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DYNAMICS OF MYCORRHIZAL ASSOCIATION IN CORN (*Zea mays* L.): INFLUENCE OF TILLAGE AND MANURE

By
Md. Zahangir Kabir

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Natural Resource Sciences
Faculty of Agricultural and Environmental Sciences
McGill University
Montreal, Canada
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SUGGESTED SHORT TITLE

CORN MYCORRHIZAE AND SOIL MANAGEMENT

ABSTRACT

Ph. D. **Md. Zahangir Kabir** **Natural Resource Sciences**

Mycorrhizal fungi are a major component of agricultural systems and play a key role in plant nutrition. Little is known about the effects of tillage practices and manuring on arbuscular mycorrhizal fungi (AMF). The purpose of this study was to evaluate the effect of soil disturbance on winter survival, development and distribution of AMF in soil and on plant nutrient uptake and productivity. This research was conducted in long-term corn plots in two soils and under controlled conditions.

A growth chamber study with field soil demonstrated that most of the fungal hyphae with mycorrhizal plants were mycorrhizal rather than saprophytic. This result was extrapolated to subsequent experiments. Soil disturbance reduced corn nutrient uptake and growth by disrupting the AMF hyphal network. Similarly, fallow periods reduced density of AMF hyphae, leading to reduced mineral nutrients uptake and plant growth. Soil disturbance was also found to severely reduce winter survival of AMF hyphae in agricultural soil. AM hyphae could survive the winter in soil, even when they were not attached to roots. Their survival however, was improved when they remained attached to roots.

Under field conditions, indigenous AMF were more abundant in no-till soil, less abundant under reduced tillage and least abundant under conventional tillage. Under all tillage systems, most of AMF hyphae were located in the top 15 cm of the soil profile suggesting that deep plowing could result in dilution of AMF propagules in the seeding zone. There was a seasonal variation in the abundance of hyphae in soil. Soil hyphae and root colonization declined after the silking stage of corn. Hyphal abundance decreased further over the winter, to reach their lowest level in the spring.

The spatial distribution of fungal hyphae in the field was not homogenous. Hyphal density was maximal directly under the corn rows and decreased linearly up to the mid-row. Marked seasonal variations in hyphal densities were observed on the row but fluctuations at mid-row were not significant suggesting that little AMF hyphae were ever present between the rows. Liquid dairy manure had little effect on the abundance of hyphae and spores.

RÉSUMÉ

Ph. D. **Md. Zahangir Kabir** **Sciences des Ressources Naturelles**

Les champignons mycorhiziens à arbuscules (CMA) sont un élément essentiel de la durabilité des systèmes sol-plante et, de plus, il jouent un rôle important dans la nutrition des plantes. Nous savons peu de l'impact des pratiques de travail de sol et de la fertilisation organique, sur les CMA. Le but de l'étude présentée ici était de comprendre les effets du travail du sol et de la fertilisation organique sur le développement et la distribution des CMA dans le sol, et sur l'efficacité de la symbiose qu'ils forment avec le maïs grain. L'étude a été menée dans des parcelles longue durée installées depuis 12 ans sur un loam sableux St-Benoît et sur une argile Macdonald ainsi qu'en conditions controllées.

Une essai mené en chambre de croissance a d'abord servi à démontrer que la majorité des hyphes rencontrées dans le sol de la zone des racines de plantes mycorhizées appartiennent à des CMA plutot qu'à des champignons saprophytes. En extrayant et en mesurant la quantité d'hyphes recouvrée de sol ayant reçu différents traitements de boulversement mécanique, il a pu être constaté que la réduction de l'absorption de P par le maïs qui suit le bouleversement du sol, est liée au bris du réseau mycélien de CMA. Des périodes de jachère ont aussi réduit la viabilité des hyphes et leur capacité à produire de nouvelles infections et, conséquemment, ont mené à la dégradation de la nutrition et de la performance du maïs cultivé après la jachère. Au champ, les CMA sont plus abondants sous un système de production en semis direct, moyennement abondant en travail de sol réduit tandis que c'est en condition de travail conventionnel du sol que la densité de ces champignons est la plus faible. Dans tous les systèmes de travail du sol, la majorité des hyphes et des spores de CMA se retrouvent dans les premiers 15 centimètres du sol, ce qui suggère qu'un labour plus profond dilue la quatité de propagules dans un plus grand volume de sol, réduisant ainsi le potentiel mycorhizien du sol le printemps suivant. La fertilisation organique n'a pas eu d'effet différent de la fertilisation minérale.

La quantité d'hyphes de CMA dans le sol, varie avec la saison. Après le stade de

sortie des croix du maïs, la densité d'hyphes extraradiculaires et la colonisation radiculaire de la plante décroissent jusqu'à la récolte. Au cours de l'hiver, la densité des hyphes métaboliquement active ou non continue de décroitre et atteind son plus bas niveau au printemps où la venue de la cullture de maïs suivante augmentera à nouveau l'abondance des CMA jusqu'à la sortie des croix. Bien que le labour réduise la quantité d'hyphes viables dans le sol au printemps, à mesure que la saison progresse, la différence entre la densité des hyphes en sol labouré et en semis direct se rétrécie pour disparaître, la pluspart du temps, à la floraison du maïs.

La densité des hyphes de CMA varie aussi dans l'espace. Elle est plus élevée sur le rang et décroit avec la distance jusque dans l'entre rang. Les fluctuations saisonnières de la densité des hyphes extraradiculaires du sol prélevées directement sous le rang étaient marquées, tandis qu'aucune variation significative dans la densité des hyphes n'était notée dans le sol de l'entre rang, ce qui suggère qu'aucune qu'une très faible proportion des hyphes prélevées de l'entre rang proviennent de CMA.

Cette étude a aussi démontré la capacité des hyphes de CMA de survivre à l'hiver en l'absence de liens à une racine-mère, bien que la survie de ces hyphes ait été supérieure lorsque celles-ci demeuraient attachées aux racines.

PREFACE

The dissertation consists of six chapters preceded by a general introduction and a literature review and followed by a general conclusion section. Chapter I is a general introduction and literature review which establishes the research context and exposes the hypotheses and objectives of the study. Chapters II, III, IV, V, VI and VII constitute the main body of the thesis. Each of these chapters contains material which has been or will be published in refereed scientific journals and their format are conform to the specifications of the journals. This dissertation is in accordance with the guidelines for thesis preparation as published by the faculty of Graduate Studies and Research of McGill University. The following statements excerpted from the guidelines for thesis preparation:

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted or to be submitted for publication, or the clearly-duplicated text of one or more published papers. These texts must be bound as an integral part of the thesis.

If the option is chosen, ***connecting texts that provide logical bridges between the different papers are mandatory.***

The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". ***The thesis must include:*** *A Table of Contents, an abstract in English and French, an introduction which clearly states the rational and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.*

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, ***the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent.*** *Supervisors must attest to the accuracy of such statements at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all the authors of the co-authored papers.*

The first manuscript (Chapter II) entitled "*The proliferation of fungal hyphae in soils supporting mycorrhizal and non-mycorrhizal plants*", was accepted for publication by *Mycorrhiza* on September 25th, 1996, and is currently *in Press.* The second manuscript (Chapter III), entitled "*The effects of time on the viability and infectivity of extraradical hyphae of arbuscular mycorrhizal fungi detached from host plant root system*", was submitted to the editorial staff of *Soil Biology & Biochemistry* in September, 1996 and is currently *being reviewed.* The third manuscript (Chapter IV) entitled "*Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization*", was submitted to *Plant and Soil* in October, 1996. The fourth manuscript (Chapter V) entitled, "*Dynamics of the mycorrhizal symbiosis of corn: effect of host physiology, tillage practice and fertilization on spatial distribution of extraradical hyphae in the field*", was submitted to *Agricultural Ecosystems & Environment* in December, 1996 and is currently being reviewed. The fifth manuscript (chapter VI), entitled "*Vertical distribution of arbuscular mycorrhizal fungi under continuous corn in long-term no-till and conventional tillage system*", was submitted to Mycorrhiza in January, 1997 and is currently *being reviewed.* The sixth manuscript (chapter VII), entitled " Overwintering of arbuscular mycorrhizal fungi is reduced by detachment from corn (*Zea mays* L.) roots or from the mycelial web", was accepted for publication by *Mycorrhiza* on September, 1996.

The candidate is first author on all of these manuscripts. His former supervisor Dr. I. P. O'Halloran, currently in the department of Land Resource Sciences, University of Guelph, Guelph, Ontario, provided long distance supervisory guidance and research funds and is co-author of all of the manuscripts. Dr. J. W. Fyles, is co-author of articles included in chapter IV and V for his advice and review of the manuscripts. Dr. C. Hamel, is co-author

to all articles. She helped through fruitfully discussing the results with me and reviewed the manuscripts. Furthermore, Dr. P. Widden is co-author of the article included in chapter V for his technical assistance and critical reviewing of the manuscript.

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Finally, I would like to dedicate this thesis to my wife Nasreen Zahan for her understanding, patience, continuous support, love and to our son Ryan lost moments which will never be recaptured.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION

The arbuscular mycorrhizal symbiosis is a fundamental mutualistic association between fungi and plant roots. Four hundred million years ago, the advent of this symbiosis gave plants the ability to escape their aquatic environment and colonize the continents (Pirozynski and Dalpé, 1992). Since then, this association has been the most widespread plant symbiosis on earth (Taylor et al., 1995). It is found in very diverse ecosystems ranging from aquatic to arid environments, and from lowlands to high altitudes (Allen, 1991). It occurs from temperate to tropical areas (Bagyaraj, 1991). This symbiosis is formed by a wide variety of plants, including most field crops such as corn.

The fungal component of these associations obtains carbohydrates from the plants while the plant often gains several benefits including facilitated phosphorus uptake which results in improved plant growth (Cooper, 1984; Evans and Miller 1988, 1990; Jakobsen et al., 1992; Jasper et al.,1989a; Kothari et al., 1990; Li et al., 1991a; O'Halloran et al., 1986;). Decades of P application on corn monoculture, the dominant crop in the St-Lawrence valley, have increased soil P content to the extent that watersheds are threatened by eutrophication, and hence fertilizer P management is of growing concern. Increasing P uptake by corn through optimization of arbuscular mycorrhizal symbiosis would allow the maintenance of acceptable grain yield while reducing fertilizer P application.

The development of arbuscular mycorrhizal fungi (AMF) depends on several factors. Phosphorus levels in soils and in plants regulate the extent of AMF development (Abbott and Robson, 1991) and plants deficient in P tend to support more extensive AMF mycelium than plants with adequate P levels (Li et al., 1991b). The amount of mycorrhizal inoculum in soil influences the speed of colonization of crops in the spring. Crop rotations which include non-mycorrhizal plants or fallow periods reduce soil AMF propagule numbers which, in turn, can reduce colonization of subsequent mycorrhizal plants in the rotation.

Soil disturbance has a negative influence on both the formation and efficiency of the AM symbiosis (Miller et al., 1995; O'Halloran et al., 1986). The hyphal network established in the soils seems to be perennial under no-till systems, and survive after the crop plants have

died. This network quickly reestablishes functional associations with emerging crop plants the following spring (McGonigle et al., 1990). Soil disturbance destroys this hyphal network through which seedlings could otherwise derive immediate benefits. If disturbance is severe, the viability of the hyphae and the infective potential of the soil can be reduced (Jasper et al. 1989b).

In many pot studies on the impact of soil disturbance on AMF (Evans and Miller, 1988, 1990; Jasper et al. 1989a, 1989b), host plants were harvested, the soil was disturbed and seeds were sown immediately. Under these conditions, the effects of soil disturbance on subsequent plant root colonization by AMF varied from decreased colonization (Jasper et al., 1989a, 1989b) to no significant effect (McGonigle et al., 1990a). Under agricultural field conditions, the length of time elapsed between two successive crops and winter conditions are additional stresses which are likely to add to the impact of fall tillage operations on AMF vigour in spring. Although there have been studies indicating that some mycorrhizal fungi are capable of a certain level of saprophytic growth after the death of their host plant (Hepper & Warner, 1983; Tommerup & Abbott, 1981), questions remain concerning how long the hyphae stay viable in the absence of a living host plant, and how soil disturbance may affect the survival of these AMF hyphae. The disruption of AMF hyphae by fall plowing could weaken the fungi and reduce their ability to survive over the winter in agricultural soils. It is uncertain if the effect of plowing on soil mycorrhizal potential results from both disruption of AMF hyphae and from detachment of these biotrophic fungi from their food source, the root.

The AMF hyphae, which serve as a root extension that increases the volume of soil exploited by the host plant, are the major components of mycorrhizal efficiency regarding nutrient uptake. Nevertheless, the influence of crop management on the symbiosis has mainly been inferred from spore counts or from internal root colonization. Knowledge of the dynamics of mycelia development in time and space, under different production conditions would help in designing management practices which will optimize the efficiency of the AMF symbiosis in subsequent corn productions.

The vertical distribution of AMF in agricultural soils may vary with the influence of management practices and crop development. Spores of AMF are known to be generally more abundant in surface soil (An et al., 1990) but the vertical distribution of other propagules such as extraradical hyphae in the soil profile is largely unknown and so is the spatial distribution of AMF associated with row crops. The influence of tillage practices and manure application on mycorrhizal propagule number (i.e. extraradical hyphae and spores) and distribution in the soil is of considerable importance if this fungus will be considered a component of sustainable agricultural production.

The present studies address several issues concerning mycorrhizae, i.e., their distribution, effects of soil disturbance and winter survival in field conditions. The approach used was to describe the abundance and distribution of AMF hyphae in time and space and to relate these observations to corn growth and nutrition. The imposition of soil disturbance or tillage treatments created variability in the study systems allow the definition of the role and impact of the variables acting on the dynamics of field-grown mycorrhizal corn.

2. LITERATURE REVIEW

2.1. Arbuscular mycorrhizal fungi

Mycorrhiza is made of two words, mykès (greek) for fungi and rhiza (latin) for root. Arbuscular mycorrhizal fungi (AMF) derived their name from the arbuscules they formed inside the roots of their host plants. The relationship between fungus and host plant is a 'symbiosis' a beneficial association for the survival, reproduction and growth of both partners. In this process, fungus receives sugar from the plant while providing mineral nutrients to its host-plant. The intraradical phase of the fungi is restricted to the root cortex and the outside of the roots where fungal hyphae proliferate extending several centimetres into the soil (Camel et al., 1991; Li et al., 1991a). This increases the volume of soil exploited by plant root systems. Although other benefits such as protection against root disease organism (Torres-Barragán et al., 1996), improved soil structure (Bethlenfalvay, 1992), improve revegetation to polluted soil (Gildon and Tinker, 1981) and water use efficiency (Nelson, 1987; Nelson & Safir, 1982) are derived from the presence of AMF. However, the better exploitation of the soil volume by mycorrhizae is considered to be the major contribution of AMF to plant growth (Marschner, 1995) because of the consequent reduction in diffusion distance of sparingly mobile nutrients in soil, such as P (Bolan, 1991).

Arbuscular mycorrhizal fungi have been observed in about 200 families of plants representing 1000 genera and 300,000 plant species (Bagyaraj, 1991). These fungi belong to the class of Zygomycetes and form the order Glomales Morton & Benny. The order Glomales is divided into two suborders, namely the *Glomineae* Morton & Benny, forming arbuscules and vesicles, and the *Gigasporineae* Morton & Benny, forming only arbuscules (Morton and Benny, 1990). The *Glomineae* has two families, the *Glomaceae* Pirozynski & Dalpé and the *Acaulosporaceae* Morton & Benny. Each of them contains two genera namely, *Glomus* Tulasne & Tulasne and *Sclerocystis* Berkeley & Broome, and *Acaulospora* Gerd. & Trappe and *Entrophosphora* Ames & Schneider, respectively . The *Gigasporineae* has one family, the *Gigasporaceae* Gerd. and Trappe, with two genera, *Gigaspora* Gerd. & Trappe and *Scutellospora* Walker & Sanders (Bentivenga and Morton, 1994). The *Glomales* comprise

more than 150 species (Morton and Benny, 1990), all potentially able to colonize every mycorrhizal plant species.

2.2. Mycorrhizal root colonization

Communication between AMF and the host plant is an essential process in order for the organisms to recognize each other. In *Rhizobium* some flavonoids are inducers of nod genes, chemo-attractants and growth stimulants (Phillips, 1992). It has been suggested that flavonoids or other phenolic compounds could also influence AMF symbiosis formation and stimulate AMF growth (Bécard et al., 1992, 1995; Chabot et al., 1992; Giovannetti et al., 1996; Kape et al., 1992). The isoflavone formononetin, a secondary plant metabolite, was shown to increase mycorrhizal colonization at early stage of the symbiosis (Faries et al., 1996; Siqueira et al., 1991).

The proximity of the host plants root system to AMF induces branching in hyphae and penetration into the root system. The branches formed are narrower than the original hyphae (20-30 μm) (Bonfante-Fasolo, 1984). Penetration starts by the formation of appressoria by these hyphae which then make their entry into the roots. The mechanism involved in penetration through the root cell wall is unclear but it has been suggested that hydrolytic enzymes could be involved. Once the hyphae are inside a cell, intraradical hyphae remain narrow. The hyphae branch profusely in the inner cortex and grow both through and between the cortex cells. Inside of cells, these hyphae can branch repeatedly giving rise to the tree-like structures called arbuscules (Bonfante-Fasolo, 1984) offering a large surface area for contact between the fungus and the plant. The arbuscules are thought to be the preferential site for fungus/plant metabolic exchanges (Barea, 1991; Scannerini and Bonfante-Fasolo, 1983). Once formed, an arbuscule lasts 4-10 days, before being digested by the plant cell (Paul and Clark, 1989). When the internal colonization process is well established the AMF species of the *Glomineae* form vesicles which are terminal swellings of intraradical hyphae. These vesicles form between the cortical cells and occasionally within the cortical cells, serving as a storage organ and possibly as propagules (Biermann and Linderman, 1983).

Root mycorrhizal development usually follows three phases. First, during the lag phase, primary infection occurs. An abundance of fungal propagules shortens the lag phase, while high plant and soil P content increase it (Abbott and Robson, 1991). Secondly, rapid invasion of roots occurs due to the spread of increasingly abundant mycelium. Thirdly, colonization level reaches a plateau. Land and Schönbeck (1991) noted that mycorrhizal colonization increased rapidly until ear emergence of winter barley and then decreased until harvest. Similarly, Abbott and Robson (1991) reported a quick increase in the percentage of colonization of annual crops, concurrent with root elongation, followed by a decline until root senescence. Perennial plants present a different colonization pattern with little seasonal variation in mycorrhizal root colony development (Brundrett and Kendrick, 1988).

The intensity of mycorrhizal colonization varies considerably within a root system. Smith and Walker (1981) stated that the frequency of entry points was high close to the root tips. In *Trifolium*, the root tip was penetrated 10 times more frequently than the average for the whole root system. Lateral roots are more infected than first order roots, which further indicates that young tissues are preferred colonization sites (Mosse and Hepper, 1975). The data of Walker and Smith (1984) showed a marked decline in new colonization after 14 days in main roots of clover, but no such trend with lateral roots.

The spread of individual infection points in the root can vary with the fungus, the root and with plant or soil nutritional status. Schenck and Smith (1982) reported different colonization levels in soybean inoculated with six AMF species. It has been observed by several researchers that greater responses to mycorrhizal colonization occurred for coarse-rooted plant species than with fine-rooted species (Crush, 1973 and Hall, 1977). St. John (1980) proposed that coarse-rooted plants were physiologically dependent on mycorrhizae for nutrient uptake, whereas fine-rooted plants rely on their more evolved root architecture for absorptive functions. The C-4 plants are more photosynthetically efficient than the C-3 plants, and seem to be more favorable to AMF because of their higher carbon fixation ability (Hayman, 1983). Buwalda et al. (1982) reported that while increasing the levels of soil phosphate to 30 mg kg^{-1} had a slight negative effect on the AMF colonization of leek, it drastically reduced that of wheat. McGonigle et al. (1990a) observed that the addition of

phosphorus up to 400 kg ha^{-1} had no significant effect on arbuscular or vesicular colonization of corn roots.

2. 3. The extraradical hyphae of AMF

Once internal root colonization is established, the so called extraradical hyphae proliferate externally. These extraradical hyphae grow along the root surface, producing secondary infections and spreading into the surrounding soil. This increases the volume of soil exploited by the roots. These hyphae often form a fundamental bridge between host plants and soil, and translocate soil nutrients to plant, but are also a major sink for plant carbon. In addition, extraradical hyphae play an essential role in soil stabilization (Bethlenfalvay, 1992; Thomas et al., 1993).

The diameter of the hyphae ranges from 1-20 μm (Friese and Allen, 1991; Sieverding, 1991). Friese and Allen (1991) observed two types of extraradical hyphae: runner hyphae with angular projections originating from roots and growing out into the surrounding soil. Runner hyphae have thick walls (1-3 μm) and a diameter of 20 μm. The second type, absorptive hyphae are produced by the repeated branching of runner hyphae. They have short-lived (5-7 days) but efficient organs and the branching of these hyphae leads to the proportional decrease in their diameter. Hyphal diameter varies within mycelium and also varies widely from one species to another (Dodds, 1994).

In general, the diameter of absorptive hyphae are about 100 times smaller than the diameter of plant roots. The relative surface to volume ratio of the hyphae gives them a high absorptive efficiency (Dodds, 1994). Due to their increased surface for absorption and increased structural efficiency, mycorrhizae have more absorptive capacity than roots alone (Bagyaraj, 1991). Furthermore, the fine size of hyphae permit their penetration into soil pores that cannot be entered by roots (Bolan, 1991).

Extraradical hyphal density can be very high close to the root, but decreases exponentially with distance from roots (Green et al., 1994; Jakobsen et al., 1992). In plants with well established infection, each centimeter of root may have an associated 0.5-1.5 m of hyphae (Harley, 1989). Using compartmentalized containers, Li et al. (1991a) observed that

AMF could extend up to 11.7 cm in 49 days in a white clover root-free zone of soil. They further mentioned that hyphal density gradually decreased from 6 m cm^{-3} to 3.7 m cm^{-3} at 6.5 cm from the root compartment, and then increased again to about 5.5 m cm^{-3} at 11.7 cm distance. Similar results were obtained by Kothari et al., (1990) with corn, in that hyphal length gradually decreased from 4 m g^{-1} at 0-5 mm distance from the root compartment to 2 m g^{-1} at 15-25 mm distance. However, they did not observed further increase of the hyphal length at larger distances from the roots.

Density of AM fungal hyphae in soil vary greatly ranging from less than 1 m to more than 26 m g^{-1} of soil (Abbott et al., 1984; Schubert et al., 1987; Sylvia, 1986; Sylvia, 1988). Allen and Allen (1986) recorded 54 m of extraradical mycelium per gram of rhizosphere soil. Fitter (1985) reported that densities of mycorrhizal hyphae were large in sterilized soil probably as a result of reduced competition with other soil organisms, elimination of AM fungal grazers and mycopathogens.

2.4. Improvement of nutrient uptake by AMF

2.4.1. Phosphorus : The most dramatic effect of mycorrhizal root colonization is an increase in P absorption by the host plant (Koide, 1991). Phosphorus is found in very low concentrations (0.05-0.3 μg P mL^{-1}, Ozanne, 1980) in the soil solution and it is very easily fixed on soil minerals (Lambert et al., 1994). Movement of nutrients to the root occurs by three processes namely: root interception, mass flow and diffusion (Tisdale et al., 1985). Phosphorus moves to the root mostly through diffusion (Anderson et al., 1987; Koide, 1991). The rate of diffusion of phosphate ions in soil is very low (10^{-11} to 10^{-8} cm^{-2} s^{-1}) (Barber, 1984). Sanders and Tinker (1973) concluded that the extensive proliferation of AMF hyphae (Jakobsen et al., 1994) effectively reduces the distance of P diffusion and thereby facilitate P uptake by plants. The hyphae of AMF fungi allow for the exploitation of a larger volume of soil than would be exploited by roots alone. Bolan (1991) reported that the process of P uptake by AMF involves chemical modification through release of organic acids such as citrate or oxalate, which help in the desorption of chemically sorbed phosphate ions or in the dissolution of poorly soluble Ca-phosphate compounds. It seems, therefore, that AMF roots

could access absorbed P from sources not available to non-mycorrhizal roots (Sanders and Tinker, 1973).

Phosphorus uptake by mycorrhizal plants has been studied (Li et al., 1991b; Smith et al., 1985; Son and Smith, 1988) and it has been estimated that approximately 3.8×10^{-8} mole of P cm^{-2} s^{-1} pass through hyphae at the entry point (Sanders and Tinker, 1973). Stribley (1987) reported that mycorrhizal onion plants grew much better than non-mycorrhizal onions when plants were subjected to drought. The authors concluded that mycorrhizae can maintain a better P supply when the rate of diffusion of this element is markedly restricted by water shortage. Likewise, Gerdemann (1968) noted in a P-diffusion-limited soil, AMF increased P uptake by 60-folds while in a soil with good P diffusion, increased it by 10-fold.

The transfer of P from soil to the root through AMF follows three steps: i) active transport into the fungus outside the root, ii) passive transport from fungus at fungus-root interface, and iii) active transport into root. Inside the root cortex, P is thought to be transferred from the fungus to the host plant at the level of the arbuscules (Schwab et al., 1991). The concentration of inorganic P inside the hyphae is approximately 1000 times higher than that in the soil solution (Gianinazzi-Pearson and Gianinazzi, 1986). Uptake of P by AMF hyphae occurs against a concentration gradient and, therefore, involves energy expenditures.

The high surface to volume ratio of AMF hyphae compared to roots makes mycorrhizae more efficient for P uptake (Pang and Paul, 1982). Karunaratne et al. (1986) concluded that mycorrhizal roots can remove P from soil solutions having lower P concentration than non mycorrhizal roots. Li et al. (1991a) reported that P inflow rate per unit of mycorrhizal hyphae was in the range of $3.3\text{-}4.3 \times 10^{-15}$ mole cm^{-1} s^{-1}. Estimated rate of P transfer at the arbuscules is 1.3×10^{-4} mole mm^{-2} s^{-1}, which adequately meets plant requirements (Sanders and Tinker, 1973).

2.4.2. Other nutrients : Zinc and Cu in the soil move largely by diffusion and their diffusion coefficients are also low (O'Keefe and Sylvia, 1991). Faber et al. (1990) and Lambert et al. (1984) observed that mycorrhizal corn had greater Zn concentration and grew better than non mycorrhizal corn. Manjunath and Habte (1988) also reported a role for AMF in increasing the concentrations of Cu and Zn in *Leucaena leucocephala*. Using ^{65}Zn,

Swaminathan and Verma (1979) reported that mycorrhizal potato, wheat and corn utilized the same pool of soil Zn than non-mycorrhizal plants of the same species. High levels of P fertilizers can induce Zn deficiency in plants (Tisdale et al., 1985) concurrent with low mycorrhizal colonization. It seems that the improved uptake of Zn can at least, be attributed to the reduction of the distance for Zn diffusion to the extensive mycorrhizal root systems in soil, rather than to the provision of Zn from source not accessible to non- mycorrhizal roots.

Interactions between AMF and soil nutrients other than P, Zn and Cu have been reported but they are either controversial or not sufficiently substantiated. For example, Pacovsky (1986) demonstrated that AMF increased Zn and Cu concentrations in plants, but decreased those of Mn and Fe. Kucey and Janzen (1987) observed that AMF increased uptake of Fe by beans, along with P, Zn and Cu. Cress et al. (1986) reported an increase in Fe uptake due to AMF and attributed it to siderophore activity. Eivazi and Weir (1989) observed a synergistic interaction between Mo and P, resulting in a large increase in mycorrhizal corn plant dry matter.

2.5. Persistence of AMF

The persistence of AMF fungi in ecosystems depends on the formation and survival of propagules. These propagules include spores, hyphae and colonized roots. Several researchers have indicated that spores are often poorly correlated with AMF formation in soils or not correlated at all (Abbott and Robson, 1984; McGee, 1989).

The sporulation of AMF is scarce relative to that of other members of the Kingdom Fungi. However, AMF spores are thick-walled and are the largest chlamydospores known, reaching 800 μm in diameter in *Gigaspora gigantea* (Berch, 1988). These spores are filled with nutrient reserves. According to ecological principles, their individual efficiency should be quite high. These large spores are generally considered to be the propagules which ensure the survival of the species during adverse periods (Abbott and Robson, 1991; Hayman and Storeld, 1979; Kianmehr, 1981). Spore dormancy occurs in AMF. The length of the dormancy period required before germination, can vary from one AMF species to another. For example the dormancy period of *Glomus caledonium* and *G. monosporum* is

approximately 6 weeks at 5°C and -0.15 MPa, while that of *Gigaspora calospora* is 12 weeks and that of *Acaulospora laevis* lasts 24 weeks. (Tommerup, 1983).

Arbuscular mycorrhizal fungal spores can return to dormancy after germination if their germ tube does not encounter a suitable host (Mosse et al.,1981). *Gigaspora gigantea* could germinate repeatedly up to 10 times if the original germ tube was removed (Koske,1981). The spores could survive for 20 years in dry soil, and 2 years in moist soil (Tommerup 1987). Bagyaraj (1991) found that AMF spores remained viable for 2 years when stored at 45°C.

While spores of AMF are considered to be resistant structures, soil hyphae are the most important source of AMF inoculum in soils (Brundrett, 1991; Jasper et al., 1989b; McGee, 1989; Powell, 1977). Therefore, damaging the soil AMF hyphal network by tillage or other operations in agricultural fields may severely reduced the inoculum potential of these soils. It is well documented that disruption of the hyphal network reduces infectivity (Evans and Miller, 1988; McGonigle et al., 1990a; Miller et al., 1995). McGee (1987) reported that fragments of dead roots present in the soil can initiate AMF symbiosis, provided they are in close proximity to developing roots. Tommerup and Abbott (1981) observed that AMF hyphae could remain viable in dead roots long after the death of host cell and serve as infective propagules.

Significant reduction in the number of infective propagules and low levels of mycorrhizal colonization were observed after leaving the land fallow for one season preceding winter (Dodds and Jeffries, 1986, 1989), or after a long fallow (Thompson, 1987, 1994). Maximum infection level may not be reached in the crop following fallow if the lag period extends beyond a critical point. This critical point is determined by the length of the growing season and the environmental conditions. Cropping non-host plants also reduces soil mycorrhizal inoculum densities for the subsequent crops. Black and Tinker (1979) observed a reduction, of 50 % in the mycorrhizal colonization of barley when the field was left fallow or when the preceding crop was a non-mycorrhizal plant (*Brassica oleracea* L.). Harinikumar and Bagyaraj (1988) reported that growing a non mycorrhizal plant reduced AMF propagule number in the soil to 13% of the initial number, while fallow reduced it to 40%.

2.6. Quantification of AMF propagules

The relative abundance of AMF spores in soil is often used as an estimate of fungal population size (Sutton and Barron, 1972). Largest numbers of spores were found in soils when host plant roots senescence.

Numerous techniques are used to recover AMF spores from the soil. One of them is wet sieving through a series of sieves with decreasing mesh size. After sieving, the fraction containing the spores is subjected to centrifugation. Density gradient centrifugation is a widely used technique for AMF spore extraction. This method of separating spores by their density as compared to sucrose solutions was developed by Gerdemann and Nicolson (1963). The high osmotic potential of sucrose solution may affect the metabolic activity of spore (Hamel, personal communication). Tommerup and Carter (1982) suggested a dry separation technique to maintain the metabolic activity of spores. Another method, the plate method by Smith and Skepper (1979), involves mixing small quantities of soil with water and direct observation of spores under the microscope. Sutton and Barron (1972) studied spore populations using an adhesion-flotation method.

A substantial amount of spores collected from the field are usually dead (An and Hendrix, 1988). Tommerup (1992) proposed two methods to measure the viability of spores. One was a plant baiting assay and the other, a spore germination assay. These assays were questionable because of the AMF spore dormancy requirement before germination. An and Hendrix (1988) used the stain 3-(4,5-dimethylthiozol-y1)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to determine the viability of spores. With this stain, living spores become bright red and are easily distinguishable from the dead spores.

Arbuscular mycorrhizal fungi form vesicles, arbuscules and hyphae in the root cortex. These fungal components can be detect by mount the colonized roots on microscopic slides and assessed with different methods. Bevege (1968) and Phillips and Hayman (1970) developed a rapid procedure for detecting AMF fungal structures both on the surface of root and within the cortex. The methods involve clearing roots with hot KOH followed by staining of the fungal walls. Brundrett et al. (1984) proposed chlorazol black E as a superior stain for this purpose because it reveals details of arbuscules. Amber and Young (1977) proposed a

"grid intersection point" technique to quantify the length of root colonized by AMF as well as the percentage of infection. Giovannetti and Mosse (1980) compared several methods and found that grid line intersect method was the most effective in assessing AMF infection in roots. Biermann and Lindermann (1981) proposed a method with the idea of standardizing of AMF quantification. They proposed, without great success, to use the length of root containing AMF fungal structures to express mycorrhizal colonization, as it was more accurate than the percentage of root length with AMF fungal structure. Both the measurement of the percentage of colonization and root length, can be simultaneously made using the grid intersection method. McGonigle et al. (1990b) developed the magnified intersections method to more clearly detect all structural components of AMF in the root cortex. This method is very accurate but also very laborious and time consuming.

Colorimetric and autofluorescence procedures have been proposed to improve the accuracy of assessments as well as to reduce the time required for colonization measurement. Becker and Gerdemann (1977) developed a colorimetric method to measure the yellow pigmentation produced in colonized roots of certain plant species, while Hepper (1977) used a colorimetric assay to measure fungal chitin in roots. Ames et al. (1982), proposed a simple procedure involving the use of ultraviolet induced autofluorescence to reveal arbuscules in fresh mycorrhizal roots.

Extraradical mycelia are important components of the symbiosis. Numerous methods have been used to quantify hyphal length or density in the soil, including the chitin assay (Bethlenfalvay and Ames, 1987; Pacovsky and Bethlenfalvay, 1982), the quantification of specific fungi phospholipids or fatty acids (Olssen et al., 1995) and ergosterol (Frey et al., 1994). The reliability of these techniques has been criticized for several reasons. The chitin assay also measured chitin from other soil organisms such as insect or other fungi (Sylvia, 1992). Lipids occur in older infections and mature arbuscules but not in younger parts of the hyphae and arbuscules (Cox and Sanders, 1976). Finally, the ergosterol has also been observed from other fungi (Weete, 1973).

Abbott et al. (1984) used a membrane filter technique to measured mycorrhizal hyphae. This method was time consuming as it involved sonication of a soil suspension,

centrifugation and wet sieving. Jackobsen et al. (1992) reduced the time required in the process by using a filtering manifold, which allowed the preparation of 20 filtrations per hour. Recently, automated image analysis was used by Green et al. (1994) to quantify the AMF hyphae recovered on membrane filters. All of these methods can also be criticized as it is not possible to differentiate mycorrhizal hyphae from those of other fungi.

