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Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effects on Glomeromycota diversity

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Abstract: Arbuscular mycorrhizal fungi (AMF) can use different types of propagules to colonize new roots. In this work we tested different types of AMF inocula obtained from a field experiment with tilled and no-tilled soils planted with wheat as well as from nondisturbed treatments with spontaneous vegetation. AMF trap cultures were carried out with soil, mycelium, segments of roots and wheat plants from the field as sources of inocula. Then after the senescence of the trap plants Glomeromycota species that had been established from each type of propagule in the substrate from the pots were identified. In field soils the proportions of Acaulosporaceae and Gigasporaceae were relatively similar to that of Glomeraceae, mainly in conventional tillage, whereas in all trap cultures investigated the percentages of members of the Glomeraceae family were higher than 90%. Because most of the trap cultures were based on intra- and/or extraradical mycelium our results show that members of Glomeraceae have advantages in the use of these propagules over Acaulosporaceae and Gigasporaceae species. We suggest that the higher contribution of Glomeraceae previously found in no-tillage systems could be related partially to the lack of disruption of the hyphal network and the composition of the soil propagules in this system.

Key words: agricultural practices, AMF-mycelium, *Glomeromycetes,* root fragments, trap cultures

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) propagule composition has an important effect on root colonization (Klironomos and Hart 2002). AMF, which belong to phylum Glomeromycota (Schüβler et al. 2001), can colonize roots with different sources of inoculum: spores, colonized root fragments and hyphae (Smith and Read 1997). The inoculum in soils therefore may be called a propagule bank that remains until suitable conditions to germinate, grow and eventually colonize new plant roots occur (Öpik 2004, Schalamuk 2005). Studies have shown that Glomeromycota taxa may vary in their colonization strategies and that these variations could be associated with the use of different propagule types by the main AMF families (i.e. Acaulosporaceae, Gigasporaceae and Glomeraceae) (Tommerup and Abbott 1981; Biermann and Linderman 1983; Braunberger et al. 1996; Brundrett et al. 1999; Klironomos and Hart 2002; Hart and Reader 2002, 2004). Nevertheless there is conflicting evidence on the ability of each Glomeromycota family to use each propagule type.

Both no-tillage (NT) and conventional tillage (CT) may influence the AMF propagule bank composition. Numerous studies have shown that mycorrhizal colonization is negatively affected by tillage (Douds et al. 1995; McGonigle and Miller 1996; Kabir et al. 1997, 1998; Kabir 2005; Mozafar et al. 2000; Schalamuk et al. 2003, 2004). Tillage directly affects all types of AMF propagules to a greater or lesser extent through different mechanisms acting together: (i) disruption of the hyphal network; (ii) dilution of the propagule-rich topsoil; and (iii) accelerated root decomposition (Schalamuk 2005). Tillage therefore may reduce soil mycorrhizal infectivity and thereby AM root colonization at the early stages of crop growth. The main effect of soil disturbance in the reduction of AMF propagule density is the one observed when soil tillage breaks up the AMF hyphal network (McGonigle and Miller 1996). In undisturbed soils it is expected that new infection units arise primarily from extraradical hyphae and that spores are less important. Therefore in no-tillage an intact hyphal network can be present whereas soil disturbance might result in damaged hyphae that are noninfective (Jasper et al. 1989).

Further information about the propagation of Glomeromycota is necessary to understand its diversity in soils and to design soil management practices that can promote effective and infective AMF symbiosis. Little is known about the factors affecting Glomeromycota diversity in agricultural systems. Jansa et al. (2002), in an intensively used agricultural soil under long-term reduced tillage management, found that the presence of AMF spores that do not belong to *Glomus* spp. increases. However we found that the contribution of species belonging to the Glomeraceae family increases in no-tillage plots to the detriment of

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Acaulosporaceae and Gigasporaceae (Schalamuk et al. 2006). Considering that soils under CT and NT as well as those nondisturbed by agriculture may have different propagule composition and assuming a differential use of the various types of propagules by Glomeromycota families, as many authors suggest, the different proportions of spores, mycelium and root segments in soils as a result of disturbances may affect AMF diversity.

