the differences in the seasonality of plants hosting AMF.

On the other hand, it was found that the percentages of viable spores were higher in CT than in NT throughout the crop cycle, with significant differences at tillering. Although the age of spores could not be determined in this study, it can be speculated that there is a relationship between the age of spores and their viability (An et al. 1998). Assuming that a viable spore could be newly formed, then the lower percentages of viable spores in NT may suggest a higher accumulation of old residual spores in this treatment, formed during the fallow or in the previous crop.

At tillering and flowering, higher spore counts were found in NT than in CT. Studies in greenhouse pot cultures have shown that new sporulation generally starts between 4 and 8 weeks after the onset of colonization (Morton et al. 1993; Schalamuk & Cabello 2010). Although colonization of roots was not measured in this study, the lack of mechanical

disturbance in NT allows the presence of an extensive AMF hyphal network, which leads to a rapid and early root colonization (McGonigle & Miller 1996; Schalamuk et al. 2004; Kabir 2005). Thus, a higher sporulation due to an earlier colonization may be expected. Therefore, the higher spore counts in NT at the early phenological stages could be the result not only of spore accumulation from previous periods, but also of a higher sporulation in NT than in CT.

In both growing cycles, spore counts did not vary from grain filling to the following fallow in NT, whereas these counts declined until the fallow in CT. Considering that the spore counts at fallow were made after the primary tillage, it can be speculated that the dilution of AMF propagules cited above (Kabir 2005) was the most important factor affecting the spore counts found at fallow in CT. On the other hand, in NT, the lack of variation in the spore count from grain filling to the end of the fallow, which is throughout the fallow, seems to contradict several studies (Thompson 1987; Harinikumar & Bagyaraj 1988; Troeh & Loynachan 2003). However, the fallow conditions vary considerably depending on the cultivation practices, the soil and weather conditions, the vegetation cover and the duration of the fallow. Therefore, the impact of a short summer fallow in the warm temperate climate of the Pampas region on AMF communities may be different from that of winter fallows covered by snow in Canada, as studied by Kabir et al. (1999) or Troeh and Loynachan (2003), or from that of fallows lasting for 1 year or longer, as studied by Harinikumar and Bagyaraj (1988) and Thompson (1987). In this experiment, the warm temperate climate, which allowed weed growth, mainly the grass *C. dactylon*, in NT could lead to the formation of new spores, as this plant species can produce high number of AMF spores (Antunes et al. 2010).

In 2003–2004, when the NDC treatment was included, we observed different spore count dynamics mostly between cultivated and non-cultivated treatments. As spore production depends on the carbon supply from the host to the fungus (Smith & Read 2008), it can be inferred that, in cultivated soil, spore formation could be linked to the host phenology and concentrated in time, whereas in non-cultivated soils the spore formation could depend on the phenology and seasonality of different host plant species (Pringle & Bever 2002).

As a conclusion, this study found higher spore counts in NT than in CT, mainly at the early stages of the crop, but with lower viability percentages. This could be related to spore accumulation from AMF activity in previous crops or in the fallow. Considering the importance of AMF populations to support sustainable cropping systems, further studies should be carried out to better understand the influence of fallow on spore counts in the following crop in warm temperate conditions such as the Argentine Pampas.

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