Only metabolically active hyphae are involved in nutrient uptake and transfer to the host. Sylvia (1988) proposed a method to quantify metabolically active extraradical hyphae of AMF using iodo-nitro-tetrazolium (INT) reduction staining and counter staining with trypan blue. Using this method he observed that 96% of the hyphae attached to roots of *Paspalum notatum* were active from 3 to 13 weeks. Ingham and Klein (1984) observed a relationship between fungal activity and staining with fluoresceine diacetate (FDA). Hamel et al. (1990) compared three methods of staining and found that the FDA method was the best for estimating hyphal activity, followed by the INT method, while the nitro blue tetrazolium method was the least precise. Tisserant et al. (1993) used a staining technique in which a black precipitate was formed upon alkaline phosphatase activity, allowing the measurement active hyphae. Dodds (1994) used this technique with computerized image analysis and easily measured extraradical mycelium. The recent development of molecular techniques, such as the Polymerase Chain Reaction (PCR), the Randomly Amplified Polymorphic DNA Method (RAPD), and the Enzyme linked Immunosorbent Assay (ELISA) should facilitate investigation of AMF (Millner, 1991; Piché et al., 1994; Simon et al., 1992; Wyss and Bonfante, 1993).

2.7. Soil disturbance, tillage and manure effects on AMF development

The number of mycorrhizal propagules in agricultural soils can be substantially reduced by soil disturbances such as tillage practices (O'Halloran,1986; Douds et al. 1993), soil erosion or top soil removal (Call and Mckell,1985; Miller, 1979) and soil stock piling during mining activities (Rives et al.,1980; Waaland and Allen, 1987).

In crop production, tillage practices can adversely affect the physical, chemical and biological properties of soils and can also affect the growth and development of AMF. O'Halloran et al. (1986) reported a series of experiments which showed that soil disturbance

of previously untilled soils decreased early growth and P uptake in corn. They suggested that soil disturbance was adversely affecting the mycorrhizal potential of the soil. Fairchild and Miller (1988) also investigated on the effect of soil disturbance on corn plant growth, P uptake and AMF colonization. In their experiment, they grew corn in pots containing disturbed soils. After approximately 3 weeks, they harvested the corn, then either disturbed the soil or left it undisturbed, and replanted corn. Soil disturbance was then found to have an adverse effect on plant growth, on P and Zn uptake and on the percentage of root colonization.

Evans and Miller (1990) showed that disturbance of root-free soil containing only AMF hyphae detached from the host plant gave reductions in AMF infection of corn roots, decreased plant growth, and decreased P and Zn uptake. This suggested that the AMF hyphal network could serve as infective propagule and, if not disrupted, could increase the nutrient absorption capacity of the next crop as soon as it become connected to the network..

Jasper et al. (1989a, 1989b) found reductions in AMF mycorrhizal colonization of clover after soil disturbance and suggested that the major part of this reduction in colonization was due to decreased hyphal viability. However, contrasting results were obtained by McGonigle et al. (1990b) in a study on the effect of soil disturbance intensity on AMF colonization and corn growth. Compared to the undisturbed controlled treatment, P uptake and shoot dry matter were decreased by cutting the soil into 1, 2, or 4 cm cubes. Sieving the soil was found to be the most adverse treatment. However, unlike the previous study, decreases in P uptake and plant growth were not accompanied by a decrease in mycorrhizal root colonization. They proposed that if AMF were an important component of the disturbance effect, it would have to be through the dismantling of a perennial, though dynamic soil hyphal network rather than through reduction in soil mycorrhizal potential (Miller et al., 1995).

New root colonization by AMF occurs rapidly in undisturbed or natural soil systems (Jasper et al., 1989a, Read et al., 1976). This is likely due to contact with a vigorous pre-existing network of infective hyphae. However, unlike most natural ecosystems, AMF hyphae in agricultural soils must often survive a relatively long winter period without a living host plant. In addition, if the soil is tilled, AMF overwinter as fragmented hyphae and as hyphae

disconnected from the host plant roots. Thus, an important question arises as to whether soil disturbance directly harm AMF or has an adverse affect on the ability of AMF to survive winter. In all of the previously cited studies on soil disturbance effects have in common that the test crop was planted immediately after harvest and application of soil disturbance treatments. Hence only the direct effect of disturbance on AMF function was tested. The impacts of disturbance on the ability of AMF to survive for long periods without a host plant remain unknown. While short term studies conducted to date demonstrate the functional role of intact hyphal networks in soil further studies may indicate major ecological impacts of AMF survival over the winter in agricultural soils after soil disturbance.

Soil conservation practices such as reduced tillage (RT) systems involve less intensive soil disturbance or less frequent tillage operations than conventional tillage (CT). In RT systems, most crop residues remain on the soil surface over the winter, and tillage is normally done only in the spring. Thus, the soil under RT remains undisturbed throughout the winter, similar to soils under no-till (NT) systems. As a result, AMF hyphal networks remain intact, and as viable as in NT systems until spring tillage. Reduced tillage practices could be less detrimental than CT to AMF winter survival and to mycorrhizal formation the following spring. In an experiment under field conditions, McGonigle et al. (1990b) could not find a relationship between the degree of soil disturbance and the level of AMF root colonization. However, in their study the soil disturbance treatments were applied in the spring to soils which had been plowed the previous fall. Therefore, much of the AMF hyphae in the soil could already have been dead due to the combined effect of fall plowing and overwintering period.

The vertical distribution of AMF propagules probably depends on several factors, including soil tillage (Smith, 1978) as this practice has a large impact on soil properties. Most studies on quantification of AMF were based on mycorrhizal root colonization and spore density. An et al. (1990) found that AMF spores are more abundant in the surface soil of field grown soybean, while Zajcek et al. (1986) and White et al. (1989) observed spores at greater depth with perennial plants. However, the vertical distribution of extraradical hyphae in soil has never been looked at.

Johnson and Pfleger (1992) and Douds et al. (1995) noted that tillage would impose a strong selection pressure on AMF in favor of the fast-growing or resistant species. Seiverding (1991) observed that some AMF species were more aggressive than mutualistic. In a field study, he noted that *G. scintillans* was able to produce spores more quickly than other AMF species before soil plowing. Disappearance of *Gigaspora margarita* and *G. caledonium* has been observed by Hamel et al. (1994) after plowing a meadow. It seems that soil tillage could influence more than the abundance of AMF and the efficiency of the symbiosis, but also the composition of the AMF community of agricultural soils.

Application of manure has been shown to affect AMF differently under different circumstances. Harinikumar and Bagyaraj (1989) and St. John et al. (1983) observed a positive effect of manure on indigenous AMF associations, while Hayman (1982) reported a negative impact of manure. However, Mosse (1986) failed to show any relationship between soil organic matter and mycorrhizal colonization. The effect of manure on AMF growth is still a matter of controversy which needs to be clarified.

2.8. Hypotheses

The work presented in this thesis was subtended by the following hypotheses:

1) In the presence of mycorrhizal plants, most of the hyphae located in the root zone are mycorrhizal hyphae.

2) Soil disturbance reduces mycorrhizal root colonization and consequently reduces nutrient uptake and growth of corn plant.

3) Seasonal variation in hyphal densities in soil mirrors crop development. It increases throughout the growing season and decreases with plant senescence.

4) Disruption of hyphal networks by fall tillage reduces soil mycorrhizal potential the following spring.

5) Spring disking decreases hyphal viability in soil compared to NT. The relative effect of spring disking is more severe in a reduced tillage (RT) in which no fall plowing is used than in a conventional tillage (CT) system. The impact of this spring operation is large when applied to intact fields but less in fields in which much of the mycelium has already been

killed by fall plowing and overwintering.

6) The impact of soil disturbance is large in spring but the difference in soil hyphal density and root colonization between tilled and untilled plots disappears as the season proceeds.

7) Application of manure reduces AMF colonization of corn roots and hyphal densities in soil.

8) Hyphae survive the winter better when they are attached to the dead root.

9) The vertical distribution of AMF spores and root colonization changes with soil depth and tillage systems.

10) In corn fields, the distribution of AMF is heterogenous and AMF are concentrated in the root zone.

2.9. Objectives

The general objective of this research was to characterize, in two soil types, the mycorrhizal symbiosis dynamics of corn monoculture as influenced by tillage systems and organic fertilization.

The specific objectives of this research were:

1) To evaluate the proportion of mycorrhizal hyphae extracted from soil in the root zone of mycorrhizal plants.

2) To observe the effect of fall plowing and spring disking on the development of the mycorrhizal symbiosis in the next crop.

3) To observe the seasonal variation in soil hyphal densities under different tillage systems and to relate the densities of hyphae to the concentrations of P and other nutrients in corn tissues.

4) To determine the impact of the detachment of extraradical hyphae from their mother roots and from the soil mycelial web.

5) To evaluate the impact of mechanical disruption of AMF hyphae on their winter survival.

6) To assess the impact of a fallow period following soil disturbance on the viability and infectivity of AMF hyphae, and relate this impact to nutrient uptake and growth of corn.

7) To examine the influence of tillage and fertilization practices on the vertical distribution of AMF spores, fungal mycelium and mycorrhizal root colonization in corn field profiles.

8) To determine the spatial distribution of AMF hyphae under a row crop such as corn.

9) To evaluate the effect of manure and mineral fertilizers on the proliferation of hyphae in soil and on corn root colonization.

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CONNECTING PARAGRAPH I

Chapter II is taken from the manuscript by Kabir, O'Halloran and Hamel accepted for the publication in the journal Mycorrhiza. The format of the text, therefore, conforms to the specification of the journal. In this chapter, the impact of mycorrhizal plants, non-mycorrhizal plants and soil organic matter on the relative proliferation of mycorrhizal and non mycorrhizal mycelium was examined. It was necessary to demonstrate that most hyphae extracted from the root zone of mycorrhizal plants were mycorrhizal before undertaking any hyphae quantification study. The results presented in the following section confirmed that the estimations of the abundance of AMF based on measurement of hyphae recovered from corn root zone is valid.

CHAPTER II

THE PROLIFERATION OF FUNGAL HYPHAE IN SOILS SUPPORTING MYCORRHIZAL AND NON-MYCORRHIZAL PLANTS

Abstract This study investigated the impact of mycorrhizal plants, non-mycorrhizal plants and soil organic matter on the relative abundance of soil hyphae perceived to belong to indigenous arbuscular mycorrhizal plants (AMF). Mycorrhizal plants, corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.), and a non-mycorrhizal plant, canola (*Brassica napus* L.), were grown in unsterilized soil in pots inoculated with mycorrhizal corn root fragments. After 5 weeks the abundance of hyphae was measured. The response of fungal growth to the addition of corn residues in the absence of plants was also assessed. The amount of hyphae produced under these treatments was compared to that produced in control pots in which no plants were grown and no residues were added. The abundance of hyphae in soil was several times higher in the presence of mycorrhizal plants (corn and barley) than in the other treatments.

We estimated that the AM hyphae present under mycorrhizal plants accounted for more than 83% of the measured hyphae. Levels of root colonization of 32% in corn and of 27% in barley confirmed the mycorrhizal status of the experimental plants. Only a few points of entry were observed in canola, the non host plant. The percentage of mycorrhizal colonization was positively related ($R^2 = 0.85$) to the abundance of soil hyphae, indicating that AM hyphae were the major component of the soil hyphae in the presence of mycorrhizal plants in this study.

Key words Arbuscular mycorrhizal fungi . Soil hyphae . Soil fungi . Host plant . Non host plant

Introduction

While few plant species are incompatible with arbuscular mycorrhizal fungi (AMF) (Newman and Reddell 1987; Vierheilig et al. 1995), a broad range of plants naturally form a mutualistic association with AMF (Crush 1973; Harley and Harley 1987). Arbuscular mycorrhizal fungi colonize host plant roots and their extraradical hyphae proliferate within the soil to acquire mineral nutrients. The main function of these hyphae is to increase the absorptive surface area of the host plant root system (Miller et al. 1987). Host plant photosynthates are provided to the fungal symbionts in exchange for an increased absorptive surface area (Mosse et al. 1982). The needs of plants for mineral nutrients, especially P, which is determined by plant genes (Krishna et al. 1985) and soil fertility status (Hayman 1982), probably regulates the proliferation of AMF hyphae in soil. Non-mycorrhizal plants do not support AMF hyphae but could encourage the proliferation of other non symbiotic fungal mycelium through release of important amounts of carbon compounds in the vicinity of the roots (Bowen and Rovira 1991). Saprophytic hyphal proliferation is also stimulated by the addition of organic residues to soils (Broder and Wagner 1988).

Given the important role of AMF in nutrient uptake and translocation, and that AMF are a major component of soil microbial biomass (Hamel et al. 1991), their extraradical hyphae must be studied. Published methods to quantify AMF hyphae include extraction and direct measurement of extraradical hyphae from soil using the membrane filter technique (Abbott et al. 1984), the chitin assay (Bethlenfalvay et al. 1982; Bethlenfalvay and Ames 1987), and quantification of specific phospholipids or fatty acids (Olsson et al. 1995). The reliability of these techniques for measuring the extraradical hyphae of AMF is questionable. Major problems are that AMF hyphae are associated with many non symbiotic fungal hyphae in the soil. Though it may be difficult to distinguish between AMF hyphae and other fungal hyphae. It is possible to recognize AMF extraradical hyphae, which have certain morphological characteristics and this can be verified using antibody techniques. Therefore, direct microscopic observation and counting of fungal hyphae recovered from soil can provide data on total hyphal length. The chitin assay will also measure chitin from soil insects, other

fungi or other organisms (Sylvia 1992). Lipids occur in older infections and mature arbuscules but not in younger parts of the hyphae and arbuscules (Cox and Sanders, 1975). Hence, there is no reliable method to quantify AMF hyphae in the soil. In spite of these difficulties, it is essential to measure the extraradical hyphae of AMF.

The growth cabinet experiment reported here was conducted to evaluate to what extent fungal hyphae extracted from soil growing mycorrhizal plants could be non-mycorrhizal and to determine the relative abundance of AMF extraradical hyphae as compare to those of the other soil fungi.

Materials and methods

Experimental design

A sandy loam soil was collected from a corn field and passed through a 2 mm sieve, and placed in the pots. The soil had a pH of 6.2 (in water) and contained 2.56% organic carbon, 57% sand, 25% silt and Mehlich III extractable nutrients levels of 82 µg P, 81 µg K, 1365 µg Ca, 91 µg Mg, 2.5 µg Zn, 1.1 µg Cu per g of soil. Each of the 15 cm pots received 1.1 kg of soil.

The design of the experiment was a randomized complete block with five replicates and five soil treatments: (1) soil planted to corn (*Zea mays* L.), (2) soil planted to barley (*Hordeum vulgare* L.), (3) soil planted to canola (*Brassica napus* L.), (4) soil amended with 6000 kg ha^{-1} of corn residues (stover) and (5) soil supporting no plant and receiving no amendment (control). Corn supports high levels of AMF colonization compared to barley, while canola is a non-host plant for AMF. Corn residues were added at the normal field rate in an attempt to stimulate microbial growth in the absence of the plant.

Corn residues were collected from the previous year corn field, and ground and passed through a 500 µm sieve. The material was soaked in deionized water for 5 days prior to mixing with the soil and placed in the 15-cm experimental pots to which this treatment was attributed.

All pots were inoculated with mycorrhizal corn root fragments. Corn plants had been grown in 5 pots for 4 weeks in the growth cabinet. Corn roots were collected and chopped to 1.5 - 2 cm length. Seventeen percent of root length was colonized by mycorrhizal fungi. Three g of fresh roots were thoroughly mixed with the soil in each pot just before planting. Corn roots were also added to residue amended and control pots.

Six germinated seeds of corn, barley or canola per pot were planted and thinned to four plants per pot after five days. The experimental pots, including the pots without plants, were maintained in a growth cabinet for five weeks under a photoperiod of 15 hours and day/night temperatures of 25 °C/16 °C. Soil moisture was adjusted to field capacity every other day. A solution of NH_4NO_3 and KNO_3 was added to each pot at the rate equivalent to 100 kg N ha^{-1} and 50 kg K_2O ha^{-1} at the beginning of the experiment.

Hyphal extraction and measurement

At harvest, the membrane filter technique, modified from Abbott et al. (1984), was used to extract extraradical hyphae from the soil of each experimental unit. Four soil cores (1.5 x 8 cm) were taken randomly with a sampler from each of the pot to make a composite sample. The soil from each composite sample was thoroughly homogenized by mixing and two 5g sub-samples were taken for each total hyphal length and metabolically active hyphal length determination. In these sub-samples no plant roots were removed. Sub-samples were placed in a blender with 300 ml deionized water and blended for 30-60 seconds to homogenize the soil suspension. Simple blender (Drink mixer, model-31DM13) was used in our hyphal extraction and blending was done at the lowest rpm available. Preliminary studies showed that a 15-20 sec blending did not give any significant difference in hyphal length to that of a 30-60 sec blending in our soil. However, a 1-2 sec blending gave significantly lower values in our experimental soil. The blended suspension was then poured through a 250-µm sieve and washed by applying high pressure water. The residue was collected on a 40-µm sieve, transferred to a 40 ml water filled beaker and shaken for 5 seconds to resuspend the recovered mycelium. This suspension, containing the hyphae, was then decanted onto a filter

and filtered under vacuum. Each subsample was extracted three times and the measurements were combined as this had been shown, in preliminary testing, to give adequate hyphal extraction and the lowest C.V.'s.

The hyphae were stained by flooding the filter with acid fuchsin (0.2%, in equal volumes of lactic acid, glycerol and water) for several minutes before determination of total hyphal length through microscopic examination. The excess stain was removed by rinsing the hyphae and filter paper with deionized water and vacuum-filtration. The recovered hyphae were measured by the modified grid line intersect method (Tennant 1975) on a grid drawn on a small petri dish (4 cm^2 area with 2 mm squares) randomly placed over the filter. In each filter at least 50 microscopic fields were observed. A dissecting microscope was used to observe hyphae at 50 X magnification. The hyphae recovered from the other subsamples were stained by flooding the filters with a solution made of equal volumes of iodonitrotetrazolium (INT) (1 mg mL^{-1}), reduced nicotinamide adenine dinucleotide (NADH) (3 mg mL^{-1}) and 0.2 M tris buffer at pH 7.4 (Sylvia 1988) to reveal only metabolically active hyphae. The filters were incubated for 12-16 hours at room temperature. The length of these metabolically active hyphae was also measured by the modified grid line intersect method and a dissecting microscope with 50 times magnification. Though this method for the extraction of extraradical hyphae was not absolutely quantitative, it did allow relative comparisons among the treatments.

Plant root preservation and percentage of root colonization

Corn, barley and canola root systems were separated from the soil on a 850μm sieve under running water. Random samples of washed roots were collected, cut into pieces of 1 to 1.5 cm length, placed in tissue embedding capsules and kept in a formalin-acetic-acid-alcohol (FAA) solution (Phillips and Hayman 1970). Root samples were autoclaved in 10% KOH for 15 minutes, well rinsed with deionized water and stained with acid fuchsin (0.02%) in lactoglycerol (Brundrett 1994). The percentage of mycorrhizal root colonization was measured by the grid-line intersect method (Giovannetti and Mosse 1980). A dissecting

microscope was used to observed hyphae at 40-50 times magnification.

Statistical analysis

Statistical analyses of the data were performed using the general linear model (GLM) procedure in Statistical Analysis System (SAS Institute 1988). Analysis of variance was done to examine the abundance of hyphae in the soil under mycorrhizal and non mycorrhizal plants, and also in amended and non amended soil. Regression analysis was performed using mycorrhizal root colonization as the dependent variable, to examine any trends between root colonization (%) and total hyphal abundance.

Results

Within five weeks, root colonization by indigenous AMF had reached 32% and 27% in corn and barley respectively, but in canola only, few points of infection and no internal structures were observed (Fig. 1). Under corn the abundance of total and viable hyphae was 92 cm cm^{-3} and 83 cm cm^{-3} , respectively. In soil under barley, the values were 66.6 cm cm^{-3} and 50.2 cm cm^{-3}, respectively. The abundance of both total and metabolically active hyphae was very low under canola, in the soil receiving corn residues and in the control soil (Fig. 2), and there was no significant ($P < 0.05$) difference in hyphal abundance among these three treatments. There was little difference between total hyphal abundance and the abundance of metabolically active hyphae in the soil of the control treatments (Fig. 2). This suggests that few, if any, of the non-viable hyphae measured were the remains of mycorrhizal hyphae which had developed on the corn crop growing in the field where the soil had been taken and that most of these old hyphae had been degraded at the time of sampling. Regression analysis showed there was a positive significant relationship ($R^2 = 0.85$, $P<0.001$) between the percentage of root colonization and the abundance of hyphae (Fig. 3).

Discussion

Non-mycorrhizal plants can stimulate the growth of fungi through rhizosphere effects (Bowen and Rovira 1991; Veirheilig et al. 1995) but they do not allow the development of AMF, which are biotrophic (Morton, 1990). Some authors have reported that the Brassicaceae, in contrast, can inhibit fungal growth (Glenn et al. 1988; Schreiner and Koide 1993). Our results, however, showed that canola neither inhibited nor stimulated fungal growth when compared with our plant less controls.

The addition of plant residues to soil is not expected to stimulate AMF but can increase the proliferation of saprophytic fungi (Broder and Wagner, 1988). However, in our experiment the addition of corn residues to soil had little effect on the hyphal proliferation. In fact, the addition of the equivalent of 6000 kg ha^{-1} of corn residues to our pots did not increase the amount of soil hyphae over the plant less non-amended control.

We observed that over five times more hyphae were associated with mycorrhizal plants than with canola or other controls. These results can be related to the observation of Bécard and Piché (1990) who measured rapid hyphal proliferation on a host root organ cultures, but not on a non- host root culture, and suggest the prevalence of mycorrhizal hyphae in soil growing mycorrhizal plants.

Root colonization in corn, barley and canola was well correlated with the abundance of hyphae in soil. From this observation we might suggests that mycorrhizal fungi when present, are major contributors to soil hyphae. If we assume that the amount of hyphae observed in the canola, amended or control soil were non-mycorrhizal, and that the same amount of non-mycorrhizal hyphae was in the soil under corn and barley, then AMF hyphae were making up over 83% of the hyphae measured in the soil of the mycorrhizal treatments.

Our observations provide evidence that the presence of a mycorrhizal plant is by far the major determinant to hyphal proliferation in soil. Although our findings of increased fungal hyphae under mycorrhizal corn and barley do not directly prove that these hyphae are AMF, the fact that a non-mycorrhizal plant or the addition of corn residues had no effect on the observed hyphae supports the hypothesis that we are measuring mostly AMF hyphae in

soils in which mycorrhizal plants are growing. Our results agree with those of authors who previously reported indirect evidence based on chitin assay for the prevalence of mycorrhizal biomass, over that of other fungi, in soil growing mycorrhizal plants (Bethlenfalvay and Ames 1987). Unfortunately, until DNA analysis or immunological techniques are developed to unequivocally recognized the mycorrhizal character of soil hyphae, the study of the extraradical mycelium, which is a crucial component of the arbuscular mycorrhizal symbiosis, will have to be based on accumulating indirect evidence.

In conclusion, our results suggest that most of the hyphae found in this agricultural soil growing plants colonized by AMF, are mycorrhizal.

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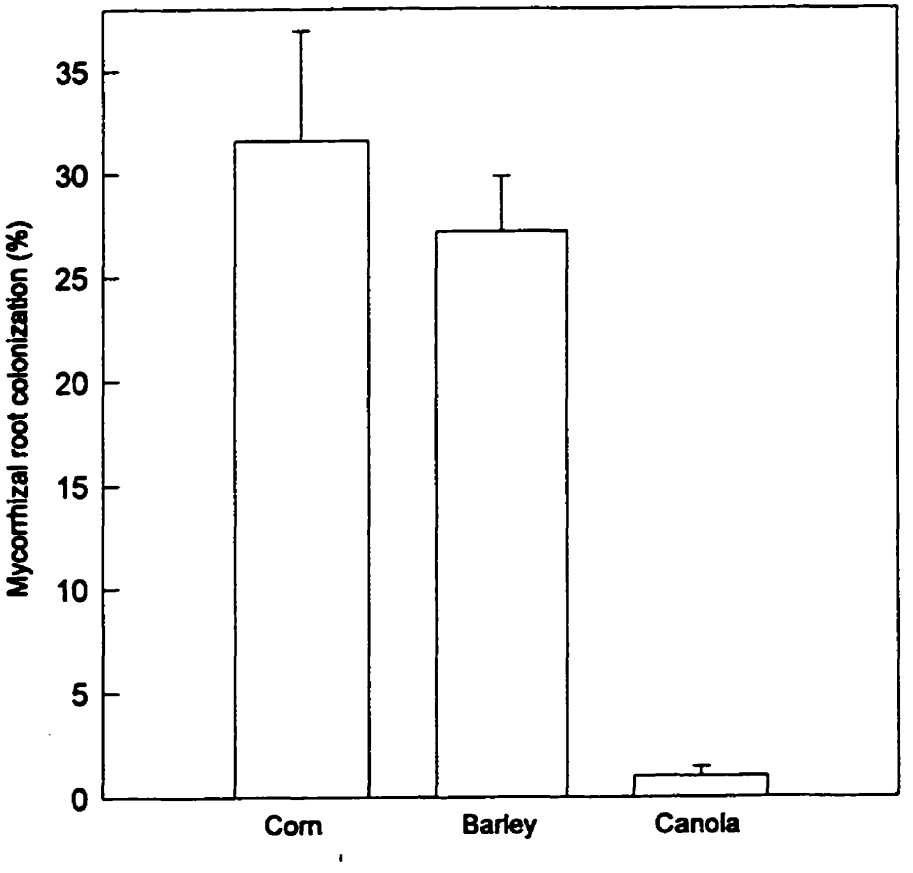

Figure 1. Percentage of root colonization of corn, barley and canola. Error bars represent the standard error.

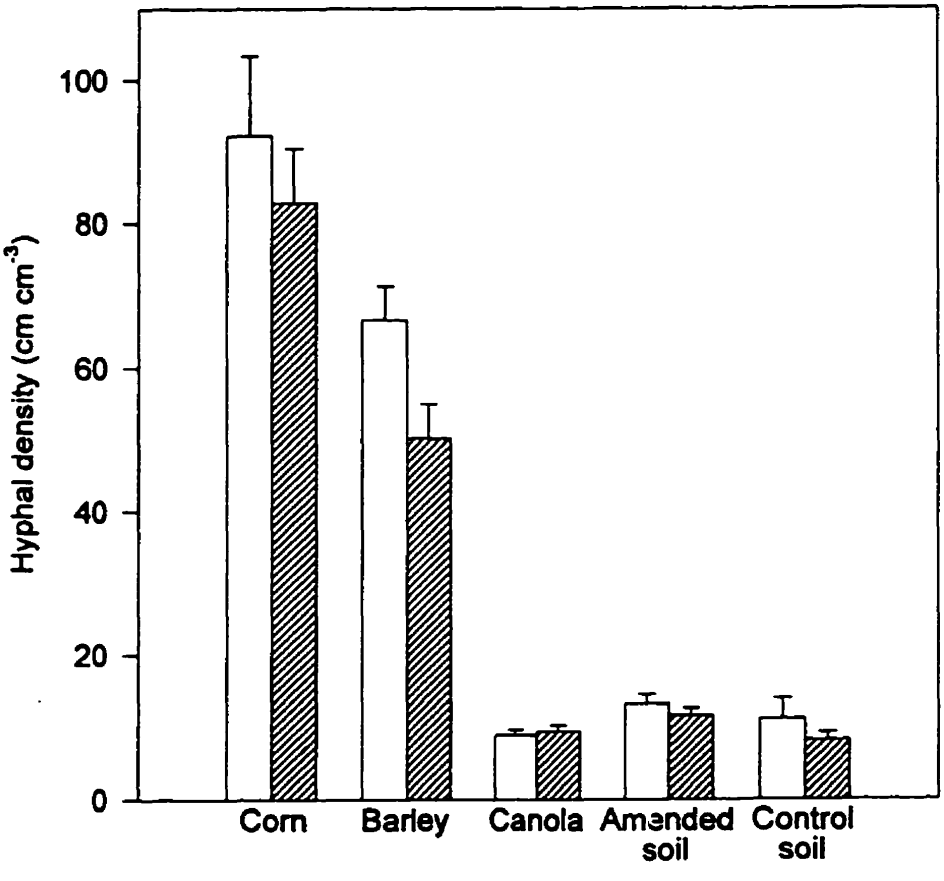

Figure 2. Abundance of total (Blank bars) and metabolically active (hatched bars) hyphae in soil growing mycorrhizal and nonmycorrhizal plants or in soil without plant and amended or not with corn residues. Error bars represent the standard error.

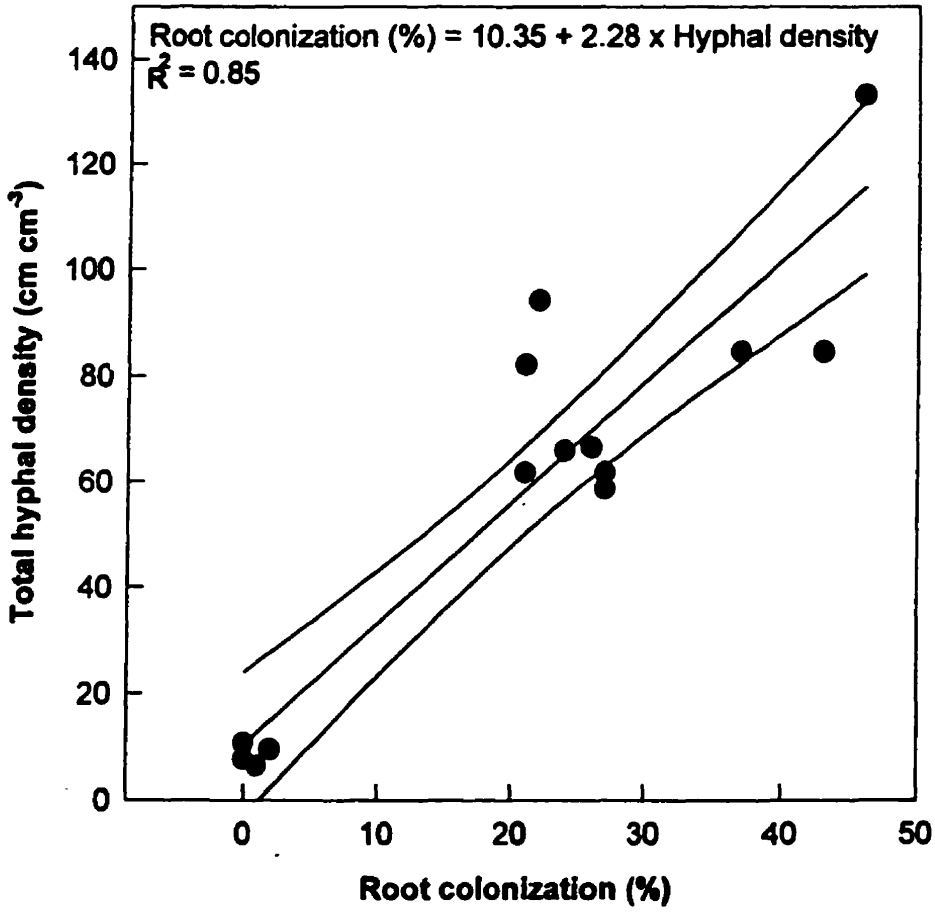

Figure 3. Relationship between the percentage root colonization and the density of soil hyphae in soil under corn, barley and canola (95% confidence limit).

CONNECTING PARAGRAPH II

In chapter II, it was concluded that AMF are the major contributors to soil hyphae in the presence of mycorrhizal plants and AMF hyphal abundance could be estimated by measurement of hyphae extracted from the root zone of corn. Chapter III addresses the impact of the interaction between soil disturbance and length of fallow on the survival and infectivity of extraradical AMF hyphae and the consequence of this interaction on corn growth and nutrient uptake. This chapter was taken from the manuscript by Kabir, O'Halloran and Hamel submitted to the journal Soil Biology & Biochemistry. The format of the text, therefore, conforms to the specification of the journal.

CHAPTER III

EFFECT OF TIME ON THE VIABILITY AND INFECTIVITY OF EXTRARADICAL HYPHAE OF ARBUSCULAR MYCORRHIZAL FUNGI DETACHED FROM HOST PLANT ROOT SYSTEM

Summary-Soil disturbance may reduce mycorrhizal plant growth and nutrient uptake through its impact on the integrity of the extraradical hyphal network. A growth-chamber experiment was conducted to evaluate the survival of extraradical arbuscular mycorrhizal (AM) fungal hyphae when detached from the host root system, and to understand the impact of soil disturbance on the ability of these hyphae to colonize plant roots and to reestablish mycorrhizal associations in previously disturbed soils. The experiment consisted of establishing AM fungi in pots divided into two compartments by a nylon mesh (37 μm), by growing corn (*Zea mays* L.) in one of the compartments for six weeks in an unsterilized agricultural field soil. The mesh prevented the growth of corn roots from one side of the pot to the other, while allowing the passage of the AM fungal hyphae. After establishment of AM fungi following treatments were performed: soil in the two compartments was either disturbed or undisturbed leading to four combined disturbance treatments: 1) both compartments undisturbed (UU); 2) root compartment disturbed and root-free compartment undisturbed (DU); 3) root compartment undisturbed and root-free compartment disturbed (UD); and 4) both compartments disturbed (DD). The effects of fallow periods of four different length; 0, 30, 60 and 90 days were also measured in the same experiment giving a total of 16 treatments. Soil disturbance was performed at the beginning of the experiment in the root compartment, and after each of the fallow periods in the root-free compartment. Immediately after disturbing the soil in the root-free compartment, corn was planted and grown for 30 days to test the effects of disturbance on efficiency of AM formation.

Soil disturbance had no adverse effect on AMF efficiency if test plants were planted immediately after disturbing the root-free compartment. However, AMF efficiency decreased with increasing length of fallow. Densities of total and metabolically active extraradical hyphae in the root-free compartments were measured before each fallow period. Significantly lower densities were observed in pots where the soil of the root compartment had been disturbed. Test plant shoot weight was highest in UU and lowest in DD treated pots. Phosphorus uptake by the test plants was twice as high in UU as in DD. Test plants in undisturbed (UU) pots had greater Zn and Cu levels than in DU, UD and DD pots. Uptake of P, Zn and Cu by test plants was reduced by about 40%, 63% and 70% respectively, by 90

days of fallow, compared to 0 days fallow.

Key words : arbuscular mycorrhizal fungi, soil disturbance, fallow period, mineral nutrient, viability, corn (*Zea mays* L.)