The aim of this study was to evaluate AMF diversity obtained in trap cultures with different types of inocula extracted from different soil management systems. Because spores can be less important than hyphae as propagules in agricultural systems we used inoculum sources containing both external mycelia, which constitute the hyphal network in soil, and internal mycelia from colonized roots. The ultimate goal of this research was to relate the propagule use strategies of different Glomeromycota taxa to changes observed in arbuscular mycorrhizal soil communities with different tillage management systems (Schalamuk et al. 2006).

MATERIALS AND METHODS

Field treatment and sampling.—Material (soil, wheat plants and roots) was collected from a long-term field tillage experiment at the agricultural experimental station Ingeniero Agrónomo Hirshhorn, School of Agriculture, University of La Plata, Argentina. The field experiment consisted of two tillage treatments: no-tillage (NT) and conventional tillage (CT). The treatments were arranged in a randomized split-plot design and replicated three times. The tillage experiment was sown with wheat in 2003. Details of the experiment are described in Schalamuk et al. (2006). In the present study we added another field treatment called the ''nondisturbed control'' (NDC) on the edges of the field experiment, which had not been cultivated more than 20 y. These plant species were present: *Ammi visnaga*, *Avena fatua*, *Briza minor*, *Cynara cardunculus*, *Cynodon dactylon*, *Deyeuxia viridiflavescens*, *Ipomea purpurea*, *Lolium perenne*, *Plantago lanceolata*, *Vicia sp* and *Xanthium cavanillesii*.

All sampling was carried out in Sep 2003, when wheat plants were 50 d old. Samples were collected with a composite random, serpentine method (Dick et al. 1996). At each sample collection location we pooled 5–6 subsamples in a 3 m^2 square.

AMF spore biodiversity was evaluated by spore extraction from field soil samples. Soil and mycelium from rhizospheric soil samples from each plot also were used to start trap cultures. Wheat plants were collected for pot cultures using transplanted wheat and trap cultures using roots. Twelve months after starting the trap cultures spore extraction was carried out with the same techniques used for field soils and AMF spore diversity generated from the different inoculum sources present in each trap culture was evaluated.

Spore extraction from field soils and trap cultures.—The AMF communities in CT, NT and NDC field samples and trap cultures were studied by spore extraction. To this end 100 g soil (dry weight) of each sample was wet seived following the methodology of Gerdemann and Nicolson (1963). Samples were centrifuged in a 60% sucrose gradient (Walker et al. 1982). Spore quantification was carried out in 9 cm diam Petri dishes with a gridline of 1 cm per side under a stereoscopic microscope at 50× (Lugo and Cabello 2002).

Glomeromycota isolations with different types of propagules.—Trap cultures with soil, colonized roots, transplanted wheat plants and mycelia from both tillage treatments (CT and NT) as well as from the NDC were carried out to identify and quantify the AMF species that can be formed from different propagule types from field soils.

Trap cultures with soil.—Field soil (250 g) (containing the three types of AMF propagules, spores, mycelia and colonized roots) collected from each soil treatment was mixed with a tindalized substrate composed of perlite-vermiculite (1:1 v/v) and placed in 2 L pots. Later a gramineae and a legume were used to host AMF in cultures, following Sieverding (1991). Then *Sorghum vulgare* (sorghum) and *Medicago sativa* (alfalfa) seedlings, which had been germinated and grown in sterile sand, were transplanted into these pots. Twelve repetitions were carried out for each field treatment. Six of the pots were transplanted with sorghum and six with alfalfa with five plants per pot. Plants were grown in a greenhouse at 24 C ± 1 C d/20 C ± 1 C night and a 16 h photoperiod provided by cool-white lamps. The pots were irrigated regularly with filtered water.

Trap cultures with AMF mycelia.—AMF mycelia extracted from soil collected from NT, CT and NDC treatments were used as inoculum source in trap cultures. The technique used for extracting spores described above was carried out in soils from each field plot with a 30 μm sieve and 50 g soil. After that all spores and small root pieces were extracted from Petri dishes containing the material derived from wet sieving with a micropipette. Later the liquid containing AMF mycelia was pipetted into pots with tindalized perlite-vermiculite substrate. Then five sorghum and five alfalfa seedlings were transplanted into these pots so that the applied AMF mycelia were the only AMF propagule source for these plants. Twelve repetitions were carried out for each field treatment. Six of the pots were transplanted with sorghum and six with alfalfa.