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are plant symbionts which benefit plant growth by improving the uptake of nutrients of low mobility in soil such as P, Zn and Cu (Eivazi and Weir, 1989; Faber *et al.*, 1990; Kothari *et al.*, 1991; Kucey and Janzen, 1987; Manjunath and Habte, 1988). The movement of P, Zn and Cu toward plant roots is often slower than the rate of plant uptake (Bolan 1991), thus creating a nutrient-depleted zone around the roots. Arbuscular mycorrhizal fungal hyphae can extend several centimetres from the root surface of AMF plants (Li *et al.*, 1991), extending beyond the zone of depletion, and can absorb and translocate these nutrients to the plants. If the soil is not disturbed, these extraradical hyphae form a permanent though dynamic web (Miller *et al.*, 1995) to which seedlings attach at a very early stage.

Soil disturbance disrupts the extraradical web and negatively influences AMF plant growth (Jasper *et al.*, 1989a, 1989b; O'Halloran *et al.*, 1986), sometimes reducing tissue P concentration, shoot dry weight (Fairchild and Miller, 1988) and degree of root colonization by AMF (Evans and Miller, 1988).

In many of the pot studies on the impact of soil disturbance on AMF soil mycelial network (Evans and Miller 1988, 1990; Jasper *et al.*, 1989a, 1989b; McGonigle *et al.*, 1990), a mycorrhizal network was established, and the soil was disturbed and immediately replanted. In those reports, effects of soil disturbance on subsequent plant root colonization by AM fungi have varied; decreased colonization levels were reported by Jasper *et al.*, (1989a, 1989b), while McGonigle *et al.*, (1990) found no effect of soil disturbance.

Land fallowing substantially reduced mycorrhizal fungal propagules (Johnson and Pfleger, 1992) because these fungi are biotrophs. Viability of AMF, crop mycorrhizal colonization, foliar concentrations of P and Zn, and crop yield were inversely related to the

length of fallow period (Thompson, 1987, 1994). Harinikumar and Bagyaraj (1988) have shown that one season of fallow can reduce the mycorrhizal propagules by 40%.

In spite of the biotrophic nature of AMF, at least some fungi are capable of limited free-living growth after the death of their host plant (Addy *et al.*, 1994; Hepper and Warner, 1983; Tommerup and Abbott, 1981; Warner and Mosse, 1980). Still, questions arise concerning how long the hyphae remain viable in the absence of a living host plant and whether the severity of the effect of disturbance would increase with time from the death of the host plant. The influence of time on the impact of soil disturbance on AMF mycelium may be important in determining the performance of mycorrhizal crops as several months often separate soil tillage and seeding of the next crop.

The present study was designed to test the effect of soil disturbance on AMF status and on the uptake of nutrients by corn (*Zea mays* L.) seedlings, after different periods of time from the death of the host plant which had been supporting hyphal proliferation in soil.

MATERIALS AND METHODS

The experiment was carried out using 80 rectangular black acrylic plastic pots divided into two compartments by a nylon mesh of 37 μm pore size. The mesh allowed the passage of hyphae from one side of the pot to the other, but prevented corn roots from passing through. The pots were filled with a 1:1 mixture of silica sand and a sandy loam soil that had been under corn production for 13 years. The soil had been sieved (2 mm) and roots and other debris carefully removed prior to being uniformly mixed with the silica sand. The mixture had the following properties: 62% sand, 22% silt, 16% clay, 0.44% organic carbon, a pH of 5.7 (in water), and Mehlich-III extractable nutrient levels (Mehlich, 1984) of 27.50 μg P, 61.53 μg K, 575 μg Ca, 112 μg Mg, 2.27 μg Zn and 1.47 μg Cu per gram of soil. Each compartment contained 1500 cm^3 of the mixture. The soil surface was covered with 2 cm of perlite to reduce evaporation, and pots were watered to field capacity every other day.

Four corn plants were grown for six weeks in one compartment of each pot, referred to as the root compartment, to establish an AMF hyphal network in the soil of the entire pot.

Solutions of NH_4NO_3 and KNO_3 were applied to the planted compartments after seeding, at rates equivalent to 100 kg N ha^{-1} and 50 kg K_2O ha^{-1}. After six weeks of growth, the shoots were harvested and treatments initiated.

Treatments consisted of a 4 by 4 factorial combination of soil disturbance and fallow treatments. Each disturbance-fallow combination was replicated five times in a randomized complete block design. Soil in the root compartment was either disturbed (40 pots) immediately after harvest of the initial host plants, or was left undisturbed. Disturbance consisted in carefully removing all the soil from the compartment, sieving it (2 mm), and putting it back into the compartment, including roots cut into 2 to 3 cm pieces. This disturbance was applied in order to detach the hyphal network in the root-free compartment from the host root system on which it had developed. Soil in the other compartment was also either disturbed or undisturbed; any disturbance of the root-free compartment was done immediately prior to planting the test-plants. The disturbance treatments were thus 1) both compartments disturbed (DD), 2) disturbance in the root compartment only (DU), 3) disturbance in the root-free compartment only (UD), and 4) both compartments undisturbed (UU). These were combined with four fallow period treatments.

Test-plants were established in the root-free compartment after a fallow period of 0, 30, 60 or 90 days following initial host-plant (root compartment) harvest. Four germinated corn seeds were planted 3 cm from the mesh dividing the pot, and thinned to two plants per pot four days after emergence. Fertilizer application was as described above. Test plants were harvested after 30 days of growth, regardless of fallow-disturbance treatment.

Hyphal density measurement

Six soil cores (1.5 x 10 cm) per pot were taken just before seeding the test plants after each fallow period. These cores were taken only from root-free compartments that were going to be disturbed. Each set of six cores were pooled and four 5 g subsamples were taken from each of these composite samples to extract AMF extraradical hyphae. The extraction technique was modified from Abbott *et al.* (1984). Each subsample was placed in a blender with 300 mL deionized water and blended for 15 to 30 seconds to homogenize the soil

suspension. This suspension was then poured through a 250 μm sieve. The fungal material, consisting almost exclusively of hyphae (few spores were encountered), was collected on a 45 μm sieve placed below the 250 μm sieve, after washing the latter with water. The recovered material was transferred to a beaker and resuspended in water, shaken for several seconds, and allowed to settle for 1 minute. The supernatant was filtered (pore size 11 μm) under vacuum. The procedure was repeated 3 times on each subsample for a thorough extraction of soil hyphae. While some hyphae could pass through the 45 μm sieve (measurements obtained after using a 30 μm sieve were about 10% higher) the nature of the soil used required a 45 μm sieve. Therefore, the values underestimate actual hyphal density, and used only to compare treatments.

The hyphae recovered from two of the four subsamples were stained by flooding the filters with a 0.2% aqueous solution of acid fuchsin. Excess stain was removed by washing with deionized water and vacuum-filtration. The length of the recovered hyphae was measured using the modified grid-line intersect method (Tennant, 1975) under a dissecting microscope at 50 X magnification.

The hyphae recovered from the two other subsamples were stained by flooding the filters with a solution containing equal parts of iodonitrotetrazolium (INT) (1 mg mL^{-1}), reduced nicotinamide adenine dinucleotide (NADH) (3 mg mL^{-1}) and 0.2 M tris buffer at pH 7.4 (Sylvia, 1988). This live stain reveals dehydrogenase activity. The filters were incubated for 12 to 16 hours at room temperature. The length of stained (metabolically active) hyphae were also measured by the modified grid-line intersect method.

Shoot weight and quality

Harvested test plant were dried at 65°C for 48 hours for dry mass determination and tissue analysis. Plant shoots were ground, digested (Thomas *et al.*, 1967) and analysed for P by the vanado-molybdate blue method on the total digest (Jackson, 1958) while Zn and Cu were determined by atomic absorption spectrophotometry.

Mycorrhizal root colonization

Test-plant roots were recovered on an 850 µm sieve, cleaned under running water. Plant roots were cut into 1 to 1.5 cm length pieces, sampled randomly, and placed in tissue embedding capsules. Root samples were preserved in a formalin-acetic acid-alcohol (FAA) solution (Phillips and Hayman, 1970) until processing. Roots were cleaned by autoclaving in 10% KOH for 12 minutes, well rinsed with deionized water, and stained with acid fuchsin (0.02%) (Brundrett, 1994) in equal volumes of lactic acid, glycerol and water. The percentage of root colonized by AMF was measured by the grid-line intersect method (Giovannetti and Mosse, 1980) under a dissecting microscope at 50 X magnification.

Statistical Analysis

Statistical analysis were done using the SAS software package (SAS Institute, 1990). The Proc univariate procedure of SAS indicated that the experimental data was normally distributed. An analysis of variance was done using the General Linear Model (GLM) procedure of SAS to test treatment effects, and a protected ($P < 0.05$) Least Significant Difference (LSD) test was used to compare treatment means.

RESULTS

Hyphal density in the root-free compartments at the end of the 30, 60 and 90 days of fallow period was significantly higher in pots where the soil of the root compartment had not been disturbed (Fig. 1). Overall, total and metabolically active hyphal density in the root-free compartment were reduced by 22% and 40%, respectively, as a consequence of soil disturbance in the root compartment. However, disturbance did not decrease hyphal density unless a fallow period was also applied.

The length of total and metabolically active hyphae in the root-free compartment gradually went down from immediate planting to 90 days of fallow (Fig. 1). Compared to immediately-planted pots, density of metabolically active hyphae in the root-free compartments were reduced by 54% after 30 days, 74% after 60 days and 86% after 90 days

of fallow in pots in which the soil in the root compartment had been disturbed, whereas in pots with undisturbed root compartment this reduction was markedly less, reaching 26% after 30 days, 40% after 60 days and 57% after 90 days of fallow.

Soil disturbance without fallow period

Phosphorus concentrations in shoots were low according to the Conseil des Productions Végétales du Québec (1985), ranging from 0.66 mg g^{-1} to 1.15 mg g^{-1}, and most plants exhibited P deficiency symptoms (data not shown). Phosphorus content was highest in the UU, intermediate in the DU and UD, and lowest in the DD treatment (Fig. 2). When the soil was not disturbed (UU), P content was about twice as high as in disturbed (DD) treatment. Soil disturbance had a marked effect on the Zn content (Fig. 3), which was significantly higher in UU and UD treatments, intermediate in DU and lowest in DD treatment. Disturbing the soil of both compartments reduced corn Zn concentration (data not shown) and content by 22% and 37% respectively, on average. Copper content also decreased with increasing intensity of soil disturbance, being greatest in UU treatment, intermediate in DU and UD treatments and lowest in the DD treatment (Fig. 4). Content of Cu was almost twice as high under UU than under DD treatment, while concentration of Cu reduced by 28% (data not shown). Plant-growth reduction due to soil disturbance followed the same pattern as that of nutrient content. Plant biomass was significantly reduced as a result of disturbance in either compartment and was lowest in DD treatment (Fig. 5). The average shoot dry weight under DD treatment was 22% less than under the UU treatment.

Soil disturbance with fallow period

A fallow period of 90 days reduced P concentration by 19% and P content by 40% (Fig. 2) as compared to the non-fallowed controls. The difference between the disturbance treatments with respect to Zn content when corn was seeded immediately after removal of the mother plant, was not statistically significant (Fig. 3). However, after 30, 60 and 90 days of fallow, Zn content was significantly lower in the DD-treated plants than in other plants. Corn Zn concentration and content decreased with the length of the fallow periods. A fallow

period of 90 days resulted in 54% and 63% reduction of Zn concentration and content respectively by corn, on average. Copper concentration in the test plants decreased by 61% after 90 days of fallow. Similarly, Cu content decreased with increasing length of fallow, and after 90 days of fallow, Cu content in test plants was to 70% less than non fallowed plants.

Plants grown in pots submitted to any one of the disturbance treatment with fallow periods of 0, 30 and 60 days produced consistently less shoot dry weight than their corresponding undisturbed controls (Fig. 5). However, after 90 days of fallow, all yields were low with no significant difference between disturbance treatments. There was no significant difference between DU and UD in any of the fallow treatments. In contrast, the DD treatment always reduced shoot dry weight ($P<0.05$). Shoot dry weight decreased with the increasing length of fallow period. Fallow alone accounted for 20% reduction in shoot dry weight.

The percentage of root length of the test plants colonized by indigenous AMF fungi ranged from 30% to 50% (Fig. 6). There were no appreciable differences in root colonization among the disturbed and undisturbed treatments when corn was sown immediately.

Fallow had larger impact on root colonization (Fig. 6). From 30 to 90 days of fallow, colonization decreased with increased length of fallow. Overall, fallow periods caused a 33% reduction in colonization. Roots grown in undisturbed soil after 30 days of fallow was significantly more colonized than roots from plants sown at the same dates in pots receiving disturbance i.e. UD, DU or DD treatments.

DISCUSSION

Soil disturbance and fallow had a negative effect on plant growth and nutrient content efficiency, especially when these treatments were applied in combination. The reduced plant development observed is probably attributable to a combination of three factors, namely, a reduction in the abundance of extraradical mycelium, a reduction in the level of root colonization, and a reduction of the efficiency of the AMF mycelium at taking up nutrient.

Fallow had a large impact on the abundance of metabolically active hyphae which dropped to only 26% of its initial value, after 90 days without a host plant. Detachment of the mycelium from the mother roots by disturbing the root compartment of the pots, led to

a large drop in the abundance of active hyphae to as low as 14% of its initial abundance. The abundance of soil hyphae as a whole, regardless of their metabolic status, was less dramatically reduced by fallow, probably because a proportion of dead hyphae which did not yet decomposed was also counted. Reduction in AMF hyphal activity was concurrent, and most likely the cause of the reduction in test-plants root colonization, as few spores were found in the soil prior to seeding. In a field study, Harinikumar and Bagyaraj (1988) reported an important reduction in the abundance of AMF in soil as indicated by a 40% reduction in spore number after leaving the land fallow for one season. The long-fallow disorders observed by Thompson (1987, 1994), were associated to a decline in mycorrhizal root colonization and AMF sporulation. Reduction in mycorrhizal colonization and sporulation was interpreted as the expression of the suppressive effect of fallow on AMF survival. Our results confirm this interpretation.

The soil in the root compartment was disturbed after the initial establishment of AMF mycelium in the pot systems to see how disruption of AMF hyphae in the root compartment and detachment of hyphae from the root could affect the survival, the effectiveness and the infertility of the AMF hyphae in the root-free compartment. Our results showed that indeed, detachment from roots could reduce the densities of total and metabolically active hyphae in the root-free compartment along with the nutrient content and shoot dry weight of the test plants, although root colonization was not significantly influenced. However, detachment from the mother root alone was not a sufficient condition to kill AMF, as revealed by the live stain and test-plant root colonization level. A fallow period between detachment and seeding of the test plants was necessary to reveal the impact of detachment on AMF hyphal vigour. In contrast, the impact of detachment of the mycelium in the root-free compartment from that of the root compartment significantly reduced test-plant growth, nutrient concentrations and content. Even if the fragmented AMF mycelium retained metabolic activity after disturbance and could act as efficient infective propagules, there was no longer a hyphal network to facilitate P, Zn and Cu content.

Jasper *et al.* (1989a) suggested that disruption of hyphae through soil disturbance may have reduced their viability. The present results provide evidence that soil disturbance

alone, i. e. without a fallow period, may reduce AMF hyphal infectivity and metabolic activity, and stresses the importance of the interaction of disturbance and fallow. Even if these two practices are commonly used in combination in agricultural fields, research had concentrated on the impact of soil disturbance on the AMF symbiosis of field crops, overlooking the importance of the length of time between two successive crops.

Shoot dry weight, nutrient content and tissue concentrations in corn were reduced by soil disturbance. This corroborates the findings of Evans and Miller (1988, 1990); Jasper *et al.* (1989a, 1989b) and O'Halloran *et al.* (1986) and further confirms the role of the extraradical hyphal network in improving the extent of soil exploitation by plant root systems. When the soil is disturbed, the AMF mycelial web is sectioned and the hyphae are disconnected from the roots causing a reduction in growth, nutrient concentrations and content as sometimes observed in plants grown in disturbed soil. It is easy to understand, then, that the impact of soil disturbance on plant growth increases with the severity of the disturbance treatment (McGonigle *et al.*, 1990). However, it was surprising to observed that disturbance of the soil in the root compartment had the same impact on corn test-plants AMF symbiosis as disturbance in the root-free compartment, the one in which test plants were grown. We would have expected a greater impact of soil disturbance when applied directly in the zone where test-plants were grown. It seems that AMF hyphae could grow from the undisturbed root compartment to the corn test-plants sown in the root-free compartment, connecting the plantlets to the extensive mycelial web which was well established in the root compartment. The results of Bürkert and Robson (1990) indicate that the majority of the extraradical hyphae develop close to the mother-plants root systems. Therefore, plants grown in pots in which the soil of the root-free compartment was disturbed but not that of the root compartment could at some point also have access to an important mycelial web.

When corn test-plants were sown after 30 and 60 days of fallow, they developed higher levels of colonization in undisturbed soil. In contrast, when no fallow period was applied, disturbance of the soil did not reduce colonization, suggesting that the AMF mycelium is more susceptible to disturbance after a period of time without a host. Therefore, hyphal mortality is higher so the ability of active mycelium to reestablish mycorrhizae is

reduced. These results also indicate that AMF mycelium fragments are as competent as an intact mycelium even when isolated from the host root, but that the detached hyphae lose their competence more rapidly than intact hyphae attached to a dead root systems.

In conclusion, our results demonstrate that soil disturbance reduces corn plant development, nutrient concentrations and content by reducing the extent of the AMF hyphal network associated with roots, and consequently the volume of soil exploited. Soil disturbance did not reduce the density of metabolic active hyphal density and their infective potential unless a period of fallow was also applied. Fallow periods seemed to increase the susceptibility of AMF mycelium to disturbance and to reduce the vigour and size of the hyphal network, leading to reduced mineral nutrient content and hampered plant development, even if the soil was not disturbed.

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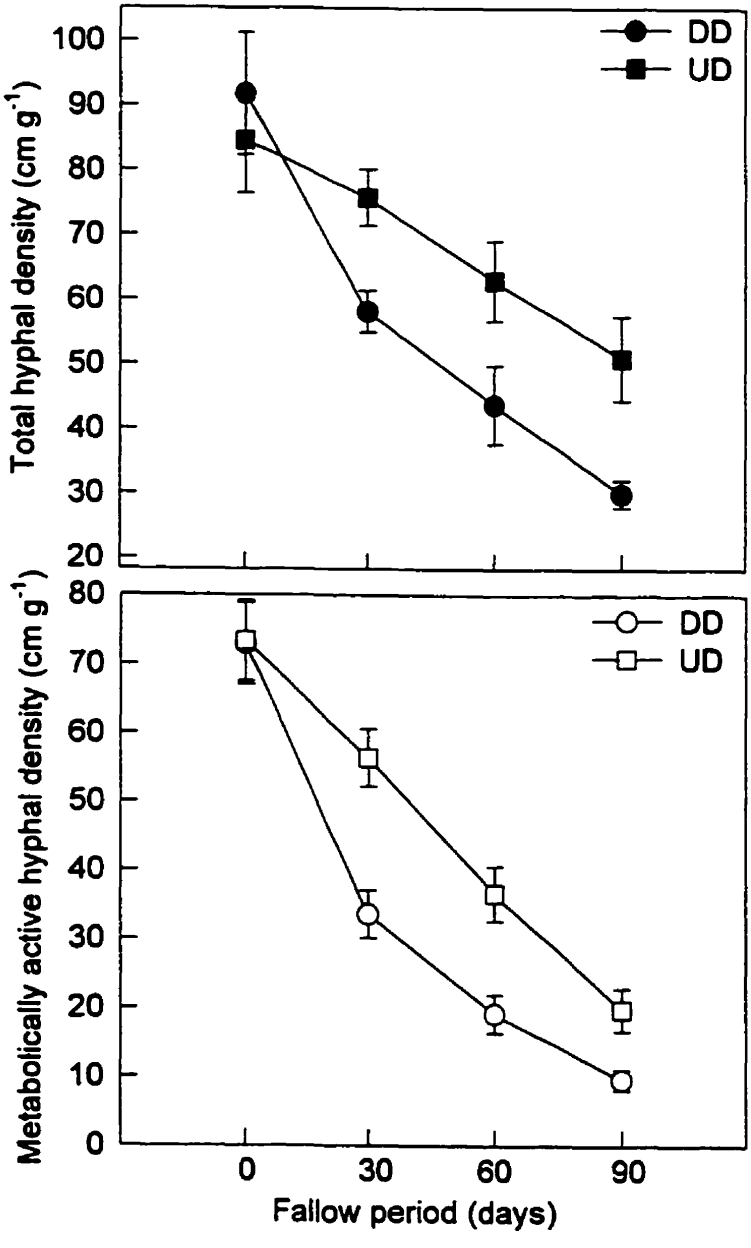

Fig. 1 Changes in the densities of total and metabolically active hyphae with disturbance (D) and without disturbance (U) of the soil in the root compartment after fallow periods of different length. Data points represent the means of 5 reps with SE bars.

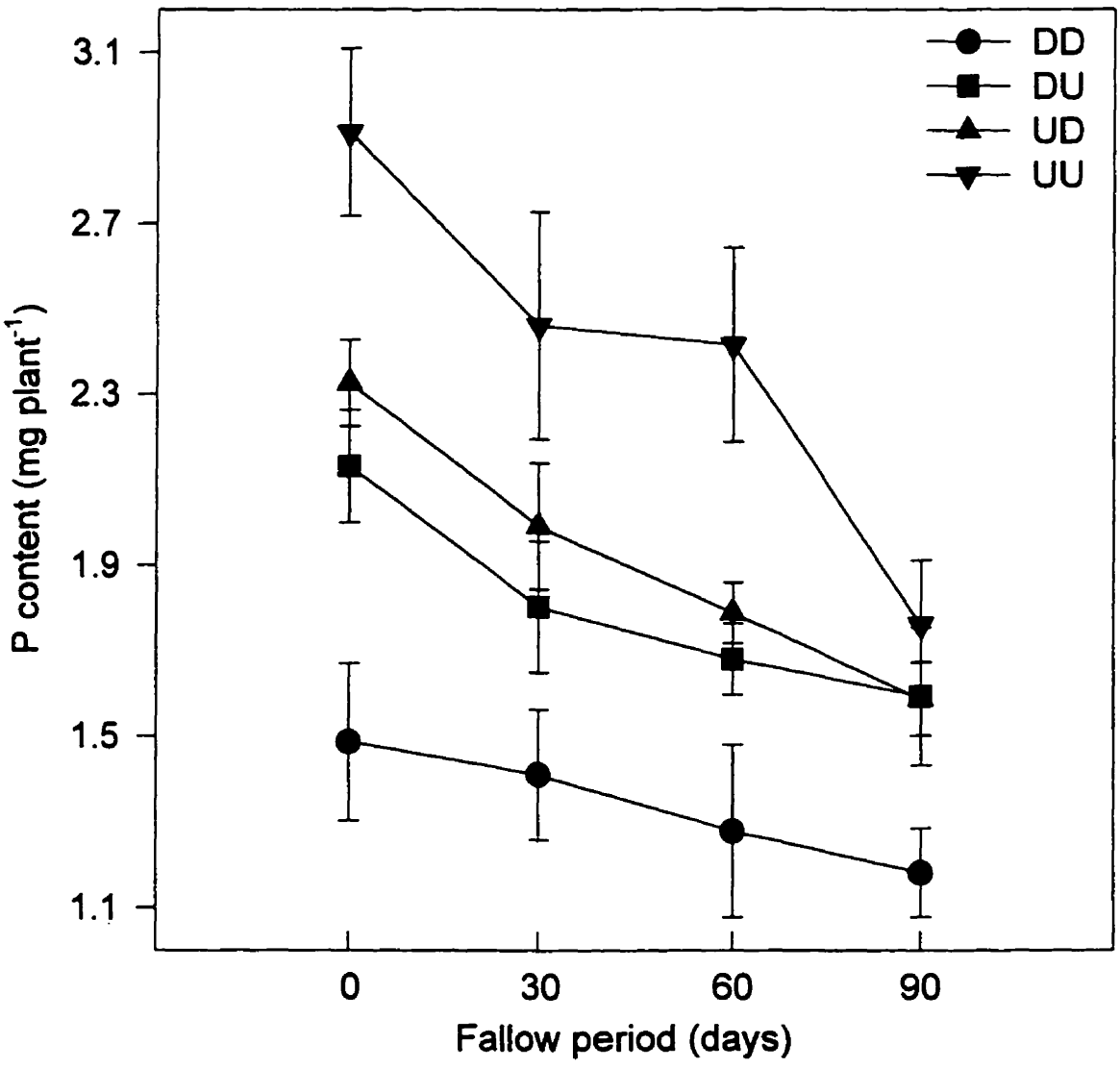

Fig. 2 Changes in phosphorus contents of plants when submitted to four soil-disturbance treatments and four different fallow periods of different length. Data points represent the means of 5 reps with SE bars.

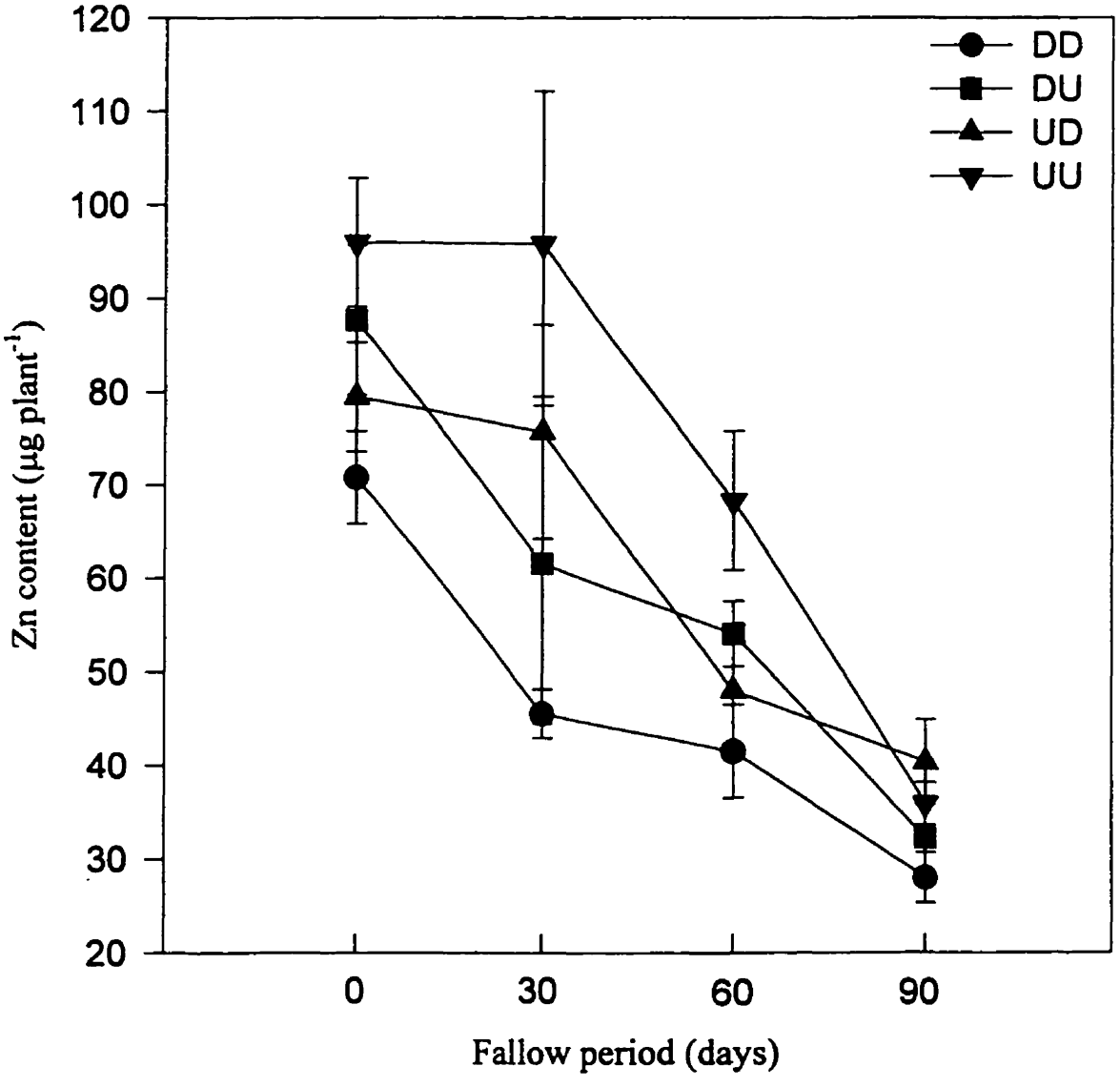

Fig. 3 Changes in zinc contents of plants when submitted to four soil-disturbance treatments and four fallow periods of different length. Data points represent the means of 5 reps with SE bars.

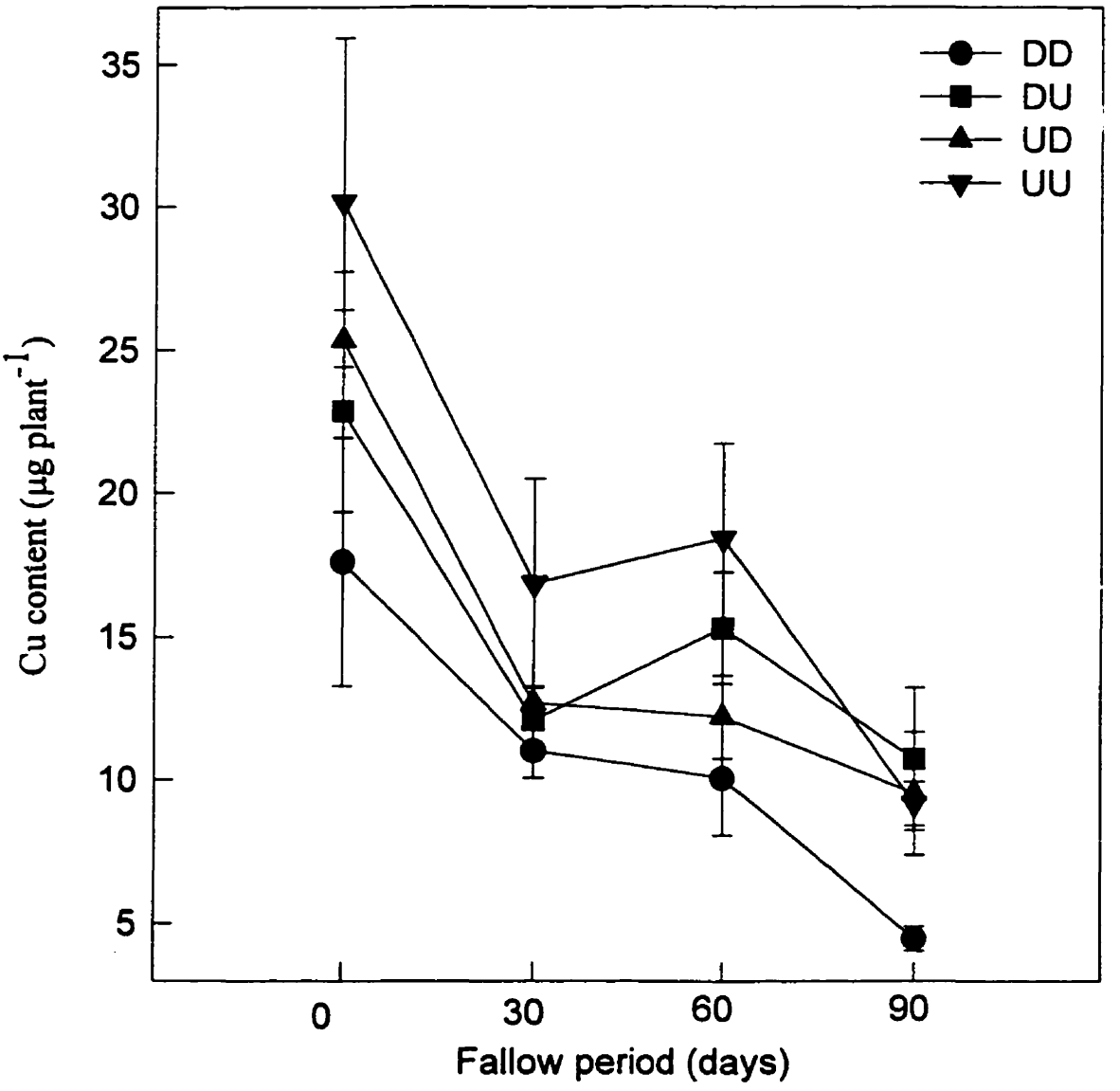

Fig. 4 Changes in copper contents of plants submitted to four soil-disturbance treatments and four fallow periods of different length. Data points represent the means of 5 reps with SE bars.

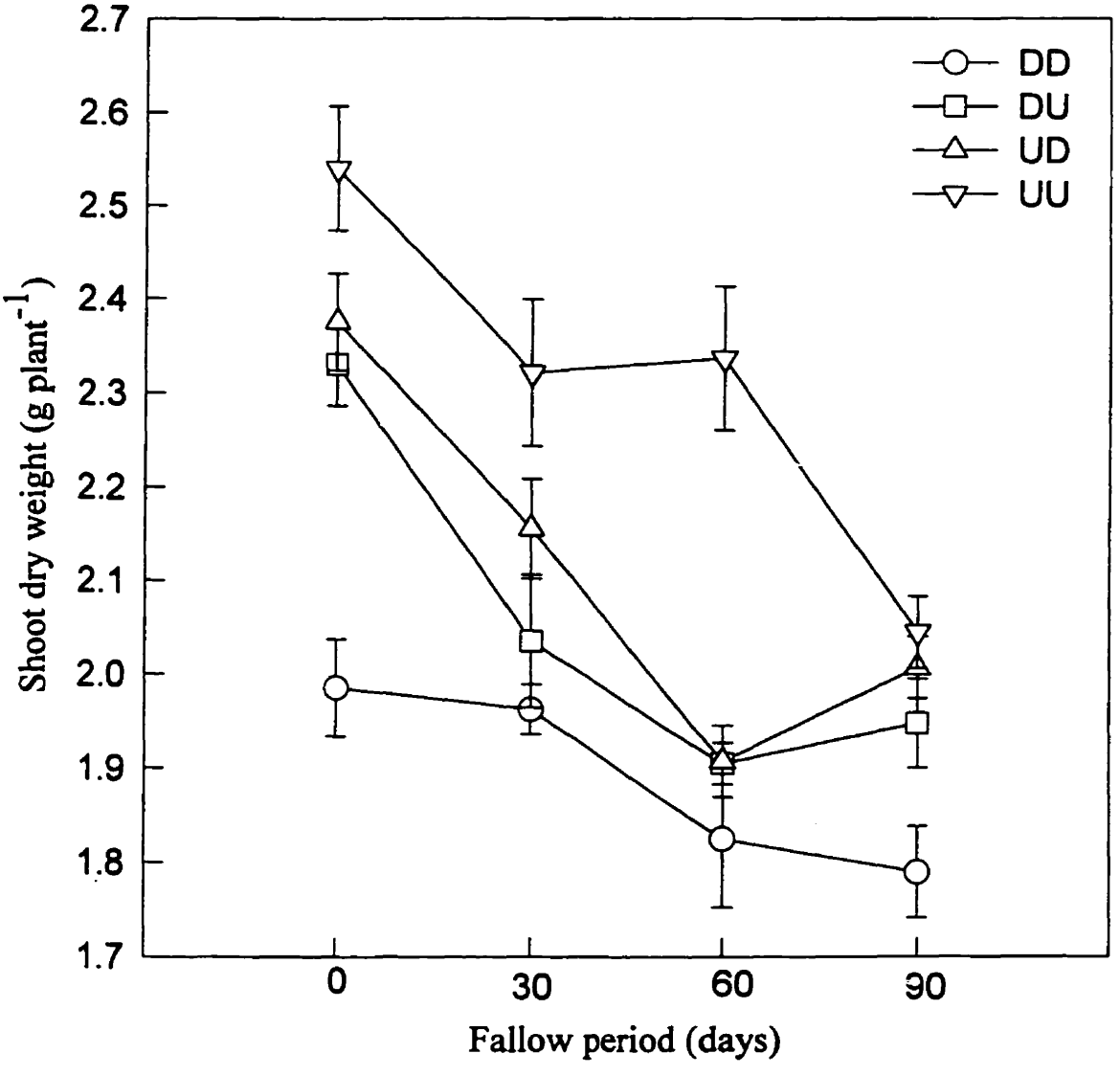

Fig. 5 Changes in shoot weight of plants submitted to four soil-disturbance treatments and four fallow periods of different length. Data points represent the means of 5 reps with SE bars.

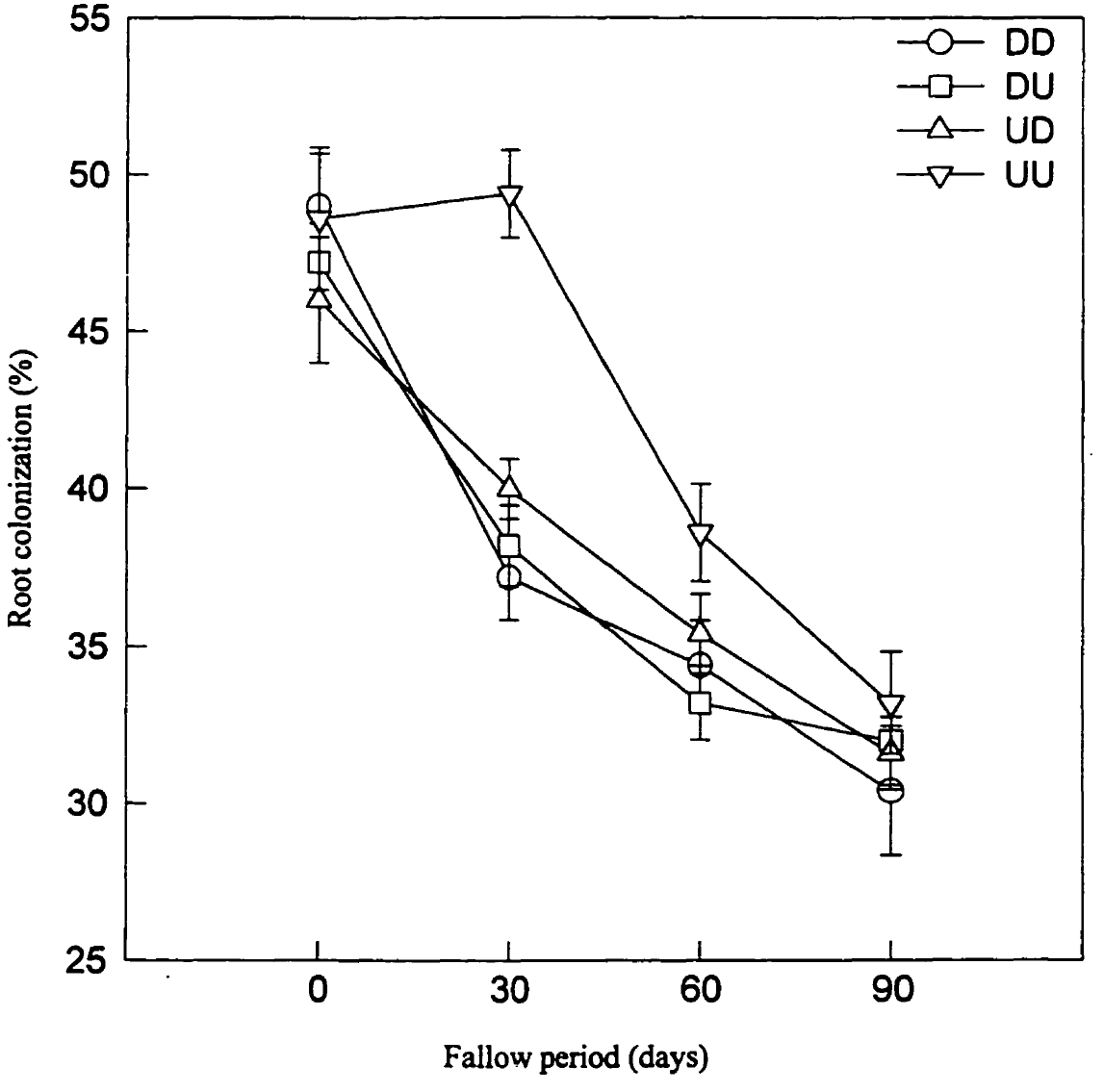

Fig. 6 Changes in mycorrhizal root colonization due to four soil disturbance treatments and four fallow period of different length. Data points represent the means of 5 reps with SE bars.

CONNECTING PARAGRAPH III

Results of the previous section suggested that soil disturbance and fellow periods reduce the activity of AMF and subsequently decreased nutrient content and growth of corn under a controlled environment. Used in combination soil disturbance and fallow interacted negatively on AMF activity. Chapter IV addresses the impact of tillage practices applied before and after winter, on the seasonal changes in AMF density in root and soil, in field soils under different fertilization regimes, from April, 1992 to April, 1994. Chapter IV was taken from the manuscript by Kabir, O'Halloran, Fyles and Hamel submitted to the journal Plant and Soil. The format of the text, therefore, conforms to the specification of the journal.

CHAPTER IV

SEASONAL CHANGES OF ARBUSCULAR MYCORRHIZAL FUNGI AS AFFECTED BY TILLAGE PRACTICES AND FERTILIZATION: HYPHAL DENSITY AND MYCORRHIZAL ROOT COLONIZATION

Abstract

The influence of tillage practices on native arbuscular mycorrhizal fungi (AMF) was studied in two consecutive years in eastern Canada, in two 11 year-old long-term tillage-fertilizer experimental field soils, a sandy loam and a clay, growing corn in monoculture. The three tillage practices were: 1) conventional tillage (CT; fall plowing plus spring disking), reduced tillage (RT; spring disking) and no-till (NT). The corn crop received either inorganic (N and K) or organic (liquid dairy manure) fertilizers. Mycorrhizal hyphal density was estimated from soil samples obtained in early spring (before plowing), at the 12-14 leaf stage, at silking, and at harvest. The percentage of corn root colonization by AMF at the 12-14 leaf stage, at silking and at harvest was also determined. The sandy loam was sampled over two consecutive seasons and the clay soil over one season.

Densities of total and metabolically active soil hyphae, and mycorrhizal root colonization were significantly lower in CT soil than in RT and NT soil. Lowest soil hyphal densities were observed in early spring. The levels of intra- and extraradical fungal colonization always increase from spring to silking and decreased thereafter. Spring disking had only a small and transient negative effect on hyphal abundance in soil. Fertilization did not influence mycorrhizal colonization of corn or abundance of soil hyphae in the sandy loam soil, but in the clay, soil metabolically active hyphae were more abundant with manure application than with mineral fertilization.

Key words : arbuscular mycorrhizal fungi, extraradical hyphae, manure, root colonization, seasonality, tillage,

Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in agricultural soil and play an important role in plant nutrition. Extraradical hyphae of AMF originate from the root cortex and proliferate in the surrounding soil from which they absorb nutrients of low mobility in soil such as P, Zn and Cu (Bürkert and Robson, 1994; Johansen et al., 1993; Kucey and Janzen, 1987; Li et al., 1991), that otherwise would be inaccessible to the plant (Bolan, 1991). Extraradical hyphae are also very important in soil conservation as they are a major factor of soil stable aggregate formation (Miller and Jastrow, 1992). Well aggregated soils are more resistant to erosive forces, have good gas exchange, water infiltration, water and nutrient storage capacity, and offer heterogenous microsites favouring microbial diversity (Blevins et al., 1984). Moreover, AMF hyphae constitute an important input of C and energy in soil; they are a major component of soil biomass (Hamel et al., 1991).

Extraradical hyphae are thought to be the main source of inoculum in soil (Brundrett et al., 1985; Kabir et al., 1996; Read et al., 1976; Sylvia, 1992;) especially in natural systems. Their role as principal propagules for the mycorrhizal colonization of AMF dependent field crops such as corn, might be of considerable importance, particularly in cool temperate climate where viable spore populations in agricultural soils may be extremely low following winter (Dalpé unpublished results). From the perspective of sustainable production, it is very important to understand the dynamics of AMF in agricultural soils as influenced by soil management strategies such as fertilization and tillage.

Soil tillage can reduce mycorrhizal colonization of roots (McGonigle et al., 1990). Greenhouse experiments have shown that extraradical hyphae of AMF are severely affected by soil disturbance (Fairchild and Miller, 1990; Jasper et al., 1989a, 1989b; McGonigle and Miller, 1996). High levels of mineral fertilization can also negatively affect AMF (McGonigle et al., 1990; Plenchette et al., 1983). The influence of manure on AMF development is less well known with manure use affecting AMF differently under different circumstances. Harinikumar and Bagyaraj, (1989) and St. John et al., (1983) observed positive effects of farmyard manuring on AMF associations whereas Hayman, (1982) reported a negative impact. Mosse (1986) reported no clear relationship between manure application level and incidence

of AMF fungal propagules.

The abundance of AMF in agricultural soil varies with the season of the year and also depends on factors such as inherent plant growth, edaphic factors, seasonal patterns in weather and seasonally applied management practices e.g. fertilization, manuring and tillage practices. Seasonal variation of AMF in spore numbers or frequency of sporulation was examined by Giovannetti (1985) and Stürmer and Bellei (1994), but spore numbers were not always related with the rate and extent of mycorrhizal formation (Abbott and Robson, 1982). Therefore, an assessment of the hyphal density and root colonization will be a more useful index to predict seasonality of AMF at the functional level under field condition.

Little is know about seasonal variations in the mycorrhizal development of agricultural crops and in the abundance of extraradical hyphae, as influenced by tillage and fertilization. Therefore, the present study was conducted to test the following hypotheses : (1) soil disturbance by fall plowing and, to a lesser extent, by spring disking reduces hyphal density in soil growing corn (2) Mineral fertilization is more detrimental to AMF development than fertilization with manure (3) Seasonal variation in hyphal mycorrhizal colonization of corn roots and in the abundance of soil hyphae is modulated by corn physiological development.

Material and methods :

Mycorrhizal development in corn was studied in an on going tillage-fertilization experiment run on two soils at the McGill University field research station ($45^{0}25'$ N longitude and $73^{0}56'$ W latitude), located at Ste-Anne-de-Bellevue, Quebec, Canada. The soils were a Macdonald clay (fine, mixed, frigid, Typic Humaquept) and a St-Benoit sandy loam (coarse-loam, mixed, nonacid, frigid Eutrochrept). The experimental plots were under timothy hay (*Phleum pratense* L.) and grain corn (*Zea mays* L.) respectively for five years prior to the initiation of the tillage-fertilization experiment in the fall of 1981. Since 1988 no phosphorus fertilizer had been applied to the fields. Variations in corn root colonization and abundance of soil hyphae were monitored from the spring of 1992 to the spring of 1994, inclusively, in the sandy loam, and the spring of 1992 to the spring of 1993 in the clay soil.

The treatments were applied to 6 x 10 m plots, arranged as split plots in a completely randomized block design with three replications. The three tillage practice treatments were randomized in the main plots and the two fertilization treatments were randomized in the sub-plots within each main plot. The three tillage practices were (1) conventional tillage (CT) consisting of fall mouldboard plowing plus spring disking, (2) reduced tillage (RT) consisting of spring disking only, and (3) no-till (NT) in which no tillage operation was performed and the corn crop was directly seeded into previous year stubble. The two fertilization treatments were: (1) inorganic fertilizer, consisting of 170 kg ha^{-1} of N (as urea for CT and RT, and as ammonium nitrate for NT) plus 75 kg ha^{-1} of K_2O (as potassium chloride), and (2) organic fertilizer, I. e. liquid dairy manure applied at a rate equivalent to 170 kg total N, 52 Kg of total P and 68 Kg of K ha^{-1}. Manure and inorganic fertilizers were applied on the soil surface and incorporated into the soil during the secondary operations of the RT and CT treatments, one to three days before planting. The mineral fertilizer and the manure applied in the NT plots were left on the surface. In May, six 75 cm spaced rows of corn (*Zea mays* L. cv. Funk 4120) per plot were planted at a rate to give a final population of 80,000 plants ha^{-1}.

Prior to initiation of the experiment soil P, K, Ca and Mg were determined with the Mehlich III extraction (Mehlich, 1984) and ions measurement by atomic absorption spectrophotometer and colorimeter. Percentage of sand, silt and clay was determined by the hydrometer method (Sheldrick and Wang, 1993). Soil organic matter (O.M.) was measured according to Nelson and Sommers (1982). Soil bulk density was determined using the method of Gee and Banders (1986). Monthly mean temperature and precipitation data were collected at Ste-Anne-de-Bellevue weather station (Macdonald Campus, McGill University, Québec, Canada), 1 km from the experimental areas.

Sampling of soil and plant material

To monitor changes in soil hyphal density, 12 soil cores (3.25 cm diameter), to a depth of 15 cm were taken randomly from each plot in early spring (April), at the 12-14 leaf stage of corn (June), at 80% silking (August) and at harvest (October). Cores from each plot were

pooled in a plastic bag and stored at 4^0C until extraction of the hyphae. Corn roots were also extracted from the soil samples taken at the 12-14 leaf stage, at 80% silking and at harvest, to measure mycorrhizal colonization. At the end of the season, corn grain and stover yield were determined from a 2 m^2 area randomly located in the centre of each plot. Grain yield data are expressed on a 15% moisture basis.

Determination of mycorrhizal root colonization

Each composite soil sample was homogenized in a bucket and root samples were collected for determination of mycorrhizal colonization levels in corn roots when applicable. The collected roots were washed on a 850 μm sieve under running water. Washed roots were cut into pieces 1 to 1.5 cm in length and placed in tissue embedding capsules. Root containing capsules were preserved in a formalin-acetic-acid-alcohol (FAA) solution (Phillips and Hayman, 1970) until processing.

The preserved roots samples were removed from the FAA solution and rinsed with water. Roots were then autoclaved in 10% KOH for 15 minutes, well rinsed with deionized water and stained with acid fuchsin (0.02%) in lactoglycerol at room temperature for at least 24 hours (Brundrett, 1994). The percentage of mycorrhizal colonization was measured by the grid-line intersect method (Giovannetti and Mosse, 1980) under a dissecting microscope with 50 X magnification.

Hyphal extraction and measurement

At every sampling date, six 5 g subsamples were taken at random from the composite soil samples to extract extraradical hyphae. The extraction technique used was modified from Abbott et al. (1984). Each subsample was placed in a blender with 300 mL deionized water and blended for 30 seconds to homogenize the soil suspension. This suspension was then poured through a 250 μm sieve and washed through with water, with hyphae collected on a 45 μm sieve placed below. The recovered material was resuspended in water, transferred to

a beaker, shaken for several seconds, and allowed to settle for 1 minute. The supernatant was filtered (pore size 11 μm) under vacuum. The procedure was repeated 3 times on each subsample for thorough extraction of soil hyphae. Although measurements obtained using a 30 μm sieve were about 10% higher than those on a 45 μm sieve, the nature of these agricultural soils (high organic residues and high clay content) necessitated the use of a 45 μm sieve.

The hyphae recovered from three of the six subsamples taken from each plot were stained by flooding the filters with a 0.2% aqueous solution of acid fuchsin. The excess of stain was removed by washing with deionized water and vacuum-filtration. The length of the recovered hyphae was measured using the modified grid line intersect method (Tennant, 1975), under 50 X magnification. At least 50 microscopic fields were observed per filter.

The hyphae recovered from the three other subsamples were stained by flooding the filters with a solution containing equal parts of iodonitrotetrazolium (INT) (1 mg mL^{-1} deionized water), reduced nicotinamide adenine dinucleotide (NADH) (3 mg mL^{-1} deionized water) and 0.2 M tris buffer at pH 7.4 (Sylvia, 1988). This stain detects dehydrogenase activity and, therefore, metabolically active hyphae. The filters were incubated for 12-16 hours at room temperature. The length of active hyphae was also measured by the modified grid line intersect method.

Statistical analysis

An analysis of variance was conducted using Statistical Analysis System (SAS Institute, 1990). Protected Least Significant Difference (LSD) tests were carried out according to Steel and Torrie (1980) to detect differences between treatment means when the analysis of variance indicated significant treatment effects. Variation in hyphal abundance and root colonization levels with time was analysed using the repeated measures analysis of variance procedure. In all procedures, probabilities less than 0.05 were considered to indicate statistical significance.

Results

Physical and chemical characteristics of both soil as influenced by 11 years of tillage-fertilization treatments is shown in Table 1, and monthly mean temperatures and cumulative monthly precipitation for the two years 1992 to 1994 of the experiment, are presented in Figure 1. The meteorological data indicate relative warm and cold months during the experimental period.

Overall, hyphal densities were slightly higher in 1992 than in 1993 (Fig. 2a, 2b). Hyphal density and root colonization (Fig. 2a, 2b, 2d and Fig.3a, 3b, 3d) was also apparently lower in the clay soil than in the sandy loam. Soil tillage reduced extraradical hyphal density. In both soils, densities of total and metabolically active hyphae were highest under NT, lower under RT and lowest in CT (Fig. 2a, 2b and Fig. 3a, 3b). The proportion of viable hyphae did not differ significantly between NT and RT tillage treatments but was always lower under CT (Fig. 2c) in the sandy loam soil. In the clay soil, the proportion of viable hyphae was highest in NT, lowest in CT and intermediate in RT (Fig. 3c).

The lowest densities of total and metabolically active hyphae were observed in spring. Hyphal densities and proportion of viable hyphae increased with plant development up to the silking stage in August in both the sandy loam (Fig. 2a, 2b, 2c) and the clay soil (Fig. 3a, 3b, 3c), and declined thereafter. In the sandy loam soil, total hyphal density averaged over treatments increased by 97% and 149% from spring to silking stage and decreased by 28% and 31% from silking to harvest in 1992 and 1993, respectively (Fig. 2a). In the clay soil, total hyphal density increased by 109% from spring to silking stage and decreased by 51% from silking to harvest (Fig. 3a). The reduction in hyphal density from fall to spring was largest under the CT treatment. Fall plowing reduced total hyphal density by 26% and 27% in the sandy loam in 1992 and 1993 respectively, while in the clay soil, this reduction was 20%.

Hyphal growth was delayed by spring disking in RT plots, but not in CT plots. As a result, in 1993, significantly less metabolically active hyphae were found in RT as compared to NT soils at the 12-14 leaf stage, in the sandy loam. In spite of the fact that metabolically active hyphae were more abundant in spring under RT than under CT, at the 12-14 leaf stage

no difference was found between the abundance of metabolically active hyphae of corn under RT and under CT in the clay soil (Fig. 3b) or in the sandy loam in 1993 (Fig. 2b). However, in 1992, in the sandy loam soil, there was no such result between RT and CT. The dynamics of mycorrhizal root colonization was similar to that of the soil hyphal abundance, being relatively low at the 12-14 leaf stage, increasing up to silking stage and decrease slightly thereafter. Overall, mycorrhizal root colonization was lower in CT than in NT and RT soils. At the 12-14 leaf stage, in the sandy loam soil, lower root colonization was found in CT than in NT or RT (Fig. 2d). Lower rate of mycorrhizal colonization in the clay soil under CT remained even after silking stage, but at harvest, the difference had disappeared (Fig. 3d).

Corn grain yield did not differ significantly among tillage treatments in 1992, in either soil (Fig. 5). In 1993, however, significantly higher yields were observed in NT and RT soil in the sandy loam soil. Treatments did not differ in stover yield (data not shown).

In the clay soil, manure significantly increased the abundance of metabolically active hyphae (Fig. 4), but no significant effect of fertilization was found in the sandy loam soil. Mycorrhizal colonization or corn yield was also not significantly differ between different fertilizer treatments.

Discussion

The seasonal variation in the abundance of soil hyphae and the intensity of mycorrhizal root colonization of corn had the was same pattern in both soils although hyphal densities and colonization levels were generally slightly greater in the sandy loam than in the clay soil. In a survey, Khan (1974) noted more AMF spores in light textured soils than in clayed textured soils. This differences may be explained, in part, by the properties conferred to the soil given by these different soil textures. In clay soils, total pore space is generally smaller than in sandy loam soils and gas exchange is reduced. A lack of drainage and aeration might restrict the development of the fungi.

The abundance of AMF hyphae fluctuated significantly within a growing season in the soils studied with lowest hyphal densities found in spring. At our latitudes, mycorrhizal

hyphae remain in the soil without a living host plant root from harvest in the fall to germination of the following crop in spring, and are submitted to adverse winter conditions. This may explain their loss of vigour. Fall plowing further stresses mycorrhizal hyphae physically disrupting them and exposing them to air or burying them to a deeper depth. In eastern Canada, Addy et al. (1994) observed that AMF hyphae in a field soil had the ability to survive and remain infective after expose to winter soil temperature -3.5^{0}C by forming 'resting hyphae'. Arbuscular mycorrhizal fungal hyphae could survive winter in agricultural soil relatively well, even when they are detached from their host roots and submitted to soil disturbance in fall. Survival is better, however, when hyphae overwinter attached to dead roots (Kabir et al. unpublished results).

Densities of total and metabolically active hyphae in both soils exhibited the same trend, increasing up to silking (August) and declining thereafter, but the increase in hyphal density lagged until the 12-14 leaf stage (June) in the clay soil. As hyphal densities were monitored during only one year in one clay soil, it is uncertain whether this growth pattern is characteristic of heavy soils which tend to be slower to warm up in spring than lighter soil. The development of mycorrhizal colonization was shown to be sensitive to low soil temperatures (Zhang et al. 1995). The lower abundance of hyphae in spring in CT soil was carried throughout the growing season. Similarly, lower mycorrhizal root colonization through out the growing season was observed in CT soil. Although the differences disappeared at harvest, the lower yield of grain obtained in CT in 1993 in the sandy loam soil suggests that the negative impact of plowing on AMF observed in the first part of the season was sometimes large enough to reduce yields.

Seasonal variation in mycorrhizal root colonization followed corn plant development, increasing up to silking (August) and decreasing thereafter. Abbott and Robson (1991) reported a rapid increase in the percentage of root colonization in annual crops, concurrent with active root growth, followed by a decline when roots senescent. Perennial plants however, seem to present a different development pattern. Diop et al. (1994) observed that mycorrhizal colonization of young Acacia trees (*Acacia albida*) was greater than older and that of potentially less vigorous trees. Brundrett and Kendrick (1988) noted with no seasonal

variation in mycorrhizal root colonization of mature sugar maple. Activity of nitrogen-fixing bacteria has also been change with plant development. Schmidt (1991) reported that populations of *Bradyrhizobium japonicum* increased in the rhizosphere of soybean during flowering and decreased thereafter. These results indicate that the development and activity of symbiotic organisms are closely associated with their host plant development, especially with annual plants. It seems that mycorrhizal development might be linked to the physiological state of the host plant more than to environmental conditions.

Tillage treatments significantly reduced densities of total and metabolically active hyphae. Soils under NT and RT practices were richer in fungal hyphae than CT soils. Our results are in accordance with the finding of Jasper et al. (1989a, b) who observed, in pot experiments, a negative effect of soil disturbance on hyphal growth. Negative effects of tillage on mycorrhizal sporulation were also reported by Douds et al. (1993). Arbuscular mycorrhizal fungi are likely to be hurt by tillage much more severely than other soil fungi because these fungi are mostly biotrophic. Hence, disruption of their extraradical hyphae and detachment from their host root system due to tillage practices is expected to reduce their growth and development.

A low proportion of viable hyphae was always associated with CT, a treatment which is thought to be particularly harmful to AMF. The proliferation of saprophytic hyphae are probably less repressed by tillage than that of AMF. If this is true, the lower proportion of viable hyphae in soil where AMF are less abundant would indicate that AMF hyphae have a slower turn over than saprophytic hyphae.

Several pot studies (Fairchild and Miller, 1988, 1990; O'Halloran et al. 1986) demonstrated clearly that mycorrhizal colonization of corn roots can be reduced by soil disturbance. In a field study, McGonigle and Miller (1993) found that mycorrhizal root colonization of corn was more extensive when plants were grown under no-till and ridge tillage than under conventional tillage until 50 days after planting, after which time the differences disappeared. We also found that mycorrhizal root colonization was delayed by tillage. Mycorrhizal root colonization levels were always lower in tilled plots although the differences between tillage treatments was not significant at later stages of corn development.

Soil disturbance due to spring disking apparently had little influence of soil hyphal proliferation in CT soil which had already been disturbed by plowing during the previous fall. In RT plots, however, disking induced a lag in hyphal development in the clay soil and in the sandy loam in 1993. The relatively larger impact of disking on hyphal development in RT plots may be due to fragmentation of the AMF mycelium and detachment of the hyphal network from the corn root systems which were yet undisturbed. It is also possible that incorporation of crop residues by fall plowing combined with spring disking in CT leads to warmer spring temperatures characteristics of plowed soils and to conditions favourable to proliferation of fungal saprophytes. Such an impact of CT on fungal saprophytes could have offset the negative impact of disking on total soil hyphae. The initial low abundance of soil hyphae measured after spring disking in RT was however, followed by a rapid increase in hyphal density, and at silking, the effect of disking had disappeared suggesting that spring disking is less detrimental to AMF than fall plowing.

In the clay, hyphal density was significantly higher with manure application than with inorganic fertilization with only N and K, although we observed same effect in the sandy loam soil in either year. This result was unexpected since manure is rich in P, and P fertilization is known to reduce mycorrhizal development (Abbott and Robson, 1991; Bethlenfalvay, 1992). No P was applied to plots receiving mineral fertilizers. It is possible that manure by improving soil biological properties favour AMF. Hepper and Warner (1983) reported the importance of organic matter in mycorrhizal development. In a field study over three consecutive seasons, an improvement in mycorrhizal populations with addition of farmyard manure as compared to mineral fertilizer application was reported by Harinikumar and Bagyaraj (1989) indicating the favourable effect of manure over inorganic fertilizers on AMF.

In conclusion, our results suggest that conventional tillage is detrimental to AMF, reducing the abundance of soil mycelium in spring, and delaying and limiting the mycorrhizal development of the following crop. Reduced tillage had much less severe negative impact on the abundance of soil hyphae and mycorrhizal colonization of corn by indigenous AMF. The development of AMF was closely associated with corn plant development under field conditions.

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Table. 1 Initial physical and chemical properties of experimental soils

Tillage	Fertilizer	Bulk density ($g\ cm^{-3}$)	Sand (%)	Clay (%)	pH in H_2O	O. M. (%)	P	K	Ca	Mg
							---------- $mg\ kg^{-1}$ ------			
				Sandy loam soil						
CT[a]	Organic	1.20	58.3	17.2	4.37	6.04	120	224	1270	127
	Inorganic	1.17	54.7	18.3	3.81	5.92	69	81	1017	45
RT[b]	Organic	1.16	56.7	16.4	6.58	4.35	142	269	1275	110
	Inorganic	1.23	57.2	16.9	6.77	5.13	91	138	1310	96
NT[c]	Organic	1.22	57.0	16.7	6.50	4.74	918	221	1228	126
	Inorganic	1.25	55.8	18.2	6.14	4.86	9	138	1107	33
				Clay soil						
CT	Organic	1.23	21.0	34.0	6.80	4.60	157	181	1805	446
	Inorganic	1.36	24.2	36.4	6.43	4.00	122	122	1102	225
RT	Organic	1.41	20.7	36.0	6.40	4.20	164	213	1423	341
	Inorganic	1.37	19.2	33.9	6.60	3.98	118	162	1019	301
NT	Organic	1.36	18.0	36.0	6.44	4.87	153	179	1300	197
	Inorganic	1.45	17.9	35.7	6.52	4.25	126	155	1259	216

[a]Conventional tillage, [b]Reduced tillage, [c]No-till

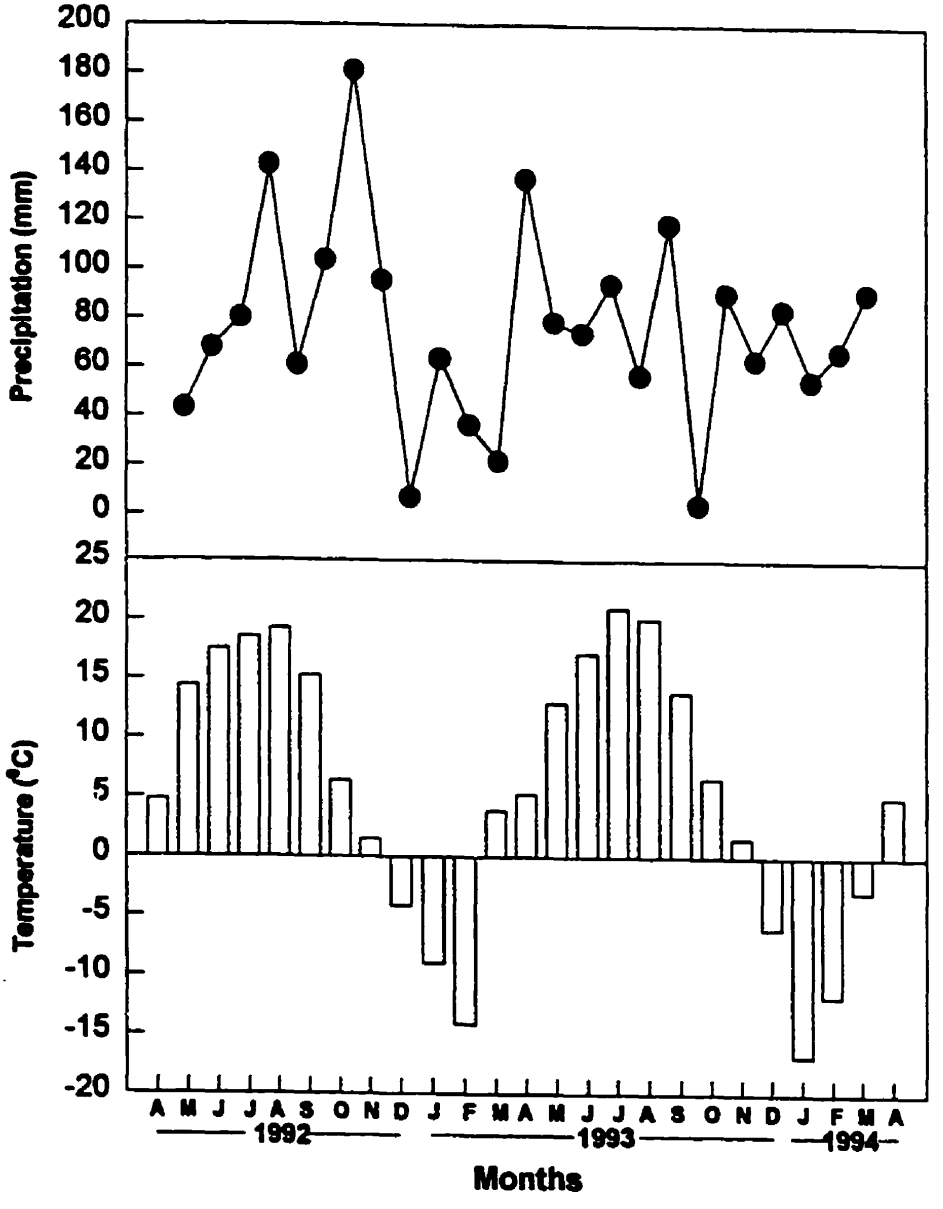

Fig. 1. Monthly mean temperatures and cumulative monthly precipitations for the length of the experiment.

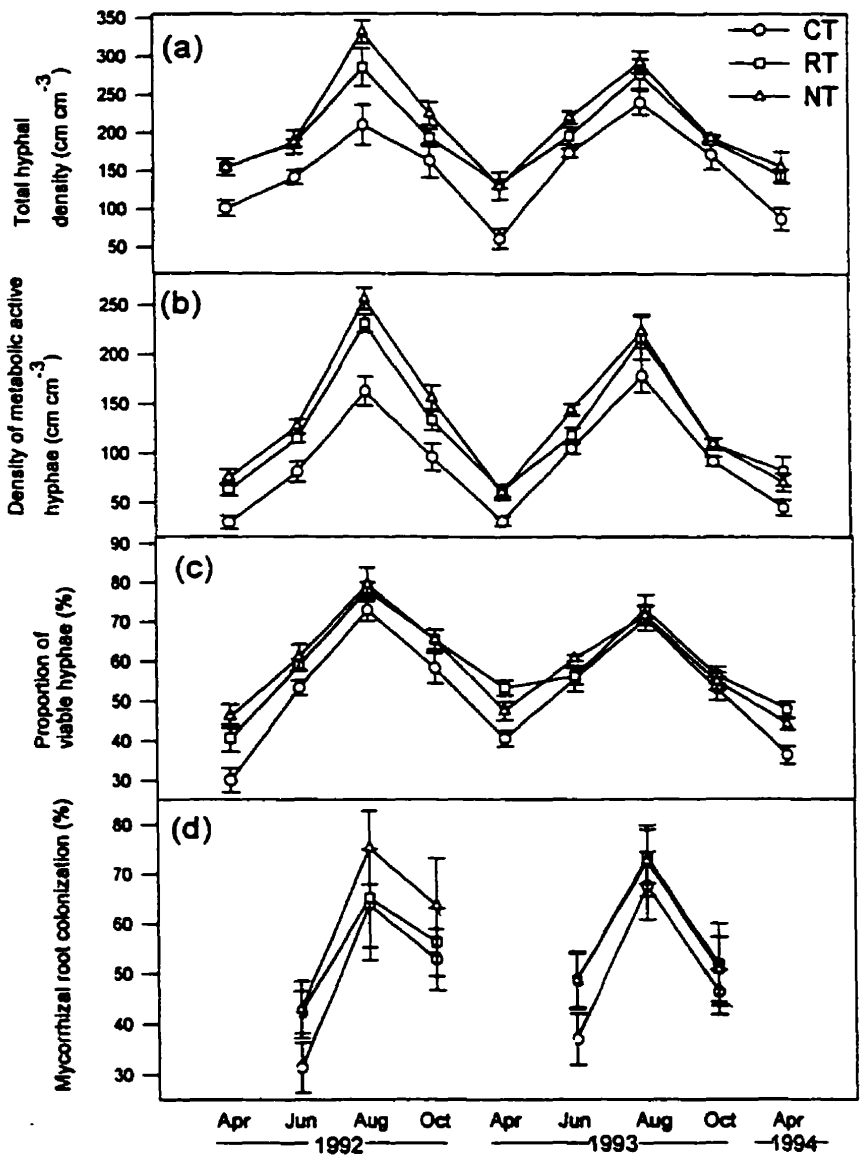

Fig. 2. Seasonal changes of total hyphal density (a), metabolically active hyphal density (b), proportion of viable hyphae (c), and mycorrhizal root colonization (d) in different tillage treatments in the sandy loam soil. Error bars represent the standard error. The month April (Apr) corresponds to spring, June (Jun) to the 12-14 leaf stage, August (Aug) to silking stage and October (Oct) to harvest.