Plants were grown in a greenhouse at 24 C ± 1 C d/20 C ± 1 C night with a 16 h photoperiod provided by cool-white lamps. In addition to irrigation with filtered water 10 mL nutrient solution (Cabello 1997) was applied to each pot weekly.

Trap cultures using roots.—Wheat plants were harvested from each field treatment 50 d after their emergence. Roots were washed, cut into 1 cm segments and surface sterilized with 5% sodium hypochloride solution, following the same criteria used to surface-sterilize spores (Wartud 1982). Root material (1–3 g) was placed in sterilized perlite-vermiculite substrate. Twelve repetitions were carried out for each field treatment. Six of the pots were transplanted with sorghum

and six with alfalfa with five plants per pot. Plants were grown in a greenhouse at 24 C ± 1 C d/20 C ± 1 C night with a 16 h photoperiod provided by cool-white lamps.

Pot cultures from transplanted field plants.—Three 50 d old wheat plants from each field treatment were transplanted, one per pot, in a tindalized perlite-vermiculite substrate. Twelve repetitions were carried out for each field treatment. Plants were grown in a greenhouse with the same conditions as those of the trap cultures mentioned above. *Plantago lanceolata* from the NDC treatment also were transplanted but did not form AMF spores.

Glomeromycota spore identification.—Spores were extracted 12 mo after starting trap and pot cultures (Gerdemann and Nicolson 1963, Walker et al. 1982) and taxonomically identified. Glomeromycota spores were mounted on slides with polyvinyl alcohol (PVA) (Omar et al. 1979) either with or without Melzer's reagent (Morton 1988). Specimens were identified with the original descriptions and reference isolates described by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. In most cases identification of the samples was assessed by the observation of morphological features of the spores obtained after sieving and decanting. These observations were corroborated with those of freshly formed AMF spores in trap cultures. Many of these species were isolated in monospecific cultures and incorporated to the germplasm bank, the AMF living culture collection at Spegazzini Institute, La Plata, Argentina.

After identifying and counting spores the relative frequency of each AMF species occurring in each sample was calculated. The percentages of each family of Glomeromycota in fields and trap cultures were arcsin-transformed and compared with ANOVA and LSD test. The abundance or relative importance expressed as percentage of each species was plotted on a logarithmic scale against species rank from most abundant to least abundant (Zak and Willig 2004).

RESULTS

A total of 21 AMF species representing genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus* and *Scutellospora* were identified from soil samples and different types of inocula used in trap cultures.

AMF families in field soil and trap cultures.—We calculated the percentages of spores belonging to Acaulosporaceae, Glomeraceae and Gigasporaceae in the soil samples and four types of trap cultures (FIG. 1). The percentages of spores belonging to Glomeraceae found in field soils were respectively 54.36, 69.9 and 79.05% in CT, NT and NDC, whereas in the four types of cultures with different inoculum type Glomeraceae percentages were above 90% in all cases. In field soils Glomeraceae proportions were higher in NT and NDC than in CT ($P < 0.05$), whereas in trap and pot cultures no significant differences were found between the field treatments.

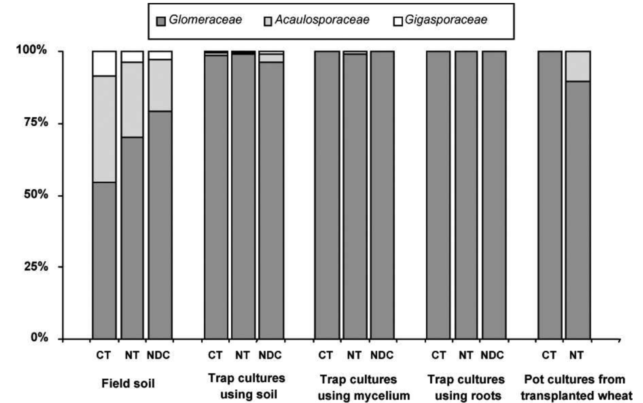

FIG. 1. Relative frequency (%) for the members of families Acaulosporaceae, Gigasporaceae and Glomeraceae in soil samples and trap cultures from each field treatment. NT: no-tillage. CT: conventional tillage. NDC: nondisturbed control.