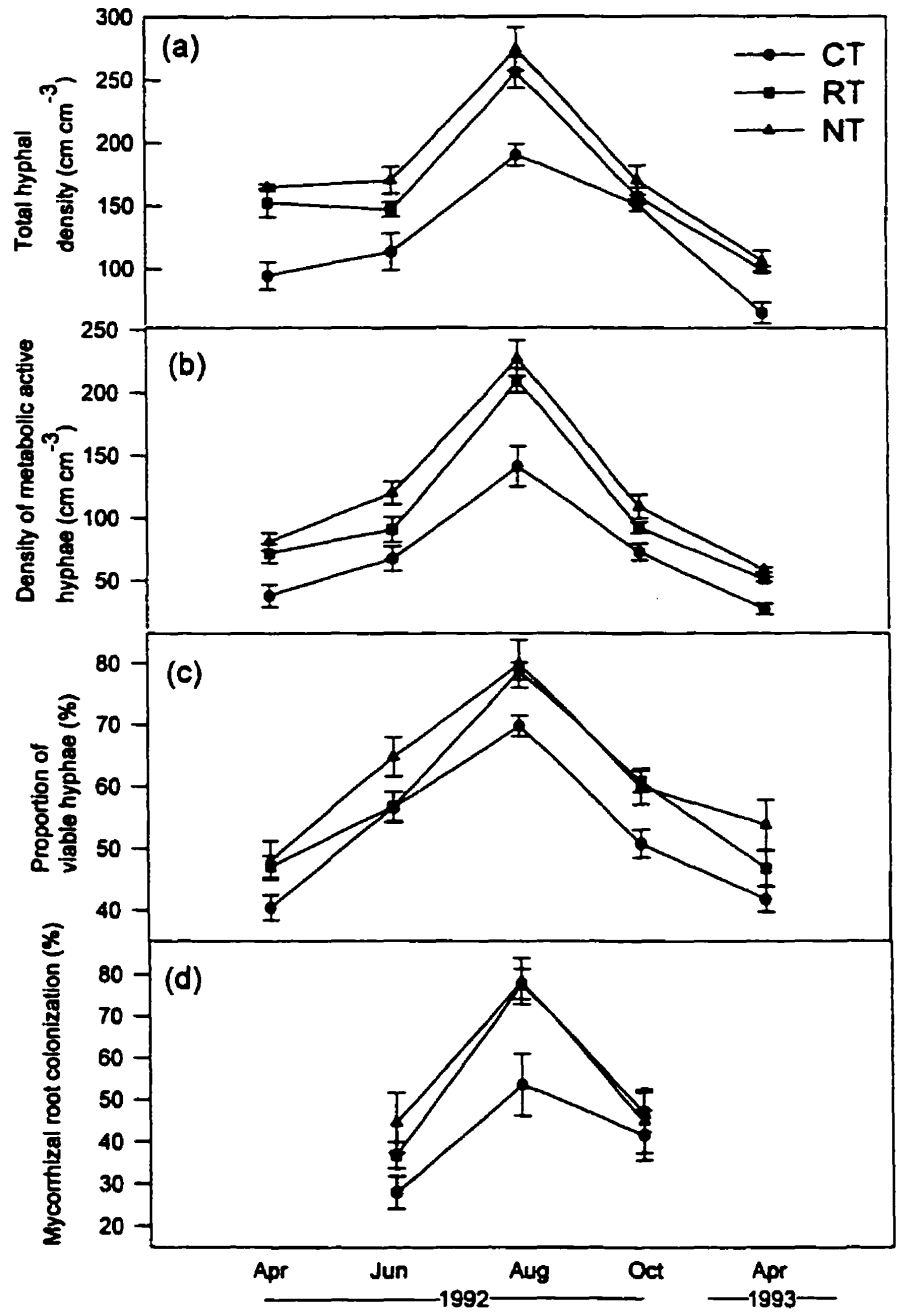

Fig. 3. Seasonal changes of total hyphal density (a), metabolically active hyphal density (b), proportion of viable hyphae (c) and mycorrhizal root colonization (d) in different tillage treatments in the clay soil. Error bars represent the standard error. The month April (Apr) corresponds to spring, June (Jun) to the 12-14 leaf stage, August (Aug) to silking stage and October (Oct) to harvest.

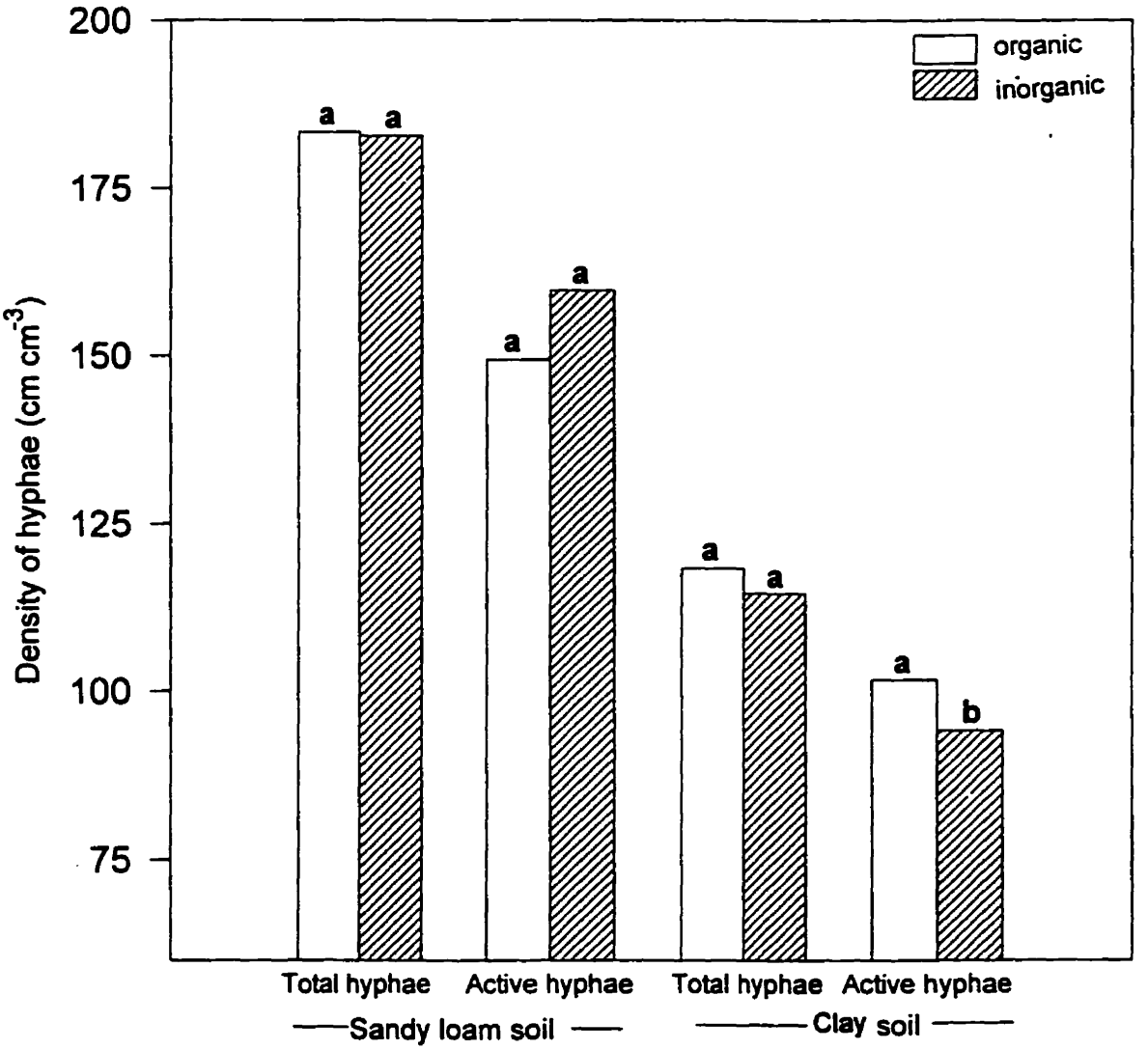

Fig. 4. Inorganic and organic fertilizer effects on sandy loam and clay soil. Values followed by the different letter within a sampling are significantly different at P = 0.05 level.

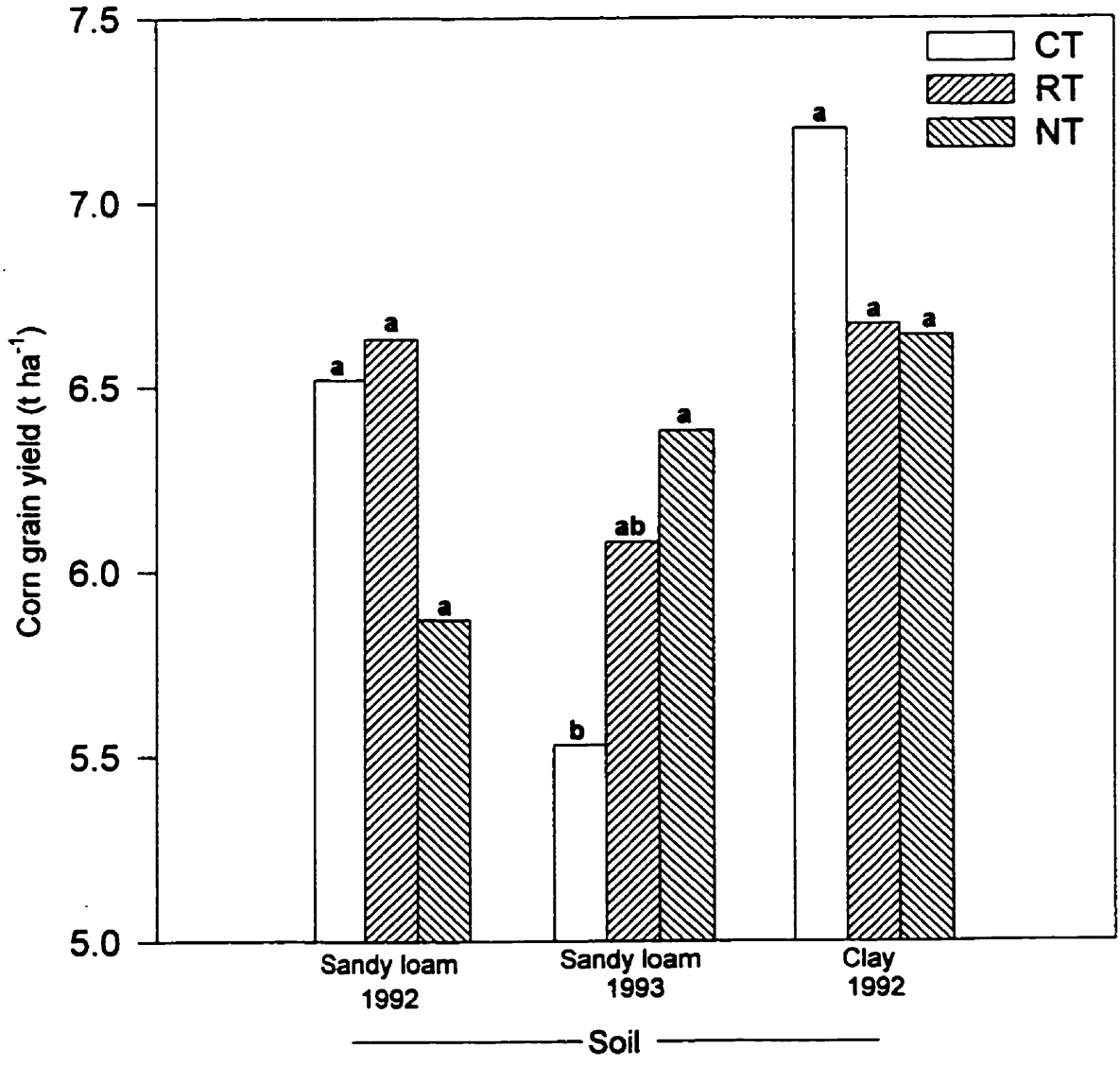

Fig. 5. Yields of corn grain harvests in 1992 and in 1993 from different tillage treatments in the sandy loam and clay soil. Values followed by the different letter/s within a sampling are significantly different at P = 0.05 level.

CONNECTING PARAGRAPH IV

The previous chapter depicted the seasonal variations in hyphal density and mycorrhizal root colonization in corn fields. Densities of hyphae were observed to be greatest at flowering of corn and declined in the fall with host plant senescence. Further decrease occurred over the winter months with the lowest values observed the following spring. Chapter V describes the changes in spatial distribution of mycorrhizal hyphae along with corn plant development and nutrient uptake of the plant during the season as influenced by tillage and fertilization. This chapter was taken from the manuscript by Kabir, O'Halloran, Fyles and Hamel submitted to the journal Agriculture, Ecosystems & Environment. The format of the text, therefore, conforms to the specification of the journal

CHAPTER V

DYNAMICS OF THE MYCORRHIZAL SYMBIOSIS OF CORN: EFFECT OF HOST PHYSIOLOGY, TILLAGE PRACTICE AND FERTILIZATION ON SPATIAL DISTRIBUTION OF EXTRARADICAL HYPHAE IN THE FIELD

ABSTRACT

Tillage and fertilization may reduce the abundance of indigenous arbuscular mycorrhizal fungi in agricultural field soils. The dynamics of hyphal abundance in soil was studied over two growing seasons in a corn crop grown in a sandy loam soil and over one growing season in a clay soil in eastern Canada. The experimental plots from a long-term tillage experiment, had been managed under no-till (NT), reduced tillage (RT) and conventional tillage (CT) for 11 years. Each of these tillage treatments had been receiving either inorganic (N and K) or organic (liquid dairy manure) fertilizer. Soil samples were collected from different places within each plot: on the row, at 18.75 cm from the row (quarter- row) and in between two rows (mid-row i.e. 37.5 cm from the row). Plant and soil samples were taken at the 12-14 leaf stage of corn (June), at silking stage (August) and at harvest (October), to measure the fluctuation in soil hyphal densities and plant nutrients concentrations during the season.

Densities of total and metabolically active hyphae were greatest in the row and lowest in the mid-row. Hyphal density on the row increased steeply from 12-14 leaf stage to silking stage and decreased thereafter, while no significant fluctuation of hyphal abundance was observed in the mid-row, suggesting a prevalence of AMF hyphae on the row and of saprophytes between the rows. Hyphal densities were higher in NT soil than in CT soil, while RT soil contained intermediate hyphal densities. The highest corn P, Zn and Cu concentrations were observed in NT and RT treatments concurrently with the highest hyphal densities. Concentrations of K, Ca and Mg did not change with tillage or fertilization type. Manure application significantly increased the densities of total and metabolically active hyphae in the clay soil.

Key words: arbuscular mycorrhizal fungi, tillage, manure, extraradical hyphal density, corn nutrition,

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) improve plant nutrient uptake (Manjunath and Habte, 1988; Li et al., 1991), water use efficiency (Hardie and Leyton, 1981; Kothari et al., 1991; Tarafdar and Marschner, 1995), and plant growth (Evans and Miller, 1988, 1990). They also contribute substantially to the maintenance of good soil structure (Bethlenfalvay and Barea, 1994; Miller and Jastrow, 1990; Tisdall, 1991). These benefits of the AMF symbiosis to plants and soils are derived from the extraradical phases of AMF.

The extraradical hyphae formed by AMF extend from the roots into the surrounding soil beyond the P depletion zone which generally develops around plant roots because P uptake by the plant is normally faster than P diffusion towards the roots. In plants with well established infection, each centimetre of root length is associated with 0.5-1.5 m of hyphae (Harley, 1989). The extraradical hyphae can also be an important source of inoculum (Brundett et al., 1985; Sylvia, 1992). Hence, the status of the extraradical mycelium development in the soil appears to be a major determinant of the efficiency of AMF to selective nutrient uptake. However, quantification of extraradical hyphae in the soil system has rarely been done (Sylvia, 1992) and the dynamic of AMF indigenous to agricultural fields as influenced by field management practices is not well understood. Most of the knowledge on the impact of soil management practices has been derived from experiments in which the impact of soil disturbance on AMF was inferred from the response of the host plant.

Spatial and temporal variation in the rate and extent of mycorrhizal hyphal development may occur concurrently with plant development. Plant metabolism most likely influences mycorrhizal development and, conversely, the spread of the mycorrhizal mycelium influences plant nutrient status. The mycorrhizal mycelium is closely linked to the plant development. The ecological significance of AMF is determined to a large extent by the spread and dynamics of AMF extraradical mycelium. Such information is essential for understanding the ecology of AMF. However, little information is available about the spatial and temporal patterns of hyphal development under field conditions.

Soil tillage has an impact on nutrient uptake by corn. Phosphorus absorption by corn

plants was reduced by soil disturbance (O'Halloran et al. 1986; Evans and Miller, 1988; Jasper et al., 1989a,b), but the soil disturbance effect disappeared when the soil was treated with γ radiation (O'Halloran et al. 1986). Similar results reported by Evans and Miller (1988) when soil was treated with benomyl, a fungicide, suggest that the observed reduction in P uptake by plants is related to harmful effects of soil disturbance on AMF. In other studies with *Spinacea oleracea* L. (Evans and Miller, 1988) and *Brassica napus* L. (O'Halloran et al. 1986), both known to be non-mycorrhizal, no difference in plant P uptake was observed when soil was disturbed, while soil disturbance reduced uptake of P by corn (*Zea mays* L.), a mycorrhizal plant. This further suggests that the disruption of extraradical hyphae of AMF by soil disturbance is the cause of reduction in mycorrhizal plant P uptake. The same mechanisms could also explain the reduction in Zn and Cu concentration in plants grown in disturbed soil (Evans and Miller, 1990; Fairchild and Miller, 1988).

Several authors (McGonigle et al., 1990; Jasper et al., 1989a,b) have proposed the involvement of extraradical mycorrhizal hyphae in the soil disturbance effect on plant growth. However, until now little attempt has been made to study the extraradical hyphae in the field as influenced by seasons and tillage practices. This experiment was conducted with the main objective to determine the spatial distribution of hyphae under corn, a row crop, and with following sub-objectives 1) the seasonal variation in soil hyphal densities under different tillage systems, and 2) to relate the concentrations of P and other nutrients to soil hyphal densities.

MATERIALS AND METHODS

The study was conducted in 1992 and 1993 at the McGill University field research station (45°25' N longitude and 73°56' W latitude), located at Ste-Anne-de-Bellevue, Quebec, Canada, in an 11-year old tillage-fertilization experiment run on two soils. Soil types were a St-Benoit sandy loam (coarse-loam, mixed, nonacid, frigid Eutrochrept) and a Macdonald clay (fine, mixed, frigid, Typic Humaquept).

The experiment had a split-split plot design in space and time (Steel and Torrie, 1980) with three replications. The main plots consisted of three levels of tillage intensity. The

practices tested were (1) conventional tillage (CT) consisting of fall mouldboard plowing plus spring disking, (2) reduced tillage (RT) consisting of spring disking only and (3) no-till (NT) in which no tillage operation was performed and the corn crop was directly seeded with a planter into previous year stubbles. Two types of fertilizer were randomized in the sub-plots. The fertilizer treatments were: (1) inorganic fertilizer, consisting of 170 kg ha^{-1} of N (as urea for CT and RT, and as ammonium nitrate for NT) plus 75 kg ha^{-1} of K_2O (as potassium chloride), and (2) organic fertilizer, i. e. liquid dairy manure was applied at a rate equivalent to 170 kg total N, 52 kg of total P and 68 kg of K ha^{-1}. Sub-subplots in space consisted of three sampling locations, on the row, at 18.75 cm from the row (quarter of the row) and at 37.5 cm from the row (mid-row), used to determined the spatial distribution of mycorrhizal hyphae. Sub-sub plots in time consisted of three sampling times, at the 12-14 leaf stage (June), at 80% silking stage (August) and at harvest (October) to determine the seasonal changes of mycorrhizal hyphae within a growing season.

Prior to initiation of the experiment soil samples were collected and air dried, crushed to pass through a 2-mm sieve. These subsamples were further ground to pass through a 40-μm sieve, and used for chemical analysis. Soil P, K, Ca and Mg were extracted with the Mehlich III extractant (Mehlich, 1984) and determined by atomic absorption spectrophotometer and colorimeter. Percentage of sand and clay was determined by the hydrometer method (Sheldrick and Wang, 1993). Soil organic matter (O. M.) Was measured according to Nelson and Sommers (1982). Soil bulk density was determined using the method of Gee and Banders (1986)

Soil and plant sampling

Soil cores (3.25 cm diameter) were taken from the top 15 cm on the row, at the quarter of the row and at mid-row in each sub-plot. At each sampling location, composite soil samples consisting of five soil cores were taken randomly for hyphal measurements. These soil samples were collected from both soils at the 12-14 leaf stage of corn (June), at 80% silking (August) and at harvest (October). Plant nutrient concentrations were determined from 5 corn

plants at the 12-14 leaf stage, from 10 ear leaves at silking, and from 1 kg of ground stover and from the seed of 10 cobs at harvest. Plant material was randomly collected from each sub-plot.

Plant analysis

Plant samples were dried at 65°C for 48 hours. Dry samples were weighed and ground to pass a 0.5 mm sieve. Plant samples were digested using concentrated H_2SO_4 and 30% H_2O_2 and their nutrient uptake was determined (Thomas et al., 1967). Total N and P was determined by colorimetry (Jackson, 1958), while K, Ca, Mg, Zn and Cu were determined by atomic absorption spectrophotometry.

Hyphae extraction and measurement

Four 5 g sub-samples were taken at random from the composite soil sample taken from each sampling location of the sub-plots, at each sampling date, to extract extraradical hyphae. The extraction technique used was modified from Abbott et al. (1984). Each subsample was placed in a blender with 300 mL deionized water and blended for 30 seconds to homogenize the soil suspension. This suspension was then poured through a 250 μm sieve and washed through with water. Hyphae were collected on a 45 μm sieve placed below. The recovered material was resuspended in water, transferred to a beaker, shaken for several seconds, and allowed to settle for 1 minute. The supernatant (containing hyphae) was filtered (pore size 11 μm) under vacuum. The shaking suspension was repeated 3 times on each subsample for thorough extraction of soil hyphae. Some hyphae could pass through the 45 μm sieve. The values obtained using a 30 μm sieve were about 10% higher. However, the nature of these agricultural soils necessitated the use of a 45 μm sieve.

The hyphae recovered from two of the four subsamples taken per plot were stained by flooding the filters with a 0.2% aqueous solution of acid fuchsin. The excess of stain was removed by washing with deionized water and vacuum-filtration. The length of the recovered

hyphae was measured using the modified grid line intersect method (Tennant, 1975), under 50 X magnification.

The hyphae recovered from the three other subsamples were stained by flooding the filters with a solution containing equal parts of iodonitrotetrazolium (INT) (1 mg mL^{-1} deionized water), reduced nicotinamide adenine dinucleotide (NADH) (3 mg mL^{-1} deionized water) and 0.2 M tris buffer at pH 7.4 (Sylvia, 1988). This stain detects dehydrogenase activity and, therefore, metabolically active hyphae. The filters were incubated for 12-16 hours at room temperature. The length of active hyphae was also measured by the modified grid line intersect method.

Though it may difficult to distinguish between AMF hyphae and other fungal hyphae in the soil. It is possible to recognize AMF extraradical hyphae, which have certain morphological characteristics (Sylvia, 1992) and this can be verified using antibody techniques. Evidence of this was provided in our previous study (Kabir et al, 1996).

Statistical analysis

All statistical analyses were carried out using general linear model procedure in the Statistical Analysis System (SAS, 1990). Protected Least Significant Difference (LSD) tests were used to compare treatment means when the analysis of variances indicated significant treatments effects. In all procedures, probabilities less than 0.05 were considered to indicate statistical significance.

RESULTS

Physical and chemical characteristics of both soil as influenced by tillage-fertilization treatments is shown in Table 1. Analysis of variance of the complete data set indicated that there were significant differences in hyphal densities and proportion of viable hyphae in both soils between tillage practices, between sampling location within each sub-plot and between sampling time (Table 2). There were several two-way interactions between the treatments and a three-way interaction between tillage, sampling location and sampling time for total and

metabolically active hyphae in the clay soil (Table 2).

The overall hyphal density was lower in 1993 than 1992 in the sandy loam soil. The hyphal density in the clay soil sampled only in 1992, was lower than that in the sandy loam. The relative treatment effects, however, were similar in both soils and both years. Application of manure significantly increased the densities of total and metabolically active hyphae in the clay soil but not in the sandy loam soil in either year (table 2). In the clay soil, manure application significantly increased the densities of hyphae only at the quarter-row.

Effects of sampling location and sampling time in the growing season on hyphal density

An interaction between sampling location and sampling time indicated greater fluctuation in hyphal densities on the row than between the rows, during the growing season in both the sandy loam (Fig. 1a, 1b) and in the clay soil (Fig. 1d, 1e). The densities of total and metabolically active hyphae first increased up to silking stage and decreased thereafter in both soils and in both years. On the row, a small increase in the proportion of viable hyphae was measured from 12-14 leaf stage to silking stage and this was followed by a large reduction in viability from silking to harvest (Fig. 1c, 1f). However, in the quarter-row and in the mid-row, the early season increase in the proportion of viable hyphae was much larger than that on the row and a large reduction was found thereafter.

Effects of tillage and sampling location on hyphal density

Densities of total and metabolically active hyphae were significantly influenced by sampling location and tillage in both soils (Table 2). There was an positive interaction between tillage and sampling location in the clay soil (Table 2). This interaction comes from the data indicating that hyphal density approached the mid-row minima closer to the row (i. e. quarter-row) in CT but was higher at quarter-row in NT (Fig. 2). In the sandy loam soil, in 1992, a similar interaction occurred but it was significant only with the densities of metabolically active hyphae. The highest densities of total and metabolically active hyphae and

the highest proportion of viable hyphae were found on the row and decreased with distance from the row (Fig. 2). About half of the total and metabolically active hyphae were observed on the row in both soil, whereas only about 22% total hyphae and approximately 16% metabolically active hyphae were observed 37.5 cm from the row (mid-row) in both soils (Fig. 2a, 2b, 2c, 2d).

Hyphal densities were highest in NT and lowest in CT in the sandy loam (Fig. 2a, 2b) and in the clay soil (Fig. 2d, 2e). The difference between NT and CT with respect to hyphal density was largest on the row, but in mid-row this difference disappeared in the sandy loam soil, in 1993 (Fig. 2a, 2b, 2d, 2e).

Effects of tillage and sampling time in the growing season on hyphal density

The significant interactions between tillage and sampling time indicated that the seasonal pattern of hyphal development changed with the tillage method used (Fig. 3). A more intensive sampling schedule would have provide a more precise picture of hyphal development and more precise values for the maximum densities reached with the different tillage systems used. For all tillage treatments, change in hyphal densities with time appeared to follow a similar trend, increasing with plant growth, and then decreasing at maturity. The proportion of viable hyphae also increased until silking stage and then decreased up to harvest (Fig. 3c, 3f). The abundance of soil mycelium, however, was higher in NT and RT than CT all over the seasons. In the RT and NT systems, the fluctuation in the abundance of total and metabolically active hyphae during the seasons were large as compared to those measured in the CT systems in both the sandy loam (Fig. 3a, 3b) and clay soil (Fig. 3d, 3e). The overall increase in total hyphal density from 12-14 leaf stage to silking stage was 57% and 30% in the sandy loam soil in 1992 and 1993, while in the clay soil, the increase was 67%.

Nutrient concentrations in corn plants

In the sandy loam soil, shoot P concentrations were significantly higher in NT and RT

treatment than in CT treatment at the 12-14 leaf stage, in 1993 (Table 3). In the same year, phosphorus concentrations in plant tissues at silking stage, and in the grain at harvest, were significantly higher in NT than in CT treatment, and intermediate in RT. The increase in corn P level in NT as compared to CT treatment was 34% at the 12-14 leaf stage and 28% at silking stage in the sandy loam soil. In 1992, P concentrations of corn plants grown on sandy loam were significantly higher in NT and RT than in CT, whereas at silking stage and at harvest these differences between tillage treatments disappeared in all soils and in all years. At the 12-14 leaf stage, P concentrations were 44% higher in NT than in CT. In the clay soil, P concentrations in corn under NT and RT were significantly higher than under CT at both the 12-14 leaf stage and silking stage (Table 4). In the clay soil, P level of corn under NT was increased by 43% as compare to CT, when measured at the 12-14 leaf stage.

A positive relationship between P uptake in corn and hyphal density in the soil was observed in the early stage of corn growth in both soils and in both years (Fig. 4a). This relationship was best in the sandy loam soil, in 1993 where it was significant at all stages except in the stover at harvest (Fig. 4b, 4c).

Plant Zn and Cu concentrations were sometimes higher in NT and RT treatments (Table 3 and 4). In the sandy loam soil, in 1993, Zn concentrations were significantly higher in NT and RT than in CT treatment at the 12-14 leaf stage but at silking stage, the difference between RT and CT had disappeared (Table 3). In the sandy loam soil, in 1992, Zn concentrations were 16% higher in NT than in CT at the 12-14 leaf stage, a difference which was statistically significant. No such trend between the tillage treatments was observed in the clay soil . Corn plant Cu concentration was higher in NT than in CT only at silking stage, in the clay soil.

Plant K, Ca and Mg levels were never influenced by the tillage treatments. Corn N concentration was lower in the NT treatment in the sandy loam soil at 12-14 leaf stage, in 1993 (Table 3). Nutrient concentrations of stover was not influenced by any of tillage systems.

DISCUSSION

The spatial distribution of fungal hyphae in the soil was a function of the distribution of the corn plants within the plots. Hyphal density was maximum directly under the corn rows and decreased logarithmically up to the mid-row, presumably, following root distribution. Anderson et al. (1987) noted that mycorrhizal abundance was related to the density of plant roots and as root systems grow, mycorrhizal hyphae proliferate. Our results confirm this observation. Bürkert and Robson (1994) also observed that the majority of extraradical hyphae of AMF were located close to the roots.

Mouldboard plowing in fall followed by spring disking reduced the abundance of extraradical mycorrhizal hyphae. The lower abundance of hyphae in CT soil was significant at all the sampling locations in 1992, in the sandy loam soil. However, in the clay soil and in the sandy loam, in 1993, this differences disappeared at the mid-row. Greater seasonal fluctuation of hyphal densities occurred on the corn row than at quarter and mid-row. If we assume that roots stimulate AMF more than other soil fungi, our observations suggest that AMF hyphae make up a larger proportion of the soil mycelium on the row than between the rows. Production of AMF hyphae is hampered after the death of the host plant and the mycorrhizal mycelium senesced. At harvest, total hyphal length had decreased markedly on the row. In contrast, in mid-row, corn root physiology had little influence on the development of the hyphae, and only a slight decrease in hyphal abundance was observed. It seems therefore, that relatively little mycorrhizal mycelium extends from one corn row to the next.

A relationship was observed between densities of hyphae and the growth stage of corn plants. In our experiment, hyphal density increased up to silking and decreased after this stage. Land and Schönbeck (1991) also observed a rapid increase in mycorrhizal colonization until ear emergence of winter barley, followed by a decreased until harvest. Likewise, Lópaz-Sánchez and Honrubia (1992) in a survey with several plant families noted a maximum colonization at flowering stage and a decrease thereafter. Mengel and Barber (1974) in a two-year field study observed exponential corn root growth up to silking stage and a decline in length thereafter. Wiesler and Horst (1993) reported that the most active CO_2 fixation rates

by a corn crop was reached at silking, the development stage at which the plant also has the highest demand for nutrients (Mengel and Barber, 1974). After silking, CO_2 fixation and nutrient uptake slow down up to plant senescence and death (Wiesler and Horst, 1993). Mengel and Barber (1974) suggested that the reduction of root length after silking is probably due to the high C demand of the ears, which reduces the translocation of C to the roots. Similarly, AMF hyphae are a sink of C and, as they are linked to the roots, they probably senesce along with them. Although mycorrhizal development could also be influenced by environmental conditions such as cold temperatures (Zhang et al., 1995), edaphic factors (Khan, 1974) or host genotype (Krishna et al., 1985), the physiological status of the host-plants certainly plays a major role.

Substantially higher hyphal densities were observed in the NT soil as compared to the CT soil, in three soil-years. It had been proposed, based on indirect evidence, that tillage practices such as plowing and disking had negative effects on AMF hyphal density by disrupting the hyphal network (McGonigle et al., 1990). After having directly observed, the negative effect of CT on soil hyphae a large proportion of which was mycorrhizal, we confirm conclusions previously drawn from a series of pot experiments conducted by Evans and Miller (1988, 1990) and Jasper et al. (1989a, b) that soil disturbance had negative impacts on mycorrhizal hyphal networks.

Densities of total and metabolically active hyphae were affected by manure in the clay soil but not in the sandy loam soil. The application of manure in the clay soil increased soil hyphal density. Manure may stimulate fungal saprophytes rather than AMF as manure is a source of P, and P is known to reduce mycorrhizal development (Abbott and Robson, 1991). It is also possible that manure favoured AMF by improving soil physical, chemical or biological properties. Several authors (Warner and Mosse, 1980; Hepper and Warner, 1983) reported the positive influence of manure on the independent growth of AMF. In a field study Harinikumar and Bagyaraj (1989) noted the favourable effect of farmyard manure over inorganic fertilizers on AMF. Although in our experiment, manure was applied on the corn row, significantly higher hyphal densities were noted only on the quarter of the row, in the clay soil. Increase in saprophytes on the row due to manure may have been masked by mycorrhizal

hyphae supported by the plant. At the quarter-row the AMF hyphae may have been reduced enough for the manure effect on saprophytes to be observed.

The higher P absorption by corn plant in NT observed at the 12-14 leaf stage in both soils are in accordance with the results of O'Halloran (1982) who also observed higher P absorption by field grown corn under NT than under CT. McGonigle et al., (1990) also obtained similar pattern of P absorption by corn in a no-till system. McGonigle and Miller (1993) in a two-year field study undertaken in eastern Canada, observed that corn shoot P concentrations were significantly greater under NT and RT than under CT. We also found that in addition of plant P, Zn and Cu concentrations were sometimes higher under NT than under CT. It was also well documented from pot studies (O'Halloran et al. 1986; Fairchild and Miller, 1990) and field studies (McGonigle and Miller, 1993; Kabir et al. unpublished data), that soil disturbance or tillage practice reduced mycorrhizal root colonization. The association of higher levels of P, Zn and Cu with higher mycorrhizal colonization of plant under reduced soil disturbance underscore the role of AMF in soil exploitation by corn root system.

The concentrations of K, Ca and Mg in different growth stages of corn were unchanged in different tillage treatments. These elements move toward the root largely by mass flow (Tisdale et al., 1985). They also move by diffusion but are much more mobile than P and, therefore, plants rely less on AMF hyphal extensions to take them up. In addition, the level of K, Ca and Mg in our soils was high. The low N concentration of corn plants was only noted at the 12-14 leaf stage, in the no-tilled sandy loam, in 1992. Our data, however, do not allow any conclusions to be drawn regarding the negative impact of NT on corn N-uptake in this study.

The abundance of hyphae were positively related with the P uptake of the plants. This suggests a role for mycorrhizal hyphae in the uptake of P from the soil. In addition, if plant requirement for P regulates mycorrhizal development (Menge et al., 1978), it seems that P levels of plants under NT and RT were suboptimal. This suggest that the yield potential of corn under conventional management was probably not reached, and, therefore, that yields could be improved by optimization of AMF symbiosis.

In conclusion, mycorrhizal hyphal proliferation under field conditions was related to spatial distribution of corn roots and plant phenology. High densities of AMF hyphae in NT were associated with higher concentrations of P in corn plants. Reduction of the intensity of tillage could promote the favourable effects of AMF, an impact of which is important in developing sustainable agricultural systems.

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Table 1. Initial physical and chemical properties of soil

Tillage	Fertilizer	Bulk density ($g\ cm^{-3}$)	Sand (%)	Clay (%)	pH in H_2O	O. M. (%)	P	K	Ca	Mg
							——— $mg\ kg^{-1}$ ———			
				Sandy loam soil						
CT[a]	Organic	1.20	58.3	17.2	4.37	6.04	120	224	1270	127
	Inorganic	1.17	54.7	18.3	3.81	5.92	69	81	1017	45
RT[b]	Organic	1.16	56.7	16.4	6.58	4.35	142	269	1275	110
	Inorganic	1.23	57.2	16.9	6.77	5.13	91	138	1310	96
NT[c]	Organic	1.22	57.0	16.7	6.50	4.74	918	221	1228	126
	Inorganic	1.25	55.8	18.2	6.14	4.86	9	138	1107	33
				Clay soil						
CT	Organic	1.23	21.0	34.0	6.80	4.60	157	181	1805	446
	Inorganic	1.36	24.2	36.4	6.43	4.00	122	122	1102	225
RT	Organic	1.41	20.7	36.0	6.40	4.20	164	213	1423	341
	Inorganic	1.37	19.2	33.9	6.60	3.98	118	162	1019	301
NT	Organic	1.36	18.0	36.0	6.44	4.87	153	179	1300	197
	Inorganic	1.45	17.9	35.7	6.52	4.25	126	155	1259	216

[a]Conventional tillage, [b]Reduced tillage, [c]No-till

Table 2. Summary of analyses of variance for hyphal measurements in the sandy loam and clay soil

Sources	df	Sandy loam soil '92			Sandy loam soil '93			Clay soil '92		
		Total hyphae (cm cm^{-3})	Active hyphae (cm cm^{-3})	Proportion of viable hyphae (%)	Total hyphae (cm cm^{-3})	Active hyphae (cm cm^{-3})	Proportion of viable hyphae(%)	Total hyphae (cm cm^{-3})	Active hyphae (cm cm^{-3})	Proportion of viable hyphae(%)
T[a]	2	0.0001***	0.0001***	0.003**	0.0004***	0.004**	0.141	0.0001***	0.0001***	0.003**
F[b]	1	0.674	0.77	0.38	0.53	0.199	0.30	0.029*	0.032*	0.47
TxF	2	0.56	0.83	0.29	0.35	0.74	0.50	0.545	0.65	0.89
Error (a)	10									
L[c]	2	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
TxL	4	0.088	0.002**	0.69	0.19	0.43	0.48	0.006**	0.0006***	0.88
FxL	2	0.415	0.81	0.67	0.30	0.37	0.38	0.59	0.85	0.67
TxFxL	4	0.40	0.40	0.58	0.689	0.77	0.51	0.95	0.95	0.37
Error (b)	24									
S[d]	2	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
TxS	4	0.052	0.021*	0.87	0.61	0.50	0.61	0.01**	0.007**	0.13
FxS	2	0.673	0.53	0.70	0.29	0.212	0.71	0.69	0.693	0.56
TxFxS	4	0.95	0.86	0.83	0.81	0.91	0.98	0.84	0.75	0.60
LxS	4	0.0001***	0.0001***	0.055	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
TxLxS	8	0.214	0.47	0.79	0.92	0.785	0.46	0.024*	0.01**	0.376
FxLxS	4	0.813	0.647	0.27	0.60	0.40	0.54	0.95	0.71	0.40
TxFxL	8	0.85	0.88	0.95	0.99	0.94	0.41	0.90	0.72	0.16
Error (c)	72									

*,**,***Significant at $p<0.05$, 0.01 and 0.001, respectively.

[a]tillage, [b]fertilizer, [c]sampling location, [d]sampling time

Table 3. Nutrient concentrations in corn, grown in the sandy loam soil

Till age	1992							1993						
	N	P	K	Ca	Mg	Zn	Cu	N	P	K	Ca	Mg	Zn	Cu
	----------mg g^{-1}----------					----μg g^{-1}---		----------mg g^{-1} ----------					----μg g^{-1}---	
12-14 leaf stage (June)														
NT[a]	46.0a[#]	4.96a	48.0a	6.62a	2.89a	45.8a	10.2a	36.6b	4.44a	44.0a	5.30a	2.40a	47.9a	10.0a
RT[b]	43.6a	4.27a	45.8a	6.01a	2.52a	42.3a	9.5a	37.0ab	3.95a	45.8a	4.95a	2.33a	44.8a	10.0a
CT[c]	41.6a	3.44b	45.6a	5.85a	2.24a	39.4b	9.2a	38.6a	3.31b	44.3a	5.36a	2.48a	38.6b	8.6a
Silking stage (August)														
NT	30.5a	2.69a	33.8a	4.61a	1.93a	28.1a	10.2a	30.4a	4.15a	33.8a	3.50a	2.04a	34.4a	10.0a
RT	29.0a	2.65a	33.1a	4.21a	1.89a	27.1a	9.5a	26.8a	4.02a	34.3a	3.50a	2.23a	32.0ab	10.0a
CT	27.7a	2.44a	31.9a	4.15a	1.86a	26.3a	9.2a	29.5a	3.24b	31.9a	3.44a	2.08a	27.0b	8.6a
Grain (October)														
NT	14.5a	2.69a	5.4a	0.11a	1.62a	nd[*]	nd	13.1a	3.65a	5.6a	0.13a	1.55a	nd	nd
RT	15.4a	2.65a	5.0a	0.11a	1.52a	nd	nd	12.8a	3.2ab	5.6a	0.12a	1.51a	nd	nd
CT	14.5a	2.44a	4.9a	0.10a	1.49a	nd	nd	12.9a	2.87b	5.6a	0.11a	1.51a	nd	nd
Stover (October)														
NT	10.1a	0.84a	10.4a	nd	nd	12.2a	5.8a	9.7a	0.94a	11.7a	2.92a	1.22a	7.3a	3.8a
RT	9.0a	0.78a	10.4a	nd	nd	12.4a	6.3a	9.1a	0.89a	12.2a	2.75a	1.27a	6.9a	3.9a
CT	9.1a	0.66a	10.6a	nd	nd	11.7a	6.1a	11.1a	0.88a	13.0a	3.00a	1.27a	5.7a	3.8a

[a]No-till, [b]Reduced tillage and [c]Conventional tillage

[*]not determined

[#]Means in the column within same block followed by the same letters are not significant at the 0.05 level, according to Least Significant Difference (LSD) test.

Table 4. Nutrient concentrations in corn, grown in the clay soil

Tillage	1992						
	N	P	K	Ca	Mg	Zn	Cu
	——mg g^{-1}——					——μg g^{-1}——	
12-14 leaf stage (June)							
NT[a]	44.7a[d]	5.49a	46.2a	6.85a	3.67a	48.2a	16.1a
RT[b]	46.8a	5.35a	45.1a	7.27a	3.87a	46.3a	15.6a
CT[c]	47.5a	3.83b	44.9a	7.73a	4.12a	43.1a	13.8a
Silking stage (August)							
NT	28.5a	2.93a	34.9a	4.24a	2.28a	30.0a	11.2a
RT	26.8a	2.84a	35.7a	4.54a	2.26a	30.3a	10.6a
CT	28.6a	2.72b	34.5a	4.99a	2.36a	28.5a	9.8b
Grain (October)							
NT	14.2a	3.60a	4.9a	0.11a	1.68a	nd[e]	nd
RT	13.9a	3.59a	4.8a	0.11a	1.57a	nd	nd
CT	14.6a	3.37a	5.1a	0.11a	1.99a	nd	nd
Stover (October)							
NT	8.0a	1.13a	10.0a	2.87a	2.20a	7.7a	4.4a
RT	8.4a	1.08a	9.4a	2.99a	2.30a	7.9a	4.2a
CT	8.2a	0.99a	10.0a	2.94a	1.99a	7.0a	3.3a

[a]No-till, [b]Reduced tillage and [c]Conventional tillage

[e]not determined

[d]Means in the column within same block followed by the same letters are not significant at the 0.05 level, according to Least Significant Difference (LSD) test.

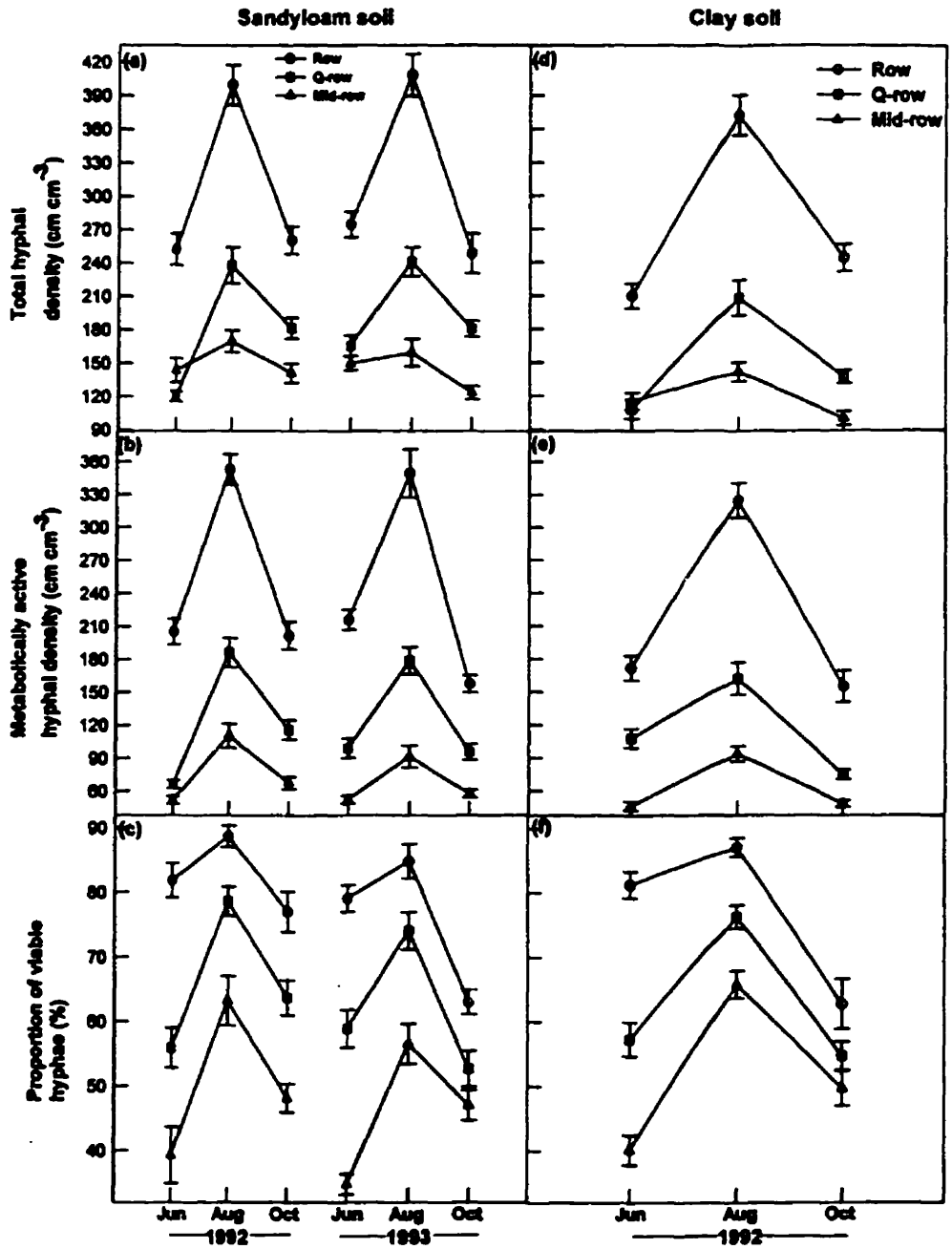

Fig. 1 Spatial distribution and seasonal variation of total hyphae (a, d), metabolically active hyphae (b, e) and proportion of viable hyphae (c, f) in different sampling locations in the sandy loam and clay soil respectively. Error bars represent the standard error. The month of June (Jun) corresponds to the 12-14 leaf stage of corn, August (Aug) to silking stage and October (Oct) to harvest.

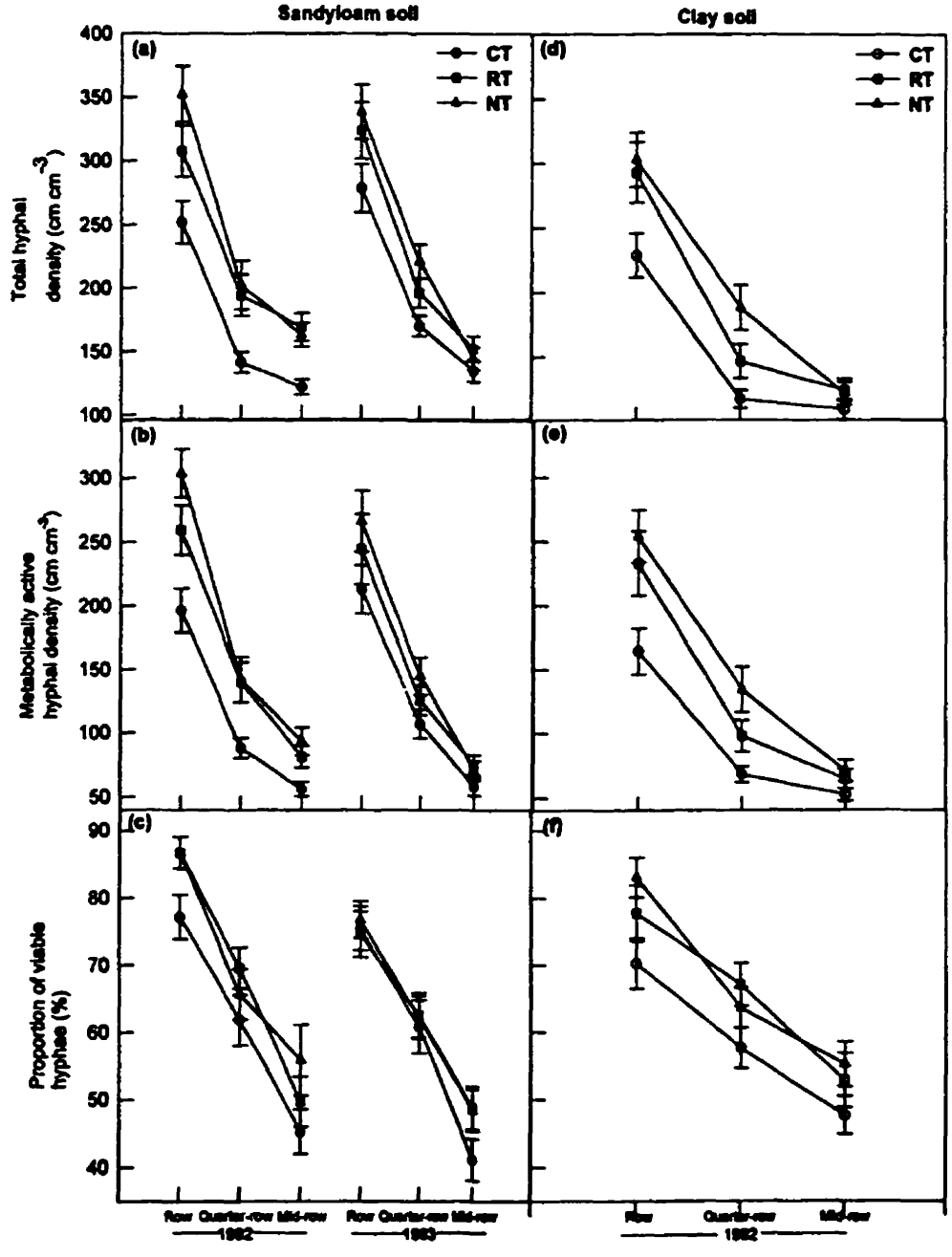

Fig. 2 Spatial distribution of total hyphae (a, d), metabolically active hyphae (b, e) and proportion of viable hyphae (c, f) in different locations in the sandy loam and clay soil respectively. Error bars represent the standard error.

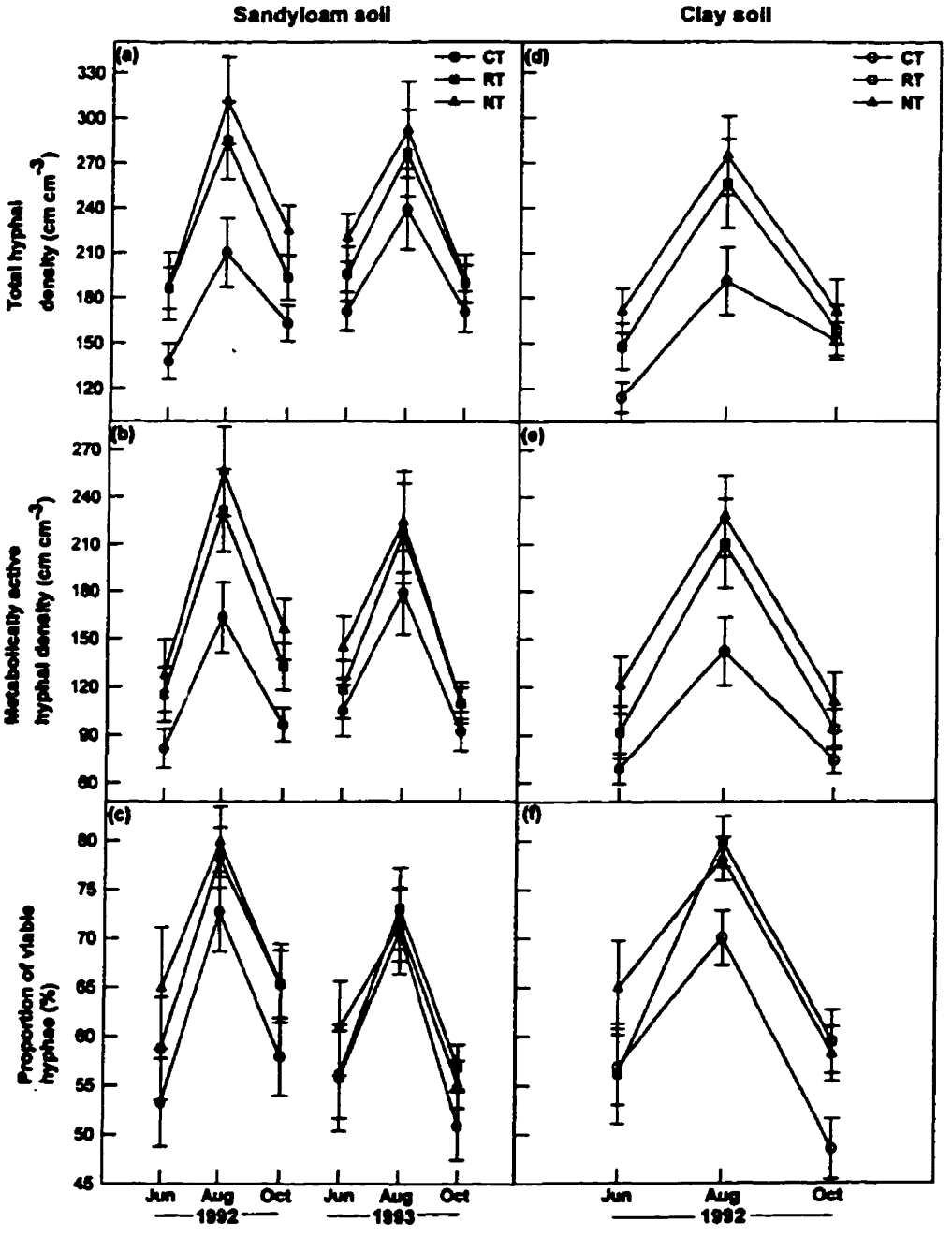

Fig. 3 . Temporal distribution of total hyphae (a, d), metabolically active hyphae (b, e) and proportion of viable hyphae (c, f) in different tillage treatments in the sandy loam and clay soil respectively. Error bars represent the standard error. The month of June (Jun) corresponds to the 12-14 leaf stage of corn, August (Aug) to silking stage and October (Oct) to harvest.

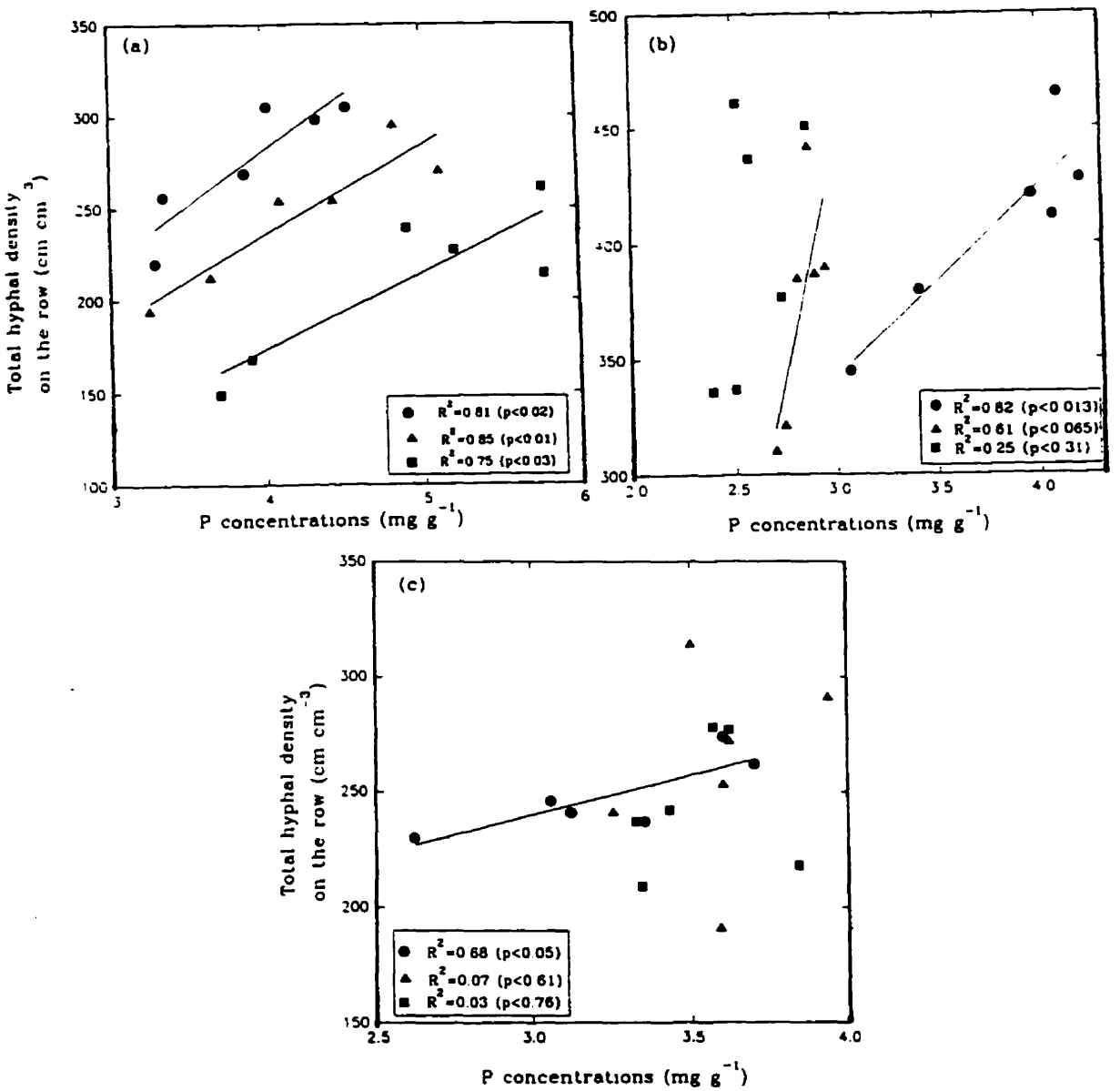

Fig. 4. Relationship between total hyphal density on the row and P concentrations in corn grown in the clay soil, in 1992 (■) and in the sandy loam soil, in 1992 (▲) and in 1993 (●), at the 12-14 leaf stage (a), at silking stage (b) and at harvest (c).

CONNECTING PARAGRAPH V

Chapter VI was taken from the manuscript by Kabir, O'Halloran, Widden and Hamel submitted to the journal Mycorrhiza. The format of the text, therefore, conforms to the specification of the journal. In chapter V, it was reported that the densities of hyphae were greatest on the corn rows and decreased linearly up to the mid-row. Larger seasonal fluctuation in hyphal abundance was observed on the row when AMF were concentrated than in the mid-row. In the following chapter, the effects of tillage and fertilization on the vertical distribution of mycorrhizal hyphae, root colonization and spores were studied.

CHAPTER VI

VERTICAL DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI UNDER CONTINUOUS CORN IN LONG-TERM NO-TILL AND CONVENTIONAL TILLAGE SYSTEMS

Abstract Agricultural practices such as tillage and manuring may affect the amount and distribution of arbuscular mycorrhizal fungal (AMF) inoculum in the soil. We examined the vertical distribution (0-25 cm) of AMF spores and soil hyphae at grain filling stage of corn subjected to either no-till (NT) or conventional tillage (CT) for 12 years. Both tillage systems had been treated with either organic (liquid dairy manure) or inorganic (N and K) fertilizers for six years. There was no difference in AMF hyphal density, spore numbers or mycorrhizal colonization associated with the application of dairy manure as compared to inorganic fertilization. Arbuscular mycorrhizal fungal spores, hyphae and root colonization were correlated and were more abundant in the 0-15 cm layer of the soil profile and decreased dramatically below this depth. Both hyphal density and spore number were significantly greater only in the 0-5 cm layer of the NT compared to that of the CT soil. The proportion of viable hyphae was not influenced by tillage and decreased linearly with depth suggesting that the quality of the environment for AMF decreases with depth. Similarly, mycorrhizal root colonization was not influenced by tillage system. Six *Glomus* species were observed in NT and three in CT soils. The vertical distribution of total, metabolically active hyphae, spores and mycorrhizal root colonization would suggest that tilling the soil at 15 cm depth in this soil would affect most of the AMF mycelia and spores and that plowing below 15 cm depth would dilute the AMF inoculum in the zone of seedling establishment.

Key words Arbuscular mycorrhizal fungi . extraradical hyphae . spore . tillage . manuring . corn

Introduction

Arbuscular mycorrhizal fungi (AMF) facilitate the uptake of non-mobile nutrients by plant (Khalil et al. 1994). The extraradical hyphae of mycorrhizae exploit a soil volume many times greater than that available to an individual root system without AMF. Tillage practices and fertilizer application can alter the vertical distribution and the size of the AMF population (Douds et al. 1993; Douds et al. 1995; Harinikumar and Bagyaraj 1989; McGonigle and Miller 1993; McGonigle et al. 1990). It has been observed in pot studies (Evans and Miller 1988; Jasper et al. 1989a) and in a field study (Douds et al. 1993) that soil disturbance reduces the AMF population. Mycorrhizal colonization of corn plants is often reduced due to tillage practices (McGonigle et al. 1990; McGonigle and Miller 1993). Quantitative studies of mycorrhizal fungi have been done either based on AMF spore numbers or on host root colonization (Vilariño et al. 1993). Several authors (Graham et al. 1982; McGonigle et al. 1990) proposed that extraradical hyphae are better determinant of mycorrhizal efficiency than root colonization. In spite that the extraradical hyphae of AMF play a fundamental role in the symbiosis, bridging the plant and the soil (O'Neil et al. 1991) and being the major agent of propagation (Brundrett et al. 1985; Read et al. 1976; Sylvia 1992), the distribution of extraradical hyphae in the soil profile has rarely been measured, if ever.

Number of mycorrhizal spores in annual crops are usually more abundant in the surface soil but in perennial plant considerable amounts of spores occur at greater depth (White et al., 1989; Zajcek et al. 1986). Studies of spore distribution (An et al.1990; Smith 1978) through the soil profile, have shown that spore number generally decreases with soil depth. Mosse et al. (1981) reported that AMF spores are not normally found below the normal plant rooting zone. Khalil et al. (1992) showed that AMF sporulation was positively correlated with environmental stress. The distribution of mycorrhizal hyphae in different management systems is important because these hyphae supply soil nutrients to the plant and improve soil aggregate stability (Miller and Jastrow 1990; Miller and Jastrow 1992).

Little information exists about the vertical distribution of mycorrhizal spores and hyphae in agricultural soil. A better understanding of how tillage and fertilization might affect

the distribution of AMF is of great importance. Therefore, an experiment was conducted to examine the influence of tillage practices and fertilization on the vertical distribution of AMF spores, fungal mycelium and mycorrhizal root colonization in the upper 25 cm of soil planted to corn.

Materials and methods

The experiment was conducted in 1993 at the McGill University research field station located at 45°25' N and 73°56' W. The soil was a St-Benoit sandy loam (Eutric Cambisol). The tillage treatments, no-till (NT) in which no tillage operation was performed and the corn crop was directly seeded with a planter into previous year stubble and conventional tillage (CT) consisting of fall mouldboard plowing followed by spring disking. These tillage treatments were established on continuous corn (*Zea mays* L.) Plots in 1981. No phosphorus fertilizer had been applied in the experimental field since 1988 at which time, mineral fertilization with N and K, and manure treatments began. Tillage treatments were randomized in the main plots which were split in half to received the fertilizer treatments. These two treatments, inorganic (170 kg ha^{-1} of N and 75 kg ha^{-1} of K_2O), and organic fertilizer (170 kg total N, 52 kg of total P and 68 kg of K ha^{-1} from dairy-cow manure) were randomized in the sub-plots. Treatments were applied to 6x10 m sub-plots. There was three repetitions. Corn was planted in early May of 1993. There was 80,000 plants ha^{-1}. Prior to initiation of the experiment, soil extractible P, K, Ca and Mg (Mehlich 1984) at 25 cm depth had been determined by atomic absorption spectrophotometry and colorimetry. Percentage of sand and silt was determined by the hydrometer method (Sheldrick and Wang 1993). Soil organic matter was measured according to Nelson and Sommers (1982) and soil bulk density was determined using the method of Gee and Banders (1986).

Soil sampling

Soil sampling was done to extract extraradical hyphae, spore and mycorrhizal root. Sampling was done with a 3.25 cm diameter soil corer to a depth of 25 cm. Soil was sampled at random locations on the corn row at grain filling stage at the beginning of September, 1993. Five soil cores were taken randomly from each plots. Each core was divided into sections corresponding to 0-5, 5-10, 10-15, 15-20 and 20-25 cm measured from the surface. The depth-specific sections from the five cores were thoroughly mixed to form five composite soil samples one for each depth in each plot. The soil samples were stored in a cold room (oC) until extraction of hyphae and spores. Hyphal extraction was undertaken immediately after collection of the soil samples.

Spore extraction and quantification

Two sub-samples were taken from the composite sample for spore extraction. A wet sieving and decanting technique (Pacioni 1992) was used for spore recovery. One hundred grams of soil were poured onto the top of a series of sieves (850 μm, 500 μm, 300 μm and 35 μm). The material retaining on the finest sieve was partitioned in two centrifuge tubes containing a 60% sucrose solution and centrifuged at 1500 r.p.m. for 20 minutes. The supernatant was collected with a pasteur pipette and filtered through a gridded millepore filter (0.45 μm). The filter was washed with distilled water to remove sucrose and to distribute spores evenly on the filter. Spores present on 10 squares randomly chosen were counted and the total number of spores on the filter were estimated. Spores were counted under a stereoscopic microscope with 100-200 times magnification. Identification of AMF species was carried out by Dr. Y. Dalpé, Eastern Cereal and Oil seed Research Centre, Agriculture and Agri-Food Canada, Ottawa and based on spore features and wall.

Root preservation and mycorrhizal colonization determinations

Root samples were separated from the soil and organic debris under running water and collected on the 850 µm sieve. Washed roots were cut into 1 to 1.5 cm pieces and placed in tissue embedding capsules. Root material was then preserved in a formalin-acetic acid-alcohol (FAA) solution (Phillips and Hayman 1970) until further processing. When roots were removed from the FAA solution, these were rinsed with water and autoclaved in 10% KOH for 15 minutes, rinsed with deionized water and stained with acid fuchsin (0.02%) in lactoglycerol at room temperature for at least 24 hours (Brundrett 1994). The percentage of mycorrhizal colonization was measured by the grid-line intersect method (Giovannetti and Mosse 1980) under a dissecting microscope with 40 to 50 X magnification.