AMF species diversity in the field soils and pot cultures.—We calculated the abundance distributions for AMF species in the different field treatments and isolation procedures (FIG. 2). In field soils species richness from the NDC treatment was higher than that from the plots with wheat. In cultivated plots more AMF species were found in CT than in NT. In trap cultures species richness decreased in this order: trap plants using soil > trap plants using mycelia > transplanted plants > trap plants using roots as source of inoculum.

Specificity in the recovery of Glomeromycota species from each type of inoculum.—Species present in the samples collected in the field and the substrate from isolation procedures tested are provided (TABLE I). Cultures with different types of AMF inocula showed distinct specificity for the recovery of Glomeromycota species. Thirteen out of the 20 species present in field soil samples were recovered from the cultures with diluted soil and 12 from the cultures with mycelia isolated by sieving. Cultures with mycelia allowed the recovery of *Glomus aggregatum*, a species that had not been found in the field samples. Cultures with roots and transplanted plants showed high specificity because three and six species were recovered respectively. In the last two treatments the recovered AMF species were present in wheat roots of 50 d old plants. *Glomus etunicatum*, *G. clarum* and *G. claroideum* were found in all types of cultures tested.

DISCUSSION

Our study shows differences between soil management practices in the contribution of Glomeromycota families. In field soils the contribution of Glomer-

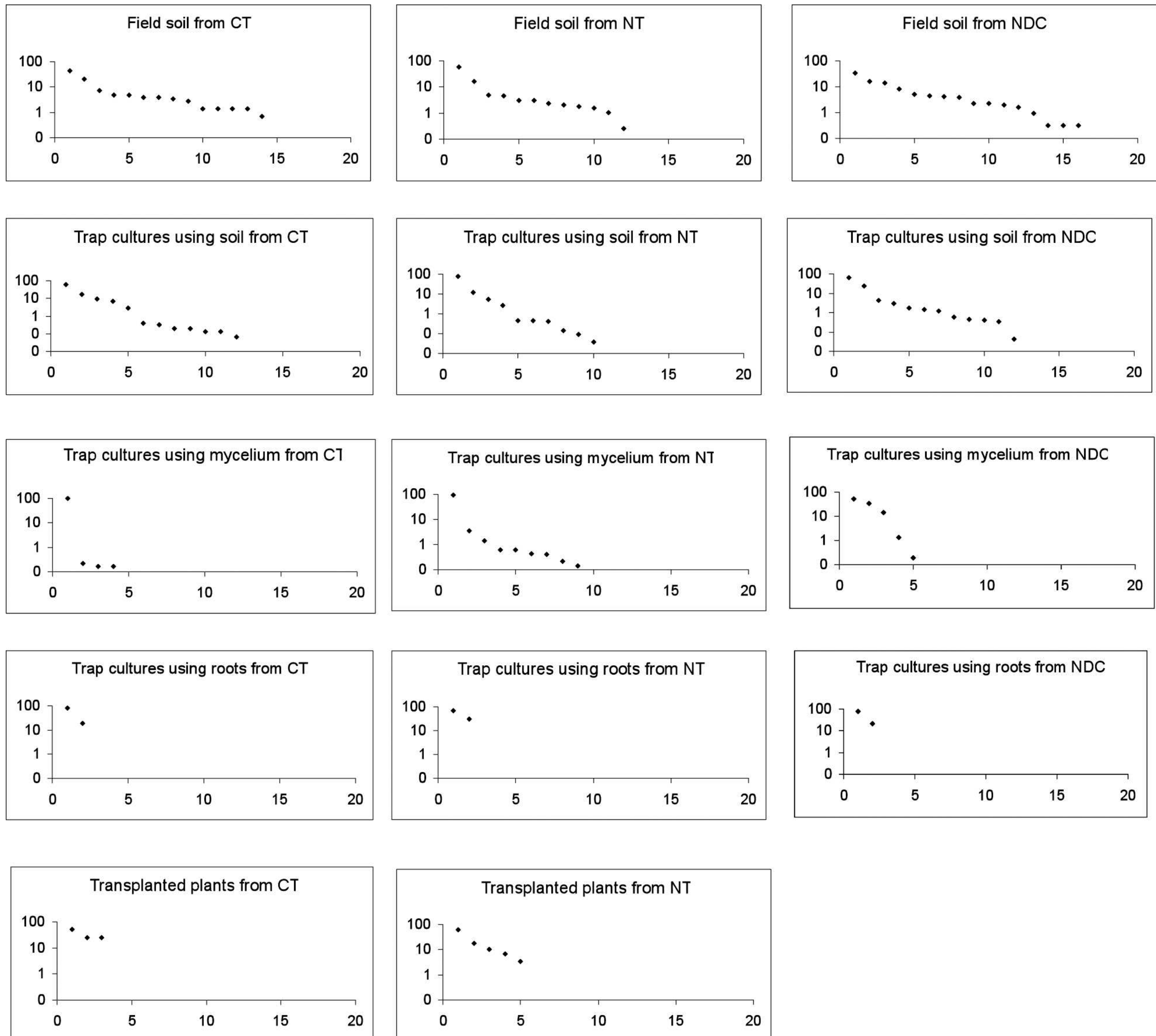

FIG. 2. Abundance distributions for fungal species in field soils and isolation from different propagule types tested. The y-axis (log scale) represents the relative importance of each species in the assemblage as a percentage. Each species subsequently was ranked from most frequent to least frequent along the x-axis.

aceae was higher in NT and NDC than in CT (FIG. 1). Proportions of Acaulosporaceae, Gigasporaceae and Glomeraceae in NT and CT were similar to those obtained in 2001 and 2002 (Schalamuk et al. 2006). As expected higher species richness was found in the nondisturbed soils (FIG. 2) because several researchers (e.g. Pringle and Bever 2002) found that various fungal species may associate preferentially with different plant species and thereby promote diversity in the plant community. Our results coincide with findings by Menéndez et al. (2001) in Argentina. However most species found in our study belonged to the Glomeraceae and the proportion of this family did not differ significantly from that of NT (FIG. 1). We speculate that in both no-tillage and nondisturbed control the lack of disturbance might allow the development of a hyphal network and therefore AMF diversity in both systems might have been influenced by the presence of intact mycelia.

In the four types of inoculum used in the trap cultures (i.e. soil, mycelia, roots and transplanted wheat) Glomeraceae were above 90%, regardless of the field treatment from which the inoculum was obtained (FIG. 1). The proportions of each family in trap cultures contrasted with those found in field soils where AMF families were much more equally distrib-

TABLE I. *Glomeromycota* species recovered from field soils and each type of propagules used. An asterisk indicates the presence of the species

Glomeromycota species	Field soil	Trap cultures with soil	Trap plants with mycelium	Trap cultures with roots	Pot cultures from transplanted wheat
Acaulospora bireticulata	*		*		
Acaulospora delicata	*	*			*
Acaulospora excavata	*	*	*		*
Acaulospora laevis	*	*	*		
Acaulospora mellea	*	*	*		
Acaulospora sp	*				
Acaulospora spinosa	*				
Entrophospora infrequens	*				
Gigaspora gigantea	*				
Gigaspora margarita	*	*			
Gigaspora sp	*				
Glomus aggregatum			*		
Glomus ambisporum	*	*	*		
Glomus claroideum	*	*	*	*	*
Glomus clarum	*	*	*	*	*
Glomus coronatum	*	*	*		*
Glomus etunicatum	*	*	*	*	*
Glomus mosseae	*	*	*		
Glomus sp	*				
Scutellospora dipapillosa	*	*	*		
Scutellospora fulgida	*	*			

uted. Therefore a modification of the inoculum source either can or cannot favor the contribution of certain Glomeromycota families.

With soils as inoculum source several Glomeromycota species were found, but most of them belonged to Glomeraceae. In several studies trap cultures with diluted soil have helped to find species that had not been observed in field samples (Miller et al. 1985, An et al. 1990, Stutz and Morton 1996, Oehl et al. 2004, Wang et al. 2008). Nevertheless in our trap cultures the number of AMF species was lower than in field soils (TABLE I) and all these species also were observed in the field, also reported by Tchabi et al. (2008). The higher proportions of Glomeraceae in these cultures coincide with the findings of Brundrett et al. (1999). Brundrett et al. (1994) and Sieverding (1991) indicated that soil-trap culture isolation procedures apparently select the most competitive AM fungi or those that can best adapt to the growth conditions of the experiment.