Hyphal extraction and measurement

Hyphae were extracted from four 5 g sub-samples taken from the composite soil samples described in the section "Soil sampling". The extraction technique use by Abbott et al. (1984) was modified. Each sub-sample was placed in a blender with 300 ml deionized water and blended for 20 seconds to homogenize the soil suspension. Blending was done at the lowest speed. This suspension was then poured through a 250 µm sieve overlaying a 45 µm sieve and rinsed with water. The material recovered on the 45 µm sieve was resuspended in water, transferred to a beaker, shaken for several seconds, and allowed to settle for 1 minute. The supernatant (containing hyphae) was filtered (pore size 11 µm) under vacuum. The 45 µm fraction was resuspended 3 times as described for a thorough extraction of soil hyphae. Some hyphae could pass through the 45 µm sieve. Measurements obtained using a 30 µm sieve were about 10% higher but the nature of these agricultural soil dictated the use of a 45 µm sieve. Therefore, the method used underestimated soil hyphal length. Comparisons of treatment effects on hyphal abundance were made assuming that the % error was the same in all samples.

The hyphae recovered from two of the four sub-samples were stained by flooding the filter with a 0.2% aqueous solution of acid fuchsin for a minute. The excess of stain was removed by washing with deionized water and by vacuum-filtration. The length of the recovered hyphae was measured using the modified grid line intersect method (Tennant 1975).

The hyphae recovered from the two other sub-samples were stained by flooding the filters with a solution containing equal parts of iodonitrotetrazolium (INT) (1 mg ml^{-1}), reduced nicotinamide adenine dinucleotide (NADH) (3 mg ml^{-1}) and 0.2 M tris buffer at pH 7.4 (Sylvia 1988). This vital stain detects dehydrogenase activity. The filters were incubated for 12-16 hours at room temperature. The length of stained and, therefore, metabolically active hyphae was also measured by the modified grid line intersect method. A dissecting microscope with 50 x magnification was used to measure the hyphae.

Statistical analysis

Statistical analysis was done using general linear model (GLM) procedure in the SAS software package (SAS Institute, 1990). When the analysis of variance showed significant ($P<0.05$) treatments effects, the protected Least Significant Difference (LSD) test was used to compare differences among treatment means. Repeated measures analysis of variance was used to detect differences in the variables at different depths. Correlation analysis was performed to test the relationship between spore number and total hyphal abundance, between mycorrhizal colonization and total hyphal density, and between mycorrhizal colonization and spore number.

RESULTS

The physical and chemical characteristics of the soil under the different tillage systems are shown in Table 1. Two unknown *Glomus* species, *G. mosseae* [(Nicol. & Gerd.) Gerd. & Trappe], *G. macrocarpum* (Tul. & Tul.), *S. rubiforme* (Gerd. & Trappe), and *G. aggregatum* (Schenck & Smith) were observed in NT soil. In CT soil only *G. mosseae*, *G.*

macrocarpum and *S. rubiforme* were found. The type of fertilization, with inorganic or organic fertilizer, did not influence the composition of the mycorrhizal population, mycorrhizal spores number, total hyphal length, metabolically active hyphal length, mycorrhizal colonization and proportion of viable hyphal length in the soil (data not shown).

In both CT and NT treatments, most of the hyphae and spores were recovered from the top 0-15 cm of soil (Fig. 1, 2 and 4). The length of total and metabolically active hyphae in the top 15 cm represented 84% and 87%, respectively, of the hyphal length measured in the entire CT profile, and 87 % and 90 % of the total measured in the NT profile. Seventy four percent and 76% of spores observed in CT and NT soil, respectively, were found in the top 15 cm. Total hyphal density, metabolically active hyphal density and spores density were declined markedly below 15 cm. Mycorrhizal colonization was also more extensive located in the upper 15 cm of the soil profile (Fig. 3). The largest differences in spore and hyphal abundance between CT and NT was found in the 0-5 cm soil layer where hyphae and spore density were, respectively, 40% and 50% lower under CT than under NT (Fig. 1, 2 and 4). The proportion of live hyphae decrease linearly with depth (Fig. 5) with no significant difference between NT and CT soils.

Correlation analysis showed that spore numbers and total hyphal length in all treatments were significantly correlated ($r = 0.80$) (Fig. 6). Density of total hyphae ($r=0.74$) and spores ($r=0.65$) were also significantly correlated with mycorrhizal root colonization (Fig. 7).

DISCUSSION

The abundance of mycelium and spores was positively correlated with mycorrhizal roots colonization, suggesting that most of the hyphae measured were mycorrhizal. It had also been shown in a controlled environment study that most of the hyphae recovered from a soil growing mycorrhizal plants were mycorrhizal hyphae (Kabir et al. 1996). Densities of total and viable hyphae and density of spores measured at the grain filling stage of corn crop differed markedly between NT and CT only in the 0-5 cm soil depth. Below 5 cm, the amount

and distribution patterns of spores and hyphae were very similar under both tillage systems, i.e. the AMF were abundance up to 15 cm and thereafter their abundance decreased sharply. Smith (1978) also observed a similar pattern of distribution of AMF spores in wheat growing soil under both no-till and conventional tillage in Australia.

Two factors may explain the lower density of AMF spores and hyphae in the top 5 cm of the conventionally tilled soil: the direct impact of tillage on mycelium integrity or the indirect effect of tillage on the quality of the soil environment which is particularly marked in the surface layer of the soil. The soil under CT was more intensively disturbed at the 0-5 cm depth than at lower depths as this layer was also disked in the spring. The more intensive soil disturbance in the first 5 cm of CT soil may explain why spores and hyphae are less abundant in this zone, while the soil underneath might have not been disturbed intensively enough to create an impact on AMF abundance. Negative effects of soil disturbance on hyphal density are well documented (Jasper et al. 1989a, 1989b; McGonigle and Miller 1996). Negative effects of tillage on mycorrhizal sporulation were also observed by Douds et al. (1993).

The residue cover in the NT soil is a protection against sun rays, extreme temperatures and drying of the surface soil (Lal 1989). Mycorrhizal fungi may be negatively affected by the adverse physical conditions prevailing at the surface layer of tilled soils in the same way as other soil organisms are affected (Kirchner et al. 1993). Furthermore, organic debris and organic matter have been reported to increase the proliferation of AMF hyphae (Hepper and Warner 1983; St. John et al. 1983). The top 5 cm soil layer under NT contained 48% more organic mater than CT, (Table 1) and was, therefore, more favourable to AMF proliferation. The residue cover was not measure in this study but the effect of tillage systems on residue cover in corn fields is well documented (Cassel et al. 1995; Shelton et al. 1995). Mouldboard plowing incorporated corn residues within the entire soil profile, while under NT residues accumulate on soil surface where conditions are less favourable to decomposition.

In both tillage systems, it was shown that AMF abundance population drops in soil below 15 cm depth. Similar results have been obtained with AMF spores by Redhead (1977) in a survey in Nigerian soil. In a study involving soybeans grown on a silty loam soil, An et al. (1990) also observed that AM fungal spores were more abundant in the upper 15 cm of the

soil. We found most total hyphae, metabolically active hyphae and spores in the top 15 cm of soil in both tillage systems. Physical, chemical and gas properties of soil change with depth and influence the distribution of soil organisms. Fungi are particularly sensitive to the low partial pressure of oxygen which prevail in deeper soil layer (Brady and Weil 1996). The reduced quality of the soil environment most likely influence AMF (Bethlenfalvay et al. 1984). The reducing proportion of the hyphae which are viable with depth further support that lower soil layers are less favourable to AMF and may results in higher rate of mortality. It is also possible that the rate of decomposition of death hyphae is less in the deeper profile. In addition, AMF are also likely to be rare where root density is sparse (Anderson et al. 1987). Root density was not measured in this study, but corn root density is known to decrease below 15 cm (Mengel and Barber 1974).

Although several researchers have observed a positive correlation between AMF spore number and manure application (Harinikumar and Bagyaraj 1989; St. John et al. 1983), no significant difference was found in our study, after six years of treatment. In a low-organic-matter field soils, Harinikumar and Bagyaraj (1989) observed a positive effect of farmyard manure over inorganic fertilizers on AMF development. We did not observed such effects. Manure is a good source of P and high soil P levels often reduce AMF development (Abbott and Robson 1991).

Twelve years of tillage treatments had influenced the AM fungal population of the soil. We observed reduction of mycorrhizal species diversity under CT treatments. Our results suggest that agricultural practices may select AMF species with certain characteristics and exclude others. For example, soil disturbance created by tillage operation may select fast growing species that might be less mutualist rather than efficient species (Johnson and Pfleger 1992). Sieverding (1991) observed that *G. scintillans* was sporulating earlier and was able to produce more spores than other AMF species in plowed soil. The author reported that 75% of the spores in plowed soil was belonging to this species while *G. scintillans* accounted for only 5% of spores in the NT soil. Hamel et al. (1994) reported the disappearance of *Gigaspora margarita* and *G. caledonium* 3 years after plowing and putting an old field into cultivation.

Abundance of hyphae, spore and mycorrhizal colonization at the 0-15 cm soil depth indicates high mycorrhizal activity at this level. Our results therefore, suggest that deeper mouldboard plowing (>15 cm) will reduce AMF density by diluting mycorrhizal propagules to a greater volume of soil.

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Table 1. Initial physical and chemical properties of experimental soils

Soil depth (in cm)	Sand (%)	Silt (%)	pH in H_2O	O. M. (%)	Bulk density g cm^{-3}	P	K	Ca	Mg
						μg g^{-1}			
No-till									
0-5	57	25	6.26	5.45	1.18	167.5	262.6	1434	97.3
5-10	57	25	6.30	4.42	1.30	177.1	219.8	1088	78.7
10-15	57	25	6.03	4.47	1.28	144.8	215.4	1018	70.2
15-20	58	20	5.85	4.21	1.26	131.3	159.7	1025	53.1
20-25	59	21	6.05	4.03	1.33	96.3	135.4	1230	85.2
Conventional tillage									
0-5	63	21	6.18	3.68	1.01	120.1	143.2	1070	73.8
5-10	58	26	5.78	4.53	1.21	139.3	154.4	1137	85.3
10-15	59	23	5.65	4.60	1.30	162.5	180.2	1140	95.5
15-20	58	25	5.95	5.04	1.25	163.5	169.5	1090	79.5
20-25	58	26	6.16	4.15	1.28	113.8	158.6	1123	78.5

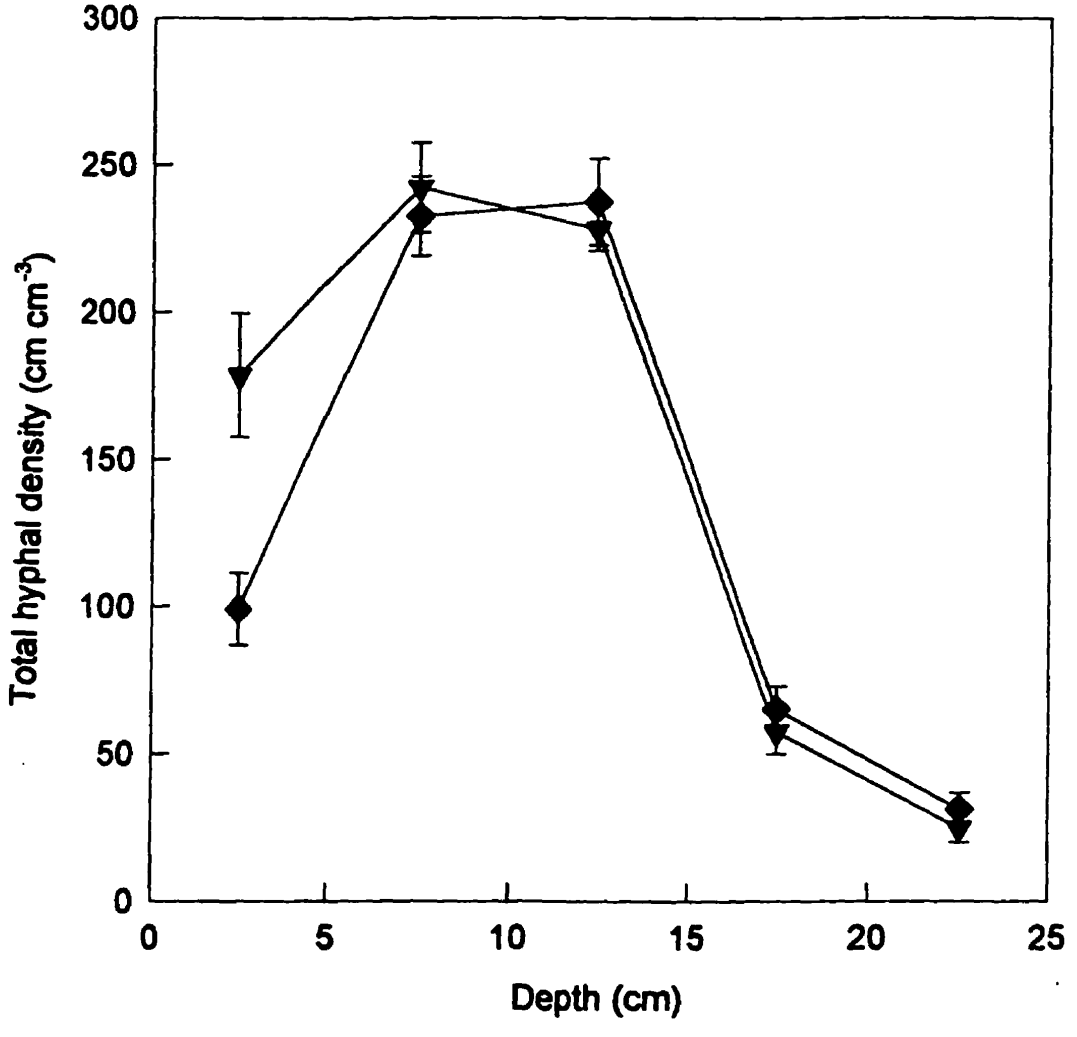

Figure 1. Total hyphal distribution in ▼ no-till (NT) and ◆ conventional tillage (CT) systems in a 25 cm deep soil profile. Error bars represent the standard error.

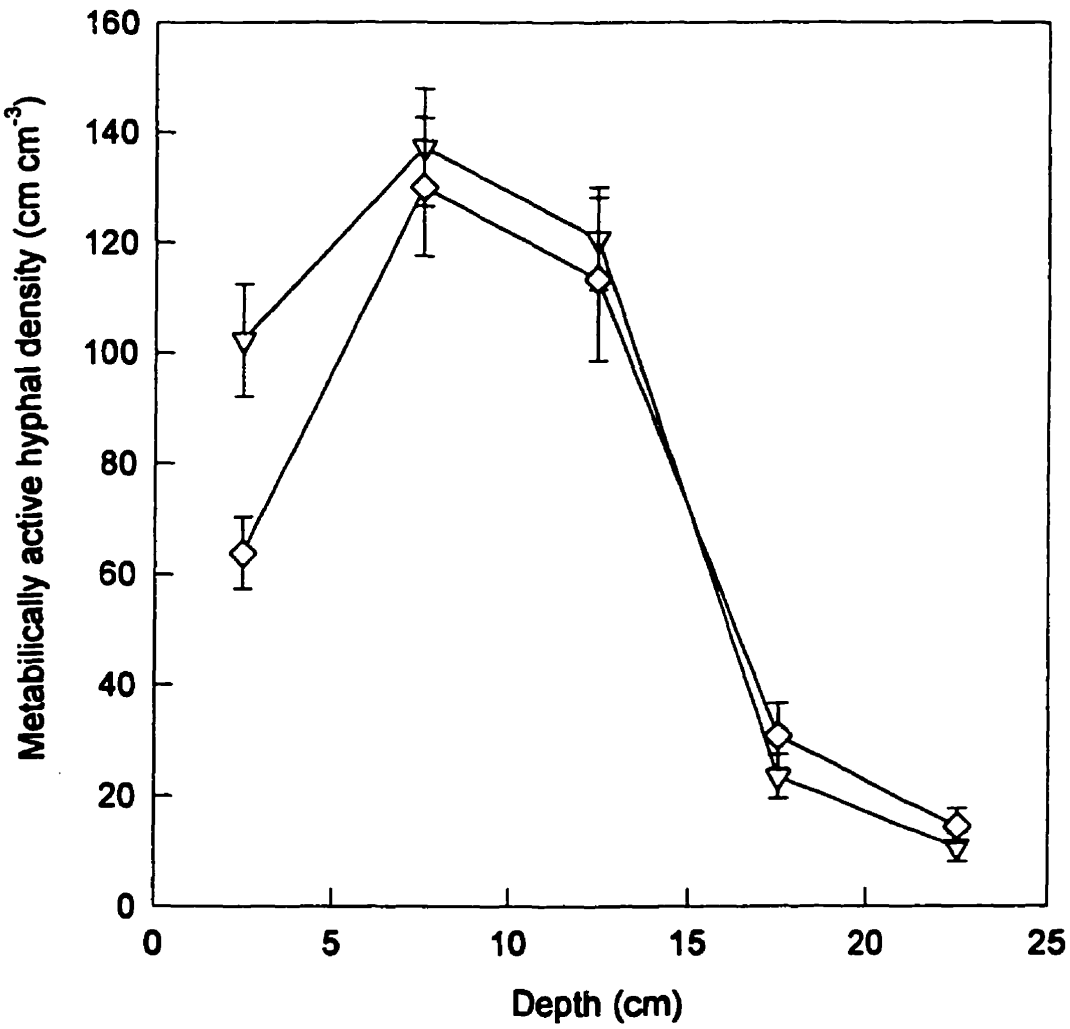

Figure 2. Metabolically active hyphal distribution in ∇ no-till (NT) and ◊ conventional tillage (CT) systems in a 25 cm deep soil profile. Error bars represent the standard error.

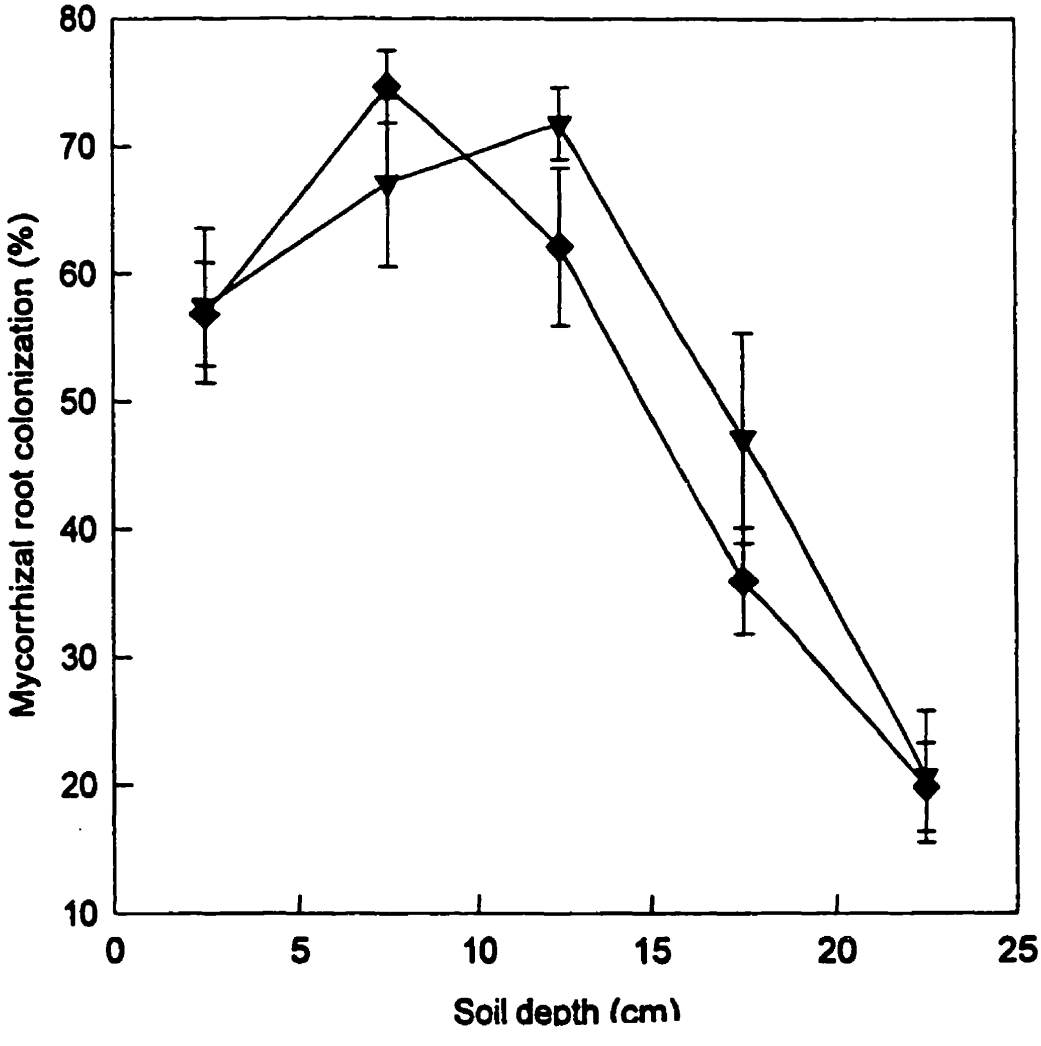

Figure 3. Mycorrhizal root colonization in ▼ no-till (NT) and ◆ conventional tillage (CT) systems in a 25 cm deep soil profile. Error bar represent the standard error

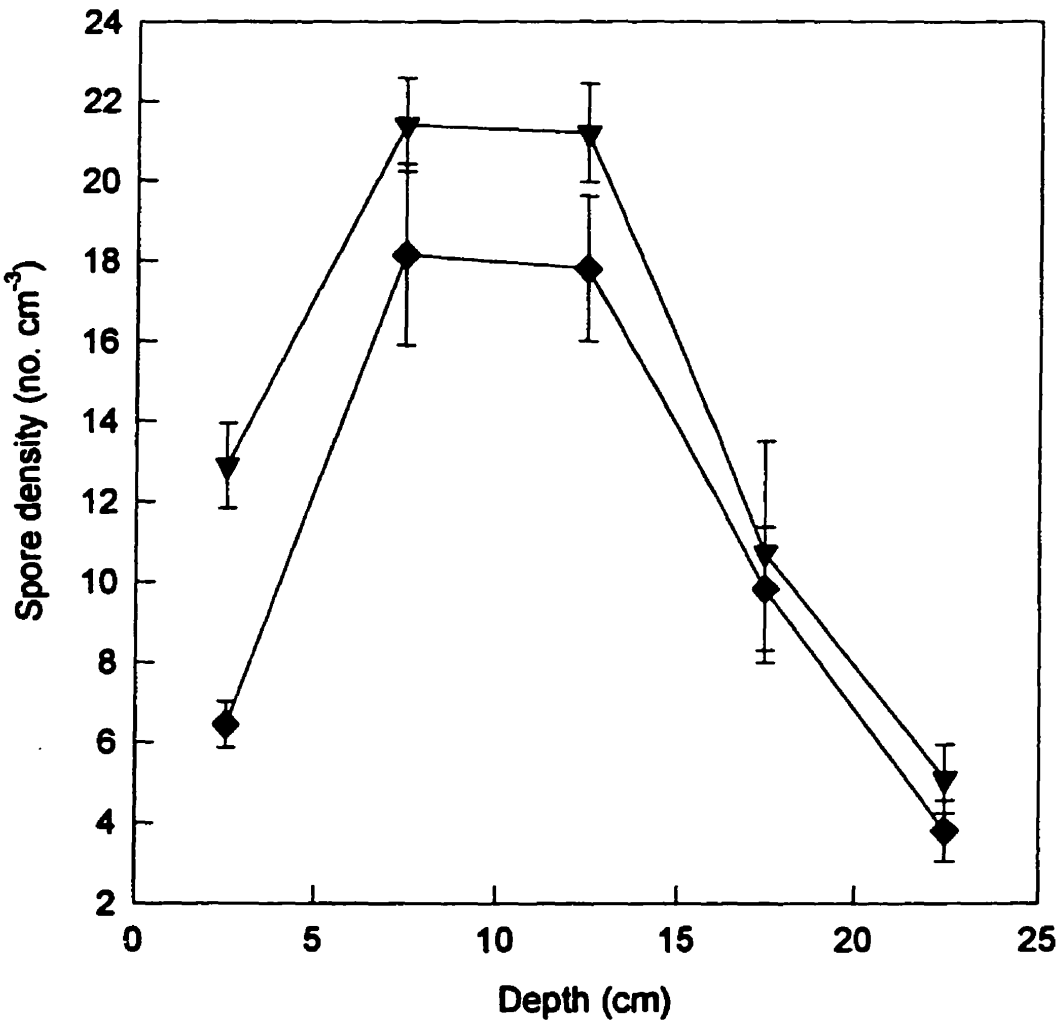

Figure 4. Mycorrhizal spore distribution in ▼ no-till (NT) and ◆ conventional tillage (CT) systems in a 25 cm deep soil profile. Error bars represent the standard error

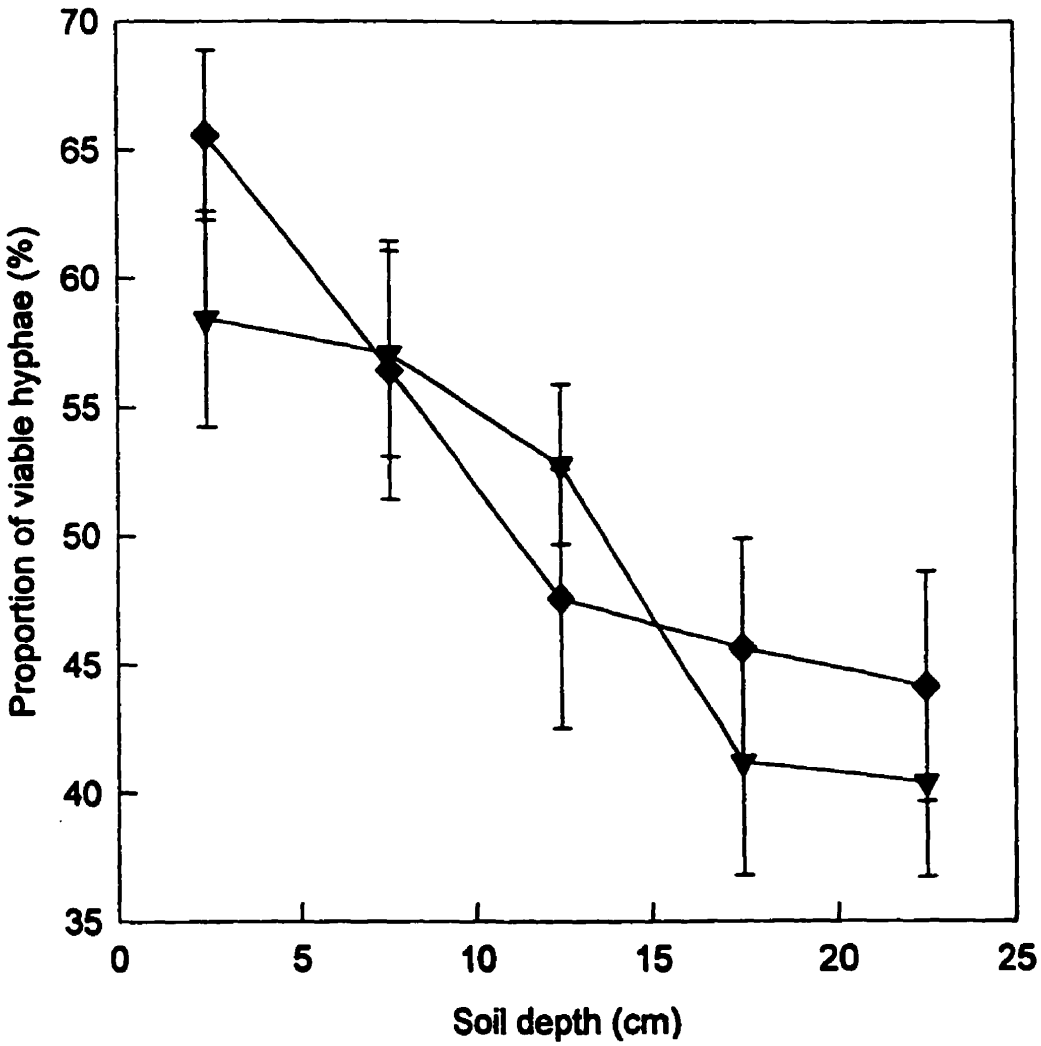

Figure 5. Influence of depth on the proportion of viable hyphae in ▼ no-till (NT) and ◆ conventional tillage (CT) systems in a 25 cm deep soil profile. Error bars represent the standard error

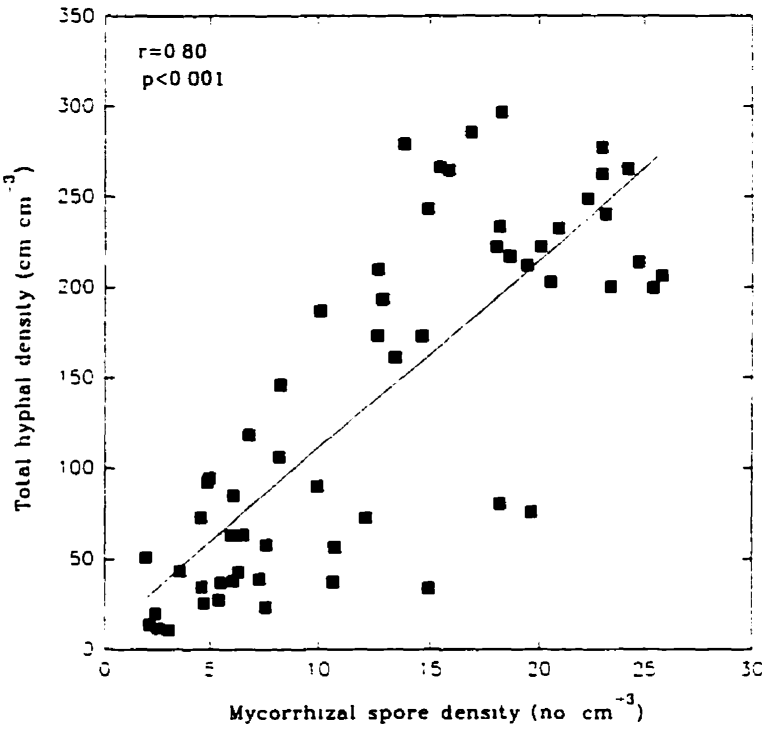

Figure 6. Correlation between the spore densities and the abundance of total hyphal density in different tillage systems and different soil depths

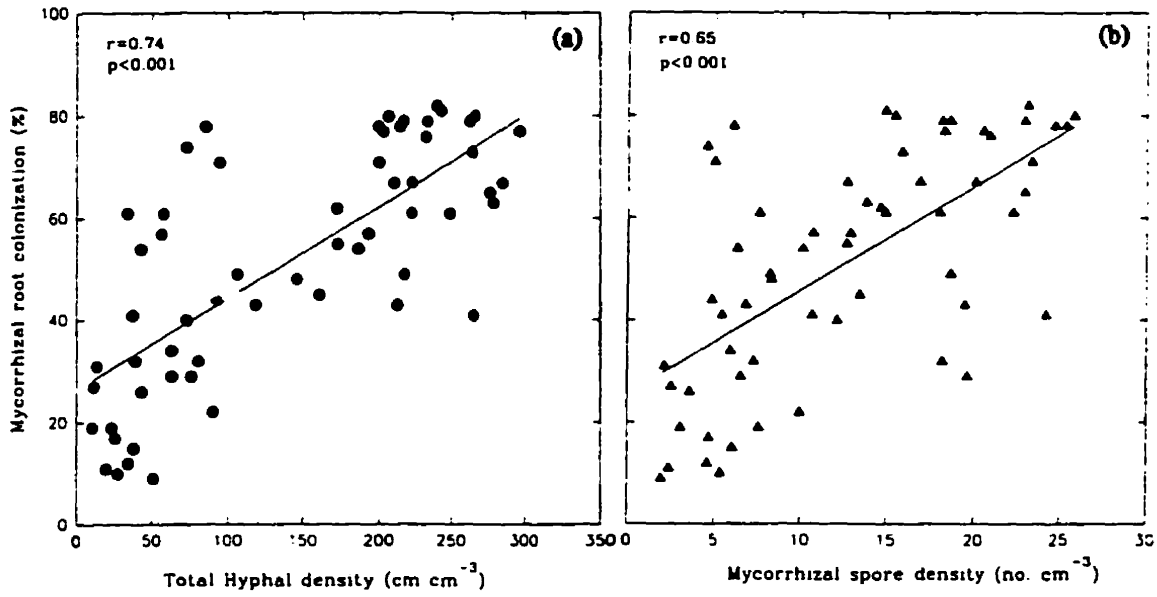

Figure 7. Correlation between mycorrhizal root colonization and spore (a), and total hyphal density (b) in different tillage systems and different soil depths.

CONNECTING PARAGRAPH VI

Chapter VII was taken from the manuscript by Kabir, O'Halloran and Hamel accepted for publication in the journal Mycorrhiza. The format of the text, therefore, conforms to the specification of the journal. In chapter IV, it has been observed that mycorrhizal hyphae can survive in the winter in agricultural soil. This observation led us to design an experiment investigating the mechanism of winter survival in field soil in the temperate region. The following chapter describes the impact of detachment of the hyphae from the host root on the winter survival of these hyphae.

CHAPTER VII

OVERWINTERING OF ARBUSCULAR MYCORRHIZAL FUNGI IS REDUCED BY DETACHMENT FROM CORN (*Zea mays* L.) ROOTS OR FROM THE MYCELIAL WEB.

Abstract

Soil disturbance adversely affects mycorrhizal fungi. The disturbance can result from disruption of fungal hyphae or from their detachment from host roots. The experiment was run in eastern Canada to investigated the survival of indigenous arbuscular mycorrhizal fungal (AMF) hyphae when they overwinter connected or detached from corn roots, and when submitted to three levels of soil disturbance. Soil filled pouches were buried in the root zone of a field grown corn crop when the plants were at the 10-12 leaf stage. Half of the pouches were permeable to roots (2-mm mesh) while the others (37-μm mesh) allowed the entry of hyphae only. Three soil disturbance treatments were applied to these pouches in the fall following crop harvest. They were: 1) no disturbance (UU), 2) disturbance outside but not inside the pouch (DU), and 3) disturbance inside and outside the pouch (DD). Hyphae abundance in the soil contained in the pouches was measured in the fall and in the spring after overwintering of the treated pouches at 3 cm below the surface. The abundance of total and metabolically active hyphae were determined. Total and metabolically active hyphae measured in the undisturbed control were 20% and 33% less abundant respectively in the spring than in the fall. This reduction was attributed to natural death occurring during the unfavourable conditions created by winter conditions. The proportion of live hyphae were more abundant in the coarse mesh pouches (2 mm) than in the fine mesh pouches (37 μm), suggesting that AMF hyphae survived winter better when they were close or attached to corn roots. In the spring AMF were more abundant and their metabolic activity was greater in soil that had not been disturbed. Abundance and metabolic activity of AMF hyphae was intermediate in the DU, indicating an effect of detachment from the soil web on AMF winter survival, and lowest in the DD treatment indicating a direct effect of disturbance on hyphal integrity and, possibly indirect effects through soil microbial activity or soil physics after mixing the soil in the pouches.