It is important to emphasize that the cultures using mycelia, colonized roots and transplanted wheat all were based on external and/or internal mycelia and that AMF spores were absent. In these cultures the dominance of *Glomus* species revealed a higher ability of the members of Glomeraceae to use these types of propagules, whereas Acaulosporaceae and Gigasporaceae species that had been found in field soil showed little success in their establishment from the inoculum sources tested. Brundrett et al. (1999) and Klironomos and Hart (2002) found that the ability of AMF taxa to use different types of propagules differs between genera or families. The remarkably high Glomeraceae proportions in these cultures show that the species belonging to this AMF family were not affected by the absence of spores and were able to use mycelia as a sole propagule source. Our results agree with research that has shown that spores seem to have little relevance as propagules for some species of *Glomus* (Brundrett et al. 1999).

The trap culture technique with mycelia isolated by sieving to our knowledge is a new technique carried out for the first time in this investigation. Thereby we found species belonging to Acaulosporaceae and Gigasporaceae, although the proportions of spores belonging to these families were much smaller when compared with those obtained for Glomeraceae species. The contribution of species belonging to genera *Glomus* thus was favored by the presence of mycelia and the absence of spores.

In the cultures using wheat roots and transplanted plants a low number of species and no *Acaulospora*, *Gigaspora* and *Scutellospora* species were observed, as also found by Brundrett et al. (1999) with inoculum from tropical Australia. The absence of Gigasporaceae is in accordance with other investigations that

have shown that the members of this family colonize primarily from spores (Biermann and Lindermann 1983, Hart and Reader 2004).

The species recovered in the two above-mentioned cultures, *Glomus etunicatum*, *G. clarum* and *G. claroideum*, were present in wheat roots of 50 d old plants from the field trial. Taking into account the fact that AMF species can differ in their colonization rate (Sieverding 1991) and considering the short time of the wheat plants in the field, these Glomeraceae species showed high infectivity. Spores of these species were found in the four types of trap cultures tested. These three species, along with *Glomus mosseae*, were dominant in the Glomeromycota community of the field soil from the present experiment (Schalamuk et al. 2006, 2007). Of interest Tchabi et al. (2008) found that from 59 AMF species found in Benin, Africa, only five were recovered with trap cultures using soil and that *Glomus etunicatum* and *G. claroideum* were among these species. Assuming that soil-trap culture isolation procedures select the most competitive AM fungi present in a soil (Brundrett et al. 1994, Sieverding 1991) and taking into account the findings by Tchabi et al. (2008) and our results, we consider *Glomus etunicatum*, *G. clarum* and *G. claroideum* to be highly competitive species both in field soils and trap cultures, possibly through the effective use of intra/extraradical mycelia.

Our results and previous research in the same plots (Schalamuk et al. 2006) show that AMF diversity was influenced by soil management. Glomeraceae proportions were higher in NT and NDC, where soils and the extramatrical hyphae were not disturbed by tillage. The importance of the extraradical mycelia as a propagule source in no-tillage has been largely recognized (e.g. Miller et al. 1995). On the other hand, considering the inoculum composition of the trap cultures with mycelia isolated by sieving, roots and transplanted plants, our investigation shows the ability of the species belonging to Glomeraceae to propagate by intra- and/or extraradical mycelia. There is evidence that AMF families use different strategies for colonizing new roots (e.g. Hart and Reader 2002, Allen et al. 2003), and in addition de Souza (2005) based on life history strategy studies has suggested that, in contrast to *Glomus* species, members of Gigasporaceae are ''*K*'' strategists. Hart and Reader (2004) stated that Gigasporaceae is less sensitive to soil disturbance than Glomeraceae because after disturbance some hyphal fragments lose viability due to cytoplasmic leakage, whereas spores are not greatly affected, and that Gigasporaceae colonize roots primarily from spores. Assuming a higher ability of Glomeraceae to propagate through mycelia, we could speculate that differences in the strategies of each AMF family might explain at least partially the higher proportions of the members of Glomeraceae found in untilled soils, where the disruption of the hyphal network does not occur and the mycelium is probably the most important type of propagule.

In summary in the agricultural systems here analyzed we found that the lack of disturbance favors the dominance of Glomeraceae and that mycelium is the preferential inoculum for this family, as cited by other investigations (e.g. Brundrett et al. 1999, Klironomos and Hart 2002, Hart and Reader 2004).

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