Key words: Arbuscular mycorrhizal fungi - soil disturbance - overwintering- survival - extraradical hyphae

Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with plants. They benefit plants mainly by facilitating their uptake of phosphorus and other nutrients (Evans and Miller 1988; Manjunath and Habte 1988; Rhodes and Gerdeman, 1975). The extraradical hyphae of AMF are commonly regarded as an efficient organ for nutrients uptake by plant, but their role as important source of inoculum in soils is often overlooked. However, among the other known AMF propagules, for instance extraradical hyphae, spores, vesicles and root fragments, extraradical hyphae might be the principal source of inoculum in soil (Brundrett et al. 1985; Read et al. 1976). We know that several AMF are capable of some free living growth (Bierman and Linderman 1983; Hepper and Warner 1983; St. John et. al. 1983; Warner and Mosse 1980) after the death of their host plant (Tommerup and Abbott 1981). Mycorrhizal colonization of crops can proceed quickly in undisturbed soil (Jasper et al. 1989 a, b,) but can be delayed if the soil is disturbed (Douds et al. 1995; Evans and Miller 1990; Jasper et al. 1989 a, b; Jasper et al. 1991). Such a delay in colonization is often accompanied by the reduction in both P uptake by plants and plant growth (McGonigle et al. 1990). Moreover, It has been suggested that the soil disturbance-induced reduction in mycorrhizal colonization was linked to the fragmentation of the AMF hyphal network in soil (Evans and Miller 1988; Fairchild and Miller 1988; O'Halloran et al. 1986). It clearly appears that hyphal fragments can act as infective propagules. A recent study (Addy et al. 1994) has shown that AMF hyphae detached from plant roots could survive winter and retain their infectivity even after exposure to winter conditions with a minimum temperature of -3.5° C. It is quite possible that breakage of the connections between old host root system and extraradical hyphae could affect their overwinter survival in agricultural fields which are commonly plowed prior to winter. It is not clear if the effect of soil disturbance on soil mycorrhizal potential results both from disruption of AMF hyphae and from their detachment from their food source, the roots. In an attempt to answer this question, we studied the impact of the detachment of extraradical hyphae from their mother roots, the impact of detachment from the soil mycelial web, and the impact of mechanical disruption of AMF hyphae on their survival overwinter. To investigate this question, soil filled pouches either permeable to root and hyphae or to hyphae only were

buried under a corn crop and, in the fall, different levels of soil disturbance were applied to the systems.

Materials and methods

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 % of organic carbon, 87 mg P, 114 mg K, 1565 mg Ca, 91 mg Mg, 3.3 mg Zn, 1.3 mg Cu per Kg of soil (Mehlich III extractable) and 55% sand; 28% silt and 17% clay. No phosphorus fertilizers had been applied in the experimental site since 1988. *G. mosseace* Gerdemann & Trappe, *G. aggregatum* Schenck & Smith, *G. macrocarpum* Tul. & Tul. *and G. rubiforme* Gerd. & Trappe, were forming the mycorrhizal community of this soil.

The experiment had a 3 by 2 factorial design with three levels of disturbance and two mesh sizes for the buried soil-containing pouches. Treatment combinations were randomized in five complete blocks. The mesh size treatments allowed the penetration and inclusion of either roots and hyphae (2-mm) or hyphae (37-μm) only, into the pouches. These were buried such that the top of the pouch was 3 cm below the surface and the bottom of the pouch was 18 cm below the surface, in the root zone of a field grown corn crop at the 10-12 leaf stage. The volume of the pouches was 424 cm^3. They contained 490 g of soil taken from the same field, which had been sieved to pass through 2mm-seive to remove debris. One pouch of each mesh size per block were collected just after the harvest of corn to measure hyphal density at time zero (referred to as fall values), and disturbance treatments were applied to the remaining pouches which were left in place over winter. The three disturbance treatments were applied as follows: The soil was 1) left undisturbed (UU), 2) was disturbed outside but not inside the pouch (DU) to detach pouch uptakes from the external network or 3) was disturbed both inside and outside the pouch (DD) to both detach and disrupt the pouch uptakes. These treated pouches were left in the field during winter and recovered in spring. The hyphae contained in the pouches were extracted and their abundance and metabolic activity were

measured. The comparison of the fall and spring values obtained within each mesh treatment was used to assess the effect of attachment of hyphae to the root and the attachment of hyphae to the soil network of root and mycelium on their survival over winter.

Hyphal extraction and measurement

The membrane filter technique was modified from Abbott et al. (1984) to extract extraradicular hyphae from soil samples taken from within the pouches. Each pouch uptake was thoroughly mixed and four 5 g subsamples were taken. Each subsample was placed into a blender with 300 ml deionized water and blended for 30 seconds to homogenize the soil suspension. This suspension was then poured through a 250-µm sieve. The hyphae were collected on a 45-µm sieve placed below the 250-µm sieve, after washing with water the upper sieve on which the soil suspension was poured. The recovered material was resuspended in water, transferred to a beaker, shaken for several seconds, and allowed to settle for 1 minute. The supernatant was filtered (pore size 11µm) under vacuum. The procedure was repeated 3 times on each subsample for a thorough extraction of soil hyphae. Some hyphae could pass through the 45 µm mesh sieve. The measurement obtained using a 30 µm mesh sieve were about 10 % higher, but soil type dictated the use of a 45 µm mesh seive.

The hyphae recovered from two of the four subsamples taken from each pouch were stained by flooding the filter with a 0.2% aqueous solution of acid fuchsin. The excess of stain was removed by washing with deionized water and vacuum-filtration. The length of the recovered hyphae was measured using the modified grid line intersect method (Tennant, 1975).

The hyphae recovered from the two other subsamples were stained by flooding the filters with a solution containing equal parts of iodonitrotetrazolium (INT) (1 mg ml^{-1}), nicotinamide adenine dinucleotide in the reduced form (NADH) (3 mg ml^{-1}) and 0.2 M tris buffer at pH 7.4 (Sylvia, 1988). This live stain detects dehydrogenase activity of hyphae. The filters were incubated for 12-16 hours at room temperature. The length of stained and,

therefore, metabolically active hyphae was also measured by the modified grid line intersect method.

Statistical analysis

Statistical analysis of the variance was performed using the general linear models (GLM) procedure in SAS (SAS, 1988). Protected Least Significant Difference (LSD) test was used to determine the significance at the 5% level of the differences between treatment means at the 5% level. The Proc Univariate procedure indicated that the data was normally distributed. T-test was used to compare the effects of hyphal density on fall values and spring UU treatment values.

Results

Analysis of variance is shown in Table 1. There was no difference between coarse-mesh and fine-mesh with respect to proliferation of hyphae within the pouches (Fig. 1). However, it appears that hyphae survived winter better in the presence of roots as shown by a greater ($P<0.05$) abundance of metabolically active hyphae in coarse-mesh pouches than in the fine-mesh pouches (Fig. 2) in the spring.

In between fall values and spring undisturbed (UU) treatments, the abundance of hyphae was significantly reduced from fall to spring (Fig. 1 and Fig. 2). In the spring, the abundance of hyphae was least following application of the DD treatment in the fall, intermediate following the DU treatment and greatest when no disturbance had been applied prior to overwintering.

The DU treatment accounted for about 34% and 49% of the reduction in abundance of total and metabolically active hyphae overwinter, while 67% and 77% of reduction in total and metabolically active hyphae was attributable to the DD treatment. About 20% and 33% reduction in the abundance of hyphae was attributable to the winter period alone in total and metabolically active hyphae respectively (Fig. 1 and Fig. 2). The proportion of live hyphae decreased with increasing intensity of soil disturbance (Fig. 3).

Discussion

The experiment was conducted to study the importance of attachment of the mycelium to roots and to the network of roots and mycelia in soil, on the winter survival of AMF hyphae under field conditions. Our results show that AMF hyphae survived better when close or attached to corn roots. The proportion of live hyphae was consistently higher when roots were also present in the soil of the pouches. Our results do not reveal the cause of this observed improved survival when roots are present, but we hypothesize that plant assimilate concentrations are higher near the roots and decreases with distance as they are utilized by more proximal zones of the mycelium. Larger food reserves in hyphae near the roots may facilitate their survival.

It has been shown recently that AMF hyphae have the ability to survive in frozen soil even when they are on their own and that they can maintain their infectivity over winter (Addy et al. 1994). Addy et al. (1994) found shrunken beads of cytoplasm in AMF hyphal tubes extracted from frozen soil, and suggested that hyphae survive stresses associated with freezing (such as dehydration) by forming "resting hyphae". The potentially low level of host assimilates in the cytoplasm beads of the distal region of the cytoplasm as compared to the region close to the root could be insufficient to permit winter survival and recovery of activity in spring or may act as antifreeze.

Nicolson (1959) showed that oil globules within the cytoplasm of thick walled hyphae of AMF could be the possible nutrient storage available for the extraradical hyphae to survive in absence of living plants. The lipid rich AMF vesicles, when found in roots, might also supply energy to attached hyphae (Bierman and Linderman 1983; Millner 1991), and in this way, improve their overall strength and their survival during winter.

While these hypotheses are still a matter of speculation, our data show that AMF hyphae benefit from being attached to roots, and emphasize the importance of the host plant contribution to AMF propagule vigour. They also clearly indicate that attachment is not a necessary condition for the metabolic activity of hyphae to be retained, as about 34% of the detached hyphae stained with INT.

Disturbance had a striking effect on hyphal metabolic activity in our experiment. The percentage of viable hyphae found in the soil in spring was markedly reduced by soil disturbance and went from 64% in undisturbed soil, to 44% for the maximum disturbance treatment indicating a reduced resistance to winter in disturbed soil. In this study, the destruction of the extraradical hyphae through soil disturbance (DD) was so important that the difference in survival attributed to the presence or absence of host root system became negligible in disturbed systems. Our findings explain results such as those of Evans and Miller (1990), who observed higher corn shoot P and Zn concentrations and percentages of root infection when host root system attached with the extraradical hyphae.

The reduction in total hyphal density in spring in the disturbed treatments indicates that the hyphae killed in the fall by these treatments had already disappeared. This suggest that measurements of total hyphal length alone, would have provide a good assessment of the mycorrhizal status of the soil.

Our results have shown that extraradical AMF hyphae can survive winter in agricultural soil, even when they are not attached to roots, but that their survival is improved by attachment to roots and most importantly to the soil network of roots and hyphae. The disruptive effect of soil disturbance was also found to severely reduce winter survival of AMF hyphae in field conditions. Survival of AMF extraradical hyphae over winter is important under cool climates which limit crop production to only few months of the year. Disturbance of the soil by fall plowing probably physically disrupts the root hyphal network. Our results show that both detachment from the web and disruption of mycorrhizal hyphae in the fall reduces their resistance to winter and, in this way, explain the previously observed reduction in the soil mycorrhizal potential of fields plowed in the Fall (McGonigle et al. 1990; McGonigle and Miller 1993; O'Halloran et al. 1986).

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Table 1. Analysis of variance for total and metabolically active hyphal density, and proportion of viable hyphae

Source	df	Probability of F value		
		Total hyphae	Metabolically active hyphae	Proportion of viable hyphae
Pouch	12	0.19	0.035*	0.106
Treatment	2	0.0001***	0.0001***	0.004**
Pouch x treatment		0.38	0.24	0.93

*, ** and *** significant at the 0.05, 0.01 and 0.001 probability level respectively

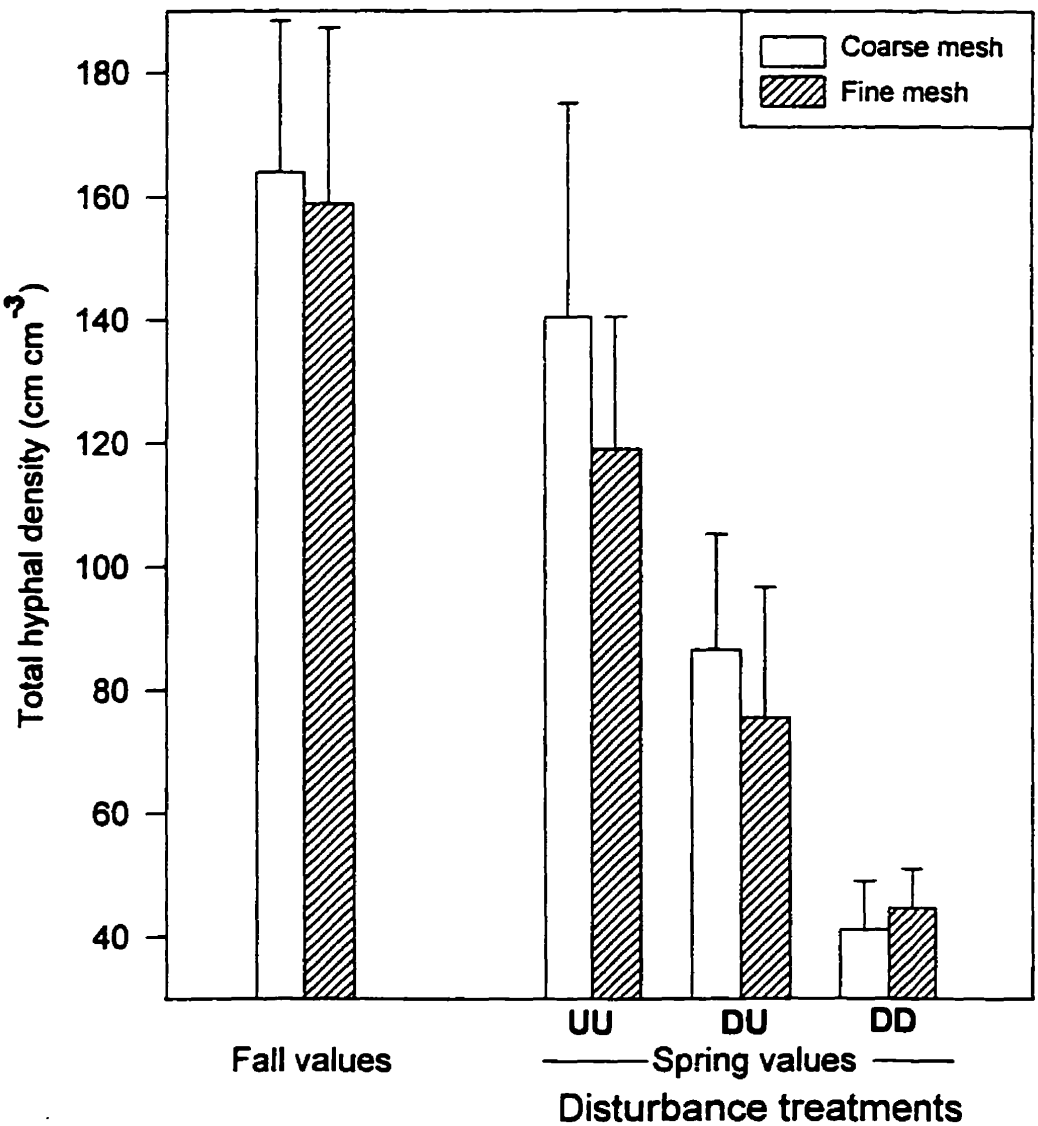


Figure 1. Total hyphal density in soil within the coarse-mesh and fine-mesh pouches before winter in fall or in spring after having received various disturbance treatments prior to overwintering. The disturbance treatments: "UU" no disturbance, "DU" the soil was disturbed outside of the pouch but not inside, "DD" the soil was disturbed both inside and outside the pouch. Vertical lines represent sd (n=5) of each point.

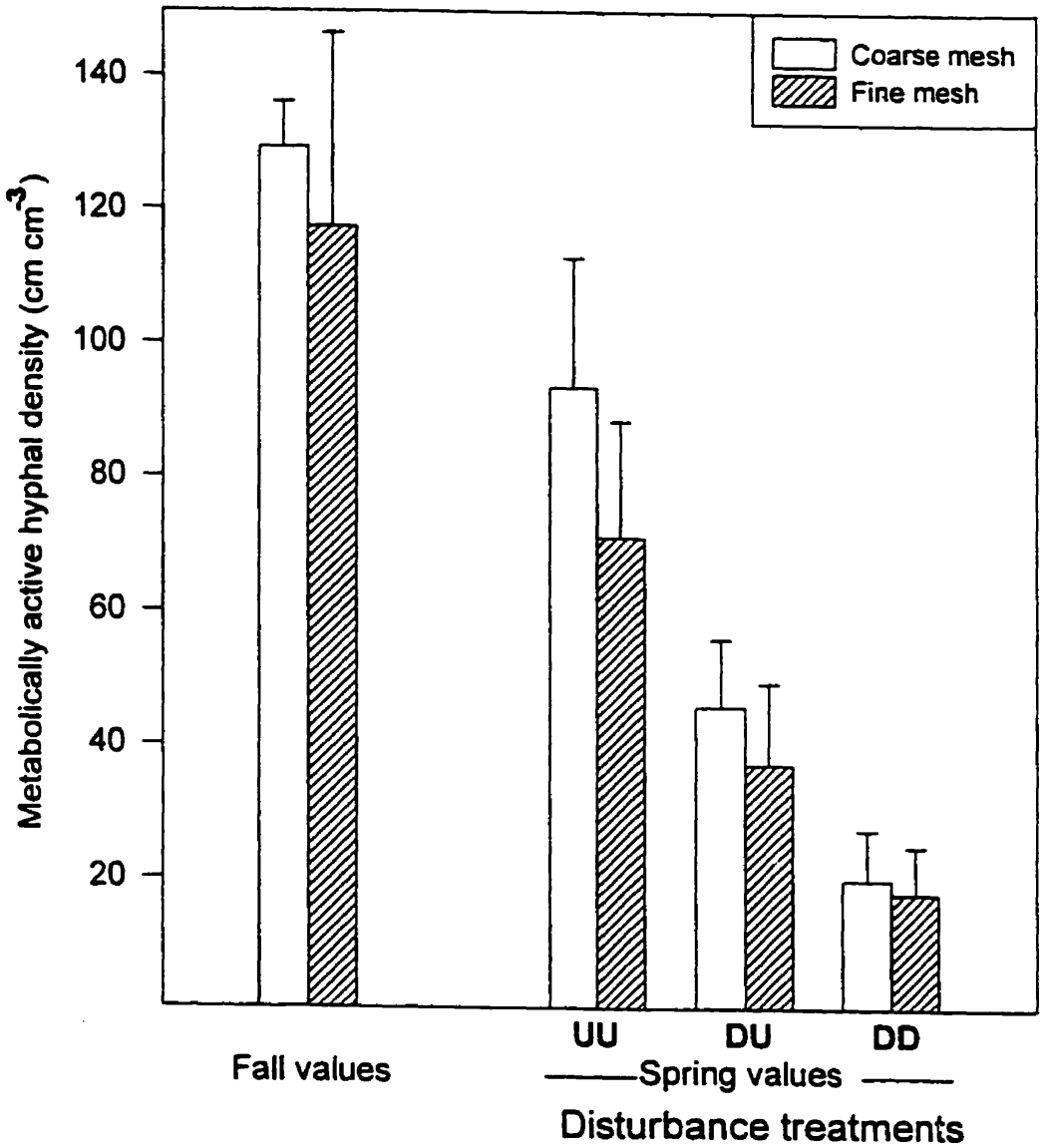


Figure 2. Metabolically active hyphal density within the coarse-mesh and fine-mesh pouches before winter in fall or in spring after having received various disturbance treatments prior to overwintering. Vertical lines represent sd (n=5) of each point. Abbreviations for disturbance treatments are as in figure 1.

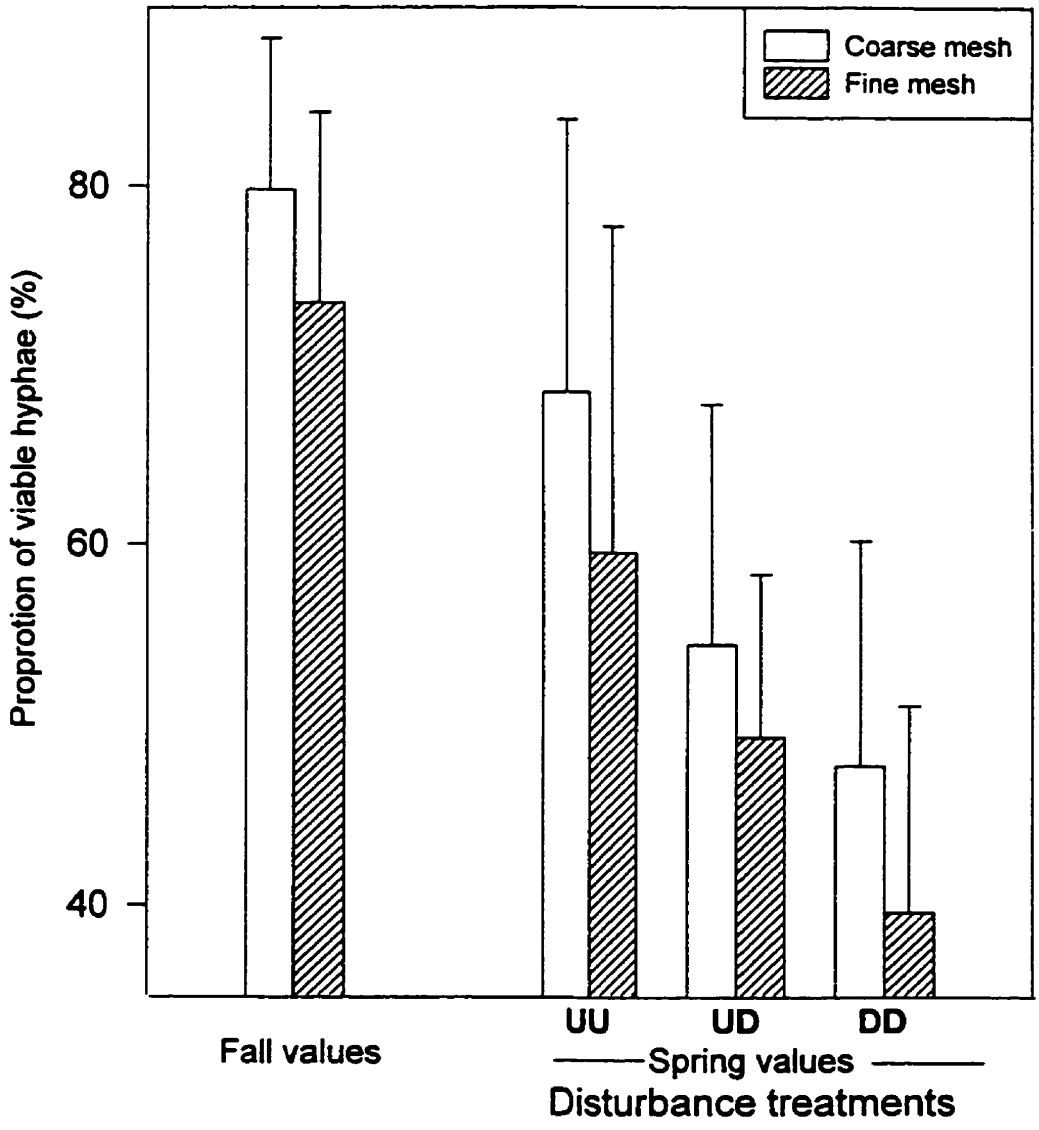


Figure 3. Proportion of viable hyphae in soil within the coarse-mesh and fine-mesh pouches before winter in fall or in spring after having received various disturbance treatments prior to overwintering. Vertical lines represent sd (n=5) of each point. Abbreviations for disturbance treatments are as in figure 1.

CHAPTER VIII

GENERAL CONCLUSION

GENERAL CONCLUSIONS

The study of AMF extraradical mycelium has been delayed by the lack of a reliable method to recognize AMF hyphae from those of saprophytes. Using non-sterilized field soil we have shown that, under a controlled environment, most of the hyphae recovered from the root soil volume of mycorrhizal plant were AMF hyphae. Very little hyphal growth was found associated with the root zone of non-mycorrhizal plants, and mycelia proliferation was not stimulated by addition of organic residues to the soil.

After fall tillage, AMF must survive several months before being able to associate again with the subsequent host plant. The effect of soil disturbance on the ability of AMF to survive fallow periods of up to 90 days was tested. It was found that both soil disturbance and length of fallow period had a negative effect and the combination of these treatments was more detrimental to AMF survival than the sum of their single effects. Only 14% of AMF hyphae could survive 90 days of fallow in previously disturbed soil, but their survival was much (43%) better in undisturbed soil.

Seasonal variation in the abundance of mycorrhizal hyphae and intensity of mycorrhizal colonization of corn plants was observed. The lowest hyphal densities were found in the spring. The abundance of soil hyphae and the proportion of viable hyphae increased with corn plant development up to the silking stage and declined thereafter. The dynamics of mycorrhizal root colonization had the same pattern as hyphal abundance in the soil.

Conventional tillage (CT) reduced the abundance of mycorrhizal hyphae in the spring and slowed the mycorrhizal development of the subsequent crop. Reduced tillage (RT) had a smaller negative impact than CT on the abundance of soil hyphae and mycorrhizal colonization. The impact of tillage on AMF abundance was larger in the spring, persisted up to the silking stage, but disappeared at harvest. Corn plant P and sometimes Zn and Cu concentrations were higher under no-till (NT) and RT than under CT system. The different corn nutrient levels associated with tillage practices were closely related to differences in hyphal densities in the soil. Liquid dairy manure sometimes increased AMF hyphal growth but had little effect overall.

The diversity of the AMF community decreased under the CT system. After 12 years of conventional tillage treatments only three AMF species *G. mosseae*, *G. macrocarpum* and *G. rubiforme* were present in soil while under the NT system six species were found namely *G.mosseae*, *G.macrocarpum*, *G. aggregatum*, *G. rubiforme* and two unknown *Glomus* species. Tillage practices possibly exerted a selective pressure on the AMF community. It remains to be seen whether stress-resistant or fast-growing species, or both, are dominant in CT soils.

Spatial and temporal distributions of mycorrhizal hyphae were related to corn plant distribution and development. Maximum hyphal density was found on the corn rows and decreased linearly with distance from the row. Fluctuations in hyphal abundance were more marked on the row and were not significant on the quarter-row suggesting that AMF are concentrated under the row and are very scarce between the rows where saprophytes may dominate.

Arbuscular mycorrhizal fungal mycelium and spores were more abundant in the upper 15 cm of the soil profile indicating that deep moldboard plowing will dilute AMF propagules into a larger soil volume, thus reducing their density in the seeding zone. Densities of hyphae and spores in the first 5 cm of the profile were significantly higher under NT soil than under CT soil. Higher intensity of soil disturbance was associated with lower organic matter uptake (48% less). Low organic matter level and the absence of residue to protect the soil against abrupt variation in temperature and moisture in the upper 5 cm of CT soil, may explain the lower AMF abundance in CT soil. The abundance of hyphae and spores were consistently correlated with mycorrhizal root colonization, thus suggesting that either mycorrhizal spores or hyphae could be used as indicators of AMF abundance, at least within the conditions of our study.

Arbuscular mycorrhizal hyphae had the ability to survive winter relatively well (67% survival in spring), especially when hyphae were attached to dead corn roots. Food reserves present in the root vesicles or in root tissues may facilitate the survival of extraradical hyphae. The negative impact of soil tillage in the fall was so large that the positive impact of attachment to roots on overwintering hyphae became insignificant.

Contribution to knowledge

The research presented in this thesis has answered several questions relative to the basic biology of AMF and to ecological aspects of the symbiosis in corn fields. This research contributed to the basic knowledge needed by agronomists to exploit the potential offered by AMF in their quest for sustainable agricultural production.

Original contributions to knowledge include:

1. The impossibility of differentiating with certainty, mycorrhizal hyphae from hyphae of other fungi was a serious impediment to the study of extraradical hyphae. In the present research, it was demonstrated that the hyphae in the rooting zone of mycorrhizal plants are almost entirely mycorrhizal and, therefore, the measurement of hyphae extracted from this zone represented an acceptable estimation of AMF density.

2. All the previously published research on the effect of soil disturbance on corn P nutrition used indirect approaches to conclude that soil disturbance disrupt mycorrhizal mycelium. Using a direct approach, we confirmed this detrimental impact of soil disturbance on AMF hyphae.

3. We further demonstrated that the combination of soil disturbance and fallow, which is common in agricultural fields of cool temperate climates where several winter months separates two crops, had a much larger impact that soil disturbance alone. We found that these two factors had an additive effect in reducing soil mycorrhizal potential.

4. The spatial distribution of AMF hyphae across rows in the soil profile of an agricultural field was described for the first time.

5. The impact of different tillage intensities on vertical distribution of AMF hyphae, spores and mycorrhizal root colonization was described.

6. Seasonal variation in distribution of hyphae on and between the rows in corn field were observed for the first time. These results on AMF spatial distribution provide very valuable information for the management of AMF in sustainable agricultural production.

7. Seasonal variation in the development of the mycorrhizal symbiosis of field grown corn submitted to different soil management systems was revealed.

8. The demonstration of the impact of tillage on the mycorrhizal symbiosis of corn in systems at equilibrium (after 12 years of treatments) is unique.

9. The negative influence of detachment of AMF hyphae from dead host root on overwintering of hyphae was first shown. The relative importance of detachment from host root was also evaluated.

Direction of future research

This research has answered significant questions regarding the behavior of indigenous AMF mycelium in corn field soils, the development of the symbiosis under different soil conditions, and the survival of AMF hyphae over the winter, but also raised questions. The following research should be done to better understand this system.

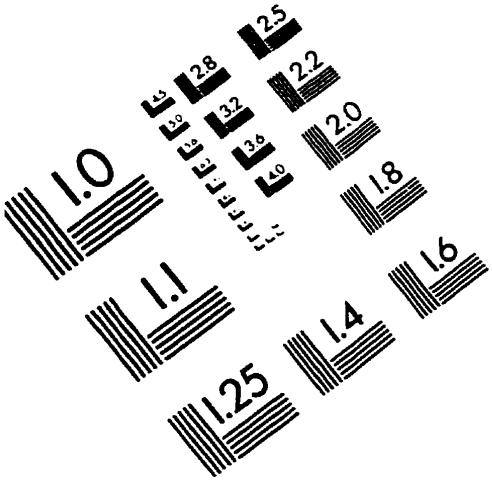
1. Reliable methods for quantification of hyphae in soil need to be developed. Such methods could be based on DNA finger printing or could involve the further refinement of the method used in this research. A computer-based image analysis system could also facilitate data collection.

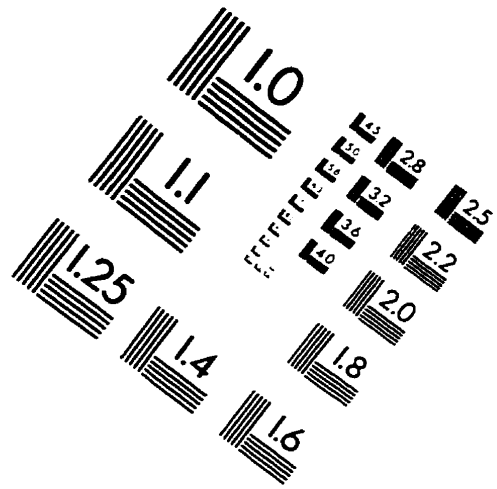
2. Experiments in a controlled environment demonstrated that the combination of fallow and soil disturbance interacted negatively on survival, mycorrhizal development, plant nutrient uptake and plant growth. More research is needed to define the impact of soil disturbance and fallow interaction in field crop production where winter conditions may also influence AMF survival and infectivity in spring.

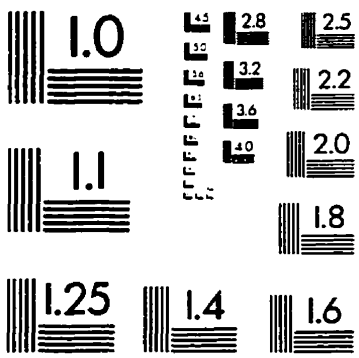
3. Seasonal variations in the abundance of mycorrhizal hyphae, root colonization and nutrient concentrations of corn were derived from few sampling dates. A more frequent sampling schedule would provide a more precise picture of mycorrhizal development.

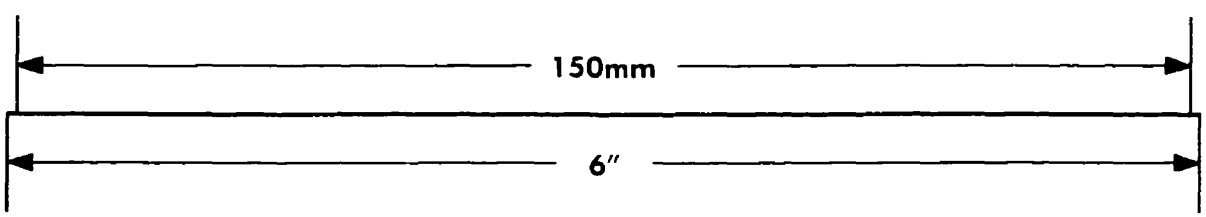
4. The impact of tillage practices had induced change in the composition of AMF populations. It would be interesting to see if the AMF populations in other monoculture corn field under conventional or no tillage are often composed of the same species. It would also be interesting to evaluate these species for symbiotic efficiency.

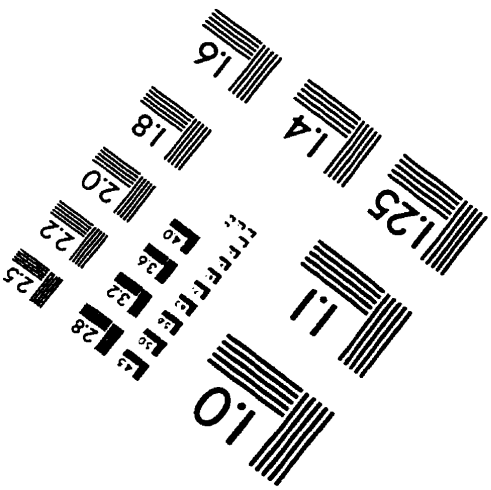
IMAGE EVALUATION TEST TARGET (QA-3)

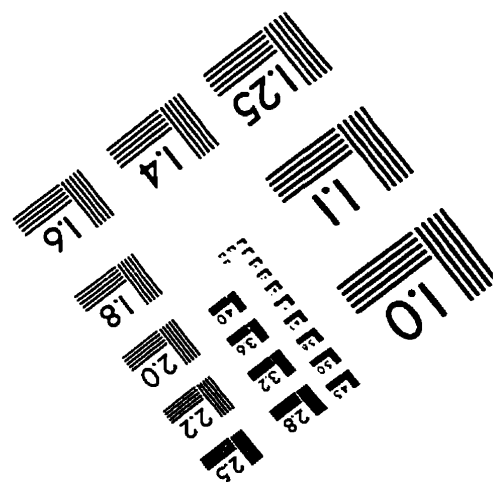












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989